Poster Session Abstracts

The 4th Annual IIIS Symposium

February 26, 2016

Data Blitz by Poster Presenters	15:00 – 15:25
Poster Session	15:25 – 16:15

Poster list

No.	Presenter	Affiliation	Title	Page
P01	Carsten T. Beuckmann	Eisai Co., Ltd.	Preclinical pharmacological characterization of lemborexant, a novel dual orexin receptor antagonist for insomnia treatment	1
P02	Carsten T. Beuckmann	Eisai Co., Ltd.	Lemborexant, a novel DORA, promotes physiological sleep in mice and rats without causing motor impairment or alcohol interaction	1
P03	Yo Oishi	WPI-IIIS (Lazarus Lab)	Indirect pathway in the nucleus accumbens core regulates slow-wave sleep	2
P04	Haruhisa Kawasaki	AIST	Sleep abnormality of *Drosophila* model for Gaucher's disease and Parkinson's disease	2
P05	Kazuki Sakata	AIST	Inositols affect close-proximity rhythm of *Drosophila* mating circadian behavior	3
P06	Tomomi Okajima	Life and Environmental of Science, University of Tsukuba	Molecular basis of microglial activation regulated by hypoxanthine	3
P07	Takuma Aihara	Life and Environmental of Science, University of Tsukuba	New insight into the effect of cold shock protein on autophagy in brain	4
P08	Kristopher McEown	WPI-IIIS (Lazarus Lab)	The Role of Sleep Restriction on Appetite for Highly Palatable Foods	4
P09	Yoan Cherasse	WPI-IIIS (Urade Lab)	Zinc, a novel modulator of sleep	5
P10	Masashi Furukawa	Nishikawa Sangyo Co., Ltd.	Influences of in-bed temperature on sleep	5
P11	François Grenier	WPI-IIIS (Greene/Vogt Lab)	Studying the locality of sleep slow wave activity and its homeostasis	6
P12	Tomasz M. Rutkowski	Life Science Center of TARA, University of Tsukuba	Automatic sleep staging and apnea events classification from EEG and multimodal physiological signal – Synchrosquezing transform processing and Riemannian geometry classification approaches	6
P13	Liqin Cao	WPI-IIIS (Liu Lab)	Understanding the molecular basis of fear and the role of emotion in sleep regulation	7
P14	Takato Honda	WPI-IIIS (Yanagisawa/Funato Lab)	Systematic behavioral screening of *Sleepy* and *Dreamless,* newly identified mouse pedigrees with sleep abnormalities	7
P15	Makoto Taniguchi	Haravard Medical School	*Npas4* , novel HDAC5 target gene, mediates its role in limiting cocaine reward-related behavior	8
P16	Zhiqiang Wang	WPI-IIIS (Liu Lab)	Sleep widespread remodeling brain phosphoproteome implicate sleep homeostasis restoration mechanism	8
P17	Yoshimasa Koyama	Fukushima University	Mesopontine-amygdala mechanisms inducing induce blood pressure fluctuation during REM sleep	9
P18	Haojun Xu	WPI-IIIS (Yanagisawa/Funato Lab)	Alterations in microglia in response to sleep loss	9
P19	Mohammad Sarowar Hossain	WPI-IIIS (Yanagisawa/Funato Lab)	Identification of *Sim1* and *Mc4r* mutations through obesity screening of randomly mutagenized mice	10
P20	Tomoyuki Fujiyama	WPI-IIIS (Yanagisawa/Funato Lab)	Identification of a single nucleotide substitution specific to the *Dreamless* mutant mouse by linkage analysis and whole exome sequencing, and its genetic verification by CRISPR	10
P21	Shinichi Miyazaki	WPI-IIIS (Hayashi Lab)	Analyses of the *C. elegans sleepy-homologue* mutants suggest a deep conservation of sleep-regulating genes	11
P22	Chika Miyoshi	WPI-IIIS (Yanagisawa/Funato Lab)	Sleep screening of randomly mutagenized mice established a long sleep pedigree	11
P23	Deependar Kumar	WPI-IIIS (Sakaguchi Lab)	The role of adult born neurons in memory consolidation during sleep-wake cycles	-

P01

Preclinical pharmacological characterization of lemborexant, a novel dual orexin receptor antagonist for insomnia treatment

Carsten T. Beuckmann[1], Michiyuki Suzuki[1], Makoto Nakagawa[1], Shigeru Akasofu[1], Takashi Ueno[2], Tohru Arai[3], Hiroyuki Higashiyama[1]

[1] *Global Discovery Research, Neuroscience & General Medicine PCU, Eisai Product Creation Systems, Eisai Co., Ltd.; Tsukuba, Japan*

[2] *Global Drug Metabolism and Pharmacokinetics, Biopharmaceutical Assessments CFU, Eisai Product Creation Systems, Eisai Co., Ltd.; Tsukuba, Japan*

[3] *Biomedicinal Screening, Next Generation Systems CFU, Eisai Product Creation Systems, Eisai Co., Ltd.; Tsukuba, Japan*

Dual orexin receptor antagonists (DORA) promote sleep in animals and humans, offering a new treatment option for insomnia patients. Here we describe in vitro and initial *in vivo* characterization of the novel DORA lemborexant.

