World Premier International Research Center Initiative (WPI)
Executive Summary (For Final Evaluation)

Host Institution	University of Tsukuba	Host Institution Head	NAGATA Kyosuke
Research Center	International Institute for Integrative Sleep Medicine (IIIS)		
Center Director	YANAGISAWA Masashi	Administrative Director	KOKUBO Toshio

Instruction:
Based on the Center's Progress Report and Progress Plan, prepare this summary within 6 pages.

A. Progress Report of the WPI Center

I. Summary

International Institute for Integrative Sleep Medicine (IIIS) has been established *de novo* in University of Tsukuba as the WPI center for sleep science, aiming to solve the medical and social issues related to sleep by elucidating mechanisms of sleep/wake regulation and molecular pathogenesis of sleep disorders, and developing treatments for sleep disorders. Despite the fact that most of its PIs were recruited from institutions outside University of Tsukuba at/after its inauguration, IIIS has succesfully achieved four objectives of the WPI program.

Advance cutting-edge research: Numerous ground-breaking discoveries have been accomplished. The research achievements of IIIS are highly recognized in and outside Japan, and IIIS researchers have received many awards. Besides many articles published in scientific journals, 80 patent applications have been filed so far, aiming at practical applications of research results.

Establish international research environment: More than 30% of researchers from overseas actively participated in the research activities at IIIS. We host International Symposium every year from FY 2013 to FY2019. On the other hand, we canceled it in FY 2020 because of the outbreak of COVID-19.

Reform research organization: The basic concepts of the organization and the operation of IIIS involve creating a new style of research center by learning from the merits and virtues in the organization of "departments" in major US universities. We continue the efforts of system reforms in cooperation with University of Tsukuba. In the University, IIIS is positioned as a pioneering model of the forefront research organization the mid-term plan targets.

Create interdisciplinary domains: We conduct wide-ranging sleep research, covering a scope from a) basic biology such as neuroscience, molecular genetics and molecular cell biology to b) pharmaceutical science, and further to c) experimental medicine. We create the new interdisciplinary research domain, "sleep science" by fusing 3 research fields.

II. Items

1. Overall Image of Your Center

Sleep is a behavior that everyone experiences daily and takes up as much as one third of one's entire lifetime. However, the very fundamental mechanisms of sleep and its raison d'être remain still unknown today. While sleep has been a black box stubbornly resisting scientists' challenges, its medical and social importance is very clear. Healthy sleep is necessary for maintaining our mind and body fitness; lack of sound sleep not only causes a reduction in higher brain functions including memory and decision making, but also increases the risk of mood disorders such as depression as well as metabolic syndrome, etc.

In developed countries, the prevalence of sleep disorders is around 15%, with the lifetime prevalence more than 30%. The underlying factors behind this problem include an increase of the elderly population and the nocturnal lifestyle. The deficiencies in healthy sleep cause significant social losses, and are linked to a decrease in working efficiency and an increase in accidents due to excessive sleepiness. Domestic economic loss caused by sleep disorders in Japan was estimated by RAND Europe in 2016 as ¥15.4 T/year, which corresponded to 2.92% of GDP. Indeed, a lack of sleep in Japanese working population is the worst among advanced nations. Such a "sleep underdeveloped country" definitely needs a world-class research institute for sleep medicine. International Institute for Integrative Sleep Medicine (IIIS) of University of Tsukuba has served the role to solve sleep-related issues.

To solve the sleep-related issues, we set out our major research objectives as follows.

1. To elucidate the fundamental mechanisms of sleep/wake regulation
2. To elucidate molecular pathogenesis of sleep disorders and related diseases
3. To develop and verify treatment strategies for sleep disorders
To achieve these objectives, we have conducted wide-ranging sleep research, covering a scope from a) basic biology such as neuroscience and molecular genetics to b) pharmaceutical science, and further to c) human physiology. We thus aim to create the new interdisciplinary research domain, "sleep science" by fusing these research fields.
In FY2020, the Vice Center Director found a group of neurons in hypothalamus whose forced-activation induces hibernation in mice. Besides sleep, hibernation is another behavior that is characterized by regulated hypomobility. Revealing similarities and differences between sleep and hibernation would lead us to better understanding of both hypomobile behaviors. We have thus added the following goal as the fourth objective of our research in IIIS.
4. To elucidate the fundamental mechanisms of hibernation regulation

To achieve these objectives, we continue the efforts to secure sufficient external research funds and to increase and expand collaboration/research alliances especially in the field of translational research with outside groups including the collaboration groups in University of Tsukuba, the Satellites, external research institutions, and many companies.

The basic concepts of the organization and the operation of IIIS are to create a new style of research center by learning from the merits and virtues in the organization of "departments" in major US universities. In addition to the strong leadership of the "Department Head," other characteristics including the flat organization, the appointment of PIs regardless of their age and career stage, and flexible/dynamic allocation of the floor space and other resources, have been implemented in IIIS.

The PI meeting chaired by the Center Director is held every month and serves as the decision-making body of IIIS. There are 9 labs operated by 14 PIs (5 Co-PIs) in the basic biology. 2 PIs presides a lab of medicinal chemistry with 3 assistant professors, and 4 PIs run 4 labs in human physiology. The Core of IIIS is thus comprised of 14 labs with 20 PIs and 12 non-PI faculties, 30 researchers, and 65 graduate students. IIIS attracts many students for their dissertations and the number of students is still increasing.

The Administration of IIIS headed by Administrative Director offers the accounting and secretary services to PIs and assists the Center Director to operate IIIS in terms of personnel management, recruiting, budgeting, accounting, maintenance, grant application, outreach, public relations, etc. Further, it also functions as a local office of intellectual property right/legal/business development for alliances.

In 2018, R&D Center for Frontiers of MIRAI in Policy and Technology (F-MIRAI), established as the joint project between Toyota Motor Corporation and University of Tsukuba in April 2017, moved into the unfurnished space (365 m^2) held for future expansion on the 4th floor of IIIS Building. As the first collaboration with F-MIRAI, a mobile sleep lab was developed by renovating a Toyota fuel cell bus.

Expansion of the studies of human physiology in IIIS had been a pending issue, and we thus newly established a facility for human sleep research, Human Sleep Lab in Innovation Medical Research Institute located in Kasuga Campus of the University as of March 31, 2019. This 211 m^2 wide facility near Tsukuba station has 4 beds of sleep recording chambers equipped with a bath room, and another bed separately placed in a human calorimeter chamber (for whole room indirect calorimetry during sleep), which was moved from the IIIS Building.

Before launching IIIS, the Center Director Yanagisawa had been running the Funding Program for World-Leading Innovation R&D on Science and Technology (FIRST) that prominently helped the establishment of IIIS. Although the total amount of external funds available for researchers in IIIS (Core Group) in FY 2014 was reduced at the end of FY 2013 after the termination of FIRST, great efforts to raise research funds were made. Since then, our funding records have marked rapid increases as ¥282 M in FY 2015, ¥611 M in FY 2016, ¥667 M in FY 2017, ¥770 M in FY 2018, and ¥808 M in FY 2019. In FY 2020, IIIS earned ¥659 M, which is 20% less than previous annual income due to fewer opportunities of joint or commissioned research projects affected by a pandemic outbreak of COVID-19. Nevertheless, we gained KAKENHI from ¥280 M in FY 2019 to ¥285 M in FY 2020 and even primed greater success in Moonshot granting ¥3,000 M for the next 5 years. Hence, IIIS is one of the most successful research centers within University of Tsukuba.

