Poster Session Abstracts

The 7th Annual IIIS Symposium ~ Solving the mystery of sleep ~

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Mammalian sleep contains rapid eye movement (REM) and non-REM sleep, each employs different mechanisms for episodic memory consolidation. Previous reports showed that boosting slow oscillations or inhibiting sharp wave-ripples during non-REM sleep potentiated or interfered with memory consolidation1,2. On the other hand, disruption of REM-related theta rhythm impaired memory consolidation3. However, the memory circuit that is responsible for memory consolidation during each sleep stage has not been clear. We have shown that hippocampal adult-born neurons are incorporated in memory circuits after learning4. Therefore, we silence the activities of the adult-born neurons during specific stage of sleep after learning using optogenetic intervention, which provides reversibility, higher time resolution and target specificity. It revealed that the activities of the adult-born neurons are necessary for memory consolidation during sleep.

2. Girard et al., Nat Neurosci, 2009, v12, p1222

We thank all Sakaguchi lab and WPI-IIIS members, K.G. Akers, S. Yaghisita, Y. Hayashi, K. Kobayakawa, R. Kobayakawa, M. Hayashi, X. Hayashi, M. Oishi, G. Chiangarathil, S. Narita, Y. Hirose, M. Sezaki, T. Hosokawa, K. Shikama, S. Sugimori, Y. Mimura, N. Hasegawa, H. Obo for technical assistance, N. Kobayashi and M. Adachi for secretarial assistance. This work was partially supported by grants from the World Premier International Research Center Initiative from the Japan Ministry of Education, Culture, Sports, Science, and Technology (MEXT); JST CREST Grant Number JPMJCR1655; Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (JSPS, KAKENHI grant numbers 16K18359, 15F15408, 26115502, and 25116530); The Takeda Science Foundation; Shimadzu Science Foundation; Kanae Foundation for the Promotion of Medical Science, Research Foundation for Opto-Science and Technology; The Ichiro Kanehara Foundation; Kato Memorial Bioscience Foundation; Japan Foundation for Applied Enzymology; Senshin Medical Research Foundation; Life Science Foundation of Japan; Uehara Memorial Foundation; Brain Science Foundation; Kowa Life Science Foundation; and 2016 Inamori Research Grants Program to MS, JSPS (KAKENHI grant numbers 25000015, 18H04012) to M.K, JSPS FPD; and the University of Tsukuba to DK, the Tokyo Biochemical Research Foundation to SSR, NIH-NCI CCSG: P30 014195, NINDS R24 Core Grant and NEI to SK.
Cumulative phosphorylation of SNIPPs as the molecular substrate of homeostatic sleep need

Zhiqiang Wang1, Jing Ma1, Chika Miyoshi1, Yuxin Li2, Makito Sato1, Yukino Ogawa1, Tingting Lou1, Chiyu Lee1, Xiaojie Yang1, Noriko Hotta-Hirashima1, Daniela Klewe-Nebenius1, Aya Ikkyu1, Miyo Kakizaki1, Satomi Kanno1, Liqin Cao1, Junmin Peng2, Yonghao Yu1, Hiromasa Funato1, Masashi Yanagisawa1, Qinghua Liu1,3

1International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba
2St. Jude Proteomics Facility, St. Jude Children’s Research Hospital
3University of Texas Southwestern Medical Center

The sleep-wake homeostasis is maintained by generation of a sleep need that accumulates during waking and dissipates through sleep. In mammals, the best known measurable index of sleep need is the slow wave activity (SWA) or EEG delta power (1-4 Hz) during non-rapid-eye-movement sleep (NREMS). To investigate the molecular basis of sleep need, we performed quantitative phosphoproteomic studies of two opposite models of increased sleep need in mice. Sleep deprivation induces cumulative phosphorylation of brain proteome, which dissipates during recovery sleep. Strikingly, Sleepy (Sik3Slp+/+) mutant brains, with an elevated sleep need despite chronic hypersomnia, exhibit a hyper-phosphoproteome mimicking sleep-deprived brains, owing to a gain-of-function mutation of protein kinase SIK3. Comparison of two models identified 80 mostly synaptic Sleep-Need-Index-PhosphoProteins (SNIPPs), whose phosphorylation states closely parallel changes of sleep need. Mutant SIK3 (SLEEPY) kinase preferentially associated with and phosphorylated SNIPPs. Inhibition of SLEEPY/SIK3 activity reduced phosphorylation state of SNIPPs and SWA of NREMS in both Sleepy and sleep-deprived mice. Our results expose an unexplored brain phosphoproteome landscape and suggest that SNIPPs gradually accumulate/dissipate phosphorylation as a function of homeostatic sleep need. While waking encodes memories by potentiating synapses, sleep consolidates memories and restores synaptic homeostasis by globally downscaling excitatory synapses. Thus, we propose that phosphorylation and dephosphorylation of SNIPPs may represent a major regulatory mechanism that underlies both synaptic homeostasis and sleep-wake homeostasis.

Dopaminergic activity-dependent astrocytic glycogenolysis producing lactate in the exercising hippocampus

Takashi Matsui1,2,3, Ignacio Torres-Aleman3, Hideaki Soya1,2
1Laboratory of Exercise Biochemistry and Neuroendocrinology, Faculty of Health and Sport Sciences, University of Tsukuba;
2Sport Neuroscience Division, Advanced Research Initiative for Human High Performance (AHIIP), University of Tsukuba; Tsukuba 305-8574, Ibaraki, Japan;
3Neuroendocrinology Laboratory, Cajal Institute, CSIC, Avda Dr Arce 37, 28042 Madrid, Spain.

