

In this research project, we aimed to elucidate artificial intelligence and brain function by developing a tissue called "connectoid" that mimics neural circuits by connecting neural organoids made from human iPS cells and functionalizing them. To achieve this, we modeled the complexity of brain activity beyond ordinary neural organoids by imitating the "connections" between regions in the brain, assigning different roles to each organoid. We aimed to construct a circuit that performs motor processing by stimulating an organoid expressing channelrhodopsin with a specific shape (spatial pattern) of light to provide a sensory input role and making the activity of the connected organoid a motor output. To accomplish this, we applied multiple patterns of light stimulation to the sensory organoid and obtained neural activity patterns on the motor output side using a multielectrode array. We amplified and digitized the obtained signals and examined the relationship between the given signal patterns and the output activity patterns. Upon repeated analyses using deep learning, we observed a trend of increasing input-output relationship strength with each test. This is thought to be due to the primitive circuit enhancement and learning-like changes caused by the strengthened circuit and increased pattern discrimination ability through repeated stimulation. Furthermore, we developed a schizophrenia model and analyzed changes in neural activity and gene expression. We are also developing a technique to cut neural organoids gently and quickly. While the low efficiency of recording neural organoid activity on multielectrode arrays is recognized as an issue, we have been developing a simple method to improve the recording efficiency of neural organoid activity. The future possibilities of this research project are expected to contribute significantly to various fields, not only the advancement of artificial intelligence research but also applications to brain-machine interfaces, the creation of bio-AI, and the development of therapeutic drugs for mental disorders. We will continue to develop and pursue the elucidation of the network functions of neural organoids.

#### Publications

1: Saito H, Osaki T, Ikeuchi Y, Iwasaki S. High-throughput Assessment of Mitochondrial Protein Synthesis in Mammalian Cells Using Mito-FUNCAT FACS. *Bio Protoc.* 2023 Feb 5;13(3):e4602. doi: 10.21769/BioProtoc.4602. PMID: 36816992; PMCID: PMC9909305.

2: Murata K, Saibe Y, Uchida M, Aono M, Misawa R, Ikeuchi Y, Ishii K. Two-photon, red light uncaging of alkyl radicals from organorhodium(III) phthalocyanine complexes. *Chem Commun (Camb).* 2022 Oct 6;58(80):11280-11283. doi: 10.1039/d2cc03672j. PMID: 36124703.

- 3: Chow SYA, Nakayama K, Osaki T, Sugiyama M, Yamada M, Takeuchi H, Ikeuchi Y. Human sensory neurons modulate melanocytes through secretion of RGMB. *Cell Rep.* 2022 Sep 20;40(12):111366. doi: 10.1016/j.celrep.2022.111366. PMID: 36130522.
- 4: Chow SYA, Hu H, Osaki T, Levi T, Ikeuchi Y. Advances in construction and modeling of functional neural circuits in vitro. *Neurochem Res.* 2022 Sep;47(9):2529-2544. doi: 10.1007/s11064-022-03682-1. Epub 2022 Aug 9. PMID: 35943626; PMCID: PMC9463289.
- 5: Beaubois R, Khoyratee F, Branchereau P, Ikeuchi Y, Levi T. From real-time single to multicompartmental Hodgkin-Huxley neurons on FPGA for bio-hybrid systems. *Annu Int Conf IEEE Eng Med Biol Soc.* 2022 Jul;2022:1602-1606. doi: 10.1109/EMBC48229.2022.9871176. PMID: 36083914.
- 6: Kimura Y, Saito H, Osaki T, Ikegami Y, Wakigawa T, Ikeuchi Y, Iwasaki S. Mito-FUNCAT-FACS reveals cellular heterogeneity in mitochondrial translation. *RNA.* 2022 Jun;28(6):895-904. doi: 10.1261/rna.079097.122. Epub 2022 Mar 7. PMID: 35256452; PMCID: PMC9074903.
- 7: Wu X, Park J, Chow SYA, Kasuya MCZ, Ikeuchi Y, Kim B. Localised light delivery on melanoma cells using optical microneedles. *Biomed Opt Express.* 2022 Jan 31;13(2):1045-1060. doi: 10.1364/BOE.450456. PMID: 35284152; PMCID: PMC8884222.
- 8: Chow SYA, Nakanishi Y, Kaneda S, Ikeuchi Y. Modeling Axonal Degeneration Using Motor Nerve Organoids. *Methods Mol Biol.* 2022;2515:89-97. doi: 10.1007/978-1-0716-2409-8\_6. PMID: 35776347.
- 9: Misawa R, Ikeuchi Y. Light-Induced Differentiation of Forebrain Organoids by NVOC-SAG. *Methods Mol Biol.* 2022;2374:185-194. doi: 10.1007/978-1-0716-1701-4\_16. PMID: 34562253.
- 10: Kashiwagi K, Shichino Y, Osaki T, Sakamoto A, Nishimoto M, Takahashi M, Mito M, Weber F, Ikeuchi Y, Iwasaki S, Ito T. eIF2B-capturing viral protein NSs suppresses the integrated stress response. *Nat Commun.* 2021 Dec 7;12(1):7102. doi: 10.1038/s41467-021-27337-x. PMID: 34876589; PMCID: PMC8651795.
- 11: Mitsuzawa S, Suzuki N, Akiyama T, Ishikawa M, Sone T, Kawada J, Funayama R, Shirota

M, Mitsuhashi H, Morimoto S, Ikeda K, Shijo T, Ohno A, Nakamura N, Ono H, Ono R, Osana S, Nakagawa T, Nishiyama A, Izumi R, Kaneda S, Ikeuchi Y, Nakayama K, Fujii T, Warita H, Okano H, Aoki M. Reduced PHOX2B stability causes axonal growth impairment in motor neurons with TARDBP mutations. *Stem Cell Reports*. 2021 Jun 8;16(6):1527-1541. doi: 10.1016/j.stemcr.2021.04.021. Epub 2021 May 27. PMID: 34048688; PMCID: PMC8190591.

12: Misawa R, Minami T, Okamoto A, Ikeuchi Y. Light-inducible control of cellular proliferation and differentiation by a Hedgehog signaling inhibitor. *Bioorg Med Chem*. 2021 May 15;38:116144. doi: 10.1016/j.bmc.2021.116144. Epub 2021 Apr 2. PMID: 33845416.

13: Chanda A, Ikeuchi Y, Karve K, Sarkar A, Chandhoke AS, Deng L, Bonni A, Bonni S. PIAS1 and TIF1 $\gamma$  collaborate to promote SnoN SUMOylation and suppression of epithelial-mesenchymal transition. *Cell Death Differ*. 2021 Jan;28(1):267-282. doi: 10.1038/s41418-020-0599-8. Epub 2020 Aug 7. Erratum in: *Cell Death Differ*. 2020 Aug 18;: PMID: 32770107; PMCID: PMC7853041.

14: Shaik FA, Ihida S, Ikeuchi Y, Tixier-Mita A, Toshiyoshi H. TFT sensor array for real-time cellular characterization, stimulation, impedance measurement and optical imaging of in-vitro neural cells. *Biosens Bioelectron*. 2020 Dec 1;169:112546. doi: 10.1016/j.bios.2020.112546. Epub 2020 Aug 30. PMID: 32911315.

15: Osaki T, Chow SYA, Nakanishi Y, Hernández J, Kawada J, Fujii T, Ikeuchi Y. Three-Dimensional Motor Nerve Organoid Generation. *J Vis Exp*. 2020 Sep 24;(163). doi: 10.3791/61544. PMID: 33044443.