2. Advancing Research of the Highest Global Level

The four objectives described above are actually the issues of a global level that the Center has challenged, and we provide 20 representative research results to achieve them as follows;
[1] Identification of novel sleep-regulating genes

[2] Elucidation of an intracellular signaling that regulates sleep need
[3] Molecular mechanisms of homeostatic sleep regulation
[4] A discrete neuronal circuit induces a hibernation-like state in rodents
[5] Orexin modulates behavioral fear expression through the locus coeruleus
[6] Identification of a neural circuit that regulates REM and non-REM sleep
[7] Role of sleep in functional brain regeneration
[8] The gating of sleep homeostasis by motivation
[9] Neural circuits controlling sleep and mania-like behaviors
[10] Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors
[11] Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia
[12] Intracellular signaling of sleep function
[13] Cortical organization in waking and NREM sleep
[14] Thalamic regulation of wake and sleep
[15] A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila
[16] Novel delta opioid receptor agonists with oxazatricyclodecane structure
[17] Novel method for tracking vigilance decrement during sleep deprivation
[18] Energy metabolism during sleep
[19] Effect of orexin receptor antagonist on sleep, sleeping energy metabolism and physical/cognitive functions
[20] Cerebrospinal fluid orexin measurements in various disorders

Efforts have been continued to increase and expand collaboration/research alliances especially in the field of translational research with outside groups including groups in University of Tsukuba, the Satellites, and external research institutions.

3. Feeding Research Outcomes Back into Society

Tsukuba Global Innovation Promotion Agency (TGI), which is the organization established by University of Tsukuba, Tsukuba City and Ibaraki Prefecture as the hub of alliances among research institutions in Tsukuba, made a proposal to start 2 R&D projects for Regional Innovation Ecosystem Program initiated by MEXT in FY2016, and the proposal was adopted successfully. One of the R&D projects was the development of an in-home sleep measuring system. In FY 2017, the project succeeded in developing the first prototype of the device with 3 channels of EEG and the sleep staging model of polysomnography (PSG) data on the basis of deep learning. Based on this achievement, a startup, S'UIMIN Inc. was established as the spin-out of IIIS in October 2017 and succeeded in raising fund of ¥900 M in capital as Series A in December 2018. S'UIMIN has taken over the development of the device from Cyberdyne and also has born responsibility for the development of the system, to provide users with the sleep measuring/examination services. IIIS, Center for Computational Science (CCS) and S'UIMIN continue the collaboration to develop the EEG-analyzing model based on deep learning, which consists of the core of the system "InSomnograf" as the analysis engine. S'UIMIN started to provide a closed β service of the sleep measurement in September 2020, and will initiate a sleep examination service in the complete check-up in University of Tsukuba Hospital in April 2021. Improving and expanding the services, the in-home sleep measuring system could bring innovation to sleep medicine.

4. Generating Fused Disciplines

To achieve the three interrelated objectives of IIIS, we have to conduct wide-ranging sleep research, covering a scope from basic biology such as neuroscience and molecular genetics to pharmaceutical science and further to experimental medicine. It is the new interdisciplinary research domain, "sleep science," we aim to create by fusing 3 research fields.

Collaborative research among labs in IIIS is crucial to fuse 3 research fields into "sleep science." The internal collaborations are becoming more active recently, owing to physically and psychologically open atmosphere created/enhanced by 2 factors, i.e., the open structure of the IIIS building and the open communication through unique IIIS-wide meetings such as the Work in Progress (WIP) meeting, the Dojo journal club, and B&B.

In the fused research fields we provide 12 representative research results as follows;

[1] MC-SleepNet: Large-scale Sleep Stage Scoring in Mice by Deep Neural Networks
[2] Novel Tools to Analyze Sleep Quality
[3] Label-free imaging of neurons by multimodal nonlinear optical imaging
[4] Technical developments for sleep research
[5] Structure-activity relationship between thiol group-trapping ability of morphinan compounds with a Michael acceptor and anti-*Plasmodium falciparum* activities
[6] Discovery of attenuation effect of orexin 1 receptor to aversion of nalfurafine and

structure-activity relationship between orexin 1 receptor antagonist YNT-707 and orexin receptors
[7] Design and synthesis of potent and highly selective orexin 1 receptor antagonists with a morphinan skeleton and their pharmacologies
[8] Design and Synthesis of Non-Peptide, Selective Orexin Receptor 2 Agonists
[9] Novel treatment of insomnia by enhancing adenosine A2A receptor signaling
[10] Molecular/neural mechanisms of predator odor-induced innate fear
[11] Effect of food ingredients on sleep and sleeping energy metabolism
[12] Sleep disorder risk factors among athletes

5. Realizing an International Research Environment

Liu and Greene, the overseas PIs, have actively participated in research activities at IIIS through The University of Texas Southwestern Medical Center (UTSW) and National Institute of Biological Science, Beijing (NIBS), respectively. While appointed as Satellite PIs, they have established their own labs in the Center's core group since IIIS was launched in FY 2013. Liu stayed at IIIS for 571 days during 39 visits to Japan totally, though he couldn't visit IIIS even once in FY 2020 due to the COVID-19 epidemic unfortunately. In the meantime, Greene stayed at IIIS for 213 days on 21 visits to Japan totally. He was also unable to visit IIIS during the outbreak of COVID-19 in FY 2020. Both Greene and Liu have contributed to the management of IIIS by joining the PI meeting held monthly, even when absent from the institute, via online from UTSW or NIBS and also take an active part in important events including the symposia hosted by IIIS and the annual site visits for over 8 years.

To WPI-IIIS Symposia, totally 43 outstanding foreign researchers have been invited from abroad each year in order to present the latest achievements in sleep research or relevant fields. On each following day, we have offered them the post-symposium seminar at IIIS onsite to have researchers in the Tsukuba community share the recent progresses in sleep research.

Consequently, we hosted 168 WPI-IIIS Seminars where we invited domestic and foreign researchers in sleep/neuroscience fields almost every other week; 68 speakers from overseas gave us lectures and the ratio of foreign researchers was 41% of the total seminar speakers since the inauguration in December 2012.

6. Making Organizational Reforms

The basic concepts of the organization and the operation of IIIS involve creating a new style of research center by learning from the merits and virtues in the organization of "departments" in major US universities. In addition to the strong leadership of the "Department Head," other characteristics including the appointment of independent PIs regardless of their age and career stage, and a flexible and dynamic allocation of the floor space to each lab, have been consistently implemented.

We continued the efforts of system reforms in cooperation with University of Tsukuba as follows.

1. Introduction of a system to evaluate research results and ability-linked salary system
2. Authority over personnel matters and simplification of the appointment system
3. Joint appointment system
4. Tsukuba Short-term Study Program (TSSP)
5. Establishment of the spin-out of IIIS, S'UIMIN Inc. as a IIIS TLO

During the third mid-term plan of University of Tsukuba starting from FY 2016, the university aims to develop a globally unrivaled frontier research of 2 objectives, i.e., research for the quest for truth and research for innovation contributing to society, in wide-ranging disciplines and research fields. To realize these objectives, the university is making a plan of reorganization/restructuring/merger of all research centers and will implement it during the period of the 3rd mid-term plan. IIIS is positioned as a pioneering model of the forefront research organization the mid-term plan targets, attesting the "ripple effect" of IIIS management.

