

The 5<sup>th</sup> Annual IIS Symposium • The 32<sup>nd</sup> Wako Workshop • Joint Meeting

# Solving the mystery of sleep

December 12, 2016

Tokyo Conference Center Shinagawa

Tokyo, Japan

## Organizer

**Masashi Yanagisawa**

International Institute for Integrative Sleep Medicine (WPI-IIS)  
University of Tsukuba

## Organizing Committee

WPI-IIS / Wako Pure Chemical Industries, Ltd.

## Supporting Organization

Ministry of Education, Culture, Sports, Science and Technology



# Timetable

## Opening

9:00 - 9:15	Welcome	Masashi Yanagisawa	Director, IIS, University of Tsukuba
	Opening Address 1	Toshio Kuroki	WPI Program Director
	Opening Address 2	Masami Watanabe	Research Promotion Bureau, Ministry of Education, Culture, Sports, Science and Technology (MEXT)

## Session 1 | Chair: Hiromasa Funato

9:15 - 10:00	Keynote Lecture: Evolutionarily conserved role of the habenula in resolution of social conflict	Hitoshi Okamoto	RIKEN BSI	Page 4
10:00 - 10:20	IIS Updates: Forward genetic analysis of sleep in randomly mutagenized mice	Masashi Yanagisawa	WPI-IIS, University of Tsukuba	Page 5
10:20 - 10:30	Break			

## Session 2 | Chair: Michael Lazarus

10:30 - 11:00	Circadian rhythm and sleep disturbances in discrete <i>Zfhx3</i> mouse mutants	Patrick Nolan	MRC Harwell	Page 6
11:00 - 11:30	Brainstem circuitry regulating slow-wave-sleep	Christelle Anaclet	University of Massachusetts Medical School	Page 7
11:30 - 11:50	IIS Updates: Sleep-regulatory mechanism of the nucleus accumbens	Yo Oishi	WPI-IIS, University of Tsukuba	Page 8
11:50 - 13:00	Lunch & Poster Viewing			

## Session 3 | Chair: Qinghua Liu

13:00 - 13:30	Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation	Antoine Adamantidis	University of Bern	Page 9
13:30 - 14:00	Dynamical neural circuitry underlying working memory	Chengyu Li	Shanghai Institutes for Biological Sciences	Page 10
14:00 - 14:20	IIS Updates: Synaptic phosphoproteome as a molecular correlate of sleep need	Zhiqiang Wang	WPI-IIS, University of Tsukuba	Page 11

## Poster Session & Tea Break

14:20 - 16:20	Data Blitz and Poster Presentation
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## Session 4 | Chair: Kaspar Vogt

16:20 - 16:50	Hypothalamic neurons regulate sleep/wakefulness and memory	Akihiro Yamanaka	Nagoya University	Page 12
16:50 - 17:20	Top-down mechanism for consolidation of associative memory	Jin-Hee Han	Korea Advanced Institute of Science and Technology	Page 13
17:20 - 17:50	Cortical top-down input for perceptual memory consolidation	Masanori Murayama	RIKEN BSI	Page 14

## Closing

17:50	Closing	Masahiro Miura	Wako Pure Chemical Industries, Ltd.
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## Reception

18:00 - 20:00	Reception
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## Information

### Precautions

1. No outlets are available nearby audience seats
2. No smoking in symposium venues: please smoke at the designated area on each floor
3. Wireless LAN is unavailable in the venue. We apologize for the inconvenience.