Hippocampal lactate produced from astrocytic glycogen is a critical energy source and/or a neuromodulator in memory functions. Although exercise, even in mild intensity, activates hippocampal neurons and enhances memory functions, hippocampal glycometabolism during exercise is less clear. Here we reveal the dopamine (DA) D2 receptor (R)-mediated astrocytic glycogenolysis to produce lactate in the exercising rat hippocampus. Running exercise decreased hippocampal glycogen and increased its lactate in a running speed-dependent manner associated with DAergic activation, independent of noradrenaline and serotonin. DA challenge and an agonism of DAD2R, but not DAD1R, decreased glycogen via Ca2+/cAMP/PKA signaling in primary cultured rat astrocytes, but DA did not serve in astrocytes from mice lacking DAD2R. An antagonism of DAD2R, but not DAD1R, inhibited glycogenolysis and lactate production in mild exercising hippocampus. Our findings demonstrate the DAergic activity-dependent astrocytic glycogenolysis producing lactate in the running hippocampus, providing insights into energetics and neurotrophicity underlying exercise-enhanced memory functions.
P04

Sex difference in sleep architecture, thermal regulation and energy metabolism in human

Simeng Zhang1, Haruka Oosumi2, Akiko Uchizawa2, Haruka Hamada3, Insung Park1, Yoko Suzuki3, Yoshikazu Tanaka2, Asuka Ishihara1, Katsuhiko Yajima2,4, Makoto Satoh1, Naomi Omi2, Kumpei Tokuyama2
1International Institute for Integrative Sleep Medicine, University of Tsukuba
2Graduate School of Comprehensive Human Sciences, University of Tsukuba
3School of Health and Physical Education, University of Tsukuba
4Faculty of Health and Nutrition, Tokyo Seiei College

Human sleep is generally consolidated into a single prolonged period, and its metabolic consequence is to impose an extended period of fasting. The energy metabolism during sleep does not simply reflect the extension of fasting time. It has been assumed that oxidized substrate during sleep shift from carbohydrate to fat as sleep continues, but the time course of respiratory quotient (RQ) indicates that carbohydrate oxidation begins to increase prior to awake. During sleep, core body temperature decreases followed by a gradual increase before wake. Gender difference in time course of core body temperature during sleep is known: nadir of core body temperature appears earlier in female than male.

The purpose of this study is to confirm whether there is a gender difference in the time course of energy metabolism during sleep. Energy metabolism was measured by a whole room indirect calorimetry with improved time resolution, core body temperature and skin temperature was also monitored during sleep. Sleep was monitored using a standard PSG recording.

Sleep architecture was similar between male and female, and between follicular and luteal phase. Time course of RQ during sleep showed a gender difference, and nadir of RQ appeared earlier in female than male. Core body temperature during the luteal phase was higher than that of the follicular phase. In the present study heat production (energy expenditure) was similar between follicular and luteal phase, but heat loss, estimated from the distal-proximal gradient of skin temperature, was smaller during the luteal phase.

P05

Large Scale Forward Genetic Screening Identifies Trpα1 as a Chemosensor for Predator Odor-evoked Innate Fear Behaviors

Chia-Ying Lee1, Yibing Wang1,2, Liqin Cao1, Tomohiko Matsuo1, Kejia Wu1,2, Greg Asher3, Lijun Tang1, Tsuyoshi Saitoh1, Jamie Russell4, Daniela Klewe-Nebenius1, Li Wang2, Shingo Soya2, Emi Hasegawa1, Yoan Chérassie5, Jianmin Zhou1, Yuwenbin Li6, Tao Wang2, Xiaowei Zhan2, Chika Miyoshi2, Yoko Irukayama2, Jie Cao2, Julian P. Meeks5, Laurent Gautron1, Zhuqiang Wang2, Katsuyasu Sakurai1, Hiromasa Funato1, Takeshi Sakurai1, Masashi Yanagisawa1,2, Hiroshi Nagase1, Reiko Kobayakawa4, Ko Kobayakawa4, Bruce Beutler1 & Qinghua Liu2,3,5,6,10
1National Institute of Biological Sciences, 102206 Beijing, China.
2Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
3International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan.
4Functional Neuroscience Lab, Kanazawa Medical University, Hirakata, Osaka 573-1010, Japan.
5Center for Genetics of Host Defense, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
6Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
7Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
8Department of Anatomy, Faculty of Medicine, Toho University, Ota-Ku, Tokyo 143-8540, Japan.
9Life Science Center; Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan.
10Tsinghua Institute of Multidisciplinary Biomedical Research, Tsinghua University, 100084 Beijing, China.

Innate fear behaviors have been believed to be genetically encoded in all organisms. But their underlying molecular mechanisms remain largely unknown. Predator odor 2,4,5-trimethyl-3-thiazoline (TMT) and its potent analogue 2-methyl-2-thiazoline (2MT) elicit innate fear/defensive behaviors in naive mice. We conduct a large-scale recessive genetics screen based on the innate defensive responses to 2MT in ethylthiosourea (ENU)-mutagenized mice. We find that Trpα1, a pungency/irritancy receptor, is related to both 2MT and snake skin-evoked innate fear/defensive responses. Accordingly, Trpα1-knockout mice (TRPA1 KO) are unable to effectively activate known fear/stress brain centers upon 2MT exposure, despite their apparent ability to smell and learn to fear 2MT. Moreover, in vitro calcium influx tests indicate Trpα1 acts as a chemosensor for 2MT. Interestingly we find trigeminal ganglia (TG) neurons are highly activated in 2MT stimulations. Unilateral lesion of TG reduced innate freezing and TG specific AAV-Trpα1-expression rescued freezing behavior in TRPA1 KO mice under 2MT exposure. These results suggest trigeminal ganglion neurons contribute critically to 2MT-evoked freezing.
Rapid stimulation of hippocampal memory circuit with acute mild exercise

Kazuya Suwabe1,2, Kyeongho Byun2,3, Kazuki Hyodo1, Zachariah M. Reagh3, Jared M. Roberts3, Akira Matsushita1,5, Kousaku Saotome4, Genta Ochi1, Takemune Fukuie1, Kenji Suzuki4, Yoshiyuki Sankai4, Michael A. Yassa2,3, and Hideaki Soya1,2

1Laboratory of Exercise Biochemistry and Neuroendocrinology, Faculty of Health and Sport Sciences, University of Tsukuba
2Sports Neuroscience Division, Advanced Research Initiative for Human High Performance (ARIHHP), Faculty of Health and Sport Sciences, University of Tsukuba
3Department of Neurobiology and Behavior, Center for the Neurobiology of Learning and Memory, University of California, Irvine
4Center for Cybernics Research, University of Tsukuba, Ibaraki 305-8574, Japan
5Department of Neurology, Ibaraki Prefectural University of Health Sciences

Physical exercise has beneficial effects on neurocognitive function, including hippocampus-dependent episodic memory. Exercise intensity level can be assessed according to whether it induces a stress response, the most effective exercise for improving hippocampal function remains unclear. Our prior works using special treadmill running model in animals have shown that stress-free mild exercise increases hippocampal neuronal activity and promotes adult neurogenesis in the dentate gyrus (DG) of the hippocampus, improving spatial memory performance. However, the rapid modification from mild exercise on hippocampal memory function and exact mechanisms for these changes, in particular the impact on pattern separation acting in the DG and CA3 regions are yet to be elucidated. To this end, we adopted an acute-exercise design in human, coupled with high-resolution functional MRI techniques, capable of resolving hippocampal subfields. A single 10-minute bout of very light intensity exercise (30% VO2peak) results in rapid enhancement in pattern separation and an increase in functional connectivity between hippocampal DG/CA3 and cortical regions (i.e., parahippocampal, angular, and fusiform gyri). Importantly, the magnitude of the enhanced functional connectivity predicted the extent of memory improvement at an individual subject level. These results suggest that brief, very light exercise rapidly enhances hippocampal memory function, possibly by increasing DG/CA3-neocortical functional connectivity.