7. Others

R&D Center for Frontiers of MIRAI in Policy and Technology (F-MIRAI) supported by Toyota Motor Corporation has moved into the south side on 4th floor of IIIS Building. We are planning a long-term collaboration with them and, as the first step, we are developing the Mobile Sleep Lab based on a fuel cell bus lent by Toyota at no charge, taking advantage of its characteristics; the fuel cell bus can supply bulk power for air conditioning and sleep measurement without noise and vibration, and access easily to subjects at living/working environment.

To avoid research misconduct, we have launched educational campaigns for research ethics since FY 2015. We have held total five seminars in the series of Research Ethics Seminars. In addition, we introduced the official laboratory notebook of IIIS to formalize and let everyone use a common hard-covered laboratory notebook for better data management and prevention of research misconduct.

In FY 2018, under the leadership of the Center Director and in close cooperation with key members of the Faculty of Medicine, University of Tsukuba, including the Dean and Associate Dean of the Faculty, we applied for the Doctoral Program for World-leading Innovative & Smart Education (WISE Program) of MEXT, and presented a proposal to create the Ph.D. Program in Humanics. Fortunately, our application was adopted in October 2018. The Ph.D. Program in Humanics aims to create a new academic discipline called "Humanics," which merges high levels of expertise in a) biomedical sciences and in b) physical sciences/engineering /informatics.

B. Progress Plan

1. Mid- to Long-term Research Objectives and Strategies Based on the Center's Research Results to Date

Long-term objectives we aim to achieve in the future by 2040

A. To realize a society where various issues of sleep are well managed to prevent diseases caused/worsened by severe sleep debts

Sound sleep is essential for maintaining physical and mental health; losing sleep chronically poses immense, medical, and social problems. We thus aim to overcome sleep disorders and resulting sleep debts from which more than 20% of people in developed countries suffer, in order to let them enjoy the lives with relief and release from health concerns until 100 years old. To achieve this vision for 2040, we set out the long-term objective A as shown above.

B. To realize a society where innovative emergency medical care based on hibernation is implemented to save lives even in disasters

Last year, the Vice Center Director of IIIS discovered a group of neurons (Q-neurons) in the hypothalamus whose forced-activation induces a hibernation-like state in mice. Besides sleep, hibernation is another behavior that is characterized as regulated hypomobility. The hypomobile behavior adapted to the rotation of the earth is sleep, while another hypomobile behavior accommodated to the revolution of the globe is hibernation. Revealing similarities and differences between sleep and hibernation would lead us to better understanding of both hypomobile behaviors. The characteristics of hibernation, hypometabolism reduces systemic oxygen demand drastically and could offer an effective critical care to avoid tissue injuries and necrosis under hypoxia/anoxia. We thus set the 2nd long-term objective B as indicated above.

Expanding our research subject from sleep to hypomobile behaviors (sleep and hibernation), we aim for ensuring to achieve our vision, having people enjoy their lives with relief and release from health concerns until 100 years old.

Five specific goals we aim to reach in 2040

To achieve our vision and 2 long-term objectives mentioned above, we broke down them into 5 specific goals as described below.

1. To develop methods to control homeostasis of sleep to save more time to improve QOL
2. To develop preventive measures against diseases caused/worsened by severe sleep debts
3. To develop methods to predict risks to be suffered from diseases caused/worsened by sleep debts
4. To build a model of the medical network offering sufficient cares for sleep disorders to everyone in the world
5. To develop innovative emergency medical care using the hibernation technology to save lives even in disasters

2. Management System of the Research Organization

To implement the research strategy and plans outlined above, as of April 1, 2021, we will organize a new project team, consisting of all the PIs in Core Group of the Center (IIIS), and PIs of new Collaborative Groups in University of Tsukuba and new Satellites (refer to Appendix 1-1 for the details). The new project shall be financed mainly by **Moonshot R&D Program** operated by Japan Agency for Medical Research and Development (AMED). The Center Director, Masashi Yanagisawa stays in the position as it is and will also serve as the project manager (PM) of Moonshot R&D Program.

To strengthen our capability of systems biology/mathematical analyses further, we invite to the project 3 more computational scientists in University of Tsukuba as new members of the Collaborative Groups. We nominate also a specialist of non-human primate in University of Tsukuba as a new member of the Collaborative Group for the hibernation study, while we keep good relations with the PIs of the current Collaborative Groups and continue on-going collaborations with them. Among current Satellite PIs, R. Greene, The University of Texas South Western Medical Center (UTSW) and Q. Liu in National Institute of Biological Sciences, Beijing (NIBS) will also join the new project.

Regarding a sustainable positioning of the Center, University of Tsukuba has established Organization for Development of Global Research Centers in March 2020 and included IIIS among their supporting system to develop world-class research centers in the University together with Center for Computational Science (CCS) and Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA). Through the Organization, much is expected of IIIS to share experiences and knowledge/know-how on the operation of the World Premier International Research Center such as internationalization, system reforms, interdisciplinary research, and strategies of intellectual property right and alliance.

As means of fostering and securing the next generation of researchers, IIIS played an active role in the application to Doctoral Program for World-Leading and Smart Education (WISE) program started in FY2018 by MEXT. The Center Director led the effort, in close cooperation with the deans and associate deans of Faculty of Medicine as well as Faculty of Pure and Applied Science and Faculty of System and Information Engineering to propose the launch of the "Ph.D. Program in Humanics." This program aims to foster future leaders with doctorate level training in both a) biomedical science and b) mathematics, physics, chemistry, engineering or informatics. Introducing the double mentorship system, M. Yanagisawa serves as the de facto leader of the program, Program Coordinator, and several IIIS PIs functioned as mentors. The unique and original program contributes to the reform of the graduate schools of the University as well as the promotion of the interdisciplinary research significantly.

3. Center's Position within the Host Institution and Measures to Provide It with Resources

IIIS has given a major impact on the reform of University of Tsukuba. During the third mid-term plan starting from FY 2017, the University aims to pursue the globally unrivaled frontier research, i.e., the research for quest for truth and innovation contributing to society. To achieve the goals, the University has made a plan of reorganization/restructuring/merger of all research centers and is implementing it during the period of the 3rd mid-term plan. Based on the plan the research centers have been classified by function into the Advanced Research Centers and the Research Support Centers. The former has been further classified as R1 (World-class Research Center), R2 (National-class Research Center), R3 (Developing Research Center), and R4 (Research Unit) to facilitate strategic resource allocation. CCS and TARA are classified as the R1 status of World-class Research Center in physics and bioscience, respectively. In addition to the research center's reorganization and classification, the University established Organization for Development of Global Research Centers in March 2020 to implement its objectives through the comprehensive support provided by the creation of an 'On-campus Special Zone for Research Strategy' and strategic allocation of the University's research resources. IIIS and R1-accredited CCS and TARA, are the initial group of the research centers to be supported by the Organization, which aims to expand horizontally among these centers the tasks/achievements of promoting advanced/interdisciplinary researches, internationalization, and reforming systems thus far headed by IIIS.

To make the foundation of IIIS sustainable, the President of University of Tsukuba has repeatedly stated at the WPI Program Committee that PIs with a proven track record of achievement should be promoted to receive the status of 'tenure.' The Center Director and the Deputy Director have already acquired this status, and in FY 2018, with the cooperation of the Faculty of Medicine, a female PI (A. Hirano) was appointed to a tenure track assistant professor by using the strategic position secured by the University. Recently, President Nagata has led the strategic process of initiating the tenure reviews of 4 PIs, whose terms of employment contracts are approaching to the renewal limitation stipulated by the Labor Contract Act. By the termination of the supporting period of the WPI program on March 31, 2022, 7 PIs of IIIS will have received the status of tenure, pending successful reviews. Accordingly in the next fiscal year, the Center Director will nominate additional PIs for tenure reviews to the Personnel Committee under the approval by Vice President for Human Resources.

