



Director  
**Masashi Yanagisawa**

## Sound sleep for everyone in the world ~ Solving the mystery of sleep ~

We spend nearly one-third of our lives asleep. Although sleep is a quite common phenomenon in vertebrates including human beings and other animals, it still remains one of the greatest mysteries in today's neuroscience. Masashi Yanagisawa's discovery of neuropeptide "orexin," which plays important roles in sleep/wake regulation, opened a new era of sleep science, and led to the elucidation of neural circuits and neurotransmitters controlling sleep/wakefulness. However, there are two major and important questions unanswered; why do we sleep, and what are fundamental mechanisms that regulate sleep/wakefulness (i.e. what is "sleepiness")?

IIS, a unique world-premier institute specialized in the fundamental studies on sleep medicine, was established to uncover the mystery of sleep, and to develop therapeutic means for sleep disorders and associated metabolic syndromes and/or mental disorders.

### ■ Research Center's Information (FY 2015)

Center Director: Masashi Yanagisawa

Principal Investigators (PI): 22 (including 8 overseas researchers and 2 female researchers)

Other Researchers: 29 (including 10 overseas researchers and 13 female researchers)

Research Support Staff: 11

Administrative Division:

Administrative Director: Toshio Kokubo

Administrative Staff: 17 (percentage of bilingual staff:71%)

**Satellites and Cooperative Organizations:** University of Texas Southwestern Medical Center, USA; University of California, Berkeley, USA; Akita University, Japan; RIKEN Brain Science Institute, Japan; Japan Aerospace Exploration Agency (JAXA), Japan; Kyoto University, Japan; and others

URL: <http://wpi-iiis.tsukuba.ac.jp/>

## Major Research Achievements

1

### Discovery of genes regulating sleep/wake network

Yanagisawa/Funato laboratory discovered mouse mutations affecting sleep/wake, and identified responsible genes. This will be a huge first step to uncover the entire signaling network of sleep/wake regulation, potentially leading to diagnostic and therapeutic targets for sleep disorders and related diseases.

2

### Development of the orexin receptor agonist

Nagase's group developed a compound that acts as orexin receptor agonist, which significantly induces wakefulness and ameliorates narcoleptic symptoms in model mice. This finding holds intriguing implications for developing remedies for the sleep disorder.

3

### Why do we dream? Exploring the role of REM sleep

Hayashi and his colleagues identified the brain region switching REM/NREM sleep, and developed a transgenic mouse whose REM sleep can be manipulated arbitrarily. They found that REM sleep enhances the slow wave during NREM sleep, which is important for memory formation and restoration of brain function.

4

### The 'pacemaker' cells for the circadian rhythm

Researchers identified specific neurons that function as pacemakers of the circadian rhythm in the suprachiasmatic nucleus (SCN). Those neurons play an important role in synchronizing the circadian rhythm via the intercellular neurotransmission.

5

### Discovery of a novel neural network connecting wakefulness and memory regulation

Greene *et al.* revealed that dopamine released from neurons in the locus coeruleus (LC) plays an important role in the hippocampus in memory retention. LC is known to control wakefulness, and found to be important for regulating memory as well.



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Sleep is an essential behavior that everyone experiences everyday and it takes up one-third of the entire lifetime. However, the very fundamental mechanisms of sleep still remain unknown. While underlying principles of sleep has been a complete black box, its medical and social importance is clear. Healthy, natural sleep is essential for maintaining our mind and body fitness, and lack of sleep causes severe economic loss every year. Masashi Yanagisawa (Director of IIS) discovered a neuropeptide "orexin", which plays an important role in sleep/wake regulation, opened up the avenue of sleep science, and greatly contributed the development of sleep medicine. However, the questions of how is sleep/wake regulated, why two sleep phases – rapid eye movement (REM) sleep and deep NREM sleep – exist, what is the neural substrate for "sleepiness", and even why we have to sleep, remain unanswered.

International Institute for Integrative Sleep Medicine (IIS) in University of Tsukuba, launched in 2012, focuses on sleep neurobiology and aim to solve one of the biggest black boxes of today's neuroscience. IIS gathered prominent scientists globally from various research fields contributing to neurobiology of sleep, to elucidate the fundamental principles of sleep/wake regulation, and develop new strategies to diagnose and treat sleep disorders as well as closely associated metabolic /mental disorders.