Where does the Dreamless mutation take effect? - Generation of Nalcn-FLEx and Nalcn-flox Knock-In Mice

Tomoyuki Fujiyama1, Seiya Mizuno2, Manabu Abe3, Satomi Kanno1, Miyo Kakizaki1, Kanako Iwasaki1, Aya Ikkyu1, Noriko Hotta-Hirashima1, Mana Yamada1, Chika Miyoshi1, Makito Sato1, Takeshi Kanda1, Kenji Sakimura3, Satoru Takahashi2, Hiromasa Funato1,4, Masashi Yanagisawa1,5

1International Institute for Integrative Sleep Medicine (WPI-IIS), University of Tsukuba, Ibaraki, Japan
2Laboratory Animal Resource Center, University of Tsukuba, Ibaraki, Japan
3Department of Cellular Neurobiology, Japan Neuroscience Research Institute (JNRI), Ibaraki, Japan
4Department of Anatomy, Faculty of Medicine, Toho University, Tokyo, Japan
5Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX, USA

Although sleep is a ubiquitous animal behavior, the molecular/neural basis mechanism of REM sleep (REMS) remains unknown. We performed high-throughput screening of ENU-mutagenized mice in order to identify genes regulating sleep/wake behavior, and established the Dreamless mutant pedigree that shows about 50% reduction in 24-h REMS time. We identified a nucleotide change specific to Dreamless mutant mice within the exon9 in Nalcn gene. The single nucleotide substitution leads to a single amino acid substitution (N315K) of the gene product leak cation channel, NALCN, that we termed “Dreamless”. We adopted CRISPR/Cas system to recapitulate Dreamless mutant mice bearing flox knock-in and FLEx (flip and excision) knock-in mice for loss-of- and gain-of-function studies, respectively. Now we are planning to analyze sleep and wakefulness by using these two mutant mouse lines.
Impaired Sleep Architecture and Learning ability in an App Knock-in Mouse Model of Alzheimer’s Disease

Sakura Eri Maezono¹,², Mika Kanuka¹, Chika Tatsuzawa¹, Miho Morita¹, Mitsuaki Kashiwagi¹, Pimpimon Nondhalee¹, Masanori Sakaguchi¹, Takashi Saito³, Takaomi Saido³, Yu Hayashi¹
¹International Institute for Integrative Sleep Medicine, University of Tsukuba.
²School of Integrative and Global Majors, University of Tsukuba
³Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science

Alzheimer’s disease (AD) is the major cause of dementia. It is a neurodegenerative disease characterized by aggregation of amyloid-beta (Aβ) peptides. In addition to cognitive decline, AD patients often suffer from sleep disorders. These symptoms not only affect their quality of life (QOL) but also those of the primary caregivers. Moreover, increasing studies support that sleep deficits can contribute to the progression of AD, consistent with the roles of sleep in memory and brain homeostasis. Thus, it is crucial to understand the underlying mechanisms of sleep disorders in AD. Here, we unveil the sleep abnormalities exhibited by an AD mouse model. We chose the App knock-in mouse (Saito et al., Nature Neuroscience, 2014). In this AD mouse model, Aβ accumulates in a manner similar to human AD via mutation in the gene encoding amyloid-beta precursor protein (APP), and thus, various aspects of human AD are faithfully recapitulated. In these mice, at 6 months of age, when cognitive deficits become apparent, impairment in various aspects of the sleep architecture were detected. These phenotypes became more severe at 12 months of age. Consistent with the notion that sleep is important for memory consolidation, the AD mouse model also exhibited learning deficits. We expect that our results provide a start point for addressing how sleep is impaired in AD and how it can affect cognitive functions.

Dissection of prefrontal cortex circuit in guiding the extinctions

Akira Uematsu¹, Mika Hamada-Uematsu², Tomoya Duenki¹, Joshua Johansen¹,³
¹RIKEN Center for Brain Science
²Department of Surgery and Bioengineering, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo
³Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo

Emotional memory is crucial to guide behaviors for individual’s survival. Organisms need to respond to stimuli that predict dangerous or desirable outcomes. When this contingency changes, organism can extinguish emotional memory, the process called extinction. Extinction is essential for mental health in both aversive and appetitive domains and malfunction of extinction results in mental disorders. More than decade of research indicates that the ventromedial subregion of the prefrontal cortex (vmPFC) is important for extinctions of both appetitive and aversive memories. Anatomically the vmPFC sends broad projections and each set of projecting neurons forms distinct population in vmPFC. However, little is known about how each population or combination of them encodes aversive and appetitive extinction and how they differentiate distinct forms of extinction. To understand these questions, we first performed a series of optogenetic experiments by targeting 5 projection-specific populations in rats. Animals were learned to associate auditory cue with sucrose solution or foot shock and then presented auditory cue alone repeatedly with optogenetic manipulation. We found that each population has distinct role on appetitive and aversive memory extinctions. Next, to examine neural representations of each population, we recorded calcium dynamics in vmPFC excitatory neurons with miniature microscope and tracked same neurons during appetitive and aversive extinctions. Our preliminary data show that vmPFC neurons show orthogonal coding for appetitive and aversive extinctions with the population level analysis. Further results will be discussed in the poster.
Longitudinal EEG/EMG measurement in mice show sleep/wakefulness maturation from infant to young adult

Noriko Hotta-Hirashima¹, Chika Miyoshi¹, Aya Ikkyu¹, Satomi Kanno¹, Hiromasa Funato¹,², Masashi Yanagisawa¹

Although it is postulated that sleep/wakefulness behavior changes along with the postnatal development of brain, there have been very few studies on EEG/EMG-based sleep study during infancy due to technical difficulties in obtaining EEG/EMG signaling from a very small pups during breast-feeding. Here, we have established EEG/EMG electrode implantation surgery for pre-weaned mice (postnatal days P12) and a recording system optimized for breast-fed pups. We obtained 24h EEG/EMG signaling from the same mouse once a week from P15 to P57, and at P70. We observed a drastic change in sleep/wake pattern between P15 and P22. At P15, sleep/wake stages were very unstable and rarely continued for longer than 10 seconds so that it was very difficult to stage reliably. At P17, the duration of each sleep/wake stage became more stable and longer, which enabled us to determine sleep stage. The circadian change in sleep/wake behavior was getting obvious towards P36. We also observed the upward shift of the theta wave peak during REM sleep toward P29. Thus, the maturation of sleep/wakefulness progresses during infancy and after weaning with a different time-course depending on sleep parameters.