### Lunch

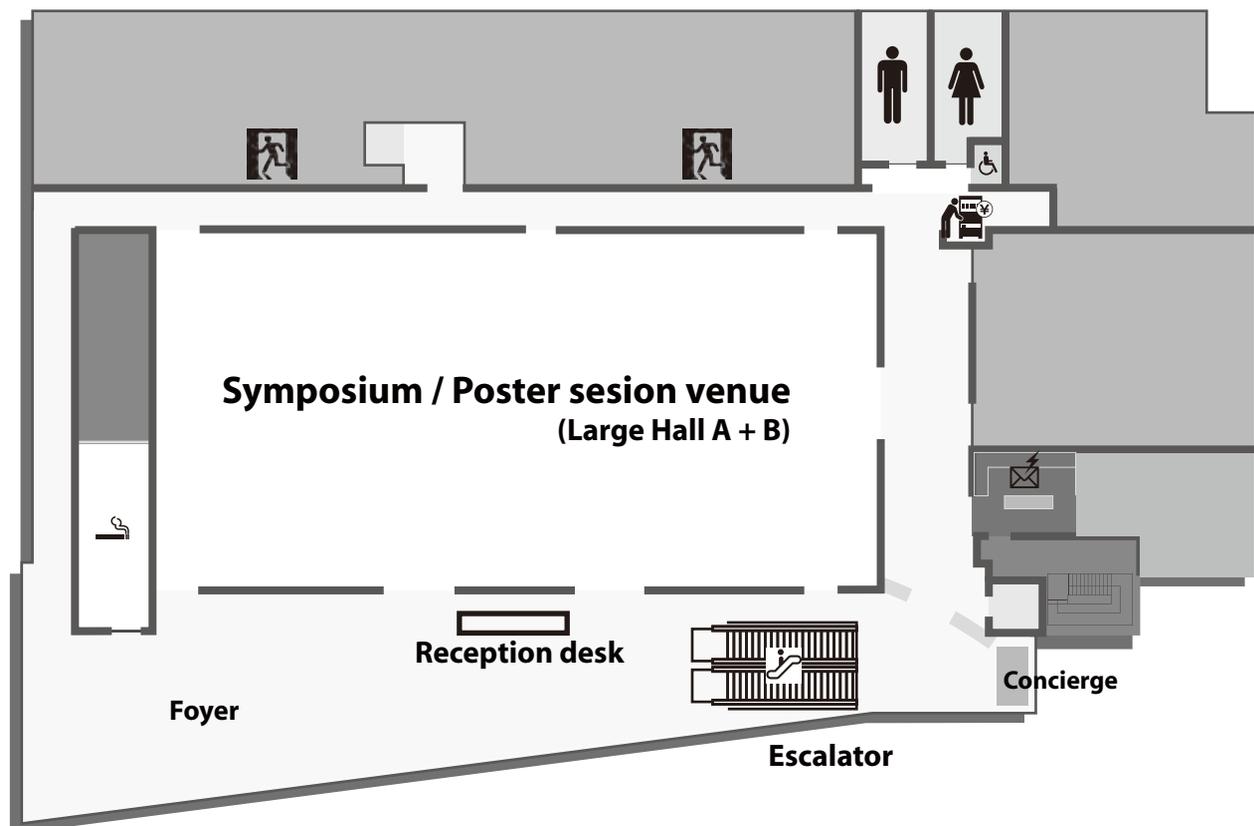
Free lunch will be provided for pre-registered participants. Some extra may be available for non-pre-registered participants on a first-come-first-served basis (will be announced).

### Reception

**18:00 - 20:00** at **“Something Delicious”** (restaurant on 3<sup>rd</sup> floor)

We still welcome walk-in participants. Please pay participation fee in advance (2,000 yen) at the reception desk.

### Floor Map (5<sup>th</sup> floor) | Tokyo Conference Center Shinagawa



## Keynote Lecture:

**Evolutionarily conserved role of the habenula in resolution of social conflict****Hitoshi Okamoto**

*RIKEN Brain Science Institute,  
Japan*

**■ ABSTRACT**

Aggression is an evolutionarily conserved behavior which is critical for animals' survival. To compete for limited resources, animals exhibit aggressive behaviors that lead to displacing, dominating, and harming another individuals. During animal conflicts, experiences influence the outcome of contests such that previous winning experience increases the probability of winning a subsequent interaction, whereas previous losing experience has the contrary effect. These winner and loser effects exist in different animal taxa including insects, fish, lizard, and mammals. Zebrafish also exhibit aggressive behaviors after isolation for 24 hours and winner-loser effects. Winner fish showed higher tendency to win a subsequent fight while loser fish tended to lose again. We found that the two adjacent neural pathways, i.e. one from the lateral subnucleus of the dorsal habenula (dHbL) to the dorsal part of the interpeduncular nucleus (dIPN) and the other from the medial subnucleus of the dorsal habenula to the ventral part of the interpeduncular nucleus (vIPN), play the antagonistic roles in the regulation of the competitiveness in the social conflict.

We are now gaining the evidence that these functions of Hb are conserved between fish and mouse, supporting the idea that the habenula as an evolutionarily conserved switchboard for controlling win or defeat in the social conflict.