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(For Final Evaluation)

Host Institution	University of Tsukuba	Host Institution Head	NAGATA Kyosuke
Research Center	International Institute for Integrative Sleep Medicine (IIIS)		
Center Director	YANAGISAWA Masashi	Administrative Director	KOKUBO Toshio

Common Instructions:
* Unless otherwise specified, prepare this report based on the current (31 March 2021) situation of your WPI center.
* As a rule, keep the length of your report within the specified number of pages. (The attached forms are not included to this page count.)
* Use yen (¥) when writing monetary amounts in the report. If an exchange rate is used to calculate the yen amount, give the rate.

1. Overall Image of Your Center (write within 2 pages including this page)

Describe the Center's current identity and overall image.
・ List the Principal Investigators in Appendix 2, and enter the number of center personnel in Appendix 3-1, 3-2, diagram the center's management system in Appendix 3-3, draw a campus map in Appendix 3-4, and enter project funding in Appendix 3-5, 3-6.

1-1. Background

Sleep is a behavior that everyone experiences daily and takes up as much as one third of one's entire lifetime. However, the very fundamental mechanisms of sleep and its raison d'être remain still unknown today. While sleep has been a black box stubbornly resisting scientists' challenges, its medical and social importance is very clear. Healthy sleep is necessary for maintaining our mind and body fitness; lack of sound sleep not only causes a reduction in higher brain functions including memory and decision making, but also increases the risk of mood disorders such as depression as well as metabolic syndrome, etc.

In developed countries, the prevalence of sleep disorders is around 15%, with the lifetime prevalence more than 30%. The underlying factors behind this problem include an increase of the elderly population and the nocturnal lifestyle. The deficiencies in healthy sleep cause significant social losses, and are linked to a decrease in working efficiency and an increase in accidents due to excessive sleepiness. Domestic economic loss caused by sleep disorders in Japan was estimated by RAND Europe in 2016 as ¥15.4 T/year, which corresponded to 2.92% of GDP. Indeed, a lack of sleep in Japanese working population is the worst among advanced nations. Such a "sleep underdeveloped country" definitely needs a world-class research institute for sleep medicine. International Institute for Integrative Sleep Medicine (IIIS) of University of Tsukuba has served the role to solve sleep-related issues.

1-2. Research Objectives and New Interdisciplinary Research Domain

To solve the sleep-related issues, we set out our major research objectives as follows.

1. To elucidate the fundamental mechanisms of sleep/wake regulation
2. To elucidate molecular pathogenesis of sleep disorders and related diseases
3. To develop and verify treatment strategies for sleep disorders

To achieve these objectives, we have conducted wide-ranging sleep research, covering a scope from a) basic biology such as neuroscience and molecular genetics to b) pharmaceutical science, and further to c) human physiology. We thus aim to create the new interdisciplinary research domain, "sleep science" by fusing these research fields.

Since the first objective provides the establishment of sleep science, we place the biggest resources to basic biology research, especially for neuroscience. Our studies of neuroscience are making very rapid progress with cutting-edge technologies such as opto- and chemogenetics. Applying those experimental manipulations, quite a few types of neurons in various brain regions have been identified and validated by us to play important roles in the sleep/wake regulation. Novel neural circuits crucial for generating sleep-wake cycles are being elucidated in near future.

Forward genetics is our characteristic approach to elucidate the homeostatic mechanisms of sleep/wake regulation. It is indeed evident that this method has given us innovative clues to elucidate the mechanisms, *i.e.,* identification of genes such as *Sik3* and *Nalcn*.

Drug discovery to develop novel treatments for sleep disorders such as narcolepsy is another characteristic of IIIS. Likewise in the US, we have been trying to develop academia-driven drug

candidate compounds and license them to pharmaceutical companies for further non-clinical and clinical approaches, contributing to the innovation in treatments of intractable diseases. We aim to be a pioneer in the academia drug discovery in Japan to overcome sleep disorders.

1-3. Organization and Personnel

The basic concepts of the organization and the operation of IIIS are to create a new style of research center by learning from the merits and virtues in the organization of "departments" in major US universities. In addition to the strong leadership of the "Department Head," other characteristics including the flat organization, the appointment of PIs regardless of their age and career stage, and flexible/dynamic allocation of the floor space and other resources, have been implemented in IIIS.

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The Administration of IIIS headed by Administrative Director offers the accounting and secretary services to PIs and assists the Center Director to operate IIIS in terms of personnel management, recruiting, budgeting, accounting, maintenance, grant application, outreach, public relations, etc. Further, it also functions as a local office of intellectual property right/legal/business development for alliances.

Upon agreement with University of Tsukuba, IIIS organizes its own personnel committee, which is chaired by the Center Director and comprised of 6 members including Dean of Faculty of Medicine, Provost of the Graduate School of Comprehensive Human Sciences, and Associate Dean of Biomedical Science, Faculty of Medicine. Decisions made by the IIIS Personnel Committee are subjected to approval by Headquarters Personnel Council.

1-4. Facilities and Equipment

The construction of the IIIS Building (6-stories with 8,000 m^2 of floor space) was completed in June 2015. It is located at the north corner of the Hospital Area, as shown in Appendix 3-4. The IIIS Building serves as a globally unrivaled venue for conducting interdisciplinary sleep research under one roof, covering 3 different research fields. It accommodates 2,600 m^2 of the vivarium exclusive to IIIS on the 5th and 6th floors, and the breeding area on the 6th floor accepts up to 6,000 individually ventilated cages, and there are 7 sleep recording labs and 6 behavior labs on the 5th floor, cementing its role as a leading global research resource.

In 2018, R&D Center for Frontiers of MIRAI in Policy and Technology (F-MIRAI), established as the joint project between Toyota Motor Corporation and University of Tsukuba in April 2017, moved into the unfurnished space (365 m^2) held for future expansion on the 4th floor of IIIS Building. As the first collaboration with F-MIRAI, a mobile sleep lab was developed by renovating a Toyota fuel cell bus.

Expansion of the studies of human physiology in IIIS had been a pending issue, and we thus newly established a facility for human sleep research, Human Sleep Lab in Innovation Medical Research Institute located in Kasuga Campus of the University as of March 31, 2019. This 211 m^2 wide facility near Tsukuba station has 4 beds of sleep recording chambers equipped with a bath room, and another bed separately placed in a human calorimeter chamber (for whole room indirect calorimetry during sleep), which was moved from the IIIS Building. It allows overnight polysomnography in multiple subjects (up to 5) in parallel, which improves efficiency of our human sleep research significantly. It also enables us to conduct intervention tests such as the constant routine protocol.