Sleep/wakefulness in neuron-specific SIK3-deficient mice

Fuyuki Asano¹, Tomoyuki Fujiyama¹, Chika Miyoshi¹, Miyo Kakizaki¹, Noriko Hotta-Hirashima¹, Aya Ikkyu¹, Satomi Kanno¹, Seiya Mizuno², Fumihiro Sugiyama³, Satoru Takahashi¹,², Hiromasa Funato¹,² and Masashi Yanagisawa¹,⁴,⁵

Sleep is a fundamental behavior conserved from vertebrates to invertebrates. SIK3, a member of AMP-activated protein kinase (AMPK) family, was identified as a novel sleep-regulating molecule. Alanine substitution at serine551 in SIK3 resulted in longer NREM sleep time with increased NREM sleep delta density, which indicates an enhanced sleep need in Sik3(S551A) mice. Conversely, flies and round worms expressing a hypomorphic SIK3 orthologue reduced sleep-like behavior. Therefore, the long sleep phenotype of Sik3(S551A) mice may be due to a gain-of-function effect of the mutant SIK3 protein. However, the effect of the endogenous SIK3 protein on sleep/wakefulness regulation in mice is still unknown because almost all of Sik3-deficient mice died on the day of their birth and a few survived mice suffered from severe growth retardation and malnutrition.

Here, we established Sik3-flox mice, in which exon 3 of Sik3 gene is flanked by lox P sites. Cre recombinase excises the exon 3, which results in frameshift and creation of premature stop codon. Nestin-Cre; Sik3(flox/flox) mice are healthy and fertile. They didn’t have detectable SIK3 protein in their brain. We examined sleep/wakefulness in neuron-specific SIK3-deficient mice. They showed a decreased sleep need.
Forgetting during REM sleep by hypothalamic MCH neurons

Shuntaro Izawa¹, Ryo Inoue¹, Srikanta Chowdhury¹, Yasutaka Mukai¹, Daisuke Ono¹, Akihiro Yamanaka¹,²
¹Research Institute of Environmental Medicine- Nagoya University, Neuroscience Research, Nagoya, Japan.
²Start of Core Research for Evolutional Science and Technology CREST, Japan Science and Technology Agency JST, Tokyo, Japan.

Sleep is assumed to not only consolidate memory but also impair memory. However, the neural mechanisms behind it has been unclear. Here we found that hypothalamic melanin concentrating hormone (MCH) producing neurons impair memory via the hippocampus during rapid eye movement sleep (REMS).

Using retrograde tracing beads injection into the hippocampus, we found that MCH neurons have dense projections from the hypothalamus to the hippocampus. MCH neurons are known to regulate REMS, and we identified that MCH neurons were active during REMS using fiber photometry calcium imaging. To reveal the role of MCH neurons in hippocampus-dependent memory, MCH neurons manipulated mice were subjected hippocampus-dependent memory tests. The pharmacogenetic activation of MCH neurons with hM3Dq impaired memory ability, whereas the inhibition with hM4Di improved it. MCH neurons-ablation also induced memory improvement, suggesting MCH neuronal activity induces memory impairment. Optogenetic stimulation of MCH neurons reduced memory score with the stimulation during memory retention, but not encoding nor retrieval period. The slice patch clamp recording in the hippocampus showed that the light stimulation of MCH nerve terminals hyperpolarized and decreased firing frequency of hippocampal pyramidal neurons. In the behavior tests, memory ability was impaired by the optogenetic stimulation of MCH nerve terminals in the hippocampus. During memory retention period, MCH neurons inhibition in each sleep-wakefulness states (Wakefulness, REMS, and Non-REMS) were performed with optogenetic inhibition triggered by the automatic sleep staging system. The inhibition during REMS improved memory ability compared with Wakefulness or Non-REMS, indicating MCH neurons induce forgetting during REMS.

Adenosinergic mechanisms of sleep control in the nucleus accumbens

Xuzhao Zhou, Yoan Cherasse, Yo Oishi and Michael Lazarus
International Institute for Integrative Sleep Medicine (IIIS), University of Tsukuba

We have revealed a prominent role of adenosine A2A receptor (A2AR)-expressing neurons in the nucleus accumbens (NAc) in sleep/wake regulation and proposed a novel brain circuit for sleep control by motivated behavior (Oishi Y., et al., Nat. Commun., 8:article 734, 2017). This brain circuit may explain the tendency to fall asleep in the absence of motivating stimuli, i.e., when bored. We hypothesized that the ability of the NAc to induce sleep is mediated by the classic somnogen adenosine. Adenosine can be formed by various processes in all types of cells but the neurodynamics of adenosine (release, receptor activation, etc.) in controlling sleep are still unclear. In this study, we ablated glial fibrillary acidic protein (GFAP)-positive cells in the NAc of mice by virus-mediated expression of diphtheria toxin (DT) receptors and intraperitoneal administration of DT. After analyzing electroencephalogram and electromyogram recordings of the mice, we found a remarkable increase in slow-wave sleep (SWS) one week after DT treatment that was, surprisingly, accompanied by an increased number of GFAP cells in the NAc. In-vivo microdialysis one week after DT treatment revealed a significant increase of extracellular adenosine in the NAc. Moreover, we found that chemogenetic activation of NAc GFAP cells strongly increased the total amount of SWS. Our current results may suggest that adenosine from NAc glial cells plays an important role for sleep control.
Exploration of the intracellular molecular mechanisms for homeostatic sleep/wake regulation

Tomohiro Kitazono¹, Taeko Matsuoka¹, Aya Ikkyu¹, Zhiquang Wang¹, Jing Ma¹, Hiromasa Funato¹,², Masashi Yanagisawa¹,³
¹WPI-HIS, Univ of Tsukuba, ²Dept of Anat, Sch of Med, Toho Univ, ³HHMI, Univ of Texas Southwestern Medical Center at Dallas

