Poster Session Abstracts

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

Data Blitz by Poster Presenters	**13:15 – 13:45**
Poster Session	**13:45 – 14:15**

Poster list

P01

Function of the adult-born neurons in memory consolidation during 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 consolidation[1,2]. On the other hand, disruption of REM-related theta rhythm impaired memory consolidation[3]. 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 learning[4]. 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.

1. Marshall et al., Nature. 2006, v444, p610
2. Girardeau et al., Nat Neurosci, 2009, v12, p1222
3. Boyce et al., Science. 2016, v352, p812
4. Arruda-Carvalho et al., J Neurosci., 2011, v31, p15113

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P02

Cumulative phosphorylation of SNIPPs as the molecular substrate of homeostatic sleep need

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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.

P03

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

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Hippocampal lactate produced from astrocytic glycogen is a critical energy source and/or a neuromadulator 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* Ca^{2+}/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

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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 Trpa1 as a Chemosensor for Predator Odor-evoked Innate Fear Behaviors

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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 ethylnitrosourea (ENU)-mutagenized mice. We find that Trpa1, a pungency/irritancy receptor, is related to both 2MT and snake skin-evoked innate fear/defensive responses. Accordingly, Trpa1-knocknout 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 Trpa1 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-Trpa1-expression rescued freezing behavior in TRPA1 KO mice under 2MT exposure. These results suggest trigeminal ganglion neurons contribute critically to 2MT-evoked freezing.

P06

Rapid stimulation of hippocampal memory circuit with acute mild exercise

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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%V_{O2peak}) 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.

P07

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

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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* phenotype, then confirmed that the one base substitution was responsible for REMS abnormality, suggesting that the identified gene is related to the regulation of daily REMS time. To elucidate the molecular/neural basis of REMS regulation by NALCN, we generated genetically-modified *Nalcn* 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.

P08

Impaired Sleep Architecture and Learning ability in an *App* Knock-in Mouse Model of Alzheimer's Disease

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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.

P09

Dissection of prefrontal cortex circuit in guiding the extinctions

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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.

P10

Longitudinal EEG/EMG measurement in mice show sleep/wakefulness maturation from infant to young adult

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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.

P11

Sleep/wakefulness in neuron-specific SIK3-deficient mice

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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.

P12

Forgetting during REM sleep by hypothalamic MCH neurons

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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 by 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.

P13

Adenosinergic mechanisms of sleep control in the nucleus accumbens

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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 neuro-dynamics 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.

P14

Exploration of the intracellular molecular mechanisms for homeostatic sleep/wake regulation

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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 be also 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.

P15

Synthesis and evaluation of ^{18}F-labeled tetrahydroisoquinoline derivatives as novel PET probes for imaging orexin 1 receptors

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Introduction: Orexin 1 receptor (OX_1R) is thought to be involved in various body functions, including arousal maintenance and emotional control, but the full details of its function remain unknown. OX_1R imaging with positron emission tomography (PET) would be useful in elucidating the orexin system including OX_1R. However, no PET probes targeting OX_1R have been reported. Therefore, we designed and synthesized tetrahydroisoquinoline (THIQ) derivatives as novel PET probes targeting OX_1R, and evaluated their utility.
Methods: We succesfully synthesized THIQ-1 and THIQ-2. To quantify their affinities for OX_1R and OX_2R, we carried out *in vitro* competitive binding assays using OX_1R or OX_2R expression cells and [^{125}I]Orexin A. After radiosynthesis of [^{18}F]THIQ-1 and [^{18}F]THIQ-2, we investigated the cellular binding of [^{18}F]THIQ-1 and [^{18}F]THIQ-2 to cell expressing OX_1R or OX_2R. Finally, to evaluate the brain uptake of [^{18}F]THIQ-1 and [^{18}F]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 OX_1R (IC_{50} = 30 and 31 nM, respectively) than OX_2R (IC_{50} = 160 and 332 nM, respectively). These features were also observed in the cell binding assay using [^{18}F]THIQ-1 and [^{18}F]THIQ-2, demonstrating their OX_1R-specific binding property *in vitro*. In the biodistribution study using normal mice, [^{18}F]THIQ-1 and [^{18}F]THIQ-2 showed brain uptake at 2 min post-injection, but the uptake levels were not sufficient for *in vivo* imaging with PET.
Conclusion: [^{18}F]THIQ-1 and [^{18}F]THIQ-2 have the potential to become useful imaging probes for PET targeting the OX_1R.

