(NIPS)

Department of Molecular and Cellular Physiology

The Division of Biophysics and Neurobiology (Prof. KUBO, Yoshihiro and Assoc. Prof. TATEYAMA, Michihiro) aims to clarify the mechanisms of ion channels, receptors, and G proteins, which are key elements of the nervous system. The Division approaches structure-function relationships and structural dynamics of the elements through biophysical analyses with the electrophysiological and optical techniques in in vitro expression systems.

The Division of Membrane Physiology (Prof. FUKATA, Masaki and Assoc. Prof. FUKATA, Yuko) uses a unique biochemical method to identify synaptic protein complexes in brain tissue, and then uses the complexes in conjunction with a hippocampal neural cell primary culture system, genetically modified mice, etc., to clarify the mechanism that controls synaptic transmission efficiency. In addition, focusing on palmitoylation, the Division identifies specific palmitoylating enzymes and analyzes the localization and kinetics of synaptic proteins mediated by these enzymes.

The Division of Structural Biology (Material-Life Boundary Research Group, Exploratory Research Center on Life and Living Systems (ExCELLS)) (Project Prof. MURATA, Kazuyoshi) strives to elucidate the functions of biomolecular complexes from a structural perspective. The Division uses a 200-kV cryo-electron microscope to perform structural analyses of biomolecular complexes. In addition, electron tomography and serial block-face scanning electron microscopy (SBF-SEM) is used for morphological and structural analyses of intracellular biomolecular complexes.

The Division of Neural Development & Regeneration (Adjunct Division, Adjunct Prof. SAWAMOTO, Kazunobu) studies the mechanism whereby neurons and glial cells are generated during brain development and regeneration after brain injury. The Division also tries to stimulate these regeneration processes.

Department of Homeostatic Regulation

The Division of Cell Structure (Prof. FURUSE, Mikio and Assoc. Prof. IZUMI Yasushi) focuses on the molecular basis of cell-cell junctions involved in epithelial barrier function and passive transfer via paracellular pathways. In addition to basic analysis using cultured epithelial cells, the Division is proceeding with individual-level analysis using genetically modified mice and Drosophila in conjunction with techniques in the fields of cell biology and physiology.

The Division of Cell Signaling (Thermal Biology Group, ExCELLS) (Prof. TOMINAGA, Makoto, Assoc. Prof. SOKABE, Takaaki, Project Assoc. Prof. KASHIO, Makiko and Project Assoc. Prof. MARUYAMA, Kenta) aims to clarify the molecular mechanisms of thermosensitivity and

pain sensitivity centering on TRP channels, which serve as biomolecular sensors necessary for cells survival. In addition to analyses using electrophysiological, molecular cell biological, and biochemical techniques, the Division conducts studies at an individual level using genetically modified animals. Such studies include behavioral analysis of temperature, taste, and mechanical stimulus reception using Drosophila, and analysis of effects of pesticides and repellents. In addition, the Division analyzes the evolution of temperature-sensitive TRP channels because it is considered that organisms evolutionarily adapted to changes in environmental temperature by dynamically changing their temperature sensitivities.

The Division of Cardiocirculatory Signaling (Cardiocirculatory Dynamism Research Group of the ExCELLS) (Prof. NISHIDA, Motohiro and Project Assoc. Prof. NISHIMURA, Akiyuki) aims to clarify the mechanism that controls the cardiovascular adaptation or maladaptation to hemodynamic load. Specifically, it strives to elucidate the molecular mechanism of cardiovascular homeostasis from the viewpoint of signal transduction by using a wide range of techniques, including creation of model mice for human cardiovascular disease, measurement of cardiovascular functions using isolated organs, signal transduction analysis using primary cultured cardiomyocytes, and in situ imaging of post-translational protein modification based on chemical principles.

The Division of Endocrinology and Metabolism (Prof. MINOKOSHI, Yasuhiko) studies the control mechanisms, mainly involving the hypothalamus, that underlie the regulation of whole-body energy metabolism. Specifically, the Division aims to elucidate the mechanism of eating and food preference and the means by which taste sensitivity is controlled by the hypothalamus, as well as the mechanism whereby metabolic homeostasis is maintained by the autonomic nervous system at the molecular, tissue, and individual levels. The Division also studies the relationship between disordered homoeostasis and obesity, diabetes, and lifestyle-related diseases.