Sleep is a ubiquitously conserved behavior in animals with central nervous system, but intracellular mechanisms regulating sleep/wake homeostasis remain unknown. Our previous study suggested that SIK3 protein kinase plays a central role in sleep/wake regulation (Funato et al., 2016). Here, we further analyzed the upstream and downstream regulatory mechanisms involving SIK3. 1) Upstream analysis: Ser551 is one of the phosphorylation sites of SIK3, and we recently found that phosphorylation of this site was important in sleep/wake regulation (Honda et al., 2018). Therefore, we investigated the regulatory mechanisms of S551 phosphorylation in SIK3, and found that PKA could phosphorylate this site, and phosphorylation of PKA could also be regulated by SIK3. 2) Downstream analysis: Although we previously reported that a splicing mutant of Sik3 gene, Sleepy mutant brains exhibited a markedly altered phosphoproteomic status and mimicked sleep-deprived brains (Wang et al., 2018), the downstream signaling pathway of SIK3 in sleep regulation is still unclear. We recently found that Sleepy and null mutant mice of Sik3 showed opposite phenotype in sleep, thus, we examined phosphoproteomic status of Sik3 null mutant brain. We found ~200 phosphorylation sites showing significant phosphorylation state change in both mutant brains, and, surprisingly, phosphorylation of almost all sites increased in both. In addition, we examined brain interactome of SLEEPY2, which was a novel regulator of sleep homeostasis found in our genetic screening, and found that SLEEPY2 was highly associated with multiple cell adhesion molecules. These findings will enable us to provide new insights on the homeostatic sleep regulation.

Synthesis and evaluation of [18F]-labeled tetrahydroisoquinoline derivatives as novel PET probes for imaging orexin 1 receptors

Hiroyuki Watanabe¹*, Kengo Fukui¹, Yoichi Shimizu¹,², Hideo Saji¹, Masahiro Ono¹,²
¹Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadaichi-cho, Sakyo-ku, Kyoto 606-8501, Japan. ²Department of Diagnostic Imaging and Nuclear Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan.

Introduction: Orexin 1 receptor (OX₁R) is thought to be involved in various body functions, including arousal maintenance and emotional control, but the full details of its function remain unknown. OX₁R imaging with positron emission tomography (PET) would be useful in elucidating the orexin system including OX₁R. However, no PET probes targeting OX₁R have been reported. Therefore, we designed and synthesized tetrahydroisoquinoline (THIQ) derivatives as novel PET probes targeting OX₁R, and evaluated their utility.

Methods: We successfully synthesized THIQ-1 and THIQ-2. To quantify their affinities for OX₁R and OX₂R, we carried out in vitro competitive binding assays using OX₁R or OX₂R expression cells and [125I]Orexin A. After radiosynthesis of [18F]THIQ-1 and [18F]THIQ-2, we investigated the cellular binding of [18F]THIQ-1 and [18F]THIQ-2 to cell expressing OX₁R or OX₂R. Finally, to evaluate the brain uptake of [18F]THIQ-1 and [18F]THIQ-2, we performed a biodistribution study in normal mice.

Results: In an in vitro competitive binding assay, THIQ-1 and THIQ-2 showed significantly higher binding to OX₁R (IC₅₀ = 30 and 31 nM, respectively) than OX₂R (IC₅₀ = 160 and 332 nM, respectively). These features were also observed in the cell binding assay using [18F]THIQ-1 and [18F]THIQ-2, demonstrating their OX₁R-specific binding property in vitro. In the biodistribution study using normal mice, [18F]THIQ-1 and [18F]THIQ-2 showed brain uptake at 2 min post-injection, but the uptake levels were not sufficient for in vivo imaging with PET.

Conclusion: [18F]THIQ-1 and [18F]THIQ-2 have the potential to become useful imaging probes for PET targeting the OX₁R.
Mice lacking orexin peptides, orexin neurons, or orexin receptors recapitulate human narcolepsy phenotypes, further highlighting a critical role for orexin signaling in the maintenance of wakefulness. However, although the lack of orexin signaling causes the sleep disorder narcolepsy, the precise neural mechanisms by which orexin neurons prevent narcolepsy remain unclear. In a previous study, we found that targeted restoration of orexin receptor expression in the dorsal raphe (DR) and in the locus coeruleus (LC) of mice lacking both of these receptors inhibited cataplexy and pathological fragmentation of wakefulness (i.e., sleepiness), respectively. These results suggested that DR serotonergic and LC noradrenergic neurons play differential roles in orexin neuron-dependent regulation of sleep/wakefulness. As a next step, we used optogenetic and chemogenetic approaches to demonstrate that DR serotonin neurons suppress cataplexy by reducing the activity of the basolateral/lateral amygdala that plays an important role in emotional processing, as consistent with the fact that strong emotion often triggers cataplexy. Our results suggest that the orexin neuron–DR serotonin neuron–amygdala pathway is a critical circuit for preventing cataplexy. Furthermore, we identified a neuronal pathway that induces cataplexy when activated by optogenetic manipulation. We will discuss the role of this pathway in emotional processing as well as in REM-related muscle atonia.
Roles of orexin neurons in motivated behaviors in rats

Hiroyuki Mizoguchi1, Ayumu Inutsuka2, Kentaro Katahira3, Kiyofumi Yamada4, Akihiro Yamanaka5
3Dept Psychol, Grad Sch Inform, Nagoya Univ, Nagoya, Japan

Orexin neurons regulate physiological functions, including not only energy homeostasis and wakefulness, but also motivated behaviors. These neurons seem to play important roles in linking metabolic need to motivated behaviors via the dopaminergic system. Recently, we developed a rat model that expresses the Cre recombinase specifically in orexin neurons. Moreover, we established the gambling test for assessing reward motivation and decision-making under conditions of uncertainty, as well as a touch-screen system for assessing reward sensitivity and craving. Here, we examined the roles of orexin neurons in reward motivation and decision-making when orexin neurons are manipulated using pharmacogenetics approaches. In the gambling test for rodents, cell-specific excitatory manipulation of orexin neurons of rats using the DREADD technology resulted in risky arm-choice. Positive, but not negative, reward prediction error may contribute to reward-based risky choice when orexin neurons are activated. The motivational values of a large reward was increased when orexin neurons were activated by the DREADD technology. In the probability reversal learning test, cell-specific excitatory manipulation of orexin neurons resulted in low performance in rats. These results suggest that activated orexin neurons affect motivational processes, and may alter strategy in reward-based choice behavior, potentially leading to an action that fails to yield rewards and detect changes in reinforcement contingencies.

