# The 4th Annual

# IIIS Symposium

*~ Solving the mystery of sleep ~*

**February 26, 2016**

**IIIS Building, University of Tsukuba**
**Tsukuba, Japan**

**Organizer**


## Masashi Yanagisawa

**Director**
**International Institute for Integrative Sleep Medicine (WPI-IIIS)**
**University of Tsukuba**


**Supporting Organization**

**Ministry of Education, Culture, Sports, Science and Technology**

wpi

IIIS
INTERNATIONAL INSTITUTE FOR INTEGRATIVE SLEEP MEDICINE

# Timetable

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|---|---|---|---|---|
| **Opening Remarks** | | | | |
| 10:00 ~ 10:15 | **Masashi Yanagisawa** | Director, IIIS, University of Tsukuba | Welcome Address | |
| | **Kozo Kaibuchi** | WPI Program Officer | Opening Address 1 | |
| | **Masami Watanabe** | MEXT | Opening Address 2 | |
| **Session 1** | **(Chair: Kaspar Vogt)** | | | **Page** |
| 10:15 ~ 10:45 | **Hailan Hu** | Zhejiang University | Neural circuit mechanism of emotional and social behaviors | 4 |
| 10:45 ~ 11:15 | **Ko Kobayakawa** | Kansai Medical University | Htr2a-expressing cells in the central amygdala control the hierarchy between innate and learned fear | 5 |
| 11:15 ~ 11:45 | **Qinghua Liu** | UTSW / IIIS, University of Tsukuba | A fear screen to uncover the molecular bases of fear and fear/anxiety disorders | 6 |
| 11:45 ~ 12:15 | **Hao Wang** | Zhejiang University | Identifying neural circuitry for olfactory cue-induced innate fear in mice | 7 |
| 12:15 ~ 12:30 | Break & Lunch Preparation | | | |
| **Luncheon Keynote Lecture** | **(Chair: Masashi Yanagisawa)** | | | **Page** |
| 12:30 ~ 13:30 | **Clifford B. Saper** | Harvard Medical School | Chemogenetic stimulation of hypothalamic circuitry regulating sleep and wakefulness | 8 |
| **Session 2** | **(Chair: Masanori Sakaguchi)** | | | **Page** |
| 13:30 ~ 14:00 | **Robert Greene** | UTSW / IIIS, University of Tsukuba | Dopamine evoked long-term synaptic potentiation mediated by locus coeruleus | 9 |
| 14:00 ~ 14:30 | **Deependra Kumar** | IIIS, University of Tsukuba | The role of adult born neurons in memory consolidation during sleep-wake cycles | 10 |
| 14:30 ~ 15:00 | **Genevieve Konopka** | UTSW | Evolution of human-specific gene coexpression networks | 11 |
| **Poster Session** | | | | |
| 15:00 ~ 16:15 | Data Blitz and Poster Presentation | | | |
| **Session 3** | **(Chair: Michael Lazarus)** | | | **Page** |
| 16:15 ~ 16:45 | **Seung-Hee Lee** | KAIST | A neural circuit for auditory dominance on visual perception | 12 |
| 16:45 ~ 17:15 | **Yu Hayashi** | IIIS, University of Tsukuba | Identification of a sleep regulatory circuit and implications for the function of REM sleep | 13 |
| 17:15 ~ 17:45 | **Yi Rao** | Peking University | Molecular genetic analysis of sleep | 14 |
| **Closing** | | | | |
| 17:45 ~ | **Yasuo Miake** | Vice President, University of Tsukuba | Closing | |
| **Reception** | | | | |
| 18:00~20:00 | Reception | | | |