### 1 Discovery of genes regulating sleep/wake network

Masashi Yanagisawa (PI), Hiromasa Funato (PI)

The principles of regulating sleep/wakefulness are still in a black box. Substantial mechanisms of 'sleepiness' in our brains or the mechanism to determine daily sleep amount are completely unknown. Yanagisawa/Funato laboratory has been tackling this great mystery by using forward genetic screening, in which researchers explore mutagenized animals showing characters or phenotypes of interests.

They scrutinized sleep behaviors by examining electroencephalogram (EEG) and electromyography (EMG) of more than 8,000 randomly mutagenized mice to seek for individuals with sleep/wake abnormalities, resulted in the discovery of two important gene mutations in two pedigrees: *Sleepy* and *Dreamless*. They found that *Sleepy* pedigree has a mutation in the *Sik3* gene, whereas *Dreamless* pedigree has a mutation in the *Nalcn* gene.

*SIK3* is a kinase and has a protein kinase A (PKA) phosphorylation site in the middle part, which is deleted in *Sleepy* mutant. This PKA phosphorylation site

is well conserved in roundworm *C. elegans* and in fruit fly *D. melanogaster*, and also found to regulate sleep-like behaviors in those animals. It is quite interesting that the sleep-like behaviors of invertebrates are also regulated by the same molecular mechanism via *SIK3* orthologues as in vertebrates. Sleep-deprived mice (i.e. mice with increased 'sleepiness') showed an increased phosphorylation in the kinase domain, which regulates enzymatic activity of *SIK3*, suggesting that *SIK3* comprises the intracellular signal transduction of 'sleepiness'.

*NALCN* is an ion channel, and one of amino acids in transmembrane region is changed due to the *Nalcn* mutation. The mutation causes increased ion conductance through the channel and increases the activity of REM sleep-terminating neurons in the brain stem, which leads to REM sleep reduction.

The *Sik3* and *Nalcn* genes have never been annotated with sleep behavior, and this milestone discovery will give a huge impact on future sleep studies. The researchers will be keen to elucidate intercellular signal transduction pathways involved in switching between wake and sleep or between REM sleep and NREM sleep, and the entire network of sleep/wake regulation.

*Funato et al.: Nature 539: 378, 2016.*

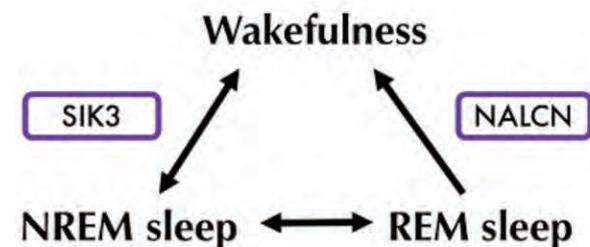


Fig. 1 Sleep states regulated by two proteins discovered in this study. *SIK3* determines the amount of NREM sleep, whereas *NALCN* regulates the termination of REM sleep.

### 2 Development of the orexin receptor agonist regulating wakefulness

Hiroshi Nagase (PI), Masashi Yanagisawa (PI)

In 1998, Yanagisawa and his colleagues revealed that the neuropeptide orexin and its receptor play central roles in maintaining and stabilizing wakefulness, and their deficiencies in brain cause a serious sleep disorder narcolepsy, in which patients experience excessive daytime sleepiness that may occur at any time. The discoveries of orexin and its receptor promoted research for developing a new type of drug that specifically inhibits the receptor (i.e. antagonist). On the other hand, the development of a small molecule mimicking

orexin (i.e. agonist), which promotes wakefulness and may potentially be useful as a therapeutic agent for narcolepsy, has been hampered by several technical reasons.

Hiroshi Nagase, a professor of IIS, and his colleagues successfully developed a potent compound that promotes wakefulness and remedies narcolepsy in mouse models in collaboration with Yanagisawa. They screened more than 250 thousand compounds to discover multiple candidate molecules that show activities on type 2 orexin receptor, which plays an important role in regulating sleep/wakefulness. Through designs and syntheses of more than 2,000 derivatives from those candidates followed by pharmacological assays, they found key structures that are relevant to the agonistic activity. Further optimization resulted in a small compound named YNT-185, with high activity and selectivity on type 2 orexin receptor. Both intracerebroventricular and intraperitoneal administrations of this compound significantly induced wakefulness in mice, and also ameliorated narcoleptic symptoms in model mice. They are accelerating the research to obtain more potent compound, which will lead to the specific cure for narcolepsy through animal and clinical studies.