IC_{50} values of lemborexant on human orexin-1 receptor (hOX1R) and hOX2R are 6.1 and 2.6 nmol/L, respectively. Among 88 off-targets, lemborexant interacted only with human melatonin 1 receptor as a weak antagonist (K_i 922 nmol/L). Lemborexant displayed fast-on and fast-off binding kinetics on the hOX2R, indicating potential for fast onset of action and reduced risk of next-morning drowsiness. Lemborexant is a competitive antagonist, with K_i values of 14.1 and 0.391 nmol/L for hOX1R and hOX2R, respectively, and binds to hOX2R in an orthosteric fashion. From 3 mg/kg on, lemborexant dose-dependently counteracted the OX2R-selective [Ala^{11}, D-Leu^{15}]-orexin-B-induced increase in plasma adrenocorticotropic hormone concentration. At doses of 30 and 100 mg/kg, lemborexant significantly decreased locomotion of wild-type mice, while it had no effect on orexin-neuron deficient *orexin/ataxin-3* Tg/+ mice. In mice, 10 mg/kg lemborexant significantly increased non-REM and REM sleep time in a ratio of about 3:1, without changing composition of promoted sleep, as judged by REM sleep time ratio to total sleep time.

These preclinical data demonstrate lemborexant to be a potential novel therapeutic for the treatment of insomnia.

P02

Lemborexant, a novel DORA, promotes physiological sleep in mice and rats without causing motor impairment or alcohol interaction

Carsten T. Beuckmann, Shigeru Akasofu, Makoto Nakagawa, Michiyuki Suzuki

Global Discovery Research, Neuroscience & General Medicine PCU, Eisai Product Creation Systems, Eisai Co., Ltd.; Tsukuba, Japan

As clinical candidate for insomnia treatment, we describe the preclinical characterization of novel dual orexin receptor antagonist lemborexant in regard to rodent sleep and some safety pharmacology aspects.

In rats, the ED_{50} of oral lemborexant for dose-dependent increase in total sleep time was 4.4 mg/kg, without changing REM sleep ratio and therefore indicating physiological sleep, in contrast to zolpidem, which suppressed REM sleep. When rats were chronically treated with lemborexant, sleep time-increasing effect and reduction of sleep latency were constant, indicating neither tolerance nor sensitization. Upon discontinuation, sleep returned to pre-dosing values without rebound, different from zolpidem. In acute and chronic rat experiments, no direct transition from wakefulness to REM sleep was observed. In mice, oral lemborexant increased total sleep time from 1 mg/kg on without influence on REM sleep ratio, and reduced sleep latency. 30 mg/kg almorexant and 3 mg/kg zolpidem also promoted sleep with comparable effect sizes. Lemborexant did not promote sleep in orexin-neuron deficient mice. Up to 300 mg/kg, oral lemborexant in mice did not impair motor coordination nor did it significantly interact with ethanol, while 100 mg/kg zolpidem showed clear detrimental effects.

These data demonstrate the potential of lemborexant to promote physiological sleep in humans without impairing motor coordination or showing a strong interaction with alcohol, offering potential advantages over non-benzodiazepine gaba-ergics.

P03

Indirect pathway in the nucleus accumbens core regulates slow-wave sleep

Yo Oishi[1], Qi Xu[2,3], Lu Wang[2], Zhi-Li Huang[2] and Michael Lazarus[1]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan*

[2] *State Key Laboratory of Medical Neurobiology, Department of Pharmacology, Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, Shanghai Medical College of Fudan University, Shanghai 200032, China*

[3] *Department of Physiology, School of Basic Medical Sciences, Anhui Medical University, Heifei 230032, China*

The arousal effect of caffeine depends on adenosine A_{2A} receptors on neurons in the nucleus accumbens (NAc). Those NAc inhibitory medium spiny neurons also express dopamine D_2 receptors and are involved in the dopaminergic control of motor function and motivational behavior. However, their role in the regulation of sleep is unclear. We found that optogenetic stimulation of the indirect pathway in the NAc induced robust slow-wave sleep, whereas slow-wave activity was not affected. As the NAc contains two anatomically and functionally different components, shell and core, we examined whether these structures have distinct roles on sleep regulation and found that the optogenetic stimulation of the indirect pathway in the NAc core, but not shell, promoted slow-wave sleep. Next, we examined the output pathways of the NAc core by anterograde tracing and found that the core heavily innervated the ventral pallidum in the basal forebrain and moderately projected to well-known arousal-related areas, such as the lateral hypothalamus (orexin), the tuberomammillary nucleus (histamine) and the ventral tegmental area (dopamine). Finally, we examined effects of photostimulation of ChR2-expressing NAc core terminals in their target areas on sleep/wake behavior in mice. We will discuss the data for the terminal photostimulation in our presentation.

P04

Sleep abnormality of *Drosophila* model for Gaucher's disease and Parkinson's disease

Haruhisa Kawasaki[1], Kunpei Ito[1, 2], Takahiro Suzuki[1] Kazuki Sakata[1, 2] and Norio Ishida[1, 2]

[1] *Ishida Group of Clock Gene, Biomedical Research Institute, National Institute of Advanced Science and Technology (AIST) 6-5 Central, Tsukuba, Ibaraki 305-8566, Japan*

[2] *Graduate School of Life and Environmental Sciences, Tsukuba University, 1-1-1 Tennoudai, Tsukuba 305-8576, Ibaraki, Japan*

Parkinson's disease (PD) is the second most common neurodegenerative disorder which associated early of sleep disturbed regulation. But the molecular mechanism of this sleep defects is unknown. Here we show sleep defects was observed at early stage than climbing abnormalities (movement disorder) in Parkinson's disease model *Drosophila* which was overexpressed human α-synuclein A30P gene. Furthermore, we detected abnormal regulation of several sleep-related genes at early stage of this model.

