

The 6<sup>th</sup> Annual IIS Symposium

# Solving the mystery of sleep

December 14, 2017

Tokyo Conference Center Shinagawa

Tokyo, Japan

**Organizer**

**Masashi Yanagisawa**

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

**Supporting Organization**

Ministry of Education, Culture, Sports, Science and Technology



## Timetable

Opening Address				
9:30-9:40	Welcome	Masashi Yanagisawa	WPI-IIS, University of Tsukuba	
	Opening address 1	Akira Ukawa	WPI Program Director	
	Opening address 2	Tetsuya Kishimoto	Ministry of Education, Culture, Sports, Science and Technology (MEXT)	
Session 1   Chair: Sakiko Honjoh				
9:40-10:10	Fast high-resolution miniature two-photon microscopy for brain imaging in freely-behaving mice at the single-spine level	Liangyi Chen	Peking University	Page. 4
10:10-10:40	Sleep deprivation, circadian rhythms, and alcohol	Joshua J. Gooley	Duke-NUS Medical School	Page. 5
10:40-11:10	Hypothalamic circuits for predation and evasion	Minmin Luo	National Institute of Biological Sciences, Beijing	Page. 6
11:10-11:40	Cell assembly mechanisms underlying memory association	Kaoru Inokuchi	University of Toyama	Page. 7
11:40-11:50	Break & Lunch preparation			
Lunchon Session   Chair: Yu Hayashi				
11:50-12:20	The neural correlates of dreaming	Francesca Siclari	Lausanne University Hospital	Page. 8
12:20-12:50	Chrono-nutrition and -exercise studies from mice to human	Shigenobu Shibata	Waseda University	Page. 9
12:50-13:00	Break			
Session 2   Chair: Robert Greene				
13:00-13:30	Molecular mechanism of sleep disorder under neuropathic pain	Minoru Narita	Hoshi University	Page. 10
13:30-14:00	Critical time window for the improved spatial memory by optogenetically-induced slow wave sleep	Shu-min Duan	Zhejiang University	Page. 11
Poster Session & Tea Break				
14:00-15:10	Data Blitz and Poster Session			
MSD Afternoon Seminar   Chair: Masashi Yanagisawa				
15:10-15:40	Arc -haeology of long-term memory	Haruhiko Bito	The University of Tokyo	Page. 12
15:40-16:10	Spatio-temporal dynamics of brain activity during sleep	Vladyslav Vyazovskiy	University of Oxford	Page. 13
16:10-16:40	Neural circuits of orexin neurons: interface of systems of emotion and arousal/vigilance	Takeshi Sakurai	WPI-IIS, University of Tsukuba	Page. 14
16:40-16:50	Break			
16:50-17:20	Pathophysiology of circadian rhythm sleep-wake disorder	Akiko Hida	National Center of Neurology and Psychiatry	Page. 15
17:20-17:50	Understanding of the impact of light on circadian rhythms: Efficacious phototherapy of circadian sleep disorders	Jamie M. Zeitzer	Stanford University	Page. 16
17:50	Closing			
18:00-20:00	Reception			