1-5. Financial Status

Before launching IIIS, the Center Director Yanagisawa had been running the Funding Program for World-Leading Innovation R&D on Science and Technology (FIRST) that prominently helped the establishment of IIIS. Although the total amount of external funds available for researchers in IIIS (Core Group) in FY 2014 was reduced at the end of FY 2013 after the termination of FIRST, great efforts to raise research funds were made. Since then, our funding records have marked rapid increases as ¥282 M in FY 2015, ¥611 M in FY 2016, ¥667 M in FY 2017, ¥770 M in FY 2018, and ¥808 M in FY 2019. In FY 2020, IIIS earned ¥659 M, which is 20% less than previous annual income due to fewer opportunities of joint or commissioned research projects affected by a pandemic outbreak of COVID-19. Nevertheless, we gained KAKENHI from ¥280 M in FY 2019 to ¥285 M in FY 2020 and even primed greater success in Moonshot granting ¥3,000 M for the next 5 years. Hence, IIIS is one of the most successful research centers within University of Tsukuba.

2. Advancing Research of the Highest Global Level (within 15 pages)

2-1. Research results to date

Describe issues of a global level that the Center has challenged, and give the results. Select 20 representative results achieved during the period from 2012 through March 2021. Number them [1] to [20] and provide a description of each. Place an asterisk (*) in front of those results that could only have been achieved by a WPI center and explain the reason in the description.

- In Appendix 1-1, list the papers underscoring each research achievement (up to 40 papers) and provide a description of each of their significance. And in Appendix 1-4 list the center's research papers published in 2020.

Although sleep is a fundamental behavior, its regulatory mechanism and physiological function are still not yet fully uncovered. Sound sleep is essential to maintain physical and mental health. Accumulation of sleep insufficiency, so-called sleep debt, increases risks for depression, obesity, dementia and even cancer. Sufficient sleep is requisite also for efficient daytime performances. A lack of sleep in Japanese working population is the worst among advanced nations, costing the economy up to $138 billion a year. Such a "sleep underdeveloped country" definitely needs a world-class research institute for sleep medicine. International Institute for Integrative Sleep Medicine (IIIS) of University of Tsukuba has served the role.

To accomplish world-leading research on sleep, IIIS has given continuous challenges of solving the mystery of sleep with cutting-edge technologies and committed our research outcomes to social implementation. During the past 8.5 years since the Center launching, IIIS led by Center Director Yanagisawa has accumulated an official reputation and by now is globally acknowledged as one of the leading sleep research institutes. One of the approaches the Director made for the success was aggressive recruitment of principal investigators (PI) who have a variety of skills and knowledge on sleep research around the world (see below, **Fig. 1**). Especially recruiting young PIs who are returner to Japan has become a powerful addition scientifically and internationally. Secondly, IIIS has expanded to include human sleep lab and established a subset of the translational research division. Opportunities have opened up by searching biochemical changes underpinning sleep-wake cycles to applied physiology targeting human health and treatment options.

Further, we must mention the discovery of an inducible hibernation-like state in non-hibernator rodents, by the research group of Vice Center Director Sakurai. The second hypomobile behavior, hibernation, is another mystery in neuroscience. This finding last year was a breakthrough to realize human synthetic hibernation. If the application of this technique becomes possible, emergency medical care gains significant benefits to reduce mortality and sequelae dramatically even in a disaster

Selected scientific achievement is listed as follows, by the order of basic biology (molecular genetics/neuroscience/neurobiology), medicinal chemistry, and human sleep physiology and pathophysiology.

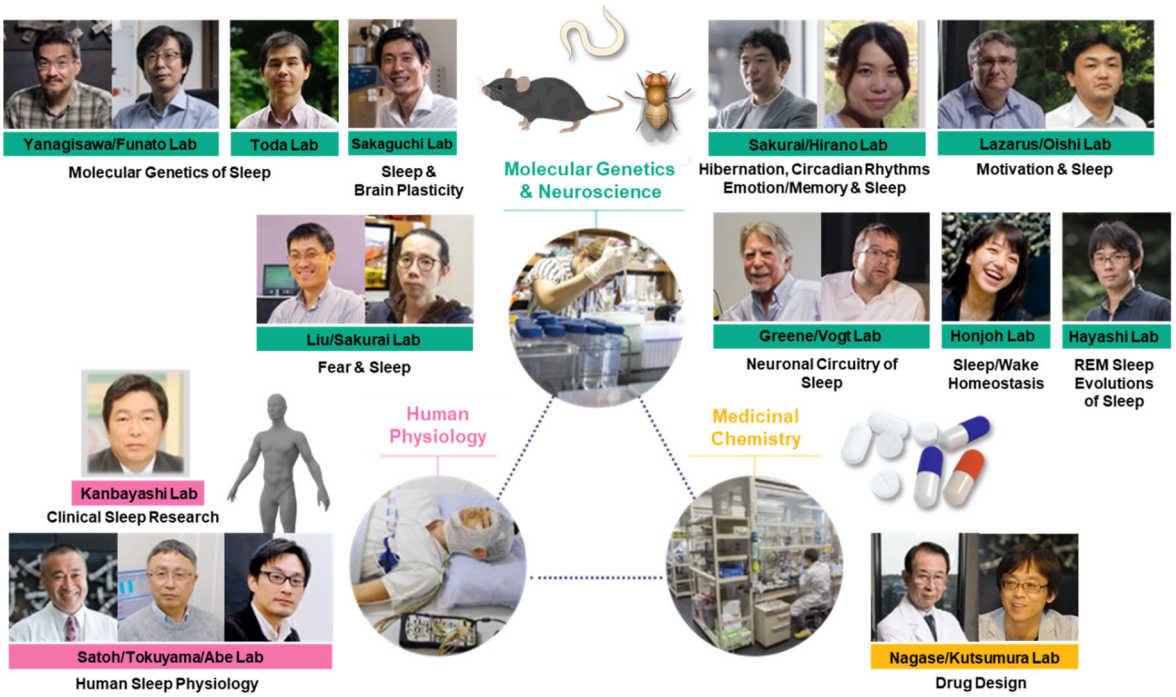

Fig. 1. Principal Investigators of IIIS and the scientific structure facilitating transdisciplinary interactions.

*[1] Identification of novel sleep-regulating genes (Yanagisawa/Funato Lab)

We have conducted EEG/EMG-based screening of randomly mutagenized mice for sleep abnormality. This un- precedented project led to the identification of novel sleep-regulating genes such as SIK1, SIK2, SIK3, NALCN and CACNA1a (**1-3**) (**Fig. 2**). *Sleepy* mutant mice have a splice mutation in *Sik3* gene and exhibited increased sleep need in terms of sleep amount and sleep quality or EEG delta power during NREM sleep. Subsequently, SIK1 and SIK2 have been shown to be involved in sleep regulation. *Dreamless* mutant mice exhibited short total REM sleep time and short REMS episode duration. The causative mutation was found in *Nalcn* gene that cause a single amino acid substitution and increased firing rate of the deep mesencephalic nucleus. Another long sleep pedigree, *Drowsy*, has been shown to have a mutation of *Cacna1a* that encodes a voltage-gated calcium channel.