Acute social defeat stress increases sleep in mice

Shinya Fujii1, Mahesh K. Kaushik1, Xuzhao Zhou1, Mustafa Korkutata1, and Michael Lazarus1
1International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Japan

Social stress is a major risk factor for psychiatric disorders. Social defeat stress (SDS) is often induced in animal models to study mood and anxiety disorders. Stress leads to insomnia, presumably by making it difficult to fall asleep and affecting sleep quality. The effect of SDS on sleep/wake behavior is not well understood. We established a mouse model of acute SDS based on strong aggressive mouse behavior toward unfamiliar intruders. In response to social defeat, slow-wave sleep (SWS) strongly increased for 9 h, whereas rapid eye movement sleep increased 6 h after the stress event for following 3 h. Slow-wave activity was only enhanced for a short period after social defeat and dissipated long before the SWS returned to baseline. Moreover, social defeat evoked a strong corticosterone response that may indicate a high stress level in the intruder mice after social defeat. These findings suggest that SDS increases sleep in mice and thus, insomnia does not appear to be a consequence of social stress in mice. Our social defeat model may be useful for studying the mechanisms and function of sleep in response to social stress.
Salt inducible kinase (SIK) family, one of the AMP-activated protein kinase (AMPK) related kinases, has 3 paralogs in vertebrate animals. Among SIK family, SIK3 is expressed in many organs including liver, skeleton, and brain, and known to be essential for glucose/lipid metabolism and skeletal development. Although the function of SIK3 in the brain had been virtually unstudied, forward-genetics analysis in randomly mutagenized mice disclosed the role of SIK3 in regulating sleep amount. A chemically induced nucleotide substitution at Sik3 splice donor site caused exon 13-skipping, resulted in prolonged non-rapid eye movement (NREM) sleep. The exon 13-encoded region of SIK3 encompasses protein kinase A (PKA) recognition site, but how the PKA recognition site affect the kinase activity is unknown.

Here, we newly developed Förster resonance energy transfer (FRET) probe that enable us to monitor spatio-temporal change of SIK3 kinase activity in living cells. We transfected the probe with mutant SIK3 such as PKA recognition site-deficient SIK3 to cultured cells and examined how the kinase activity is modulated by PKA signaling. In addition, we compared the basal kinase activity between wild type and PKA recognition site-deficient SIK3 in each cells with normalization of amount of SIK3.

The lateral hypothalamus (LH) is implicated in the regulation of instinctive behaviours, including sleep. Orexin-producing neurons (orexin neurons) and melanin-concentrating hormone (MCH)-producing neurons (MCH neurons) are exclusively distributed in the LH and project to whole brain to regulate sleep/wakefulness. Ablation of orexin neurons decreased wakefulness in the dark period and resulted in narcolepsy, whereas ablation of MCH neurons increased wakefulness. These indicated that orexin and MCH neurons play a different role in the regulation of sleep/wakefulness. Nevertheless, it is unclear how the interactions between orexin and MCH neurons affect sleep/wakefulness. To understand the functional interaction between orexin and MCH neurons and its regulation of sleep/wakefulness, transgenetic mice in which both orexin and MCH neurons are ablated by the Tet-off system were used. These orexin and MCH (double) ablation mice increased wakefulness and decreased non-rapid eye movement (NREM) sleep. This was similar to MCH neurons ablated mice. However, in addition to these, total time in REM sleep also diminished in double ablated mice. Total time and duration of cataplexy were exacerbated in double ablated mice compare with orexin neurons ablated mice. These results suggest that MCH neurons have a suppressive role in cataplexy. Interestingly, these mice showed new state, which was not classified with conventional criteria of sleep/wakefulness or cataplexy. Here we defined as delta/theta (DT) sleep. DT sleep was only observed in the double ablated mice during wakefulness as a sudden arresting behaviour with high EEG density of δ and θ wave with low EMG power. EEG spectrum analysis revealed that DT sleep was similar to NREM sleep just before transition to REM sleep. Taken together, MCH neurons might be involved in the regulation of NREM to REM sleep transition.
Cortical Neuronal Communication During Natural Slow Wave Sleep in Mice

Sumire Matsumoto1,2, Kaoru Ohyama2,3, Javier Diaz2, Robert Greene2,4, Kaspar Vogt2
1Ph.D. Program in Human Biology, School of Integrative and Global Majors, University of Tsukuba, Tsukuba, Japan
2International Institute for Integrative Sleep Medicine (WPI-IIS), University of Tsukuba, Tsukuba, Japan
3Japan Society for the Promotion of Science (JSPS) Research Fellow
4Department of Psychiatry&Neuroscience, Peter O’Donnell Brain Institute, UT Southwestern Medical Center, Dallas, TX, USA

The amplitude and power of slow wave activity (SWA) during slow wave sleep (SWS), recorded from electroencephalograms (EEG) is a key indicator of sleep homeostasis and therefore it likely has a central role in sleep. SWA are reflected the synchronized activity of cortical neurons. It reflects their high participation in alternating shifts between a hyperpolarized (DOWN) and a depolarized (UP) membrane potential. Synchronization of this activity can be observed over large areas of the cortex (Destexhe et al., 1999; Vyazovskiy, et al., 2009). These phenomena raise the question of how those neurons achieve the wide spread synchronization after we fall asleep. It was reported that the SWA is a traveling wave (Massimini, et al., 2004), suggesting that SWA synchronization propagates through cortical network interactions. However, the precise mechanism of how the reactivity is modulated is still unclear. Here we established local field potential (LFP) and unit recordings coupled with cortical stimulation using optogenetics to investigate cortical communication during natural waking and sleep. Channelrhodopsin 2 expression and laser stimulation were performed unilaterally to somatosensory cortex, and the signals were recorded from primary motor cortex of both hemispheres. We found that the light-evoked potential in both ipsilateral and contralateral side to the stimulation grow stronger during SWS. More unit activities were also recorded as a response to the stimulation during SWS. We also analyzed the stimulation during ON or OFF phase of the slow wave activity separately, and the larger response were observed in both phase. This data suggest that in SWS the cortical neurons are more responsive to the input both during ON and OFF phase.