## 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 available in the foyer.

### Lunch

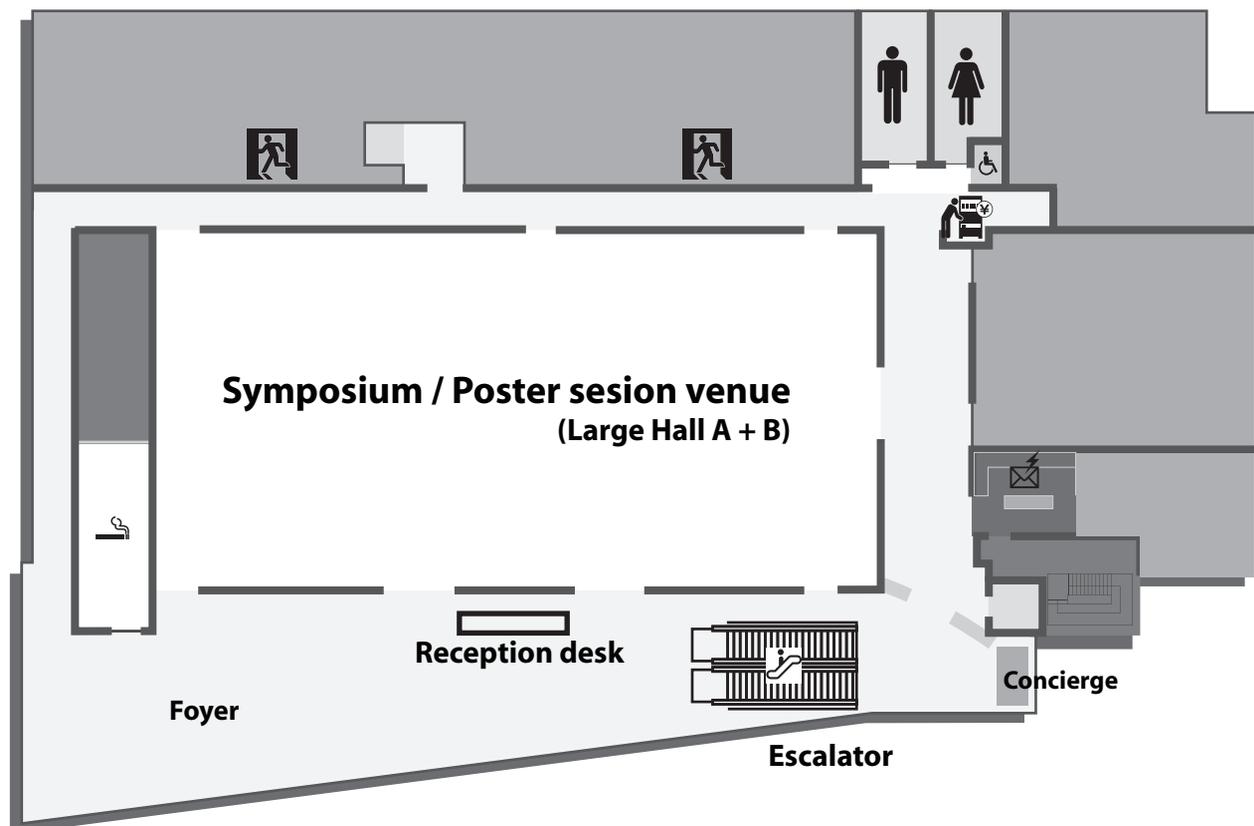
Free lunch will be provided for pre-registered participants. Some extra may be available for non-pre-registered participants on a first-come-first-served basis (will be announced).

### Reception

**18:00 - 20:00** at **Foyer** on 5<sup>th</sup> floor

For MSD afternoon seminar attendees.

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



## Fast high-resolution miniature two-photon microscopy for brain imaging in freely-behaving mice at the single -spine level



**Liangyi Chen**

*Peking University, China*

### ■ ABSTRACT

Recent developments in miniaturized microscopes have furthered the quest to visualize brain activities and structural dynamics in animals engaged in self-determined behaviors. However, it remains an unmet challenge to resolve activity at single dendritic spines, the elemental units underlying neuronal computation, in freely-behaving animals. Here, we report the design, testing, and application of a fast, high-resolution, miniaturized two-photon microscope (FHIRM-TPM) that accomplishes this goal. With a headpiece weighing 2.15 g and a new type of hollow-core photonic crystal fiber to deliver 920-nm femtosecond laser pulses, the FHIRM-TPM is capable of imaging commonly used biosensors at high spatiotemporal resolution (0.64  $\mu\text{m}$  laterally and 3.35  $\mu\text{m}$  axially, 40 Hz at  $256 \times 256$  pixels). Its micro-electromechanical systems scanner also enables random-access capability and free-line scanning at up to 10,000 Hz. It compares favorably with benchtop two-photon microscopy and miniature wide-field fluorescence microscopy in the structural and functional imaging of Thy1-GFP- or GCaMP6f-labeled neurons. Further, we demonstrate its unique application and robustness with hour-long recording of neuronal activities down to the level of spines in mice experiencing vigorous body and head movements or engaging in social interaction. Thus, our new generation miniature microscope provides neuroscientists the long-sought tool-of-choice for imaging the brain at the synaptic level in freely-behaving animals.