## IIS Updates:

**Forward genetic analysis of sleep in randomly mutagenized mice****Masashi Yanagisawa**

*International Institute for  
Integrative Sleep Medicine,  
University of Tsukuba, Japan*

**ABSTRACT**

Sleep is a behavior conserved from invertebrates to vertebrates, and tightly regulated in a homeostatic manner. The molecular and cellular mechanism determining the amount of non-REM sleep and REM sleep remains unknown. Here we identified two dominant mutations affecting sleep/wakefulness through an EEG/EMG-based screening of randomly mutagenized mice. A splicing mutation of the *Sik3* protein kinase gene causes a profound decrease in total wake time, due to an increase in inherent sleep need. Sleep deprivation affects regulatory-site phosphorylation of the kinase, suggesting a role for SIK3 in the homeostatic regulation of sleep. *Sik3* orthologues regulate sleep also in *Drosophila* and *C. elegans*. A missense mutation of the leak cation channel *Nalcn* gene reduces the total amount and episode duration of REM sleep, apparently by increasing the excitability of REM sleep-inhibiting neurons. Our results substantiate the utility of forward genetic approach for sleep behaviors in mice, demonstrating the role of SIK3 and NALCN in regulating the amount of non-REM sleep and REM sleep, respectively.

## Reference:

Forward-genetics analysis of sleep in randomly mutagenized mice. Funato H, Miyoshi C<sup>†</sup>, Fujiyama T<sup>†</sup>, Kanda T<sup>†</sup>, Sato M<sup>†</sup> et al. *Nature* 2016, doi:10.1038/nature20142 († equal contribution to this work)

## Circadian rhythm and sleep disturbances in discrete *Zfhx3* mouse mutants



**Patrick Nolan**

*MRC Harwell Institute, UK*

### ■ ABSTRACT

The complex transcription factor, zinc finger homeobox 3 (ZFHX3), has previously been implicated in neuronal differentiation and development. Its expression is high during neurulation while this declines to almost undetectable levels postnatally. However, in adults the gene maintains high levels of expression in discrete hypothalamic, thalamic and midbrain nuclei including the suprachiasmatic nucleus (SCN). Recently, using a circadian-driven forward genetics screen in mice, we identified a mis-sense mutation in *Zfhx3* that significantly shortens the free-running period in constant darkness (period ~23 hr). Using a number of molecular approaches we have established that this transcription factor regulates the expression of several neuropeptide neurotransmitter and receptor genes in adult SCN. Given the gene networks affected by the mutation, we investigated sleep in mutants and littermate controls using wireless EEG. Sleep fragmentation was evident during baseline conditions with mutants showing a significantly greater number of sleep episodes of shorter duration throughout. These changes were related to an increase in NREM sleep episodes, while REM sleep was unaltered. Moreover, EEG delta power was reduced in mutants during sleep rebound following sleep deprivation. The availability of a conditional *Zfhx3* knockout has now allowed us to investigate its function in a spatially and temporally-restricted fashion in mice. Switching off this gene in adult mice using a tamoxifen-inducible Cre driver line results in an immediate shortening of the free running period by over 1 hour in most animals while 30% become arrhythmic. Moreover, deletion of the gene in the developing hypothalamus results in complete behavioural arrhythmicity in all animals. The results support a sustained role for ZFHX3 in setting the pace of the circadian clock in adult mice. Current efforts are focused on determining additional effects of *Zfhx3* deletion on sleep and behaviour.

## Brainstem circuitry regulating slow-wave-sleep



**Christelle Anaclet**

*University of Massachusetts  
Medical School, USA*

### ■ ABSTRACT

Early transection and stimulation studies have suggested the existence of a slow-wave-sleep (SWS)-promoting / electroencephalogram (EEG)-synchronizing center in the mammalian lower brainstem, yet the location and identity of the neurons comprising this putative hypnogenic circuitry has remained a mystery for decades. We have recently shown that the medullary parafacial zone (PZ) contains sleep-active GABAergic neurons that are not only necessary for normal sleep (Anaclet *et al.*, *J Neurosci.* 2012) but also SWS-promoting in vivo (Anaclet *et al.*, *Nat. Neurosci.* 2014). Chemogenetic activation of PZ GABAergic neurons induces SWS, increases SWS amount and enhances slow-wave-activity, independent of the time of the day, providing a new and unique mouse model of SWS enhancement. Moreover, activation of PZ GABAergic neurons can counteract the wake-promoting action of psychostimulants, such as modafinil or caffeine, and induce SWS, providing additional evidences for a strong sleep-promoting action of PZ GABAergic neurons. Finally, PZ GABAergic neurons project to and inhibit the parabrachial nucleus → basal forebrain → prefrontal cortex ascending arousal system, a circuit substrate through which GABAergic PZ neurons can potentially trigger SWS and modulate cortical EEG activity.