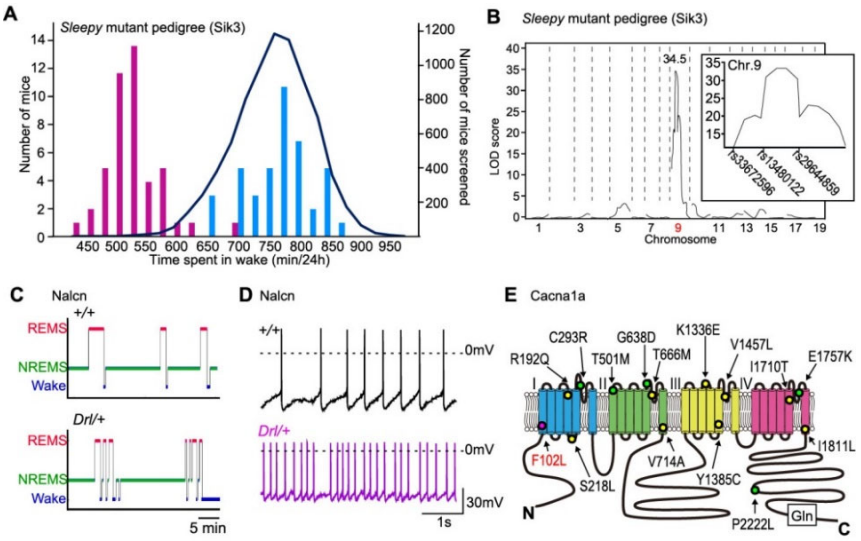

Fig. 2. Forward genetic approach has identified novel sleep-regulating genes.
A. Daily wake time of *Sleep* mutant pedigree. Littermates with *Sleepy* mutation (Magenta bars) exhibited shorter wake time than those without the mutation (blue bars) and C57BL/6 mice (dark blue line). **B**. QTL analysis of the *Sleepy* pedigree for total wake peak. **C**. Hypnogram show that frequent transitions between NREMS and REMS of Nalcn *Drl/+* mice (lower) compared to wild-type mice (upper). **D.** Representative trace of membrane potentials of DpMe neurons in Nalcn+/+ (upper) and Nalcn *Drl/+* (lower) mice. **E.** Structure of the CACNA1A protein indicating the F102L mutation (red), together with examples of human missense mutations reported in familial hemiplegic migraine (yellow) and episodic ataxia type 2 (green), as well as the polyglutamine repeat found in spinocerebellar ataxia type 6 (Gln).

*[2] Elucidation of an intracellular signaling that regulates sleep need (Yanagisawa/Funato Lab)

Since the exon skipped in *Sik3 Sleepy* mutant mice encodes 52 amino acids that include a phylogenetically conserved, protein kinase A (PKA)-phosphorylated serine residue. When mice have a heterozygous *Sik3 S551A* or *Sik3 S551D* mutation that PKA cannot phosphorylate, the mice exhibit longer total NREM sleep time and greater delta power during NREM sleep, which recapitulates sleep phenotype of *Sik3 Sleepy* mice. Thus, the PKA-SIK3 signaling is thought to be involved in the regulation of sleep need. Similarly, the lack of S551-equivalent, PKA-phosphorylated serine residues of SIK1 and SIK2 resulted in increased sleep need but to a lesser extent than *SIK3 S551A* mice. Using *SynapsinI-CreERT2* and *Sik3 Ex13 flox* mice, mutant SIK3 expressed in neuron after the infancy resulted in increased NREM sleep time and NREM sleep delta power density. These results indicate that the PKA-SIK singling constitutes a core singling pathway that regulates sleep need (**Fig. 3; 1, 3-6**).

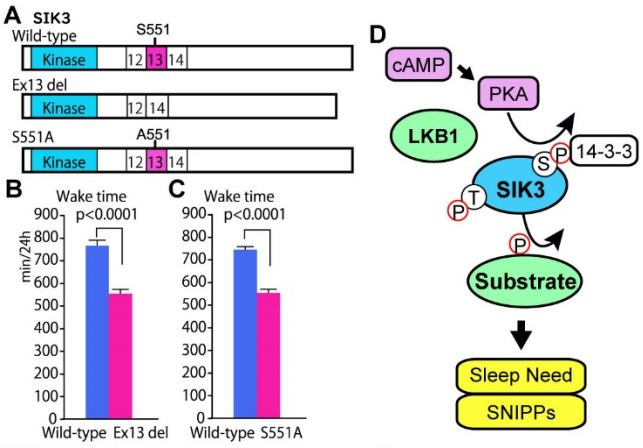

Fig. 3. PKA-SIK3 signaling regulates sleep need.
A. Scheme of wild-type, exon13-encoded region-deleted (Ex13 del), and S551A type of SIK3 proteins. **B-D.** Heterozygous Sik3 ex13 del mice (**B**) and Sik3 S551A mice (**C**) showed shorter total wake time compared to wild-type mice. **D.** PKA/LKB1-SIK3 signaling enhances sleep need and phosphorylation of sleep-need-index phosphoproteins (SNIPPs).

*[3] Molecular mechanisms of homeostatic sleep regulation (Liu/Sakurai Lab)

Through quantitative phospho-proteomic analysis of *Sleepy* (*Sik3*$^{Slp/+}$) and sleep-deprived wild-type mouse brains, we identified 80 sleep need index phosphoproteins (SNIPPs), whose phosphorylation states change in relation to sleep need in the two opposite models of increased sleep need. Preferential association between SLP (relative to wild-type SIK3) and SNIPPs suggest that SNIPPs are potentially substrates of SLP kinase and downstream effectors that mediate homeostatic sleep regulation (**Fig. 4**). Remarkably, the majority (>86%) of SNIPPs are annotated synaptic proteins, suggesting a "synaptic phosphorylation" model of homeostatic sleep regulation, wherein phosphorylation state of SNIPPs could function as the molecular substrates of sleep need, which accumulates during waking and dissipates during sleep (**3, 5**). *This work was performed in collaboration with Yanagisawa/Funato lab.

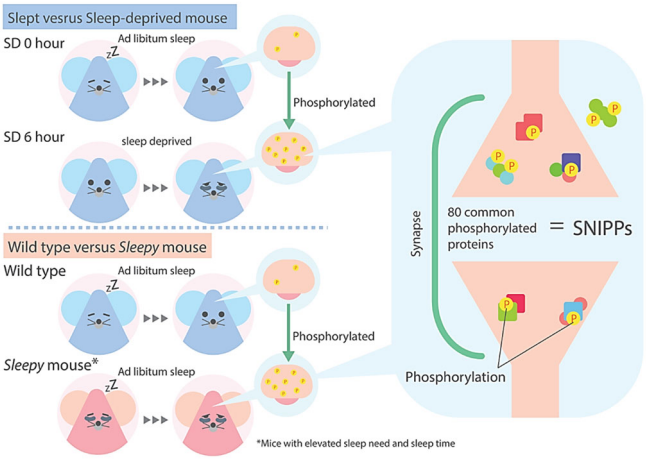

Fig. 4. By comparing the brains of sleep-deprived mice and Sleepy mutant mice, the phosphorylation state of 80 proteins, named SNIPPs (Sleep-Need-Index-Phosphoproteins), was found to increase along with sleep need.

*[4] A discrete neuronal circuit induces a hibernation-like state in rodents (Sakurai/Hirano Lab)

We show that a hypothalamic neuronal circuit in rodents induces a long-lasting hypothermic and hypometabolic state similar to hibernation (**7**). Some mammals actively lower their body temperature to reduce energy expenditure when facing food scarcity, a state known as hibernation. Hibernating animals fully recover to a normal condition with no organ or tissue damage. Because a hypometabolic state could be beneficial for many medical applications, this ability has evoked great interest. We found that chemogenetic excitatory manipulation of a neuronal population in the anteroventricular periventricular hypothalamus, that express neuropeptide Qrfp, (Quiescence-inducing neurons, Q neurons) induces a long-lasting hypothermic/hypometabolic state similar to hibernation (Q neurons-induced hypometabolic state, QIH, **Fig. 5**). Q neurons act mainly on the dorsomedial hypothalamus to induce the QIH. We also found that glutamatergic neurotransmission in Q neurons is important for inducing QIH, but GABAergic neurotransmission also plays an additional role. In the QIH, although body temperature and O_2 consumption were maintained very low, ability to regulate metabolism and behavior was conserved, showing a stark contrast to hypothermic states induced by anesthesia. No obvious tissue/organ damage or abnormalities in behavior were observed after recovery. This finding opens the door to the development of induction of a hibernation-like state, which would have potential applications in non-hibernating mammalian species including humans.