## Sleep deprivation, circadian rhythms, and alcohol



**Joshua J. Gooley**

*Duke-NUS Medical School,  
Singapore*

### ■ ABSTRACT

Most alcohol-related vehicular crashes occur at night, at a time when drivers are also likely to be sleep-deprived. It is therefore important to understand the interaction of alcohol and sleep loss on drowsiness and driving errors. My laboratory is examining whether exposure to sleep deprivation enhances the dose-dependent effects of alcohol on objective measures of sleepiness and driving simulator performance. We have implemented the alcohol clamp procedure, in which alcohol is administered intravenously to achieve a prescribed breath alcohol concentration (BrAC) while subjects undergo sleep deprivation in the laboratory. Our results indicate that combining a small dose of alcohol with partial sleep deprivation can result in profound deficits in driving performance. In other studies, we have examined circadian regulation of breath alcohol concentration by administering small doses of alcohol at different phases of the circadian cycle. Our results indicate that peak BrAC values exhibit circadian variation with lower values in the evening and higher values in the morning. Together, these studies demonstrate that sleep deprivation and circadian rhythms modulate alcohol responses. This work has potential implications for road safety and alcohol toxicity.

## Hypothalamic circuits for predation and evasion



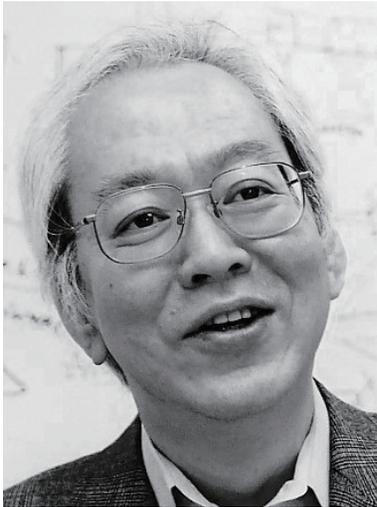
### Minmin Luo

*National Institute of Biological Sciences, Beijing, China*

#### ■ ABSTRACT

The interactions between predator and prey represent some of the most dramatic events in nature, and constitute a matter of life-and-death for both sides. The hypothalamus has been implicated in driving predation and evasion; however, the exact underlying hypothalamic neural circuits remain poorly defined. Here, we demonstrate that inhibitory and excitatory projections from the mouse lateral hypothalamus (LH) to the periaqueductal gray (PAG) in the midbrain drive, respectively, predation and evasion. Optogenetically stimulating PAG-projecting LH GABA neurons drove strong predatory attack. LH GABA neurons were activated during predation, and optogenetically inhibiting these cells blocked predation. In contrast, stimulating PAG-projecting LH glutamate neurons drove evasion. LH glutamate neurons were activated during evasion, and inhibiting them impeded the ability to make predictive evasion. Therefore, the seemingly opposite behaviors of predation and evasion are tightly regulated by two dissociable modular command systems within a single neural projection from the LH to the PAG.