Gaucher's disease in humans is considered a deficiency of glucocerebrosidase (GlcCerase) that result in the accumulation of its substrate, glucocerebroside (GlcCer). Although mouse models of Gaucher's disease have been reported from several laboratories, these models are limited due to the perinatal lethality of GlcCerase gene.

Here, we examined phenotypes of *Drosophila melanogaster* homologues genes of the human Gaucher disease gene by using minos insertion. One of minos insertion mutants showed abnormal phenotypes of biochemical markers, climbing ability, survival rate and sleep.

We propose the possibility that sleep abnormality at early stage might be a common phenotype of these two neurodegenerative disease.

P05

Inositols affect close-proximity rhythm of *Drosophila* mating circadian behavior

Kazuki Sakata[1,2], Takahiro Suzuki[1,3], Haruhisa Kawasaki[1]and Norio Ishida[1,2]

[1] *Ishida Group of Clock Gene, Biomedical Research Institute, National Institute of Advanced Science and Technology (AIST) 6-5 Central, Tsukuba, Ibaraki 305-8566, Japan*

[2] *Graduate School of Life and Environmental Sciences, Tsukuba University, 1-1-1 Tennoudai, Tsukuba 305-8576, Ibaraki, Japan*

[3] *SHIGRAY INC., Sumida, Tokyo, Japan*

Accumulating evidence indicates that the molecular circadian clock underlies the mating behavior of Drosophila melanogaster. However, information about which food components affect circadian mating behavior is scant. The ice plant, *Mesembryanthemum crystallinum* has recently become a popular functional food. Here, we showed that the close-proximity (CP) rhythm of *D. melanogaster* courtship behavior was damped under low-nutrient conditions, but significantly enhanced by feeding the flies with powdered ice plant. Among various components of ice plants, we found that $\leqq$ 0.1% myo-inositol increased the amplitude and slightly shortened the period of the CP rhythm. Real-time reporter assays showed that $\leqq$1% myo-inositol shortened the period of the circadian reporter gene Per2-luc in NIH3T3 cells. These data suggest that the ability of inositols to shorten rhythms is a general phenomenon in insects as well as mammals (Sakata *et al.*, *Front Pharmacol*, 2015). We also showed a brand-new machine, AutoCircas which can monitor and record automatically sleep, locomotor and close-proximity (CP) rhythm of small animals.

P06

Molecular basis of microglial activation regulated by hypoxanthine

Tomomi Okajima, Fuminori Tsuruta, Tomoki Chiba

Graduate School of Life and Environmental of Science, University of Tsukuba, Japan

Microglia are the resident immune cells, which monitor the brain environment. Upon stimulation, microglia changes their morphologies and migrate to the damaged regions, maintenance of brain environment. Recently, it has been suggested that microglia play important roles in regulating not only innate immune systems but also brain development. The sequential gene expressions regulate an establishment of precise and complex brain development in a fetal period. By contrast, in the postnatal brain, stimulations of external environment also regulate an establishment of brain. Microglia are thought to sense these stimulations involved in developmental brain. However, little is known about the key factors that regulate microglial functions associated with brain development.

Here, we reported that microglial properties are altered in high nutrient medium condition. BV-2 microglia cell lines exhibit slower proliferation rate and different morphology in high nutrient condition in comparison to controls. In addition, we found that one of the cadherin family proteins (Cadherin family protein 1: CFP1), which is expressed at a certain point of both pre- and postnatal brain, was increased high nutrient medium condition. Moreover, we identified that treatment with hypoxanthine, which is one of the components of high nutrient medium, promotes CFP1 expression level in BV-2 cells. Hypoxanthine is known to be an essential factor that controls the purine salvage pathway involved in regulating the brain development. Interestingly, defect in this pathway causes Lesch-Nyhan syndrome (LNS), exhibiting a mental retardation from the developmental stage of childhood. Thus, our finding could link to the onset of LNS through the regulation of microglial activation. Taken together, these data suggests that hypoxanthine regulates the developmental phase of microglia that underlie the acquirement of higher brain functions.

P07

New insight into the effect of cold shock protein on autophagy in brain

Takuma Aihara, Sarasa Yano, Fuminori Tsuruta and Tomoki Chiba
Life and Environmental of Science, University of Tsukuba, Japan

The morphological changes of neurons and remodelling of synapse along with the change in body temperature can be observed in hibernating animals during hibernation and waking state. Animals exposed to low temperature express proteins called RBM3 (RNA-binding motif protein 3) and CIRP (Cold-inducible RNA-binding protein), which are known to be cold shock proteins. RBM3 takes part in neuroprotection, while CIRP controls the response to the external stress. The precise mechanism of how these proteins act on the brain has not been understood.