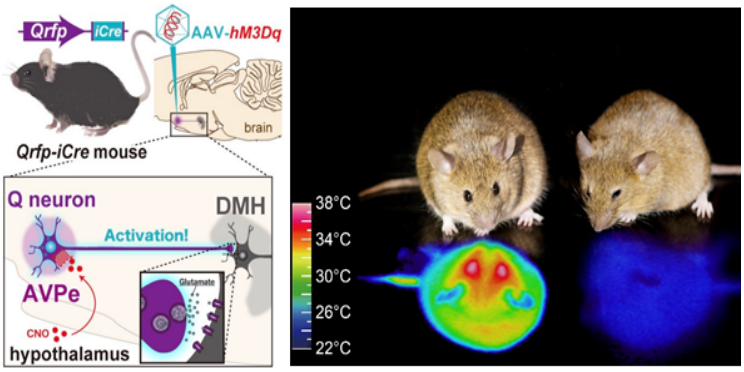

Fig. 5. Q neurons-induced hypometabolic/hypometabolic (QIH) state is induced in the mouse on the right side showing low body temperature. Right; QIH and control mice with composite images of thermographies.

***[5] Orexin modulates behavioral fear expression through the locus coeruleus (Sakurai/Hirano Lab)**

We demonstrate the circuit involving orexin, noradrenalin neurons in the locus coeruleus and lateral amygdala neurons mediates fear-related behavior and suggests inappropriate excitation of this pathway may cause fear generalization sometimes seen in psychiatric disorders, such as PTSD. (**8**). It is well-known that salient emotional information activates orexin neurons in the lateral hypothalamus, leading to increases in arousal and autonomic function. However, how this circuit alters animals' behavior remains unknown. In this study, we found that noradrenergic neurons in the locus coeruleus which project to the lateral amygdala receive direct presynaptic input by orexin neurons (**Fig. 6**). Pharmacogenetic or optogenetic silencing of this circuit inhibited sustained expression of fear responses, as did acute pharmacological blockade of the orexin receptor-1 by an antagonist administered just before the test session. In contrast, optogenetic stimulation of the orexin>locus coeruleus>lateral amygdala circuit altered fear conditioning-induced freezing behavior in a similar but distinct context. These findings demonstrate that the neural circuit that involves orexin, noradorenergic neurons and the lateral amygdala plays an important role in the regulation of fear-related behavior in response to environmental stimuli, and dysfunction of this circuit may cause inappropriate generalization of fear.

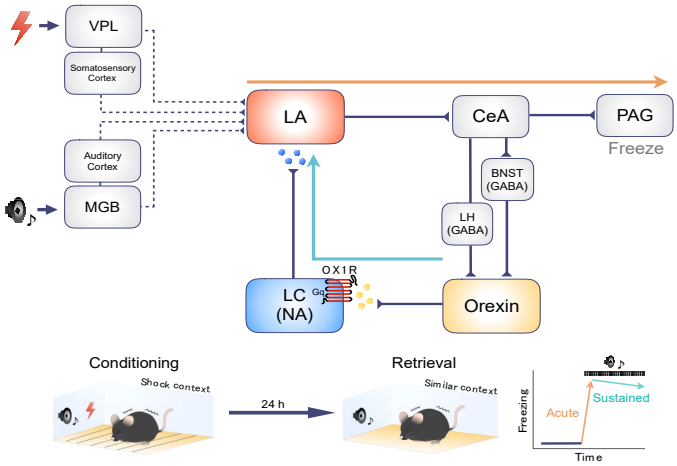

Fig. 6. Neural circuit of sustained fear expression modulated by orexin and noradrenaline signaling.

***[6] Identification of a neural circuit that regulates REM and non-REM sleep (Hayashi Lab)**

The precise mechanisms regulating the cycle of REM and non-REM sleep were poorly understood. By combining molecular, genetic, and developmental approaches, we identified multiple neuronal groups in the pons and medulla that robustly regulate REM and non-REM sleep (**9-12**).

Our sleep is composed of two stages, rapid eye movement (REM) sleep and non-REM sleep. The mechanisms and functions of REM sleep were poorly understood. The Hayashi lab has been aiming to identify neural circuits that control REM sleep and establish mouse models in which REM sleep can be manipulated (**Fig. 7**). Previous studies have shown the involvement of neurons in the pontine tegmental area. However, due to the complexity and heterogeneity of the neurons that comprise this area, the precise identity of the neurons regulating REM sleep was unknown. The Hayashi lab profiled neurons within this area based either on developmental lineage or gene expression. In the former approach, they found that glutamatergic neurons that temporally express Atoh1 during embryonic development play an important role to negatively regulate REM sleep. These neurons send axons to the deep mesencephalic nucleus (DpMEe). Within the DpMe, GABAergic neurons that negatively regulate REM sleep were also identified. Chemogenetic manipulation of this circuit allowed to either strongly decrease or increase REM sleep, respectively (**9**). By the latter approach based on gene expression, two populations of

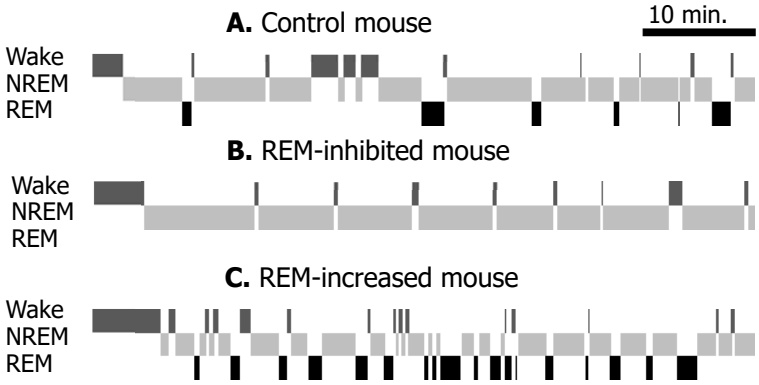

Fig. 7. Transgenic mice that enable REM sleep manipulation
A. During sleep, a wild-type mouse cycles between NREM sleep, REM sleep, and short wake. **B.** REM sleep inhibition by chemogenetic activation of brainstem REM-sleep inhibiting neurons. **C.** REM sleep increase by chemogenetic activation of brainstem REM sleep-promoting neurons.

neurons within the pontine tegmental area, each expressing Neurotensin or Copine7, respectively, were identified to regulate REM sleep. Moreover, it was revealed that Neurotensin and Copine7 are not merely molecular markers but that these factors themselves are involved in regulating REM sleep (**10, 11**). Interestingly, the neurons positive for Neurotensin projected to various neurons within the brainstem which also express Neurotensin, and these neurons were also involved in regulating REM and non-REM sleep.