## Cell assembly mechanisms underlying memory association



**Kaoru Inokuchi**

*University of Toyama, Japan*

### ■ ABSTRACT

Memories are not stored in isolation from other memories but are integrated into associative networks and are accumulated based on prior experiences throughout life. A specific neuronal ensemble represents a given memory. Recent studies suggest that synchronous activity of neuronal ensembles encoding distinct memory traces mediates the association of memories, which is accompanied by the generation of a co-sharing neuronal subpopulation. However, the functional role of this co-shared ensemble in memory association remains elusive. Here, we show the existence of a small population of co-shared neurons in the basolateral amygdala (BLA) that mediates the link between memories but is unnecessary for recalling individual memories. Using two amygdala-dependent behavioural paradigms, conditioned taste aversion (CTA) and auditory-cued fear conditioning (AFC), we find that presenting the conditioned stimulus used for the CTA task triggers the conditioned response of the AFC task, that is, freezing behaviour, after natural co-reactivation of their memory retrievals. Analyses using a cellular imaging method, Arc/Homer1a catFISH, shows an increase in the ratio of the co-shared neuronal subpopulation, an overlapping ensemble. Optical silencing of the overlapping ensemble suppresses CTA retrieval-induced freezing. However, retrieval of the original CTA or AFC memory is not affected. Thus, only the link between the CTA and AFC memories is interrupted, without impairing either original memory. Our findings provide new insight into a specific neuronal ensemble shared by two pre-stored memories, generated through consecutive recall of the distinct pre-stored memories. This finding may lead to the development of a way to dissociate unwanted connections formed between traumatic memories and related daily events without affecting the original memories.

## The neural correlates of dreaming



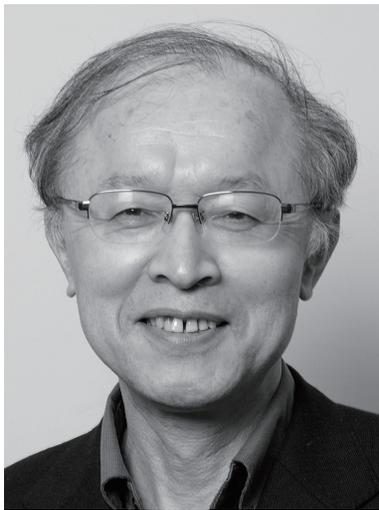
### Francesca Siclari

*Lausanne University Hospital,  
Switzerland*

#### ■ ABSTRACT

Dreaming is a form of consciousness that occurs during sleep, while we are functionally disconnected from the environment. Traditionally, dreaming has been linked to REM sleep, a behavioral state characterized by fast, desynchronized electroencephalographic activity similar to wakefulness. In recent years however, it has become clear that dreaming can also occur in NREM sleep, in which EEG activity is dominated by slow waves and spindles. This has challenged the understanding of the neural correlates of conscious experiences in sleep. In the present talk I will present a study in which we investigated the neural correlates of dreaming using a serial awakening paradigm and high-density EEG recordings. We found that dreaming, irrespective of sleep stage, is associated with a local reduction of low-frequency activity in posterior cortical regions. By monitoring brain activity in these regions in real time, we were able to predict the presence or absence of dreaming in NREM sleep with a high accuracy. In addition, we found that specific dream contents were associated with localized high-frequency increases within these posterior cortical areas. Taken together, these local EEG correlates may account for the presence of conscious experiences in behavioral states with radically different global EEG signatures.

## Chrono-nutrition and -exercise studies from mice to human



**Shigenobu Shibata**

*Waseda University, Japan*

### ■ ABSTRACT

The rapid increase in adiposity is related to significant changes in the human environment and lifestyle, including less physical exercise and high-energy dietary choices. This lifestyle is also related to locomotive syndrome, such as sarcopenia, osteoporosis and joint problems. Thus, reducing fat and/or sucrose and increasing protein and minerals in the diet and increasing physical exercise represent the primary methods for prevention of and/or recovery from lifestyle-related diseases such as obesity and locomotive syndrome in middle and old age persons. However the best timing of nutrient intake and exercise is still unknown. After discovery of molecular mechanism of circadian rhythm, research field of chrono-biology has influenced on many other research fields such as pharmacology, nutrition science and sports /exercise science.

Circadian clock systems play important roles in clock-related physiological functions including energy, protein and mineral metabolisms. Breakfast skipping and high fat diet as for late supper cause obesity. Protein rich breakfast stimulates skeletal muscle volume in mice under 2 meals per day schedule. In addition, inulin rich breakfast reduced colon pH according with increase of short chain fatty acids. Foods containing some nutrients and functional components are reported to provide phase-resetting effects on peripheral clocks. In addition, circadian rhythm system controls physical exercise. The optimal timing of exercise for protection of obesity may be late afternoon. Morning exercise and protein rich breakfast prevented the soleus muscle atrophy by tail suspension- induced muscle atrophy mouse. The present results strongly suggest that the timing of feeding and exercise is critical for protection of lifestyle related diseases such as obesity and locomotive syndromes. We are interested in elucidating how new approach such as chrono-nutrition and chrono-exercise help to promote health science using animal and observation/intervention human experiments.

