Japan Academy Prize to:

Shigeo Okabe
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for "Studies on the Mechanisms of Neural Circuit Formation through the Development of Imaging Techniques"

Outline of the work:

The development of human intelligence, personality, and emotion depends on the complex organization of neural circuits in the brain. Understanding the molecular mechanisms of neural circuit development is one of the crucial challenges in neuroscience. To investigate how neural circuits form, Dr. Shigeo Okabe examined the development of both axonal structures and synapses, which are essential components for neural circuit function. He established new imaging techniques to visualize the formation of these structures, applied them to neural circuit development, and proposed new models for axon and synapse formation.

Dr. Okabe initially developed an imaging technique to visualize dynamic changes in the cytoskeleton, which is an intracellular polymer system that is crucial for the mechanical support of axon elongation. Employing a microinjection method, he introduced fluorescently labeled cytoskeletal proteins into cells to facilitate live fluorescence imaging. Before Dr. Okabe's work, the prevailing theory indicated that the cytoskeleton was already highly stabilized within axons and transported as an interconnected polymeric structure. In contrast, Dr. Okabe has demonstrated that the cytoskeleton is actively polymerized and depolymerized within axons. The continuously formed cytoskeleton at the axon tip caused axonal elongation. The results of this study, which quantitatively revealed the intracellular dynamics of all three cytoskeleton types (microtubules, actin filaments, and neurofilaments) using fluorescent probe-based imaging techniques, have opened a new research field focused on cytoskeletal dynamics in neurons.

Dr. Okabe then recognized the potential of the fluorescent protein green fluorescent protein (GFP) for live-imaging and successfully developed a completely new technique for visualizing synapses using molecules that accumulate at these sites. Before Dr. Okabe introduced this new technique, synapse labeling was performed by tagging transmitter receptors with antibodies or fluorescence labeling of synaptic vesicles through the endocytotic process. However, these techniques could only track synapses for a short period, and a new method was required to monitor long-term changes in synapses. Dr. Okabe revealed that a GFP fusion of PSD-95, which is a protein that accumulates in the

postsynaptic density (PSD), can be expressed in neurons to identify postsynaptic sites and facilitate their long-term live-imaging. The quantitative data obtained through the GFP-based synapse live-imaging technique indicated that many of the newly formed synapses are eliminated, whereas only a subset of synapses is selectively stabilized. These results challenges the established theory indicating that newly formed synapses are highly stable and do not disappear. This compelling illustration of the rapid process of synapse formation and elimination has revolutionized our understanding of synapse development.

Dr. Okabe applied his developed imaging technology to various neuron types, reporting on the cell type-specific mechanism of synapse formation. The synapse formation process significantly differs between excitatory and inhibitory neurons, with specific changes in the membrane adhesions occurring when the synapses are stabilized. Further, he developed a new technique for measuring synaptic function, particularly a methodology for estimating the absolute number of PSD-95 and other scaffolding molecules according to the fluorescence intensity of a single GFP molecule. This method has indicated that approximately several hundred molecules accumulate in PSDs with diameters of several hundred nanometers, which provide scaffolds for retaining neurotransmitter receptors and other functional molecules.

Moreover, synapse imaging is valuable for investigating the pathophysiology of neuropsychiatric disorders. Human genome studies have revealed that the risk genes for autism spectrum disorders (ASDs) include synaptic cell adhesion molecules and PSD scaffolding proteins. Hence, the significance of analyzing synaptic pathology has gained increasing recognition. Dr. Okabe has conducted synapse imaging employing various mouse models of ASD that possess genetic mutations identified in human patients. This research aims to understand the pathophysiology of the neuronal circuits in ASD. He revealed that the increased synapse remodeling hindered the formation of accurate synaptic connections within the cortical neural circuit. Further, improved synapse remodeling was observed across multiple animal models of ASD. This work has received significant recognition because it proposes a new hypothesis concerning synaptic impairment in ASD.

Dr. Okabe's achievements exhibited a significant effect on the field of neurocircuit development, and he plays a vital role in the international community of neuroscientists. His work in determining the developmental mechanisms of neural circuits through pioneering imaging techniques has received global recognition for outstanding research that significantly contributes to neuroscience.

List of Main Publications

1. **Okabe, S**, and Hirokawa, N: Microtubule dynamics in nerve cells: analysis using microinjection of biotinylated tubulin into PC12 cells. **Journal of Cell Biology**, 107; 651–664, 1988.
2. **Okabe, S**, and Hirokawa, N: Rapid turnover of microtubule-associated protein MAP2

in the axon revealed by microinjection of biotinylated MAP2 into cultured neurons. **Proceedings of the National Academy of Sciences, USA**, 86; 4127–4131, 1989.