To understand the role of autophagy in the nervous system, we have been analysing neuron-specific Autopagy-related protein 7 knockout mice ($ATG7^{flox/flox}$: Nestin-Cre, ATG7 KO). Autophagy is an essential system to degrade the unnecessary proteins and organelle in cytoplasm. The malfunction in this system causes an accumulation of abnormal-structured proteins in brain, resulting in neurodegeneration. Previously, we revealed that the expression of RBM3 increased in ATG7 KO mice brain compared to $ATG7^{+/+}$: Nestin-Cre (WT) mice brain. In contrast to RBM3, defect in autophagy had little effect on CIRP expression. However, the mechanisms that underlie the biological relevance between autophagy and RBM3, as well as CIRP has not been elucidated.

In this study, we show the possible crosstalk between autophagy pathway and cold shock pathway. RBM3 and CIRP expression increased in cerebral cortex and cerebellum after the cold shock, although ATG7 deficiency only affects the RBM3 expression. Hence, RBM3 pathway and autophagy pathway could have a crosstalk, while CIRP pathway and autophagy pathway are less likely to have a crosstalk.

P08

The role of sleep restriction on appetite for highly palatable foods

Kristopher McEown, Yoan Cherasse, Yoko Takata and Michael Lazarus
International Institute of Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan

Animal models used to study sleep have limitations that reduce their overall utility (e.g., increased fear responses in animals). Therefore, it is nearly impossible to separate behavioral outcomes, such as increased appetite for highly palatable foods, associated with sleep restriction from outcomes produced by increased fear. We addressed this problem by developing a more sensitive model of sleep restriction that limits sleep without increasing fear in mice. In addition, we produced an altered glutamate and ivermectin (IVM) gated chloride channel (GluClαβ) that facilitated inhibition through hyperpolarizing AAV infected neurons in the medial prefrontal cortex (mPFC) of wildtype mice. We then examined the combined effect of IVM-GluClαβ mPFC inhibition and sleep restriction on appetite for highly palatable foods. We found that IVM-GluClαβ mPFC inhibition reversed the effect of sleep restriction on sucrose consumption in wildtype mice.

P09

Zinc, a novel modulator of sleep

Yoan Cherasse[1], Hitomi Saitou[2], Nanae Nagata[1], Oriana Lavielle[1,3], Kosuke Aritake[1], Michael Lazarus[1] and Yoshihiro Urade[1]

[1] *International Institute of Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *Fujifilm Corporation, Tokyo, Japan*

[3] *University of Bordeaux, France*

Zinc is an essential trace metal for all living organisms, present in hundreds of enzymes and transcription factors. It plays a critical role in functions and processes like the immune defense, taste, smell, growth, sexual maturation and brain development. In the central nervous system, zinc can be released in the synaptic cliff of some glutamatergic neurons and act as a modulator of both excitatory and inhibitory neurotransmission, but it also can modulate the activity of several voltage- and ligand-dependent ion channels. Only very recently, an increasing number of evidences suggest that the serum concentration of zinc in humans may be correlated with the amount and/or the quality of sleep.

To investigate the potential sleep-promoting effect of zinc, we orally administered zinc gluconate to mice at the onset of dark phase and examined the sleep pattern of animals for 24 hours. The animals submitted to zinc exhibited a quick increase of NREM sleep. Furthermore, c-fos mRNA expression has been monitored 1 hour after zinc administration by *in situ* hybridization. We could observe a specific activation of neurons in 4 regions of the brain. We are now investigating what is the precise neuronal network responsible for the zinc-induced sleep.

P10

Influences of in-bed temperature on sleep

Saki Shimada[1], Yoji Shimura[1], Masashi Furukawa[1], Tsutomu Nakamura[1], Shusa Hashimoto[2]

[1] *Japan Research Laboratory of Sleep Science, Nishikawa Sangyo Co., Ltd., Japan*

[2] *Hashimoto Physiologic Anthropology Research Laboratory, Japan*

Harmonizing bed linens with human physiological behaviors, in-bed temperature changes, and bedroom environment is important. In this study, we focused on thermal influences on human body and examined the influences of a constantly maintained temperature of a warm-water mat on sleep at night during the winter season.

Subjects included 6 females. The environment was set to provide an air temperature of 18 ± 2°C and a relative humidity of 50 ± 10%RH. The linen bed cover was a combination of a comforter filled with polyester batting and a towel blanket. The mattress had a warm-water mat on it. On the first night, the warm-water mat was not used. From the second to the fourth night, the temperature of the warm-water mat was set at three levels, 31°C, 33°C, and 35°C, in a random order. The in-bed temperature between the warm-water mat and the body became higher as the temperature of the mat was increased in the dorsal, lumber, femoral, and sural regions. The in-bed humidity in the dorsal region using the warm-water mat increased up to 70%RH. The frequency of body motion measured by actigraphy was higher as the set temperature of the warm-water mat increased.

Electroencephalography revealed that sleep latency tended to be longer when the set temperature was higher. In this experiment, the vicinity of the dorsum may have maintained the in-bed temperature at a range of 33 ± 1°C, which is considered to be suitable for sleep, thus enabling the subjects to experience good quality sleep.