## Molecular mechanism of sleep disorder under neuropathic pain



**Minoru Narita**

*Hoshi University School of  
Pharmacy and Pharma-  
ceutical Sciences, Japan*

### ■ ABSTRACT

It has been reported that sleep problems and daytime sleepiness are commonly found in opioid-treated primary care patients with chronic pain and cancer pain, and seem to be related mainly to depression and the severity of pain.

Cumulative evidence demonstrates that the forebrain, including neurons in the anterior cingulate cortex (ACC) plays an important role in pain-related perception and chronic pain. Sleep dysregulation was observed after sciatic nerve ligation in mice. On the basis of the results of EEG/EMG, the hypnotic effects of midazolam and zolpidem, were dramatically suppressed in mice with sciatic nerve ligation. We also found that sciatic nerve ligation caused a morphological change in GFAP-labelled astrocytes, and also GFAP-positive immunoreactivity was significantly increased in the ACC of mice. Under these conditions, membrane-bound GABA transporters (GATs) on activated GFAP-positive astrocytes were significantly increased in the ACC, and extracellular GABA levels in this area after depolarization were rapidly decreased. We next used functional magnetic resonance imaging to visualize the increased blood oxygenation level-dependent signal intensity in the ACC of mice with sciatic nerve ligation under mild noxious stimuli. Such stimuli significantly increased the release of glutamate in the ACC. In addition, sciatic nerve ligation and mild noxious stimuli changed the morphology of astrocytes in the ACC. Furthermore, glutamate induced the translocation of GAT-3 to astrocyte cell membranes using primary cultured glial cells from the mouse cortex. Moreover, the GABA level at the synaptic cleft in the ACC of nerve-ligated mice was significantly decreased exposure to mild noxious stimuli. We also demonstrated that selective optical stimulation of these astrocytes in vivo triggered sleep disturbance. Taken together, the present findings provide novel evidence that astrocytic overactivation in the ACC can mimic sleep disturbance in mice.

## Critical time window for the improved spatial memory by optogenetically-induced slow wave sleep



**Shu-min Duan**

*Zhejiang University School of Medicine, China*

### ■ ABSTRACT

Sleep is thought to be involved in memory consolidation for a long time. However, most studies investigate the correlation of sleep and memory by means of sleep deprivation, which may induce stress and thus secondarily interfere with memory formation. Furthermore, the precise time window for the correlation of sleep and memory consolidation remains unclear. We found that optogenetic stimulation of GABAergic neurons in parafacial zone (PZ) immediately induced slow wave sleep (SWS) in (VGAT)-ChR2-EYFP mice. Taking the advantage of this approach, we are able to control SWS in these mice in precise timing. The 30 min SWS induced by light stimulation of GABAergic neurons in PZ immediately or 15 min after the sample phase of the object-in-place task significantly improved the memory of mice. The improved memory was blocked when the light stimulation was coupled with gentle touch to prevent SWS induction, suggesting that SWS, but not light stimulation of PZ itself, is crucial for the improved memory. Interestingly, when SWS was induced 30 min or more after the learning phase failed to improve the memory. The light-induced SWS immediately after the learning phase also enhanced memory in Y-maze spatial memory and contextual fear memory. The light-induced SWS right before the test phase had no effect on memory performance, suggesting SWS does not affect memory retrieval. Our results indicate that SWS play a crucial role in the consolidation of hippocampus-dependent spatial memory.