# Information

## Precautions

1. No outlets are available nearby audience seats
2. No smoking at any places
3. Wireless LAN is available for guests

| Network ID: IIISGuest | Password: StMo2016 |
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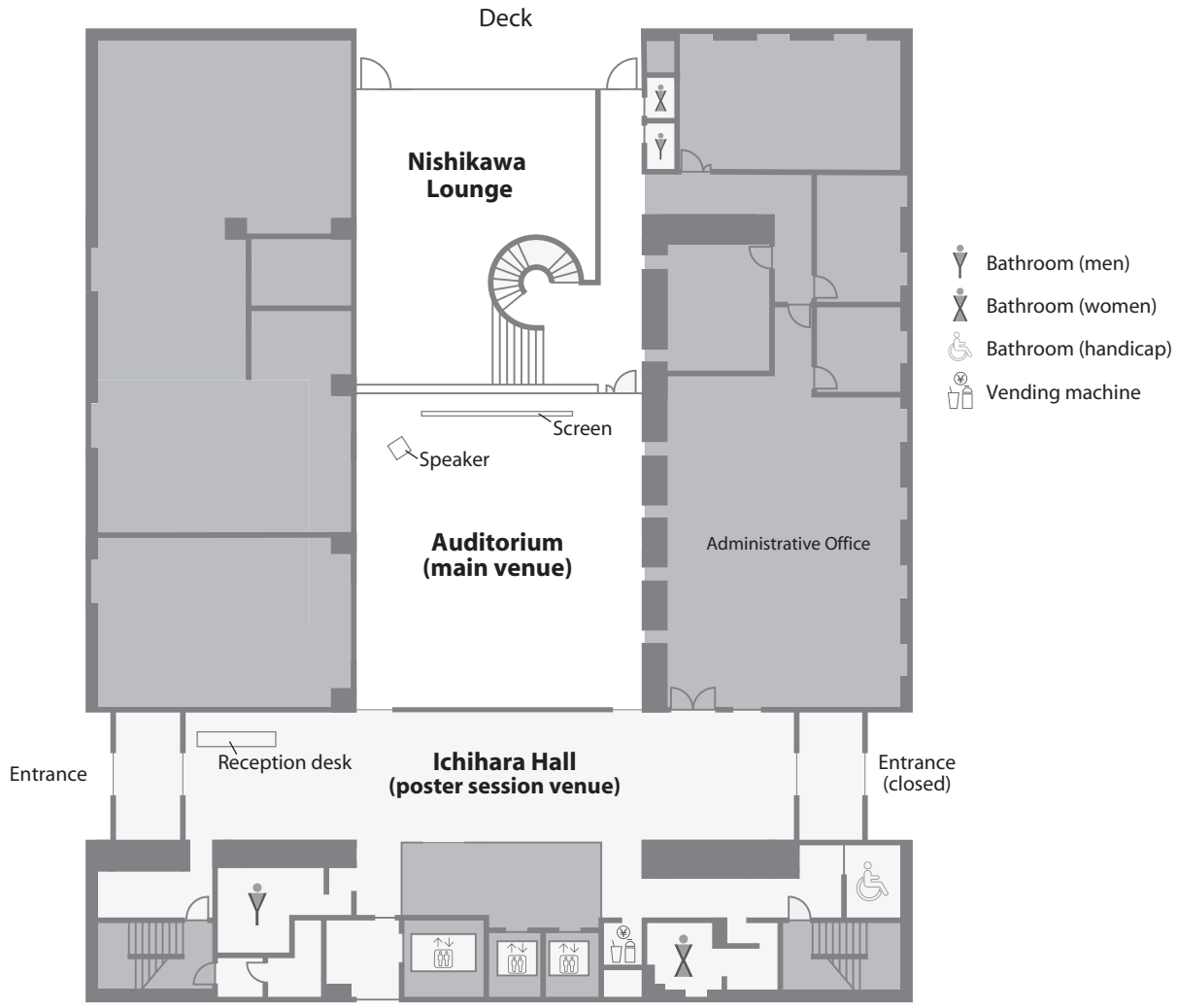
## Lunchbox

Free lunchbox will be provided for pre-registered participants. Extra may be distributed to non-pre-registered participants on a first-come-first-served basis (will be announced).