P11

Studying the locality of sleep slow wave activity and its homeostasis

François Grenier[1], Kaoru Ohyama[1], Maiko Sezaki[1], Yo Oishi[1], Mike Lazarus[1], Kaspar Vogt[1] and Robert Greene[1,2]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*

Activity in the neocortex is markedly different between sleep and wake. In particular, the neocortex displays distinct oscillations of slow wave activity (SWA; 0.5-4 Hz) during slow-wave sleep (SWS). During wake, there is a build-up of sleep pressure resulting in a stronger power of SWA in the early period of sleep resumption. As sleep is global, but SWA displays local experience-dependent variation, an important step in understanding the function of SWS-SWA is to determine if their sleep-pressure dependent homeostasis depends on local properties of the cortical network or on a more global state of the brain. We have started addressing these questions by attempting to generate local cortical areas experiencing distinct wake-sleep architectures from the rest of the neocortex using the DREADD system in specific Cre mouse lines, allowing the activation or inactivation of specific sub-groups of neurons on a scale of hours. Our results so far with surface EEG recordings indicate that, in control periods, SWS-SWA between contralateral cortical sites were highly correlated. These correlation values were stable over many days, and smaller when more local field potentials were recorded (with intracortical wires or tetrodes). In mutant "acallosal" mice, contralateral correlations were smaller, suggesting that callosal connections play a non-exclusive role in slow-wave interhemispheric synchronization. Preliminary results of DREADD activation have not shown an influence on SWS-SWA power or correlation. We hypothesize that the effects were too local to be detected with surface EEG recordings, and are in the process of repeating these experiments with more local field recordings.

P12

Automatic sleep staging and apnea events classification from EEG and multimodal physiological signal – Synchrosquezing transform processing and Riemannian geometry classification approaches

Tomasz M. Rutkowski

Life Science Center of TARA, University of Tsukuba, Japan; RIKEN Brain Science Institute, Japan

In this abstract, the author presents a multimodal approach to biomedical signal processing and classification for automatic sleep staging or apnea events classification. The presented signal processing and classification methods have been already successfully applied to real-time brainwaves decoding in brain-computer interfaces developed by the author. In the current study an extension to the multimodal physiological signals such as: EEG, ECG, EOG, EMG, oro-nasal airflow, ribcage and abdomen movements, oxygen saturation (pulse-oximetery), acoustic snoring, and body position are processed in an unified approach leading to succesful automatic classification. The employed synchrosqueezing transform (SST) method for the multimodal physiological signals outperforms the classical time–frequency analysis techniques of the non-linear and non-stationary signals such as EEG. This not only allows for examination of the spectral contents of each signal as well as the correlation between the multimodal data streams, but also complies with what clinical experts use in their visual judgment of EEG and body peripheral physiological signals used in sleep studies. For clinically, as well as home-user oriented sleep support applications, the plug & play operation is nowadays considered as a minimum requirement for any consumer devices. In the classification step, thanks to information geometry it is possible to define a metric enjoying the sought adaptive (data-driven) properties. The information geometry is a field of information theory where the probability distributions are taken as point of a Riemannian manifold (this field has been popularized by S. Amari). I present successful application of adaptive sleep staging and apnea events classification approach.

P13

Understanding the molecular basis of fear and the role of emotion in sleep regulation

Liqin Cao[1], Daniela Klewe-Nebenius[1], Andong Tang[1], Chika Miyoshi[1], Hiromasa Funato[1], Masashi Yanagisawa[1], Qinghua Liu[1, 2]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*

Sleep, emotions, and mental disorders are closely linked. It is well-known that chronic sleep loss tends to elevate negative emotions. In return, both positive and negative emotions can significantly affect sleep, resulting in acute or chronic sleep problems. Moreover, patients suffering mental disorders often have sleep and/or emotional problems. Fear is a basic emotion that enhances animal survival by triggering the "fight, flight, or freeze" response to perceived danger. For example, lab mice will freeze instantly at the first sight of a cat, despite being raised away from predators for hundreds of generations. Thus, this fear response is "innate", rather than "learned", and thus must be genetically encoded. Uncontrolled fears underlie many fear/anxiety disorders that also exhibit sleep problems. We developed a predator odor-based "innate fear" assay and conducted a forward genetic screen to isolate both "fearless" and "fearful" mutant mice. We established two inheritable mutant pedigrees after screening ~1600 ENU mice. We will identify the causative mutations for these mutants by genetic mapping and exome sequencing. This unbiased fear screen will identify core fear genes and elucidate the molecular basis of fear. The "fearless"/"fearful" mutant mice will enable us to study the role of emotions in sleep regulation. Similar to "oncomice" that will develop cancer, "fearful" mice may acquire fear/anxiety disorders as models for developing therapeutic interventions. We believe that these studies will break new grounds for us to investigate how emotions regulate sleep/wake pattern and the molecular basis of fear-related sleep and mental disorders.

P14

Systematic behavioral screening of *Sleepy* and *Dreamless,* newly identified mouse pedigrees with sleep abnormalities

Takato Honda[1,2,3], Tomoyuki Fujiyama[1], Chika Miyoshi[1], Makito Sato[1], Hiromasa Funato[1,4] and Masashi Yanagisawa[1]

[1] *International Institute for Intergrative Sleep Medicine (WPI-IIIS), University of Tsukuba*

[2] *Ph.D. Program in Human Biology (HBP), School of Integrative and Global Majors, University of Tsukuba*

[3] *Japan Society for the Promotion of Science (JSPS) Research Fellow (DC1)*

[4] *Department of Anatomy, School of Medicine, Toho University*

As a biggest unrevealed issue in sleep biology, the mechanism for homeostatic sleep/wakefulness regulation, as well as the neural substrate for "sleepiness", remains a mystery. To make a breakthrough in this question, we have initiated a large-scale forward genetic screen of sleep/wake abnormalities in mice, based on somnographic (EEG/EMG) measurements. We have so far screened >7,000 heterozygous ENU-mutagenized mice and established 10 pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and whole-exome sequencing, we have so far identified three mutations: *Sleepy* and *Sleepy2*, causing marked hypersomnia (increased non-REM sleep), and *Dreamless*, causing short and highly fragmented REM sleep. Since these dominant mutations cause very strong phenotypes, we expect that the mutated genes play central role for regulating sleep/wake amounts.