# **MSD Afternoon Seminar**

## Arc-haeology of long-term memory



**Haruhiko Bito**

*The University of Tokyo  
Graduate School of Medicine,  
Japan*

### ■ ABSTRACT

Deciphering the intricate and interactive relationship between the information encoded in the genome and the ongoing synaptic activity is critical for understanding the molecular and cellular signaling underlying long-term memory formation and maintenance of long-lasting changes within the brain. To systematically dissect this question, we investigated the molecular basis of the signaling from synapses to the nucleus and from the nucleus to the synapses, which crucially determines the persistence of synaptic plasticity. We thus uncovered an activity-dependent protein kinase cascade CaMKK-CaMKIV that critically controls the amplitude and time course of phosphorylation of a nuclear transcription factor CREB downstream of synaptic activity, thereby activating a plethora of adaptive transcriptional responses within an active neuronal circuit. We also identified a novel “inverse” synaptic tagging mechanism in which one of CREB’s target gene, *Arc*, acts as a brake that helps weaken the non-potentiated synapses during the maintenance phase of synaptic plasticity. In genomic parlance, *Arc*’s rapid induction following strong physiological stimuli is dictated by a potent synaptic activity-responsive element (SARE) present in its enhancer/promoter region, which strikingly harbors a unique cluster of binding sites for CREB, MEF2 and SRF/TCF. Based on this discovery, we created a synthetic promoter E-SARE which now allows to map, label, record and manipulate active neuronal ensembles in various areas of the brain *in vivo*. Recently, we designed a new set of genetically encoded Ca<sup>2+</sup> indicators (GECIs), such as R-CaMP2, which are molecular spies of neuronal activity with most desirable properties such as fast speed and signal linearity. This was largely achieved by exchanging the M13 sequence of classical GECIs with a sensitive CaM-binding sequence engineered based on neuronal CaMKKs. These efforts collectively start to illuminate key molecular and cellular events that are essential in neuronal coding and information processing in active neuronal circuits and systems *in vivo*.

## Spatio-temporal dynamics of brain activity during sleep



**Vladyslav Vyazovskiy**

*University of Oxford, UK*

### ■ ABSTRACT

Sleep has been found in all animal species carefully studied to date; yet, the biological function of sleep remains unclear. Sleep can be defined on at least two distinct levels: the behaviour of the whole organism and the spatiotemporal patterns of neuronal activity in the brain. Upon falling asleep, cortical networks alternate between periods of generalized population firing and periods of relative silence. This pattern of neuronal activity gives rise to electroencephalogram (EEG) oscillations at a frequency of approximately 1-4 Hz, which are termed slow waves. Although a prevailing view is that brain states are regulated in a global fashion, research over the last decades has revealed that spontaneous brain activity during sleep can be locally modulated. For example, slow wave activity (SWA) is more intense in frontal compared to more posterior areas, especially in early sleep or after sleep deprivation, and regional differences are apparent at the level of individual sleep slow waves. Although the alternation of periods of increased neuronal activity and silence is usually correlated across cortical regions and individual neurons, up-states can sometimes be seen in one region of the cortex while another region is in a down-state, with these states often spreading as travelling waves. Importantly, topographic differences in brain activity during sleep are functional, as peripheral stimulation or the spontaneous use of circumscribed cortical areas leads to more intense local EEG SWA. Such data demonstrate that not only waking duration per se, but also strong activity in specific regions affects the 'intensity' of subsequent sleep. Moreover, sleep deprivation is associated with increased low-frequency EEG activity during waking in both animals and humans, and recordings in rats suggested that this EEG pattern reflects local neuronal OFF periods. This notion suggests that sleep need accumulates at the level of local cortical networks or even single neurons. Thus, understanding the function of sleep requires one to bridge the gap between local and global levels - from individual cells to large-scale brain networks and global behaviour.