## Reception 18:00 - 20:00 at Nishikawa Lounge

We still welcome walk-in participants. Please pay participation fee in advance (Faculties & Company 3,000 yen; Postdocs & Researchers 2,000 yen; Students Free) at the reception desk.



# Neural circuit mechanism of emotional and social behaviors

**Hailan Hu**

*Zhejiang University School of Medicine, China*

## ABSTRACT

My lab is interested in the molecular and neural circuit mechanisms underlying emotional and social behaviors and psychiatric diseases.

While great advances have been made towards understanding the sensory representations of the external world, it has remained largely elusive how different emotions are represented in the brain. In one direction of our research, we introduced a dual activity mapping technique to simultaneously visualize the neural substrates of two stimuli with distinct emotional valences, with single cell resolution, With considerable precision, we have identified striking patterns of interaction between the appetitive and aversive neural ensembles, in various key limbic structures. In the nucleus accumbens (NAc), in particular, we discover topographically intermingled pattern of activation by positive and negative emotional cues, revealing the existence of a functional "valence map" (Xiu *et al*., *Nature Neuroscience*, 2014).

In a second line of research, we are investigating the neural circuit mechanism of social hierarchy, a most robust form of social behavior. We established that dominance ranking in group-housed mice is transitive, relatively stable, and highly correlated among multiple dominance measures. Using electrophysiology recording and viral-based gene manipulation, we found that social rank correlates with the synaptic strength in the medial prefrontal cortex (mPFC), and can be tweaked by molecular manipulations that alter the synaptic efficacy in mPFC (Wang *et al*., *Science*, 2011). Latest progress on optogenetical control of the dominance rank will be presented.

**Session 1** **10:45 - 11:15**

# Htr2a-expressing cells in the central amygdala control the hierarchy between innate and learned fear

**Ko Kobayakawa**

*Kansai Medical University, Japan*

## ABSTRACT

Fear is induced by innate and learned mechanisms involving separate pathways. Here, we used an olfactory-mediated innate-fear versus learned-fear paradigm to investigate how these pathways are integrated. Notably, prior presentation of innate-fear stimuli inhibited learned-freezing response, but not vice versa. Whole-brain mapping and pharmacological screening indicated that serotonin-2A receptor (Htr2a)-expressing cells in the central amygdala (CeA) control both innate and learned freezing, but in opposing directions. In vivo fiber photometry analyses in freely moving mice indicated that innate but not learned-fear stimuli suppressed the activity of Htr2a-expressing CeA cells. Artificial inactivation of these cells upregulated innate-freezing response and downregulated learned-freezing response. Thus, Htr2a-expressing CeA cells serve as a hierarchy generator, prioritizing innate fear over learned fear.

# A fear screen to uncover the molecular bases of fear and fear/anxiety disorders

**Qinghua Liu**

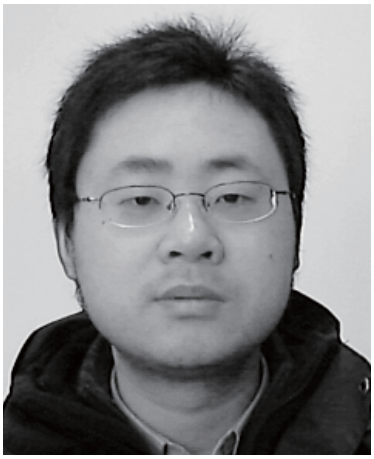
*University of Texas Southwestern Medical Center, USA*