3. **Okabe, S**, and Hirokawa, N: Incorporation and turnover of biotin-labeled actin microinjected into fibroblastic cells: an immunoelectron microscopic study. **Journal of Cell Biology**, 109; 1581–1595, 1989.
4. **Okabe, S**, and Hirokawa, N: Turnover of fluorescently labelled tubulin and actin in the axon. **Nature**, 343; 479–482, 1990.
5. **Okabe, S**, and Hirokawa, N: Actin dynamics in growth cones. **Journal of Neuroscience**, 11; 1918–1929, 1991.
6. **Okabe, S**, and Hirokawa, N: Differential behavior of photoactivated microtubules in growing axon of mouse and frog neurons. **Journal of Cell Biology**, 117; 105–120, 1992.
7. Umeyama, T, **Okabe, S**, Kanai, Y, and Hirokawa, N: Dynamics of microtubules bundled by microtubule-associated protein 2C (MAP2C). **Journal of Cell Biology**, 120; 451–465, 1993.
8. **Okabe, S**, and Hirokawa, N: Do photobleached fluorescent microtubules move? Re-evaluation of fluorescence laser photobleaching both in vitro and in growing Xenopus axon. **Journal of Cell Biology**, 120; 1177–1186, 1993.
9. **Okabe, S**, Miyasaka, H, and Hirokawa, N: Dynamics of the neuronal intermediate filaments. **Journal of Cell Biology**, 121; 375–386, 1993.
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13. **Okabe, S**, Collin, C, Auerbach, JM, Meiri, N, Bengzon, J, Kennedy, MB, Segal, M, and McKay, RDG: Hippocampal synaptic plasticity in mice overexpressing an embryonic subunit of the NMDA receptor. **Journal of Neuroscience**, 18; 4177–4188, 1998.
14. **Okabe, S**, Miwa, A, and Okado, H: Alternative splicing of the C-terminal domain regulates cell surface expression of the NMDA receptor NR1 subunit. **Journal of Neuroscience**, 19; 7781–7792, 1999.
15. **Okabe, S**, Kim, H, Miwa, A, Kuriu, T, and Okado, H: Continual remodeling of postsynaptic density and its regulation by synaptic activity. **Nature Neuroscience**, 2; 804–811, 1999.
16. **Okabe, S**, Miwa, A, and Okado, H: Spine formation and correlated assembly of

presynaptic and postsynaptic molecules. **Journal of Neuroscience**, 21; 6105–6114, 2001.

17. **Okabe, S**, Urushido, T, Konno, D, Okado, H, and Sobue, K: Rapid redistribution of the postsynaptic density protein PSD-Zip45 (Homer 1c) and its differential regulation by NMDA receptors and calcium channels. **Journal of Neuroscience**, 21; 9561–9571, 2001.
18. Nakatomi, H, Kuriu, T, **Okabe, S**, Yamamoto, S, Hatano, O, Kawahara, N, Tamura, A, Kirino, T, and Nakafuku, M: Regeneration of hippocampal pyramidal neurons by recruitment of endogenous neural progenitors: An animal model for neuronal replacement therapy for ischemic brain injury. **Cell**, 110; 429–441, 2002.
19. Ebihara, T, Kawabata, I, Usui, S, Sobue, K, and **Okabe, S**: Synchronized formation and remodeling of postsynaptic densities: long-term visualization of hippocampal neurons expressing postsynaptic density proteins tagged with GFP. **Journal of Neuroscience**, 23; 2170–2181, 2003.
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34. International Brain Initiative (J. Bjaalie and **S. Okabe**, corresponding authors). An innovative framework for coordinated global brain research efforts. **Neuron**, 105; 212–216, 2020.
35. Tamada, K, Fukumoto, K, Toya, T, Nakai, N, Awasthi, JR, Tanaka, S, **Okabe, S**, Spitz, F, Saitow, F, Suzuki, H, and Takumi, T: Genetic dissection identifies Necdin as a driver gene in a mouse model of paternal 15q duplications. **Nature Communications**, 12; 4056, 2021.
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37. Kamei, R, and **Okabe, S**: In vivo imaging of the phagocytic dynamics underlying efficient clearance of adult-born hippocampal granule cells by ramified microglia. **Glia**, 71; 2005–2023, 2023.