## IIIS Updates:

**Sleep-regulatory mechanism of the nucleus accumbens****Yo Oishi**

*International Institute for  
Integrative Sleep Medicine,  
University of Tsukuba  
Japan*

**ABSTRACT**

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 D2 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 chemogenetic and optogenetic activation of the indirect pathway in the NAc induced robust slow-wave sleep. 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. Moreover, 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). We investigated next the effects of photostimulation of ChR2-expressing NAc core terminals in their target areas on sleep/wake behavior in mice. We also chemogenetically inhibited NAc indirect pathway neurons and elucidated the effect on baseline sleep/wake behavior and rebound sleep after sleep deprivation. Our data provide evidence how sleep is controlled by the indirect pathway of the NAc.

## Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation



**Antoine Adamantidis**

*University of Bern,  
Switzerland*

### ■ ABSTRACT

Rapid-eye-movement sleep (REMs) has been linked with spatial and emotional memory consolidation. However, establishing direct causality between neural activity during REMs and memory consolidation has proven difficult due to the transient nature of REMs and significant caveats associated with REMs deprivation techniques. We optogenetically silenced medial septum GABAergic (MSGABA) neurons allowing for temporally precise attenuation of the memory-associated theta rhythm during REMs without disturbing sleeping behavior. REMs-specific optogenetic silencing of MSGABA neurons selectively during a REMs critical window after learning erased subsequent novel object place recognition and impaired fear-conditioned contextual memory. Silencing MSGABA neurons for similar durations outside REMs episodes had no effect on memory. These results demonstrate that MSGABA neuronal activity specifically during REMs is required for normal memory consolidation.

## Dynamical neural circuitry underlying working memory



**Chengyu Li**

*Shanghai Institutes for  
Biological Sciences, China*

### ■ ABSTRACT

Cognitive behavior inevitably recruits activity of multiple brain regions. However, it is unclear how neuronal activity of multiple regions is temporally coordinated. Here we simultaneously recorded neuronal activity from four associative brain regions [medial prefrontal cortex (mPFC), premotor cortex (M2), medio-dorsal thalamus (MD) and dorso-medial caudate putamen (dmCP)] of mice performing olfactory working memory tasks. Global network states can be defined by correlation and trajectory analysis from neuronal activity of these regions. We found strong correlation between the baseline global states before sensory delivery and those in sensory-delivery, delay, or decision-making periods. The baseline global network state is also gradually modulated through training and the degree is correlated with behavioral performance. Furthermore, behavior-related neuronal coding and performance are impaired by optogenetic perturbation of mPFC baseline activity that can modify initial condition of global network state. Thus the baseline state of global neuronal activity is important in orchestrating global neuronal dynamics in performing cognitive behavior.

## IIIS Updates:

## Synaptic phosphoproteome as a molecular correlate of sleep need



**Zhiqiang Wang**

*International Institute for  
Integrative Sleep Medicine,  
University of Tsukuba  
Japan*

### ■ ABSTRACT

Homeostatic sleep regulation describes a conserved mechanism to maintain a set ratio of sleep/wake time by generating a sleep need that accumulates during wakefulness and dissipates through sleep. The best known measurable index of sleep need is the slow wave activity (SWA) in electroencephalogram (EEG) during non-rapid eye movement sleep (NREMS), defined as the EEG “delta power” in the 1-4 Hz range. Although regulation of SWA is under genetic control, the molecular basis of sleep need remains unknown. Here, our quantitative mass spectrometric studies of two distinct models of increased sleep need reveal a close link between synaptic phosphoproteome and homeostatic sleep regulation. Analyses of ad-lib slept, sleep-deprived, and recovery slept mouse brains suggest that sleep and wake drive opposite remodeling of brain phosphoproteome. In *Sleepy (Sik3Slp/+)* mutant mice, dysregulation of brain phosphoproteome due to a gain-of-function mutation of SIK3 protein kinase underlies chronic hypersomnia with increased sleep need. Cross comparison of two models identifies a core set of synaptic proteins, whose phosphorylation states strongly correlate with changes in NREMS delta power. Therefore, we postulate that the generation/dissipation of NREMS delta power, a macro-electrophysiological readout of synaptic functions, may be mechanistically linked to synaptic phosphoproteome regulation.