## Neural circuits of orexin neurons: interface of systems of emotion and arousal/vigilance



**Takeshi Sakurai**

*WPI-IIS, University of Tsukuba,  
Japan*

### ■ ABSTRACT

Animals shift their sleep-wakefulness state according to their internal state and the external environment by utilizing three major influential elements, i.e., homeostatic, circadian and allostatic factors. Among them, allostatic factors include the nutritional state and external environment, which trigger emotion. For example, stressful and emotionally-salient situations such as encountering predators, adapting to novel situations or expecting a reward require animals to shift their behavior to a vigilant state, along with alteration of their physiological condition through modulation of autonomic and endocrine functions. Studies of efferent and afferent systems of orexin-producing neurons have shown that the orexin neuronal system has close interactions with systems that are involved in the regulations of emotion, energy homeostasis, reward, and arousal. Many studies have suggested that orexin neurons are activated during the behavioural expression of fear or in response to cues associated with danger or reward. These observations suggest that orexin neurons are involved in control of vigilance states in response to outer environment. On the other hand, orexin system affects expression of behavioral response against outer environment via control of monoaminergic neurons. I will discuss functional interplay between the limbic system and arousal system, and involvement of orexin and orexin receptors in these interactions.

## Pathophysiology of circadian rhythm sleep-wake disorder



**Akiko Hida**

National Center of Neurology  
and Psychiatry, Japan

### ■ ABSTRACT

Circadian rhythm sleep-wake disorders (CRSWDs) are defined by persistent or recurrent disturbed sleep-wake patterns and consist of several subtypes including delayed sleep-wake phase disorder (DSWPD) and non-24-hour sleep-wake rhythm disorder (N24SWD). CRSWDs are thought to result from impairment of the circadian clock system and/or a misalignment between the endogenous circadian rhythm and exogenous entrainment factors. In mammals, the central oscillator in the suprachiasmatic nucleus of the hypothalamus incorporates environmental cues, such as light exposure, and coordinates the phase of oscillators in peripheral tissues. The molecular mechanisms underlying the circadian clock system involve the transcription-translation negative feedback loops of multiple clock genes. Evaluating the circadian phenotype is crucial for establishing a precise clinical diagnosis and for understanding the pathophysiology of CRSWDs. we examined the circadian phenotypes of patients with DSWPD and N24SWD by evaluating *Bmal1-luciferase* (*Bmal1-luc*) rhythms in skin fibroblast cells from individual patients. The period length of the *Bmal1-luc* rhythm (*in vitro* period) varied among individuals. The N24SWD group showed a significantly longer *in vitro* period than did the control or DSWPD group. Furthermore, *in vitro* period was associated with response to chronotherapy in the N24SWD group. Longer *in vitro* periods were observed in the non-responders compared to the responders in the N24SWD group. Our findings suggest that prolonged circadian periods contribute to the onset and poor treatment outcome of N24SWD. *In vitro* rhythm assays could be useful for predicting circadian phenotypes and clinical prognosis in patients with CRSWDs.

## Understanding of the impact of light on circadian rhythms: Efficacious phototherapy of circadian sleep disorders



**Jamie M. Zeitzer**

*Stanford University, USA*

### ■ ABSTRACT

Light is the most potent influence on the timing of the human circadian clock and has been used for decades to treat circadian-based sleep disorders. Under ideal laboratory environments, light can shift the human circadian clock up to three hours in a day, though the true value is much less under real-world settings. Furthermore, light treatments in the real world often are scheduled to occur during times at which people are meant to be sleeping, further limiting its effectiveness. Based on recent advances in our understanding of the neurobiology that underlies the transfer of light information from the retina to the circadian clock, we have undertaken a series of experiments to examine if we could optimize light treatment such that larger shifts could be evoked and that such shifting could occur when people are sleeping. Our examination of sequences of brief flashes of light has satisfied both of these criteria. Flash sequences are far more potent at shifting circadian timing than is continuous light. In a first optimization step, we generated phase shifts in response to light flashes that are at least three-fold larger than those observed after traditional light therapy and are, photon-per-photon, 12,000 times more effective. Flash sequences can be administered during sleep without necessarily interfering with sleep. Our most recent work demonstrates the clinical effectiveness of this therapy in teens with delayed sleep. In conjunction with behavioral therapy, light flashes administered during sleep in teens is able to move sleep to an earlier time, increasing nightly sleep by ~45 minutes and improving subjective sleep quality. Future work focuses on further optimization of light flashes with the goal of generating very large (>8 hr.) phase shifts.