To the dissertation council

D 220.062.02 at FGBOU VO

"Stavropol State

Agrarian University"

Review for the dissertation of Evgeniya Yurevna Kirichenko "ROLE OF GAP JUNCTIONS AND PROTEIN-CONNEXINS IN NEURO-GLIAL AND NEURO-GLIO-VASCULAR INTERACTIONS IN THE THALAMOCORTICAL SYSTEM OF THE RAT'S BRAIN" submitted for the degree of Doctor of Biological Sciences, 06.02.01 - diagnosis of diseases and therapy animals, pathology, oncology and morphology of animals

Brain is a unique organ because a huge number of cells composing it communicate with one another through complicated but well-organized network that transmits signals, whereby brain can process information to execute appropriate behaviors. The cells involved in this brain activity are categorized into two groups, neurons and glia, and recent studies have shown that not only neurons but also glia can transmit signals. Specialized apparatus to establish connection between neurons is called synapse. Decades of studies have already built up detailed knowledge of the structure and function of synapses. However, most of these studies have investigated synapses of one type, the chemical synapse. Actually, brain also uses another type, the electrical synapse, which is formed by a specialized structure called gap junction. Neurons in many brain areas are connected through both chemical synapses and gap junctions, but the contribution of gap junctions to brain activities remains poorly understood. This is because detection of gap junctions requires very sophisticated morphological techniques as compared to much easier detection of chemical synapses. Recent works also demonstrate signal transmission among glial cells, and this communication is conducted by glia-specific gap junctions. The author has been exploring the role of gap junctions for many years using morphological approaches of high quality, and she has already made many important findings as fully described in the dessertation.

First, as an initial approach to reveal fundamental structure of the brain regions of interest, the author analyzed morphological characteristics of the somatosensory cortex and VPM nucleus of the thalamus using different antibodies that included those not investigated previously in these regions, such as antineurofilaments and anti-synaptophysin antibodies. This resulted in the objective discrimination of the internal structure such as barrel walls and barrel center. Another novel finding was about the non-uniform distribution of different antigens in barrels. These results facilitate understanding of the functional segregation inside barrels, the knowledge of which was further combined with subsequent analysis in the electron microscopy (EM).

Next, the author clarified specific distribution patterns of parvalbumin (PV)-positive interneurons in both barrel cortex and thalamus at the light microscopic level. PV neurons are the representative of neurons that are connected with each other through neuron-specific gap junctions. Visualization of somata, dendrites and axon terminals of PV neurons with differential patterns in each region was clearly demonstrated with sound immunohistochemical techniques.

Then, the author examined labeling patterns of connexin proteins that constitute gap junctions. Three types of connexins with different molecular weights corresponding to neuronal gap junctions (connexin 36) and glial gap junctions (connexin 43 and connexin 30) showed differential distributions at both cortex and thalamus. Of particular importance was the expression of connexin 43 as large granules associated with blood capillaries in the cortex and thalamus, suggesting the occurrence of perivascular chains of astrocytes coupled by gap junctions. Based on this observation, the author hypothesized the astrocyte-driven regulation of local blood flow in response to local neuronal activity. Another novel finding was the three-dimensional glial syncytia that corresponded to the boundaries of barrels as well as isolated module of the mouthalamic nuclei, leading to the intriguing possibility that gap junction-coupled syncytia of astrocytes contribute to the regulation of ensembles of neurons. The author further provided evidence for heterogeneity in the distribution patterns of connexin 43 and 30 in astrocytes.

As the most important part of the study, the author accomplished ultrastructural analysis of gap junctions of both neuron to neuron and glia to glia connectivity. As a morphologist in the same research field, the reviewer knows how difficult it is to acquire unambiguous ultrastructural evidence for the existence of gap junctions in EM. In this respect, the author succeeded in demonstrating gap junctions with high quality electron micrographs. The author provided several new findings in the structural features of gap junctions in the somatosensory cortex and thalamus. First, the author discovered gap junctions formed between axon and other neuronal or glial element. Second, gap junction-coupled complex of astrocytes formed between three to four thin processes of astrocytes in close vicinity to axon terminals, shown also by immunoelectron microscopy for connexin 43. This led to the formulation of a new structure represented as a "three-part synapse, consisting of astroglial processes with gap junction localizing around chemical synapses". The idea of local modulation of synaptic activities by astrocytic processes in close vicinity has been recently established, and the author further extended this concept by introducing the involvement of dense connectivity through gap junctions among multiple astrocytic processes that encapsulate the synaptic site. Importantly, gap junctions are not merely to be junctions but they allow passages of small molecules and ions, both of which can promote signal transmission through connected astrocytes. Moreover, this local phenomenon can spread broadly through gap junction-mediated "panglial syncytium", which is another important structure newly found by the author based upon both light and electron microscopic observations. Finally, the author also suggested that glial gap junctions may be one of critical components of the blood brain barrier.

In summary, the author's research into gap junctions in the brain has made a major contribution to the field. The sustained work by the author will further promote understanding of the brain in health and disease.

Head of Department of Anatomy

and Neurobiology, professor, MD, PhD

Takaichi Fukuda

Takaichi Fukuda

電子署名者 : Takaichi Fukuda
DN : cn=Takaichi Fukuda, o=Kumamoto University, Faculty of Life Sciences, ou=Department of Anatomy and Neurobiology, email=tfukuda@kumamoto-u.ac.jp, c=JP
日付 : 2021.04.01 15:35:34 +09'00'

I certify the signature of Professor T. Fukuda:

March 26, 2021

Faculty of Life Sciences, Kumamoto University

Address: 1-1-1 Honjo, Chuo-ku, Kumamoto, 860-8556 Japan

E-mail: tfukuda@kumamoto-u.ac.jp

Phone: +81-96-373-5038