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

## ABSTRACT

Emotions define the essence of being human and are powerful drivers of behaviors. And yet emotions are complex and difficult to quantify and study. Almost nothing is understood about emotions at the molecular level. Fear is a conserved basic emotion that enhances animal survival by triggering the freeze, fight, or flight response to perceived danger. For example, lab mice will instantly freeze at the first sight of a cat, despite of being raised away from this predator for many generations. Thus, this fear (of predator) is innate (heritable) and must therefore be genetically encoded. Here, we've developed a forward genetic screen in mice, based on a novel predator odor-induced “innate fear” assay, to isolate “fearless” and “fearful” mutants. This unbiased genetic screen should allow us to identify core fear genes and elucidate the molecular mechanism of fear. Furthermore, we hypothesize that the heritable fearful mice may be genetically predisposed to fear/anxiety disorders in a manner similar to the "once(gene)" mice that develop cancer with age. If true, these animal models have the potential of transformative impact on understanding molecular basis of and developing novel therapeutics for human fear/anxiety disorders.

# Identifying neural circuitry for olfactory cue-induced innate fear in mice



**Hao Wang**

*Zhejiang University, China*

## ABSTRACT

Innate fear plays a critical role in survival of animals. Unlike conditioned fear, the neuronal circuitry underlying innate fear is largely unknown. Here, we discovered that the laterodorsal tegmentum (LDT) and lateral habenula (LHb) were specifically activated by the mouse predator odorant trimethylthiazoline (TMT). Using optogenetics to selectively stimulate GABAergic neurons in the LDT immediately produced fear-like responses (freezing, accelerated heart rate, and increased serum corticosterone), whereas prolonged stimulation caused anxiety-like behaviors. Interestingly, while selective stimulation of parvalbumin (PV)-positive interneurons similarly induced fear-like responses, stimulation of somatostatin-positive interneurons or inhibition of PV neurons in the LDT suppressed TMT-induced fear-like responses without affecting conditioned fear. Finally, activation of LHb glutamatergic inputs to LDT interneurons was sufficient to generate fear-like responses. Thus, the LHb-LDT pathway plays a critical role in regulating olfactory cue-induced innate fear. Our results provide a potential target for therapeutic intervention for anxiety disorder.

**Keynote** **12:30 - 13:30**

# Chemogenetic stimulation of hypothalamic circuitry regulating sleep and wakefulness


**Clifford B. Saper**

*Harvard Medical School, USA*



## ABSTRACT

For many years, we have attempted to infer the role of hypothalamic cell groups in wake-sleep regulation from their connections or firing patterns, but causal demonstration of their functions was elusive. The availability of methods for driving hypothalamic neurons chemogenetically using the hM3Dq DREADD has made it possible to identify the effects of these cell groups more directly.

We have recently identified the supramammillary nucleus (SUM) as a site containing glutamatergic neurons with widespread cortical projections, and so hypothesized that they might be important in arousal. We placed injections of AAV-hM3Dq into the SUM in Vglut2-Cre mice, to drive the SUM neurons. Administration of 0.3 mg/kg of clozapine-N-oxide (CNO) caused up to 8 hours of continual wakefulness, confirming that the SUM is capable of providing potent wake-producing effects.

Our previous evidence indicated that galaninergic neurons in the ventrolateral preoptic nucleus (VLPO) are selectively activated during sleep, and that non-selective lesions in this area cause loss of up to 50% of sleep behavior. We placed injections of AAV-hM3Dq into the VLPO in galanin-Cre mice, to drive the VLPO neurons. Administration of 0.3 mg/kg CNO caused the animals to have up to 6 hours of increased NREM sleep, associated with a fall in body temperature to about 30 degrees C. Even when the animals were warmed by exposing them to higher ambient temperature, they remained in deep sleep. Thus the two effects of the VLPOneurons appear to be mediated separately.

The melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus have projections similar to the orexin/hypocretin neurons, with which they are intermixed. However, they have the opposite firing pattern, being most active during REM sleep, and they are thought to promote REM sleep. Recent optogenetic stimulation studies have shown contradictory changes in NREM or REM sleep, although the stimulation protocols and outcome measures were different. To drive the MCH neurons for longer periods of time and at physiological patterns of firing, we injected AAV-hM3Dq into the lateral hypothalamus in MCH-Cre mice. CNO (0.3 mg/kg) drove dramatic increases in the amount of REM sleep over the next 4-6 hours.