Prevalence of self-rated sleep apnea and related factors in a Japanese working population

Chihiro Suzuki1,2, Emi Morita2,3, Asuka Ishihara2,4, Sumire Matsumoto2,4, Yu Ikeda2, Mami Ishitsuka2, Daisuke Hori2, Shotaro Doki2, Yuichi Oi2, Shinichiro Sasahara2, Ichiro Matsumoto2,7, Masashi Yanagisawa2, Makoto Satoh2
1Master’s Program in Medical Sciences, University of Tsukuba, Japan
2International Institute for Integrative Sleep Medicine (WPI-IIS), Japan
3Forestry and Forest Products Research Institute, Japan
4Ph.D. Program in Human Biology, University of Tsukuba, Japan
5Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan
6Department of nursing, Teikyo University, Japan
7Faculty of Medicine, University of Tsukuba, Japan

Purpose: Sleep disordered breathing (SDB) is one of the risk factors for cardiovascular diseases, therefore its early detection and treatment are important. However, there are few studies investigating the self-rated prevalence of SDB. The aim of this study is to investigate the percentage of individuals who are self-aware of and treated for SDB among Japanese workers, as well as factors associated with SDB.

Methods: A survey was conducted on self-administrated questionnaire. A total of 762 participants (men 338 and women 424; mean age±SD: 43.0±11.3 years) were included in the analysis. The odds ratios were calculated by logistic regression analysis, adjusted for age, BMI (continues variables), nightcap, smoking and exercise habits.

Results: The number of participants who answered “no apnea”, “apnea rated as once a week”, and “unknown” were 142 (42.0%), 53 (15.7%), and 143 (42.3%) in men, and 257 (60.6%), 6 (1.4%), and 161 (38.0%) in women, respectively. Among potential patients (n=59), 12 participants (20.4%, 10 men and 2 women) had or are currently being treated of apnea. After excluding “unknown,”, the proportion of self-rated apnea was significantly different between men and women ($p<0.001$). The adjusted odds ratio of BMI for self-rated apnea is 1.44 (95%CI: 1.22-1.69) in men.

Discussion: Only 20.4% of participants who were aware of apnea were in treatment, suggesting that many are unaware of the importance of treatment for SDB. It is confirmed that high BMI is a risk factor for apnea in men. We will continue to analyze in detail, including data from health check-ups.
Different effects of orexin receptor antagonist and GABAA agonist on physical and cognitive functions.

Jaehoon Seol1, Yuya Fujii1, Insung Park2, Yoko Suzuki2, Fusae Kawana2, Katsuhiko Yajima1, Shoji Fukusumi2, Tomohiro Okura1, Makoto Satoh1, Kumpei Tokuyama2, Toshio Kokubo2, Masashi Yanagisawa2

1Physical Education, Health and Sport Sciences, University of Tsukuba.
2International Institute for Integrative Sleep Medicine, University of Tsukuba.

Purpose: To evaluated side-effect profile of suvorexant, compared with GABAA receptor agonists, in human cognitive and motor function during the middle of the night upon forced awakening.

Methods: The present study was a randomized, double-blind controlled, 3-way crossover study. The participants were 30 healthy males, and sleep was recorded polysomnographically. 15 minutes before bedtime, participants took a pill: suvorexant 20 mg, brotizolam 0.25 mg, or placebo. A series of physical and cognitive functions tests were performed 3.5 h before bedtime (pre-test). 90 minutes after taking a pill, participants were forced-awoken. Participants repeated the same physical and cognitive functions tests (post-test). After woke up in the morning, participants repeated the physical and cognitive functions tests (follow-test). Physical function task is adopted body sway, agility and dynamic balance test, choice stepping reaction time and Purdue pegboard test. Cognitive function task used Stroop color-word test.

Results: Body sway showed significant interaction effects (interaction P < 0.001) of time effect (P = 0.002) and main effect (P < 0.001). The post-hoc test showed that the rectangular area significantly increased in the post-test under brotizolam but returned to control value. Agility and dynamic balance significantly deteriorated in the post-treatment under brotizolam and suvorexant compared to placebo.

Conclusion: Suvorexant didn’t show significant adverse effects in static balance ability, while brotizolam reduced static balance ability evaluated after forced awakening. However, when doing intense movements or difficult cognitive task at that time, there may be adverse effects at midnight.

Reactivation of adult-born neurons for memory consolidation during sleep

Sakthivel Srinivasan1, Toshie Naot1, Masanori Sakaguchi2

1International Institute for Integrative Sleep Medicine (WPI-IIMS), University of Tsukuba, Tsukuba, Ibaraki

The mechanisms how episodic memory is consolidated during sleep is unknown. We found that the hippocampal adult-born neurons (ABNs) play crucial roles in memory consolidation during sleep1. It is suggested that memory replay contribute to memory consolidation during sleep2. However, it is not clear whether memory reactivation during sleep is necessary for memory consolidation. To clarify this point, we examine the effect of silencing the reactivation of ABN during sleep. For this purpose, we created triple-transgenic animal to specifically target ABNs, which are activated during learning. This study will provide a causal link between reactivation of memory engram with its consolidation during sleep.

2. Skaggs&McNaughton, Science, v271, p1870

We thank all Sakaguchi lab and WPI-IIMS members, D. Kumar, P. Vergara, T. Hosokawa, I. Koyanagi, Y. Mimura, K. Tanaka, T. McHugh for technical assistance, M. Adachi for secretarial assistance, This work was partially supported by grants from the World Premier International Research Center Initiative from the Japan Ministry of Education, Culture, Sports, Science, and Technology (MEXT); JST CREST Grant Number JPMJCR1655; Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (JSPS, KAKENHI grant numbers 16K18359, 15F15408, 26115502, and 25116530); The Takeda Science Foundation; Shimadzu Science Foundation; Kanae Foundation for the Promotion of Medical Science, Research Foundation for Optho-Science and Technology; The Ichiro Kanehara Foundation; Kato Memorial Bioscience Foundation; Japan Foundation for Applied Enzymology; Senshin Medical Research Foundation; Life Science Foundation of Japan; Uehara Memorial Foundation; Brain Science Foundation; Kowa Life Science Foundation; and 2016 Inamori Research Grants Program to MS, The Takeda Science Foundation (TSF); The Tokyo Biochemical Research Foundation (TBRF) to SS.
We recently reported mutant mice with significant sleep/wake abnormalities, obtained through a large-scale EEG/EMG screening of randomly mutagenized cohort (1). One of these mutants, named Sleepy, bearing a splicing mutation on the sik3 gene (with exon 13 skipping), exhibits increased daily amounts of non-REM sleep with a constitutively elevated sleep need and EEG delta power during non-REM sleep. The molecular pathway for the regulation of sleep in which sik3 takes part is yet to be determined. The EEG envelope characterization space (analysis based on the signal envelope properties) can provide valuable information about the underlying dynamics of the neuronal ensembles contributing to EEG (2). This analysis reveals that delta waves during non-REM sleep markedly differ from the behavior of rhythmic waves, showing instead clear Gaussian properties. Considering these results, delta waves can be modeled as emerging from the superposition of EEG transients, i.e., phasic activity. In the present study, unique EEG features of Sleepy mutants were explored under the light of the envelope characterization space. In this parameter space, the abnormal properties of the mutant’s EEG patterns are evident at a glance, either by visual inspection or automated analyses. We found that the high-amplitude non-REM delta waves characteristic of the Sleepy mutant are associated with an increased phasic activity in delta, theta and sigma bands. Remarkably, this augmented phasic activity is more pronounced in theta and sigma bands. These results are compatible with the hypothesis of a superposition of transient activity with broadband spectral consequences, originating from a colored spectrum as previously proposed (2), but implies different amplitudes and different temporal properties characterizing the EEG transients in the Sleepy mutant as compared with wildtype mice. Taken together, these results help to further characterize the sleep abnormalities induced by the sik3 mutation.