Furthermore, these mutant mice can be a sleep disease model to approach the relationship between sleep and other cognitive functions such as learning and memory, depression, anxiety, sociability and more. Here, we examined the following series of behavioral analyses for both *Sleepy* and *Dreamless* mutant mice: 1) Morris water maze, 2) forced swim test, 3) tail suspension test, 4) open field test, 5) novel object recognition, 6) elevated plus maze, 7) social interaction test, 8) sucrose preference, 9) nest building, and 10) fear conditioning. We detected significant behavioral phenotypes in these mice, including an impaired hippocampus-dependent memory formation in *Dreamless*, and a depression-like phenotype of *Sleepy* mutant mice. These results of behavioral test battery provide us the landmark information to approach the link between sleep and other behaviors.

P15

Npas4, novel HDAC5 target gene, mediates its role in limiting cocaine reward-related behavior

Makoto Taniguchi[1], Maria B. Carreira[1], Yonatan A Cooper[3], Evan A Balmuth[1], Jaswinder Kumar[1,2,*], Laura N. Smith[1], Nobuya Koike[2*], David W. Self[4], Tae-Kyung Kim[2], Joseph S. Takahashi[2,5], Yingxi Lin[3], Christopher W. Cowan[1,2]

[1] *Integrative Neurobiology laboratory, McLean Hospital, Harvard Medical School, Belmont, MA, USA*

[2] *Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX, USA*

[3] *Department of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge, MA, USA*

[4] *Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA*

[5] *Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Sleep deprivation and drug addiction have been reported strong association. In this study we have identified novel molecular mechanism underling drug reward related behaviors. In this symposium, I would like to discuss about a novel line of the research to elucidate a role of this molecular pathway in the sleep deprivation-induced vulnerability to drug addiction.

Cues associated with illicit drug use often develop into long-lasting triggers for drug seeking behaviors. We show here that enhanced nuclear accumulation of the epigenetic enzyme, histone deacetylase 5 (HDAC5), in the nucleus accumbens (NAc) reduces cocaine reward-context memories, and it reduces cue- and drug prime-induced reinstatement of cocaine seeking – relapse-like behaviors. Using an unbiased, genome-wide analysis of HDAC5 gene targets, we found that it binds to the activity-sensitive enhancer, and suppresses expression of, the *Npas4* gene. We find that Npas4 expression is rapidly and transiently induced in the NAc following administration of cocaine or exposure to a novel environment, and reduction of Npas4 in the NAc reduces cocaine reward-context associations without altering drug sensitivity, fear-related contextual memory, natural reward or anxiety-like behaviors. Together our data suggest that nuclear HDAC5 reduces the association between cocaine reward and drug administration cues, at least in part, through suppression of *Npas4* gene expression.

P16

Sleep widespread remodeling brain phosphor-proteome implicate sleep homeostasis restoration mechanism

Zhiqiang Wang[1], Jing Ma[1], Xiaojie Yang[1], Chi-Yu Lee[1], Hiromasa Funato[1,2], Yonghao Yu[3], Masashi Yanagisawa[1] and Qinghua Liu[1,3]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *Department of Anatomy, School of Medicine, Toho University, Japan*

[3] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*

The function and regulatory mechanism of sleep, which is conserved across all animal species, is one of the greatest mysteries in biology. To solve the mystery of sleep, Dr. Masashi Yanagisawa's laboratory is conducting an unprecedented electroencephalogram (EEG) and electromyogram (EMG) based forward genetic screen to isolate sleep mutant mice. Notably, the first "Sleepy" mutant, which carries a dominant splice site mutation and result in exon skipping and in-frame deletion of a protein kinase, exhibits the strongest hypersomnia phenotype reported to date. However, the mechanism by which a single point mutation in Sleepy kinase results causes hypersomnia phenotype is completely unknown. One possibility is the "sleep pressure" of the "*Sleepy*" mutant may be constitutively higher than that of wild-type mice. As such, the "*Sleepy*" mutant mouse model may present a unique opportunity to identify the molecular substance of "sleep pressure". By systematic analysis of whole brain proteome and phosphoproteome of the Sleepy and sleep deprived mouse brains, we report that the majority of proteome remained unchanged in both models. However, sleep appears to have a critical function in reconstruction of brain phosphoproteome to control many biological processes. In particular, the phosphorylation state of proteins in cytoskeletal and synapse organization was extensively regulated during sleep. Thus, we hypothesize that one of the vital functions of sleep is the restoration of phosphorproteome state, which correspond to the cytoskeletal reorganization and synaptic homeostasis and may represent molecular markers for the homeostatic sleep pressure.