Chemogenetic activation of these hypothalamic wake and sleep circuits permit testing of the potency of their effects in ways that traditional lesions, or optogenetic stimulation, do not.

# Dopamine evoked long-term synaptic potentiation mediated by locus coeruleus

**Robert Greene**

*University of Texas Southwestern Medical Center, USA*

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

## ABSTRACT

Neurons of the Locus Coeruleus (LC), express Tyrosine Hydroxylase to synthesize dopamine (DA). In LC axonal terminals, dopamine is actively transported into synaptic vesicles containing dopamine beta hydroxylase that converts DA to norepinephrine (NE). Thus, LC terminals contain both DA and NE but it is traditionally accepted, only NE is released. Many cortical and subcortical regions receive inputs from both the DA nuclei and the LC but there is an exception: the CA1 subfield of the hippocampus receives only LC input. Recently, we demonstrated an amphetamine-evoked release of DA from LC projections to CA1. We now show that selective optogenetic activation of LC terminals in CA1 is sufficient to cause LTP of the Schaffer Collateral synapse by specific activation of D1/5 receptors on CA1 pyramidal neurons. This effect is not antagonized by NE receptor antagonists nor mimicked by NE. However, D1/5 receptor antagonists block the LC-evoked long-term potentiation. Optogenetic LC activation also enhances tetanus evoked, weak LTP of this synapse by D1/5 receptor activation. Because D1/5 activation in CA1 is necessary for long-term memory, a critical role for LC neurons is proposed to mediate the required dopamine neurotransmission for activation of CA1 pyramidal D1/5 receptors in learning and memory.

# The role of adult born neurons in memory consolidation during sleep-wake cycles

**Deependra Kumar**

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

## ABSTRACT

Memory is suggested to be consolidated during sleep[1-3], which could occur during both rapid eye movement (REM) sleep[2] or NREM sleep[1,3]. Memory associated odor stimulation has been correlated with enhancing memory consolidation, during NREM sleep, but not during REM sleep nor awake[4]. In addition, boosting neuronal oscillations (i.e., slow oscillations) during NREM sleep has also been shown to potentiate memory consolidation[5]. Conversely, a less stressful sleep deprivation revealed a bidirectional influence of REM sleep on memory[6]. These studies examined the role of NREM or REM sleep for memory by intervening with sleep architecture or targeting unspecified neuronal circuits. We address these issues by directly intervening a memory regulatory circuit (i.e. hippocampal adult born neurons) during specific stages of sleep using optogenetics, which provides reversibility in intervention with higher time-resolution and specificity. The intervention does not affect sleep architecture and reveals that the activity of adult born neurons is necessary for memory consolidation during specific stage of sleep.

References:

1. Ji D, Wilson MA. *Nat Neurosci*. 2007; **10**:100-7.
2. Louie K, Wilson MA. *Neuron*. 2001; **29**:145-56
3. Wilson MA, McNaughton BL. *Science*. 1994; **265**:676-9.
4. Rasch B, Büchel C, Gais S, Born J. *Science*. 2007; **315**:1426-9.
5. Marshall L, Helgadóttir H, Mölle M, Born J. *Nature*. 2006; **444**:610-3.
6. Ravassard P, Hamieh AM *et. al.*, *Cerebral Cortex*. 2015 Jan 13. in press