The Division of Molecular Neuroimmunology (Prof. MURAKAMI, Masaaki and Project Assoc. Prof. HASEBE, Rie) has been analyzing tissue-specific autoimmune diseases and found two novel concepts. One is the gateway reflex, which is a novel mechanism of neuroimmune interactions, and the other is the IL-6 amplifier, which is a fundamental mechanism of inflammation in non-immune cells including tissue-specific cells. The IL-6 amplifier is activated by the simultaneous activation of STAT3 and NF-kB followed by the excessive activation of NF-kB. As for the gateway reflex, six types have been reported so far. Environmental and artificial stimulations, including gravity, electrical stimulation, pain, stress, light, and inflammation in joints, activate specific neural pathways, which induce activation of the IL-6 amplifier at specific blood vessels of target organs, particularly those with blood barriers, such as the central nervous system. Activation of the IL-6 amplifier accumulates autoreactive CD4+ T cells in the blood around the specific blood vessels, resulting in the development of tissue-specific inflammatory diseases. The lab is

investigating details about the neural pathways for the six as well as searching for new gateway reflexes.

The Division of Ultrastructural Research (Adjunct Division) (Adjunct Prof. OHNO, Nobuhiko) aims to clarify the molecular backgrounds of structural and functional changes in the nervous system in myelin diseases. To this end, the Division uses imaging techniques such as 3-dimensional microstructure analysis involving microtome-integrated serial block-face scanning electron microscopy (SBF-SEM). In addition, by combining such imaging techniques with cultured models and genetically modified animals, the Division is elucidating the mechanisms of dynamic changes in organelles (e.g., mitochondria) in the nervous system and developing technologies to control these changes.

Department of Fundamental Neuroscience

In the Division of Multicellular Circuit Dynamics (Prof. WAKE, Hiroaki), we mainly use two-photon microscopy to visualize the structure and function of neurons and glial cells in the mouse brain under awake conditions and extract their activities in physiological and pathological conditions. Furthermore, we are using holographic microscopy to manipulate neurons and glial cells activity with high spatiotemporal resolution based on this activity information.

The Division of Homeostatic Development (Assoc. Prof. NARUSHIMA, Madoka) focuses on the remodeling of neuronal circuits during the developmental and injury recovery periods. In particular, they are involved in the following: 1) electrophysiological analysis of synaptic transmission and receptor functions; (2) analysis of plastic changes in the functions of the inhibitory neurotransmitters GABA and glycine, especially from the viewpoint of the intracellular regulation mechanism for chloride ion concentration; and (3) use of in vivo multiphoton laser microscopy to determine the morphological and behavioral changes in neuronal circuits during the developmental period and in various disease states, and the contribution of glial cells to these changes.

The Division of Visual Information Processing (Prof. YOSHIMURA, Yumiko) characterizes the neural circuits of the visual cortex and elucidates the mechanisms underlying the experience-dependent development of the cortex. To this end, cortical slices and anesthetized and conscious mice are analyzed with the combined use of local laser light stimulation and electrophysiological and Ca^{2+} imaging techniques.

The Division of Biophotonics (Biophotonics Research Group of the ExCELLS) (Prof. NEMOTO, Tomomi and Assoc. Prof. ENOKI, Ryosuke) advances the development and application of cutting-edge imaging devices, including in vivo two-photon microscopes, multi-beam scanning-type two-photon microscopes, and two-photon super-resolution microscopes. The Division also conducts

research in the field of chronobiology, for instance utilizing imaging techniques to examine the neuroscientific basis of circadian rhythms.

Department of System Neuroscience

The Division of Behavioral Development (Prof. ISODA, Masaki, Project Assoc. Prof. TOMATSU, Saeka and Project Assoc. Prof. GO, Yasuhiro) aims to clarify the neural basis of social cognitive functions via studies of system neuroscience using primates. To this end, the Division conducts integrated analyses combining behavioral, electrophysiological, and neuropharmacological techniques, and also utilizes neuroanatomical methods and selectively manipulates neural circuits using viral vectors.

The Division of Neural Dynamics (Prof. KITAJO, Keiichi) aims to unveil the functional roles of diverse neural dynamics in brain information processing. In particular, experiments involving the non-invasive measurement of human brain waves and brain stimulation are used in conjunction with data analysis (nonlinear dynamics, network analysis, statistical machine learning methods, etc.) to model the information-processing mechanisms of the human brain and thereby clarify pathological conditions and individual characteristics.

The Division of Sensory and Cognitive Brain Mapping (Prof. TAKEMURA, Hiromasa) aims to investigate structure-function relationship in the human brain primarily based on neuroimaging methods. Specifically, the Division combines structural neuroimaging (diffusion and quantitative MRI) and functional neuroimaging (functional MRI) to investigate brain structure and function, in order to perform comparisons between human and animal brains as well as evaluate consequence of disorders.