H. Hiraishi$^{1,2}$, T. Ikeda$^2$, D. N. Saito$^2$, C. Hasegawa$^2$, S. Kitagawa$^2$, T. Takahashi$^3$, M. Kikuchi$^2$, Y. Ouchi$^1$
$^1$Department of Biofunctional Imaging, Hamamatsu University School of Medicine
$^2$Research Center for Child Mental development, Kanazawa University
$^3$Health Administration Center, Fukui University

Our study aimed to clarify changes in neuronal connection by examining the functional connectivity (FC) during moral judgment.

Method: Eleven male university students participated in this study. When pictures with three-frame cartoon about morality (good, bad and neutral) were presented, participants were asked to evaluate the pictures based on their morality by pushing a button and their brain activities were measured by a 160-channel Magnetoencephalography (MEG, Yokogawa). MEG-160 software was used for analyzing MEG data with sampling rate 1000Hz, and preprocessed data (classified into three time-window sets) were analyzed with a software Brainstorm.

Results: With the intensity threshold set over 0.8, the number of FC (nFC) for bad moral judgment (BMJ) was found more than that for good moral judgment (GMJ). The nFC was gradually increasing in both BMJ and GMJ with passage of time. No interhemispheric (global) connectivity was found in both judgment conditions in all three time-window sets. In the third time-window set, however, nFC for BMJ was 39 intra-hemispheric (local) connectivity (left=25) and that for GMJ was 29 (left=15).

Discussion: There was no interhemispheric connectivity in both judgments in the three time-window sets. Since lowering the threshold to 0.6 showed presence of some interhemispheric connectivity in the occipital and frontal pairs, the interhemispheric information processing might be existent but weaker than the intra-hemispheric one in moral judgment. The time-dependent greater increase in nFC in the left temporoparietal area in BMJ than in GMJ suggests dynamic changes in neuronal connections occurring in establishment of “Theory of Mind”.

J. DIAZ$^1$, K. E. VOGT$^1$, H. FUNATO$^1$, J.-C. LETELIER$^2$, M. YANAGISAWA$^1$
$^1$WPI-IIIIS, Univ. of Tsukuba, Tsukuba, Japan
$^2$Univ. of Chile, Santiago, Chile
Metabolomic screening for memory consolidation during sleep

Iyo Koyanagi1, Kazuhiro Sonomura2, Taro Tezuka3, Takaaki Sato2, Takaaki Ohnishi4, Masanori Sakaguchi1

1International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan
2Research Institute, Shimadzu co ltd, Japan
3Faculty of Library, Information and Media Science, University of Tsukuba
4ICT University of Tokyo

Sleep plays crucial roles in memory consolidation, however, its molecular mechanism is still elusive. It was shown that hippocampal theta oscillation is necessary for memory consolidation during REM sleep1. The hippocampal dentate gyrus (DG) shows pronounced theta oscillation during REM sleep2 and is sufficient to hold memory trace3. Indeed, we have demonstrated that DG neurons are necessary for memory consolidation during sleep4. Although genetic screening was conducted in the DG for memory consolidation5, there should be other molecular entities involved in memory consolidation. For example, a purine metabolite, adenosine, plays critical roles in sleep6. Therefore, we examined around 300 metabolites in the DG upon learning and sleep. We found that several purine metabolites are upregulated upon both sleep and learning. For those metabolites, we examined their function in memory and sleep. This study will show the functional significance of metabolites in memory consolidation during sleep, and shed new light on molecular mechanisms how memory is consolidated during sleep.

4. Kumar, Koyanagi et al., in prep.

We thank all Sakaguchi lab and WPI-IIIS members, K. Iio, N. Kutsurna, H. Nagase, T. Naoi, D. Kumar, P Vergara, K. Shikama, S. Sugimori, N. Hasegawa, H. Obo for technical assistance, N. Kobayashi and M. Adachi for secretarial assistance. This work was partially supported by grants from the World Premier International Research Center Initiative from the Japan Ministry of Education, Culture, Sports, Science, and Technology (MEXT); JST CREST Grant Number JPMJCR1655; Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (JSPS, KAKENHI grant numbers 16K18359, 15F15408, 26115502, and 25116530); The Takeda Science Foundation; Shimadzu Science Foundation; Kanae Foundation for the Promotion of Medical Science, Research Foundation for Opto-Science and Technology; The Ichiro Kanehara Foundation; Kato Memorial Bioscience Foundation; Japan Foundation for Applied Enzymology; Senshin Medical Research Foundation; Life Science Foundation of Japan; Uehara Memorial Foundation; Brain Science Foundation; Kowa Life Science Foundation; and 2016 Inamori Research Grants Program to MS.
Schizophrenia is a severe psychiatric disorder characterized by disturbances of thoughts, perceptions, emotions and cognition. The disease affects around 1% of the general population worldwide and is enormously costly to society. Twin and adoption studies have shown that schizophrenia is highly heritable, with disease risk resulting from the interplay of diverse genetic variants as well as environmental factors. However, the molecular and neural basis of schizophrenia remain largely unclear. Schizophrenia is often associated with emotional response deficit to fear stimuli. We developed a forward genetics screen to isolate ethylnitrosourea (ENU)-mutagenized mice with abnormal fear responses. We identified a dominant mutant pedigree, named Psycho, that exhibited "schizophrenia-like" symptoms, including hyperactivity, risk-taking behaviors, reduced motivation, and impaired cognition. We hypothesize that Psychom/+ mice may serve as an excellent model to understand the molecular and neural basis of schizophrenia.