P16

Search for neural circuits of narcolepsy-cataplexy

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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 orexin 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.

P17

A neuronal subpopulation in the bed nucleus of the stria terminalis regulates NREM sleep

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Zinc is an essential mineral that plays an important role in the body by acting as a cofactor for more than 300 enzymes and 1000 transcription factors. While zinc is naturally present in food, inappropriate or insufficient feeding behavior put more than 25% of the world population at risk of zinc deficiency. The purpose of our study was to examine the effect of zinc on sleep.

We examined the sleep-promoting activity of zinc by monitoring locomotor activity and electroencephalogram after oral administration to mice. Zinc-containing yeast extract dose dependently increased the total amount of non-rapid eye movement sleep.

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 the bed nucleus of the stria terminalis (BNST). The specific activation of these c-fos positive neurons by chemogenetics (DREADD-hM3Dq) or optogenetics (ChR_2) led to a similar increase of NREM sleep, while their inhibition (DREADD-hM4Di) resulted in an inhibition of the sleep promoting effect of zinc.

Preliminary mapping experiments confirmed an anatomical connection between zinc-sensitive neurons in the BNST and another population of neurons in the medial septum (MS). The exact role of this BNST-MS pathway in the regulation of NREM sleep is now under investigation.

P18

Roles of orexin neurons in motivated behaviors in rats

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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.

P19

Acute social defeat stress increases sleep in mice

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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.

P20

Kinase activity of sleepy mutant SIK3 visualized by newly developed FRET probe

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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.

P21

Orexin and MCH neurons double ablated mice showed severe sleep abnormality and cataplexy

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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.

P22

Cortical Neuronal Communication During Natural Slow Wave Sleep in Mice

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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.

P23

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

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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 ≥ 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.

P24

Different effects of orexin receptor antagonist and GABAA agonist on physical and cognitive functions.

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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 and Stroop task showed significant interaction and time effect; deteriorated score in post-test 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.

P25

Reactivation of adult-born neurons for memory consolidation during sleep

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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 sleep[1]. It is suggested that memory replay contribute to memory consolidation during sleep[2]. 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.

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P26

Electroencephalographic analysis of the *Sleepy* (sik3$^{slp/+}$, sik3$^{slp/slp}$) mutant mice using the envelope characterization space

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We recently reported mutant mice with significant sleep/wake abnormalities, obtained through a largescale EEG/EMG screening of randomly mutagenenized 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.

P27

Functional Connectivity during Moral Judgment -MEG study-

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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 Magneto-encepharography (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 temporo-parietal area in BMJ than in GMJ suggests dynamic changes in neuronal connections occurring in establishment of "Theory of Mind".

P28

Metabolomic screening for memory consolidation during sleep

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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 sleep[1]. The hippocampal dentate gyrus (DG) shows pronounced theta oscillation during REM sleep[2] and is sufficient to hold memory trace[3]. Indeed, we have demonstrated that DG neurons are necessary for memory consolidation during sleep[4]. Although genetic screening was conducted in the DG for memory consolidation[5], there should be other molecular entities involved in memory consolidation. For example, a purine metabolite, adenosine, plays critical roles in sleep[6]. 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.

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P29

Psycho, a potential mouse model of schizophrenia

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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 *Psycho*$^{m/+}$ mice may serve as an excellent model to understand the molecular and neural basis of schizophrenia.