Nagahara T et al.: *J. Med. Chem.* 58(20): 7931, 2015.

### 3 Why do we dream? Exploring the role of REM sleep

Yu Hayashi (PI)

REM sleep, the phase where dreams occur, was found in more than 60 years ago, but its function was a long mystery. Since REM and NREM sleep are observed only in vertebrates and Aves, those two phases have been considered to be involved in higher brain functions. The amount of REM sleep increases during neonatal period and immediately after learning, though the role of REM sleep has been unknown due to the paucity of means to effectively inhibit REM sleep. Also, the neural basis of switching between REM and NREM sleep is largely unknown.

Yu Hayashi and his colleagues used genetic engineering techniques in mice to identify neural cells that play a central role in switching between REM and NREM sleep. They manipulated those cells with the cutting-edge technologies to increase/decrease the amount of REM sleep and analyzed the effects.

When REM sleep was genetically manipulated, a brain activity termed delta activity was affected during the subsequent NREM sleep. Delta wave, another phenomenon unique to vertebrates and Aves, is known to enhance synaptic plasticity and contribute to learning and memory formation. Delta waves appear most frequently during NREM sleep. In this study, they found that elongated or shortened REM sleep resulted in correspondingly larger or smaller delta waves in the following NREM sleep. These results suggest that

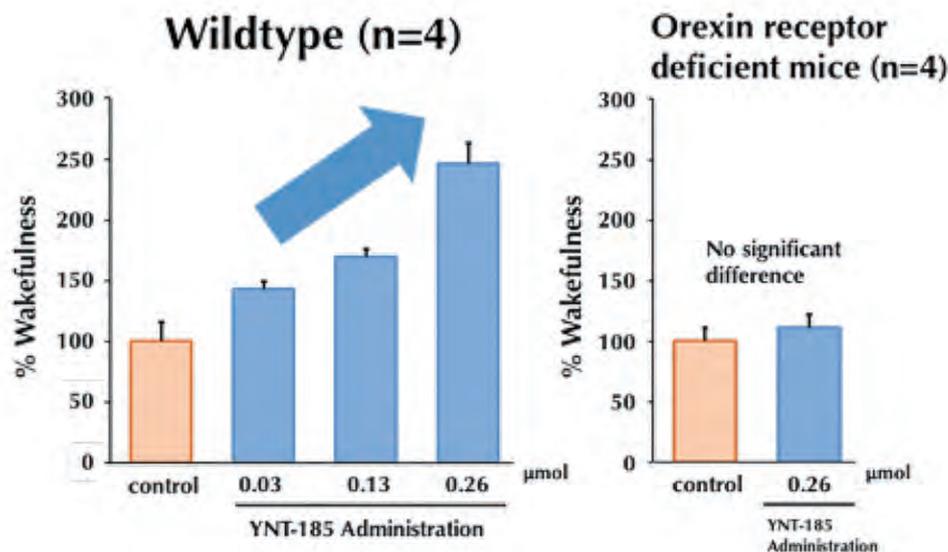


Fig. 2 YNT-185 increases the level of wakefulness in dose-dependent manner in wildtype mice, whereas no effects in orexin receptor deficient mice.

REM sleep enhances delta waves in the subsequent NREM sleep and it contributes to learning and memory consolidation.

They also succeeded in identifying neural progenitor cells in the fetus that postnatally become the neurons that function as the REM/NREM sleep switch. Interestingly, these progenitor cells not only generated neurons involved in REM/NREM sleep switching but also generated neurons involved in sleep/wake switching. This is the first discovery of a common developmental origin for cell governing switching between various brain states. It may further provide insights to how animals with REM sleep evolved from animals with more primitive sleep.

Hayashi Y et al.: *Science* 350(6263): 957, 2015.