P17

Mesopontine-amygdala mechanisms inducing induce blood pressure fluctuation during REM sleep

Y. Koyama[1], K. Nishimura[1], N. Haruyama[1], K. Takahashi[2]

[1] *Department of Science and Technology, Fukushima University, Fukushima, Japan*

[2] *Department of Neurophysiology, Fukushima Medical University, Fukushima, Japan*

The amygdala, center of emotion, is involved in expression of emotion and in activation of autonomic nervous systems associated with emotion, including blood pressure, heart rate or respiration. During REM sleep, large fluctuations of autonomic nervous system also occur, which are considered to reflect the emotional changes during REM sleep or to reflect the state failing to control the cardiovascular system. So, it is required both from physiological and clinical viewpoints, to elucidate the neural mechanisms inducing blood pressure fluctuation during REM sleep. During REM sleep, neurons in the amygdala as well as those in the mesopontine tegmental REM sleep center (laterodorsal tegmental nucleus: LDT) increase their firing in phasic way. So, it is highly probable that the autonomic changes during REM sleep is regulated by the amygdala and LDT.

Single neuronal activity in the amygdala and LDT in relation with blood pressure was examined in non-anesthetized, head restrained rats. Some neurons in the amygdala and those possibly cholinergic in the LDT displayed the firing in close correlation with blood pressure fluctuation during REM sleep. Possible role of ascending cholinergic system in the mesopontine tegmentum to the amygdala for the regulation of blood pressure fluctuation during REM sleep is discussed.

P18

Alterations in microglia in response to sleep loss

Haojun Xu[1,2], Takeshi Kanda[1], Masashi Yanagisawa[1,2]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *Ph.D. Program in Human Biology, School of Integrative and Global Majors, University of Tsukuba, Japan*

Sleep is essential for human life. Poor sleep quality or insufficient sleep disturbs several brain functions such as mood, learning and memory, and cognitive functions. In fact, sleep disruption is a risk factor for neurodegenerative disorders and psychological illnesses. Nevertheless, it is largely unknown how sleep contributes to the maintenance of brain functions. To unravel the relationship between sleep and such diseases, we focused on microglia – the brain resident macrophage. Microglia acts as the first-line-defense of the immune system in the brain by the continuous surveillance of surrounding environment with the fine processes. Recent studies implicate that microglia is involved in inflammation-mediated neurodegeneration and psychological stress. In the first place, however, it has not been well-explored whether sleep is interacted with microglia. As a first step, we examined if loss of sleep have some influence on microglia. To know the morphological changes, using CX3CR1-GFP mice expressing GFP in microglia, we compared number and size of microglia in several brain areas between mice under natural sleep-wake cycle and mice subjected to sleep deprivation (SD) for 6 hours. Furthermore, to investigate the activation of microglia, we analyzed the expression patterns of inducible nitric oxide synthase (iNos), a key enzyme for NO synthesis in activated microglia, in mice with or without 6-h SD.

P19

Identification of *Sim1* and *Mc4r* mutations through obesity screening of randomly mutagenized mice

Hossain MS[1], Asano F[1], Fujiyama T[1], Miyoshi C[1], Sato M[1], Ikkyu A[1], Kanno S[1], Hotta N[1], Kakizaki M[1], Honda T[1], Kim SJ[1], Komiya H[1], Miura I[2], Suzuki T[2], Vivek K[3], Takahashi JS[3], Wakana S[2], Funato H[1,4], and Yanagisawa M[1,3]

[1] *International Institute of Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*
[2] *RIKEN BioResource Center, Japan*
[3] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*
[4] *Toho University Faculty of Medicine, Japan*

We have screened thousands of randomly mutagenized mice for sleep abnormalities as well as obesity. In parallel with the establishment of *Sleepy1, Sleepy2* and *Dreamless* mutant pedigrees for sleep abnormalities, we have established two obese pedigrees. Linkage analysis of one obese pedigree produced a single peak of LOD score on chromosome 10. Among ~100 genes located within the mapped region of chromosomes 10, we selected a transcription factor *Single minded1* (*Sim1*) as a candidate gene because *Sim1* haploinsufficiency causes obesity. As predicted, we found a single nucleotide substitution of the *Sim1* gene which causes nonsynonymous change of a well-conserved residue in the PAS A domain, crucial for functional dimer formation with *Arnt2*. We further confirmed using luciferase assay that the mutant *Sim1* protein lacks transcriptional activity. Linkage analysis of another obese pedigree showed a single LOD score peak on the chromosome 18. We pick up *Melanocortin 4 receptor* (*Mc4r)* as a strong candidate located within the mapped region because of the well-known role in the regulation of energy metabolism and obese phenotype of heterozygous *Mc4r*-deficient mice. In fact, we found a single nucleotide substitution of *Mc4r* gene in obesity mutant mice, which results in premature stop codon in the first transmembrane domain of *Mc4r*. Because both *Sim1* and *Mc4r* are expressed in the hypothalamus, a regulatory center of feeding and energy metabolism, we examined hypothalamic gene expression of *Sim1* mutant and *Mc4r* mutant mice, and further compared with those of high-fat diet-induced obesity (DIO) mice. *Mc4r* mutant mice showed an increased in *Pomc* mRNA and *Mc4r* mRNA and a decrease in *Npy* mRNA. Both *Sim1* mutant and DIO mice had a lower level of *Orexin* mRNA, *Oxytocin* mRNA, *Sim1* mRNA and *Trh1* mRNA. All three obese mice showed a decrease in *Agrp* mRNA, *Avp* mRNA and *Ghrh* mRNA. These findings confirm that the crucial roles of *Sim1* and *Mc4r* in maintaining energy homeostasis and substantiate the sensitivity of our forward genetic screening as well. We will further discuss the probability to identify two of few genes which cause obesity by haploinsufficiency from several thousand mutagenized mice.