# Evolution of human-specific gene coexpression networks

**Genevieve Konopka**

*University of Texas Southwestern Medical Center, USA*

## ABSTRACT

Disruptions to circadian rhythms and normal sleep patterns are the most frequent quality of life issue associated with autism spectrum disorders or ASD. The identification of the molecular pathways that are at risk in these ASD-related symptoms will provide novel targets for improved therapeutics not only for ASD but also for other disorders with circadian deficits such as major depression and bipolar disorder as well as normal aging. As cognitive disorders are thought to be human-specific, the identification of human-specific brain modifications should provide insight into these disorders as well as improved therapeutics. Recent technical breakthroughs in genomics have allowed us to begin to identify genetic and molecular signatures in the central nervous system that distinguish humans from non-human primates. We have identified novel human-specific patterns of gene expression and regulation in the neocortex. These data suggest that the human brain has undergone rapid modifications of gene expression patterns to support our enhanced cognitive abilities. In addition, we identified an enrichment of cognitive disease related genes that demonstrate unique gene expression changes in the human brain. Surprisingly, we identified the circadian transcription factor CLOCK as the most connected hub gene in a human-specific frontal pole module and increased neocortical expression of CLOCK in human brain compared to non-human primates. Previously identified genes exhibiting cyclical expression patterns are not enriched in this module, suggesting that CLOCK expression in the human frontal pole has a distinct transcriptional program. We are carrying out functional follow up increased human neocortical expression of CLOCK and other genes involved in transcriptional regulation. These follow up studies have manipulated these genes in primary human neurons and rodent models followed by further genome-wide expression analyses using RNA-sequencing. These new data have uncovered additional coexpression patterns and molecular pathways that might be involved in human disorders of cognition.

# A neural circuit for auditory dominance on visual perception

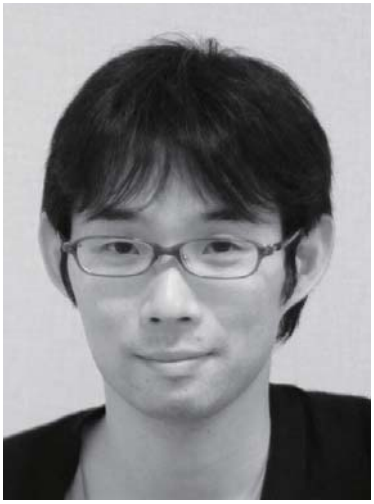
**Seung-Hee Lee**

*Korea Advanced Institute of Science & Technology, Korea*

## ABSTRACT

Sound stimuli often suppress visual processing, but the circuit mechanism of perceptual competition between visual and auditory processing is unclear. Here, we unravel a neural circuit composed of primary visual (V1), primary auditory (A1), and posterior parietal (PTLp) cortices, which mediate auditory dominance on vision. Simultaneous presentation of auditory and visual stimuli or concurrent activation of V1 and A1 elicited auditory-dominant perceptual behavior in the animal perceiving each as specific information. The auditory dominance switched to the visual dominance by inactivation of PTLp, which receives direct inputs from both V1 and A1. Supporting this, co-activation of V1 with A1 suppressed the activity of V1-selective neurons in PTLp and produced similar response to A1 activation. Our data demonstrate that converging input to PTLp from V1 and A1 determines auditory dominance on the visual perception in actively perceiving animal.

# Identification of a sleep regulatory circuit and implications for the function of REM sleep



**Yu Hayashi**

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

## ABSTRACT

Our sleep is composed of two distinct states, REM (rapid eye movement) sleep and non-REM sleep. REM sleep is the major source of dreams, whereas non-REM sleep is characterized by a synchronous brain activity called slow waves. Little is known, however, about the individual roles and neural substrates of these two states. While classical physiological studies suggest a crucial role for the brainstem, the heterogeneity and complexity of the brainstem has hampered identification of the critical neurons. Here, we developed a mouse genetics method to functionally classify neurons according to their embryonic origin. Using this method, we identified neurons that robustly regulate transitions between REM and non-REM sleep (Hayashi *et al*., *Science*, 2015). We further developed our findings to establish a mouse model in which we can induce or inhibit REM sleep. Based on analyses of our mouse model, we propose that REM sleep is involved in sleep quality control.

**Session 3** 17:15 - 17:45

# Molecular genetic analysis of sleep

**MEMO**

**Yi Rao**

*Peking University, China*