#### 4 The 'pacemaker' cells for the circadian rhythm

Masashi Yanagisawa (PI), Joseph Takahashi (PI)

Circadian rhythms, the 24-hour processes controlling sleep/wake cycles, are generated by the suprachiasmatic nucleus (SCN) in the hypothalamus of the brain. SCN controls biochemical, physiological and behavioral processes, and is regulated by feedback processes by multiple genes. SCN contains approximately 20,000 neurons secreting more than 100 neurotransmitters, neuropeptides, cytokines and growth factors, and these molecules are known to synchronize the length and phase of circadian rhythms. However, scientists have been unable to pinpoint which of those neurons are involved in time-keeping mechanisms.

Yanagisawa, Takahashi and their colleagues focused on a group of SCN neurons that express a neuropeptide, neuromedin S (NMS). By using cutting-edge genetic technologies in mice, they succeeded in constructing a system to reversibly manipulate circadian clocks of NMS-expressing neurons at arbitrary timing to remotely control their behavioral rhythms, and revealed that; (1) elongating period in NMS-producing neurons by overexpressing the molecular clock *Clock*<sup>Δ19</sup> lengthens periods of SCN- and behavioral circadian rhythms, (2) the loss of molecular clocks in NMS (*Bmal1*, *Per2*) causes losses of SCN- and behavioral rhythms, and (3) inhibition of neurotransmissions from NMS cells abolishes SCN and behavioral rhythms. Those results clearly suggest that NMS expressing neurons, which comprise about 40% of neurons in SCN, act as master pacemakers to regulate circadian clocks. The findings may offer molecular targets for the diagnosis and treatments of circadian dysfunction in the future.

Lee IT et al.: *Neuron* 85: 1086, 2015.

#### 5 Discovery of a novel neural network connecting wakefulness and memory regulation

Robert W. Greene (PI)

Both in humans and animals, episodic-like memory is enhanced when something new or emotionally salient occurs shortly before or after memorizing (memory encoding). Dopaminergic neurons originating in the ventral tegmental area (VTA) are thought to play important roles in this process, called novelty effect.

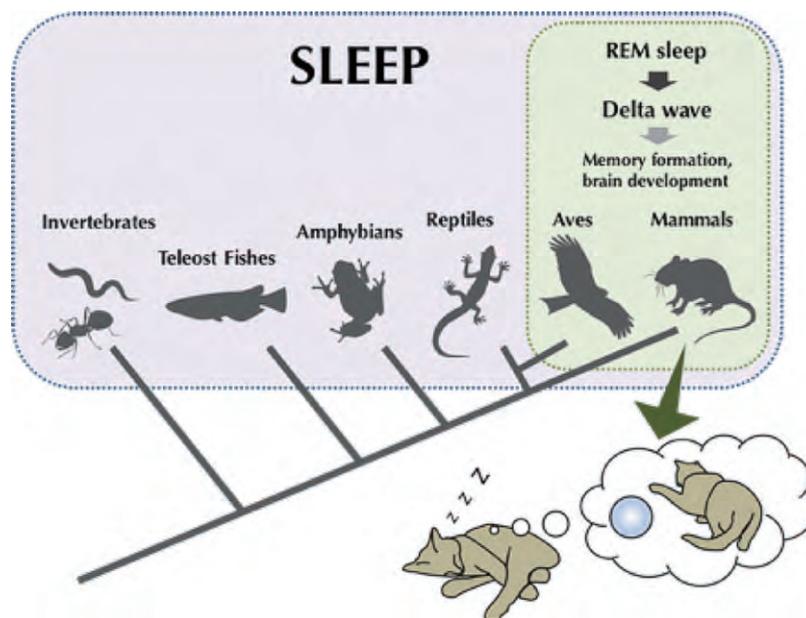


Fig. 3 REM sleep induces delta waves and thus may enhance memory consolidation, learning and brain development in vertebrates and Aves.

Dopamine and noradrenaline are monoamine neurotransmitters and both are biosynthesized from the same amino acid, tyrosine. Dopamine is a precursor of noradrenaline and essential for its biosynthesis. Tyrosine hydroxylase-positive (TH<sup>+</sup>) neurons in locus coeruleus (LC) are thought to mainly release noradrenaline and regulate higher function (e.g. wakefulness) by projecting to various parts of brain from LC. This study showed that TH<sup>+</sup> neuron is not only noradrenergic but dopaminergic, and it also regulates memory by the projection to hippocampus.