## Hypothalamic neurons regulate sleep/ wakefulness and memory



**Akihiro Yamanaka**

*Nagoya University, Japan*

### ■ ABSTRACT

Is memory naturally disappeared with time? Here we found that melanin-concentrating hormone (MCH) neurons in the hypothalamus inhibiting or erasing memory during sleep. We generated transgenic mice in which MCH neurons express channelrhodopsin2 (ChR2) and activation of these neurons using optogenetics increased time in REM sleep. MCH neurons project throughout the brain and densely innervate hippocampus. This suggests that MCH neurons have a role in the regulation of both sleep/wakefulness and memory. To reveal physiological role, MCH neurons ablated mice were subjected to memory test. Interestingly, recognition, fear and spatial learning memory of these mice were significantly increased. Sleep deprivation failed to increase memory suggests the role of MCH neurons on memory during sleep. Conversely, activation of MCH neurons using DREADD or optogenetics inhibited formation of recognition and fear memory. To reveal this mechanism, retrograde tracer was injected into the hippocampus and revealed that MCH neurons were major neurons projecting from hypothalamus to hippocampus. Previous report showed that MCH neurons in the hypothalamus firing during non-REM sleep and REM sleep. Taken together, the activity of MCH neurons works to inhibit memory or erasing memory during sleep.

## Top-down mechanism for consolidation of associative memory



**Jin-Hee Han**

*Korea Advanced Institute of  
Science and Technology,  
Korea*

### ■ ABSTRACT

Specific collection of neurons is thought to support a memory, which is called memory engram cell. Prior researches in rodent brain have demonstrated the existence of such engram cells in the particular brain regions including the lateral amygdala (LA). Recently, we and another group have shown that direct stimulation of LA engram cells alone drives fear memory recall. During memory recall, these engram cells are activated by upstream retrieval cue inputs associated with that memory. Therefore, the control of the connectivity between retrieval cue inputs and engram cells could serve as a regulatory mechanism underlying input-specific memory persistence. However, such a mechanism has been difficult to demonstrate. Here I will talk about how the retrieval of a memory engram by a selective cue is maintained over time.

## Cortical top-down input for perceptual memory consolidation



**Masanori Murayama**

RIKEN Brain Science Institute,  
Japan

### ■ ABSTRACT

Non-rapid eye movement (NREM) sleep is essential for consolidation of an animal's motor and sensory learning experiences. During sleep, bottom-up inputs from sensory organs to the brain are largely silenced and inactive. However during the NREM phase synchronous oscillations ranging from 0.5 to 4 Hz (slow wave activity) occur across cortical regions. These observations have led to the hypothesis that interregional transfer of internal information during NREM sleep has a significant role in memory consolidation. Recently, we identified a cortical top-down circuit that underlies somatosensory perception in the mouse hindpaw (Manita *et al.*, *Neuron* 2015). The circuit consisted of a long-range reciprocal projection between M2 secondary motor cortex and S1 primary somatosensory cortex. Optogenetic inhibition of M2 top-down input to S1 impaired accurate perception of tactile surfaces. However, the role of top-down cortical inputs during sleep in memory, particularly in the consolidation mechanism, has yet to be examined. We developed a novel perceptual learning task that requires sleep for memory consolidation and examined the role of M2 top-down input during sleep. During NREM sleep between the learning and retrieval periods, the optogenetic inhibition of the cortical top-down input from M2 to S1, but not vice versa, resulted in the suppression of functional communication causality from M2 to S1, the absence of reactivated S1 neurons, and behavioral deficits in texture memory consolidation. In NREM sleep and sleep-deprived states, closed-loop asynchronous or synchronous M2-S1 co-activation, respectively, reduced or prolonged memory retention. Top-down cortical information flow in NREM sleep is thus required for perceptual memory consolidation (Miyamoto *et al.*, *Science* 2016).

#### References:

1: A Top-Down Cortical Circuit for Accurate Sensory Perception. Manita S<sup>†</sup>, Suzuki T<sup>†</sup>, Homma C, Matsumoto T, Odagawa M, Yamada K, Ota K, Matsubara C, Inutsuka A, Sato M, Ohkura M, Yamanaka A, Yanagawa Y, Nakai J, Hayashi Y, Larkum ME, Murayama M. *Neuron* **86**(5) 1304-1316 Jun 2015. († equal contribution to this work)

2: Top-Down Cortical Input during NREM Sleep Consolidates Perceptual Memory. Miyamoto D, Hirai D, Fung CCA, Inutsuka A, Odagawa M, Suzuki T, Boehringer R, Adaikkan C, Matsubara C, Matsuki N, Fukai T, McHugh TJ, Yamanaka A, Murayama M. *Science* **352**(6291) 1315-1318 Jun 2016.