The Division of Cerebral Integration (Assoc. Prof. FUKUNAGA, Masaki) advances experimental studies on higher cerebral cortex activities related to cognition, memory, emotion, judgment, intention, behavior, and social ability using neuroimaging techniques. The Division aims to understand higher brain function dynamically and globally by utilizing non-invasive brain function imaging techniques to assess local circulatory changes and alterations in energy metabolism associated with cranial nerve activity.

Supportive Center for Brain Research

The Section of Multiphoton Neuroimaging (Assoc. Prof. MURAKOSHI, Hideji) explores cell functions by imaging cell morphology, signal transduction, and molecular interactions using unique two-photon microscopy techniques and two-photon fluorescence resonance energy transfer (FRET) microscopy. In addition to state-of-the-art optical technology, the Section develops novel fluorescent proteins and photoresponsive protein molecules. By combining these technologies with the patch clamp method, the Section aims to elucidate the functions of nerve

cells and cultured cells.

The Section of Electron Microscopy (Prof. FURUSE, Mikio and Assoc. Prof. KUBOTA, Yoshiyuki) has introduced a microtome-integrated scanning electron microscope (SBF-SEM) for conducting connectomics analyses. With this device, the Section automatically captures several hundred to a thousand sequential electron microscope images a day and reconstructs them into 3D models. In addition, local neural networks in the cerebral cortex are analyzed using wide-area electron microscope image datasets and correlative light-electron microscopy, the latter of which seamlessly combines *in vivo* imaging by a two-photon microscope and an automated tape-collecting ultramicrotome (ATUM)-SEM.

The Section of Brain Function Information (Adjunct Prof. INUI, Koji and Assoc. Prof. FUKUNAGA, Masaki) supports collaborative studies on brain function measurement in humans and monkeys using high-field magnetic resonance imaging (3T and 7T) devices equipped at NIPS. The Section promotes research on functional–anatomical mapping of the primate brain. The Section also promotes the elucidation of human brain functions by supporting the analysis of data obtained through the joint use of the whole-brain magnetoencephalography (MEG), which was conducted until FY2021.

The Section of Cellular Electrophysiology (Prof. YOSHIMURA, Yumiko) explores the structures and dynamic control of neural circuits and the action and control mechanisms of synaptic transmission by applying an electrophysiological method (patch clamp technique) to different brain areas (e.g., cerebral cortex, basal ganglia and cerebellum), mainly using sample slices. The Section also aims to elucidate the developmental mechanisms of brain and nervous system diseases and to develop novel treatment methods by performing pathological analyses of mice with gene mutations related to these diseases. In this Section, Assist. Professors OTSUKA, Takeshi and SATAKE, Shin'Ichiro will mainly promote joint research projects.

Center for Genetic Analysis of Behavior

The Section of Viral Vector Development (Prof. ISODA, Masaki and Assoc. Prof. KOBAYASHI, Kenta) develops high-quality and high-performance virus vectors that can be applied to: i) analysis of the neural basis of higher brain functions using model animals such as primates and rodents, and ii) pathological analysis of mental and neurological diseases. In addition, it serves as a central hub for providing viral vectors in response to requests from other laboratories. Through such efforts, the Section actively promotes joint research.

The Section of Mammalian Transgenesis (Assoc. Prof. HIRABAYASHI, Masumi) develops more efficient techniques to produce genetically modified animals and performs reproduction engineering research such as gamete preservation of genetically modified animals,

microinsemination, and establishment of rat embryonic stem cells and induced pluripotent stem cells. Its aim is to achieve further technological advances in developmental engineering techniques.

The Section of Multilayer Physiology, (Prof. NISHIJIMA, Kazutoshi) is equipped with apparatuses for chronological and automatic measurement of physiological functions and metabolic parameters of mice and rats. Using these apparatuses, the Section promotes collaborative research for the evaluation of behaviors related to emotions, learning and memories, and for the recordings and analysis of neural (single-unit, local field potential, etc.) and muscular activities in an awake state.

(Center for Animal Resources and Collaborative Study)

The Center for Animal Resources and Collaborative Study develops experimental animals (including mice, rats, rabbits, and monkeys) based on their characteristics (e.g., genetic recombination) and analyzes their phenotypes (physiological functions such as behavior, electrical activity, metabolism, etc.) from the perspectives of veterinary medicine and laboratory animal sciences. In addition, the Center develops strain preservation techniques and husbandry practices that are suitable for different animal species to improve the quality of animal experiments and animal welfare.