Greene and his colleagues used memory task experiments and the technique to control activities of specific neurons with light (called optogenetics) and revealed that: (1) the firing of noradrenergic neurons in LC occurs in novel environment and dopamine is released, (2) noradrenergic neurons in LC project more densely to hippocampus than neurons in VTA, (3) optogenetic activation of noradrenergic neurons in LC mimics novelty effect, and (4) the blockade of VTA does not affect novelty effect-related memory enhancement.

Those results suggest that dopamine, released from noradrenergic neurons from LC, not VTA, mediates the enhancement of memory after encoding. Neurons in LC are long believed to be noradrenergic, but this study showed a new role as dopaminergic neurons projecting hippocampus. It is quite interesting that LC, which

has been thought to play central roles in controlling wakefulness, also plays a critical role in regulating memory mediated by dopamine.

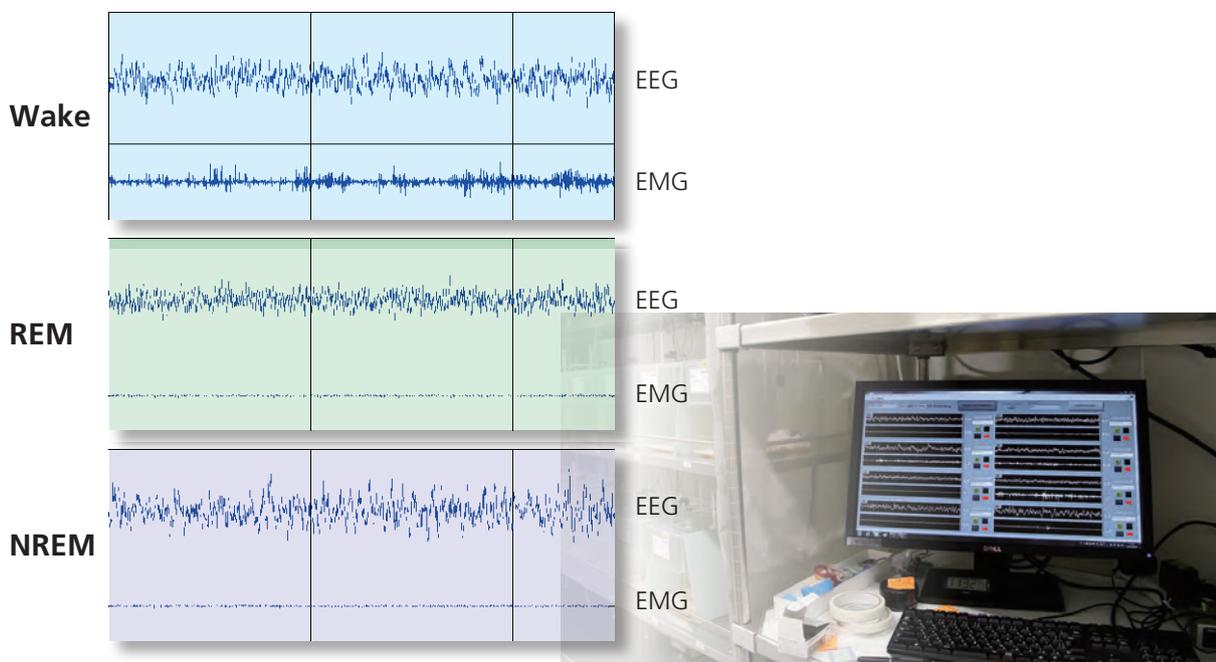
*Takeuchi T et al.: Nature 537 (7620): 357, 2016.*

## Predominant Features of IIS

Based on Yanagisawa's 20-plus-year experience at the University of Texas Southwestern Medical Center, IIS has been challenging to establish the best and unique sleep research center in Japan, by learning from the merits of academic systems and organization in the U.S. "department" style. IIS has accomplished a free and vigorous atmosphere pioneering traditional Japanese-style research organizations, in which flexible and timely appointment of independent PIs regardless of their age and career stage with necessary startup package, a flexible and dynamic allocation of floor space for each laboratory, and sharing of large facilities or capital equipment among laboratories.

Following the concepts above, IIS aims to establish a highly ambitious and novel research field, "sleep science", by integrating basic biology, experimental medicine and pharmaceutical science, under Yanagisawa's strong leadership as the driving force.

Masataka Sasabe (IIS)



Representative EEG/EMG patterns for 3 sleep stages (left) and the sleep recording room implemented in IIS Building (right).