P20

Identification of a single nucleotide substitution specific to the *Dreamless* mutant mouse by linkage analysis and whole exome sequencing, and its genetic verification by CRISPR

Tomoyuki Fujiyama[1], Chika Miyoshi[1], Makito Sato[1], Takeshi Kanda[1], Seiya Mizuno[1], Satoru Takahashi[1], Hiroki Muramoto[1], Fuyuki Asano[1], Takato Honda[1], Kanako Iwasaki[1], Aya Ikkyu[1], Miyo Kakizaki[1], Noriko Hotta[1], Satomi Kanno[1], Yukiko Ishikawa[1], Ikuo Miura[4], Tomohiro Suzuki[4], Shigeharu Wakana[4], Vivek Kumar[1,3], Joseph S Takahashi[1,3], Hiromasa Funato[1,2], Masashi Yanagisawa[1]

[1] *International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*
[2] *Department of Anatomy, School of Medicine, Toho University, Tokyo, Japan*
[3] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*
[4] *Technology and Development Team for Mouse Phenotype Analysis, Japan Mouse Clinic, RIKEN BioResource Center, Ibaraki, Japan*

Although sleep is a ubiquitous animal behavior, the molecular mechanism of sleep homeostasis remains unknown. We performed high-throughput screening of ENU-mutagenized mice in order to identify genes regulating sleep/wake behavior. We have so far analyzed EEG/EMG data of more than 7,000 mutagenized male mice. We established several pedigrees showing heritable sleep/wakefulness abnormalities. Among them, the *Dreamless* mutant pedigree shows about 50% reduction in 24-h REM sleep time. To map a chromosomal region responsible for the sleep phenotype of *Dreamless* mutant mice, we performed a linkage analysis in N2 mice, obtained by backcrossing the mutagenized founder C57BL/6J male to C57BL/6N female mice for two generations. The analysis revealed a single peak with a LOD score of more than 11. Whole exome sequencing of mutants and wild-type littermates from the *Dreamless* pedigree identified a nucleotide change specific to *Dreamless* mutant mice within the mapped chromosomal region. The single nucleotide substitution leads to a single amino acid substitution of the gene product that we termed *Dreamless*. We adopted CRISPR/Cas9 system to recapitulate *Dreamless* phenotype, confirmed that the one base substitution was responsible for REM sleep abnormality. These results suggest that the newly identified gene is related to the regulation of daily REM sleep time.

P21

Analyses of the *C. elegans sleepy-homologue* mutants suggest a deep conservation of sleep-regulating genes

Shinichi Miyazaki, Hiromasa Funato, Masashi Yanagisawa, Yu Hayashi
International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan

Sleep-like behaviors are widely observed across the animal phylum, and recently invertebrate animal models are frequently used in sleep studies. However, direct evidence that sleep in mammals and invertebrates share a conserved mechanism is lacking. The mouse *Sleepy1* and *sleepy2* genes were identified by the Yanagisawa/Funato lab by ENU mutagenesis as critical regulators of sleepiness. Here, we show that the nematode *Caenorhabditis elegans* homologues of *Sleepy 1* and *2* are also critical for the *C. elegans* sleep-like behavior, lethargus.

P22

Sleep screening of randomly mutagenized mice established a long sleep pedigree

Chika Miyoshi[1], Tomoyuki Fujiyama[1], Makito Sato[1], Aya Ikkyu[1], Satomi Kanno[1], Noriko Hotta[1], Miyo Kakizaki[1], Takato Honda[1], Staci J. Kim[1], Haruna Komiya[1], Fuyuki Asano[1], Tomohiro Suzuki[2], Kumar Vivek[3], Joseph S. Takahashi[3], Shigeharu Wakana[2], Hiromasa Funato[1,4], Masashi Yanagisawa[1,3]

[1] *International Institute of Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Japan*

[2] *Technology and Development Team for Mouse Phenotype Analysis, RIKEN BioResource Center, Japan*

[3] *University of Texas Southwestern Medical Center at Dallas, Texas, USA*

[4] *Department of Anatomy, Toho University Faculty of Medicine, Japan*

Although sleep is one of the most conserved behaviors among animal species, the molecular mechanism regulating sleep/wakefulness behavior remains unknown. In order to identify genes regulating sleep/wakefulness behavior, we have conducted EEG/ EMG-based screening of G1 mice (C57BL/ 6J(B6J)) and F1 mice (B6J/B6N F1) whose fathers were mutagenized using ethylnitrosourea (ENU). Through our screening of more than 7,000 randomly mutagenized mice, we established several pedigrees showing heritable sleep/wakefulness abnormalities. Among them, *Sleepy* heterozygous mutant mice shows reduction in 24-h wake time and increase in 24-h NREM time. *Sleepy* homozygous mutant mice showed even larger reduction in total wake time and increase in total NREM sleep time compared with heterozygous mutant mice. Time spent in REM sleep was similar among *Sleepy* mutants and wild type mice. Importantly, *Sleepy* mutant mice showed normal response to wake-promoting agents, caffeine and modafinil. Thus, the increased sleep time of the *Sleepy* mutant pedigree may be due to increased sleep need rather than disrupted wake-promoting system.