Annex 1

Overview of equipment used for analyzing metabolic physiology in mice and rats

【Major items to be analyzed and/or measured】

1) Energy intake and expenditure in free-moving animals
2) Body temperature, heart rate, and blood pressure in free-moving animals
3) Non-invasive 4-dimensional cardiac function and capillary blood flow imaging using anesthetized mice
4) Mouse temperature preference assays using a thermal gradient ring
5) Evaluation of behaviors related to emotions, leaning, and memories
6) Multicellular activity measurement and manipulation in vivo
7) Functional analysis of neuroimmune interactions in mouse models of diseases

【Equipment】

- Device for simultaneously measuring energy metabolism and activity of small animals using mass spectrometry (Arco System, Shinfactory, for mice)
- Brain wave–measuring apparatus (Nihon Kohden, AB611J)
- Electromyograph (Nihon Kohden, AB611J)
- Telemetry automatic measurement system for chronic experiments (Harvard Bioscience, mouse, rat, etc.)
- Olympus FV100, 4D ultrasound imaging device VEVO3100 (Primetech Corporation, for mice)
- Isolated heart perfusion system (Primetech Corporation, for mice and rats)
- Thermal Gradient Ring (Ugo Basile, for mice)
- Open field test analyzer (Section of Instrument Design Room of NIPS and other, for mice)
- Light/dark transition test device (O'HARA, for mice)
- Barnes circular maze test device (O'HARA, for mice)
- Elevated plus-maze test analyzer (Section of Instrument Design Room of NIPS and other, for mice)
- Forced swimming test analyzer (Section of Instrument Design Room of NIPS and other, for mice)
- Rotarod test analyzer (Ugo Basile, for mice RotaRod NG, 47650)
- Passive avoidance test analyzer (O'HARA, for mice)
- Fear conditioning test analyzer (O'HARA and other, for mice)
- Morris water maze pool (O'HARA and other, for mice)
- Nikon A1MP+holographic microscope (Nikon & Division of Multicellular Circuit Dynamics, for mice and rats)
- X-ray irradiation device (MediXtec, for mice and cells)

ANNEX 2

Overview of magnetic resonance imaging (MRI) scanners

Performance and features of the MRI scanner installed at the NIPS Supportive Center for Brain Research (two Verio scanners, 2009, Siemens Co.; one 7-T scanner, 2014, Siemens Co., Ltd.)

Verio

1. Superconducting magnet
   1) Magnetic field strength: 3 Tesla, magnet inner diameter 70 cm
   2) Magnetic field uniformity: 0.03 ppm or less (spherical range with a diameter of 20 cm, volume residual mean squared method)
   3) Shimming: Active + passive shimming, automatic shimming for each subject
   4) Liquid helium evaporation: 0.01 L/year or less

2. Imaging functions
   1) Nuclei: ^{1}H
   2) Pulse sequence: echo planar imaging, turbo spin echo imaging, etc.
   3) Slice direction: axial, sagittal, coronal, oblique
   4) Min. slice thickness: 1 mm (2-dimensional imaging), 0.3 mm (3-dimensional imaging)
   5) Gradient magnetic field: 45 mTesla/m, rise time 0.225 ms
   6) Probe: 32-channel head coil, circular polarized body coil, etc.
   7) Data processing device: Automatically saves obtained images in DICOM format via Windows network
   8) Other functions: T1, T2, T2*, proton density-weighted images, MR angiography, diffusion-weighted image, image statistical processing software, communication mediation relay system for simultaneously measuring neural activity during interaction between two individuals

7-T MRI

1. Superconducting magnet
   1) Magnetic field strength: 7 Tesla, magnet inner diameter 60 cm
   2) Magnetic field uniformity : 1 ppm or less (spherical range with a diameter of 25 cm, volume residual mean squared method)
   3) Shimming: Active + passive shimming, automatic shimming for each subject
   4) Liquid helium evaporation: 0.01 L/year or less

2. Imaging functions
   1) Nuclei: ^{1}H, ^{13}C, ^{17}O, ^{19}F, ^{23}Na, ^{31}P

2) Pulse sequence: echo planar imaging, turbo spin echo imaging etc.

3) Slice dimensions: axial, sagittal, coronal, oblique

4) Min. slice thickness: 0.5 mm (2-dimensional imaging), 0.05 mm (3-dimensional imaging)

5) Gradient magnetic field: 70 mTesla/m, rise time 0.350 ms

6) Probe: 32-channel receive-only head coil (^{1}H), circular polarized transmit/receive head coil (^{1}H, ^{23}Na, ^{31}P), transmit/receive surface coil (^{13}C, ^{17}O, ^{19}F), etc.

7) Data processing device: Automatically saves obtained images in DICOM format via Windows network

8) Other functions: T1, T2, T2*, proton density-weighted images, MR angiography, diffusion-weighted image, image statistical processing software