Identification of neurons that regulate REM sleep allowed to manipulate REM sleep. By taking this advantage, the group also found that manipulating REM sleep affects slow wave activity during subsequent non-REM sleep, suggesting that one function of REM sleep is to regulate the quality of non-REM sleep (**9**). Slow wave activity contributes to synaptic plasticity and memory consolidation. They further found that REM sleep is decreased from early stages in a mouse model of Alzheimer's disease, whereas it was increased by chronic stress (**12**, Yasugaki et al., *Frontiers in Neuroscience*, 2019). Thus, the outcome of manipulating REM sleep under stressed conditions or in dementia is another important question to be addressed in the future.

***[7] Role of sleep in functional brain regeneration (Sakaguchi Lab)**

We have shown that neurogenesis in the adult-hippocampus, a prominent mechanism of mammalian brain regeneration, plays essential roles for memory (**13-15**).

To understand the underlying mechanisms by which adult-born neurons participate in memory, we learned to perform optogenetic techniques in Dr. Edward Boyden's lab (MIT). In collaboration with Dr. Boyden and Dr. Thomas McHugh (RIKEN), we developed a method for simultaneous neural activity imaging and optogenetic manipulation (e.g., Sakaguchi et al., *PlosONE*, 2013). Combining these methods to specifically interrogate the activity and function of adult-born neurons in the in vivo brain, I investigated their roles in memory consolidation during sleep. We found that adult-born neurons that are active during learning are reactivated during REM sleep, which is necessary for memory consolidation (**Fig. 8**).

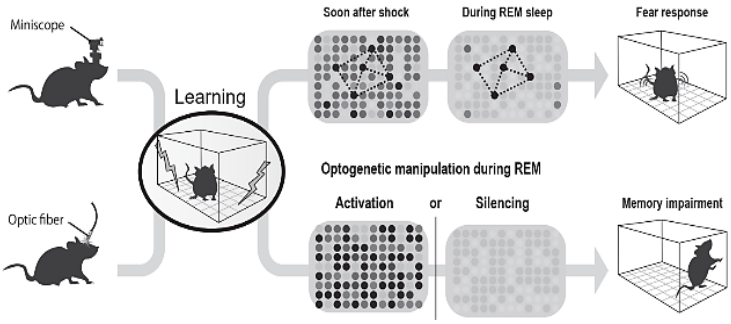

Fig. 8. Ca^{2+} imaging in freely moving mice reveals that contextual fear conditioning recruits a population of adult-born neurons that are reactivated in subsequent REM sleep (top row). Disrupting ABN activity during REM sleep by optogenetic activation or silencing impairs memory consolidation, evidenced by a reduced freezing response (bottom row).

***[8] The gating of sleep homeostasis by motivation (Lazarus/Oishi Lab)**

As humans, we often defy sleepiness and stay awake when attention is necessary, but also experience an inescapable desire to sleep in boring or pleasureless situations. The brain mechanisms governing the regulation of sleep by cognitive and emotional factors are not well understood. M. Lazarus previously demonstrated that the arousing effect of caffeine, the most consumed psychoactive compound in the world, is abolished in rodents with site-specific deletion of adenosine A_{2A} receptors in the nucleus accumbens – a part of the brain that is associated with motivation and pleasure. Recently, the Lazarus/Oishi lab also revealed that the nucleus accumbens can produce sleep. The findings may explain why we have the tendency to fall asleep in the absence of motivating stimuli, i.e., when bored (**Fig. 9; 16-18**). The achievement has initiated a new area of sleep research on hedonic motivation as a major sleep-gating factor (**19**, Lazarus M., et al. *Trends Neurosci*, 2012). The teleological problem of sleep function arises from the presumption of sleep's evolution from a default state of waking. Humans are likely biased towards this presumption by the egocentricity of waking consciousness. The achievement, however, provides further evidence that sleep is the brain's default state that is established in the absence of arousing inputs. Finally, the newly identified sleep circuit may open new therapeutic avenues for treating insomnia and other sleep or psychiatric disorders.

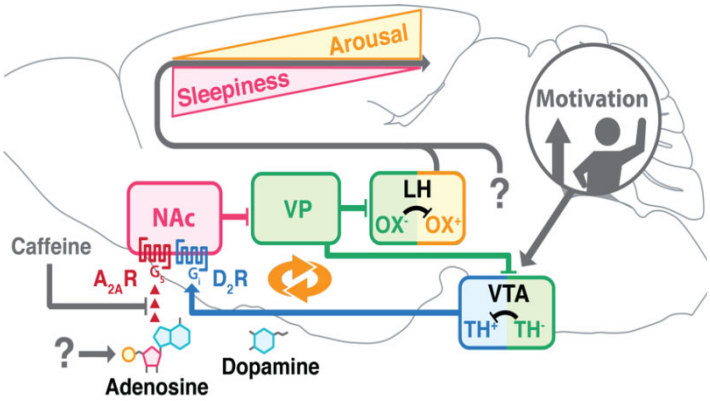

Fig. 9. The nucleus accumbens (NAc) links motivation and sleep. Motivation increases dopamine release from ventral tegmental area (VTA) neurons into the NAc, causing arousal. In the absence of dopamine release, adenosine stimulates NAc neurons to induce sleepiness via inhibition of the ventral pallidum (VP) and downstream disinhibition of neurons inhibiting orexin (OX) neurons in the lateral hypothalamus (LH) and dopaminergic VTA neurons.
A. VGAT-Cre mice were injected with AAV-FLEX-DTA into the VMP. **B**. Ablation of the VMP GABAergic neurons severely reduced slow-wave sleep. **C**. VGAT-Cre mice were injected with AAV-FLEX-hM3Dq-mCherry into the VMP. **D**. Chemogenetic activation of the VMP GABAergic neurons induced slow-wave sleep.

*[9] Neural circuits controlling sleep and mania-like behaviors (Lazarus/Oishi Lab)

Current understanding of the neuronal mechanisms and populations that regulate sleep–wake behavior is incomplete. The Lazarus/Oishi lab recently found that ventral tegmental area (VTA) dopaminergic neurons strongly induce and consolidate wakefulness, but the role of this midbrain area in sleep–wake regulation remains unclear. The Lazarus/Oishi lab discovered a neural circuit in the ventral medial midbrain/pons area (VMP) at the junction of the ventral medial midbrain and pons that controls slow-wave sleep and wakefulness (**20**).

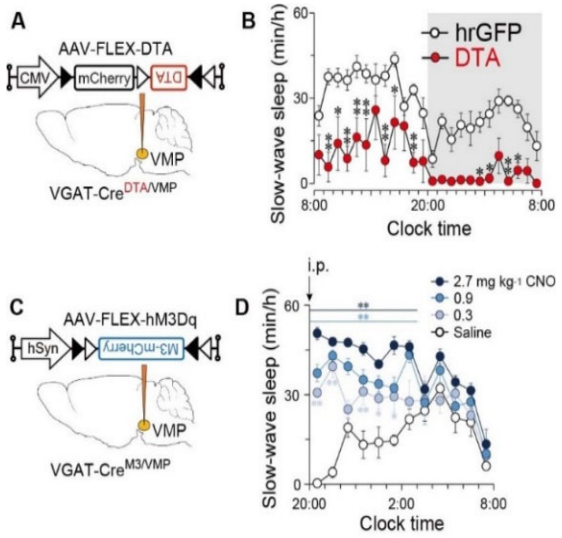

Fig.10. VMP GABAergic neurons control slow-wave sleep.
A. VGAT-Cre mice were injected with AAV-FLEX-DTA into the VMP. **B**. Ablation of the VMP GABAergic neurons severely reduced slow-wave sleep. **C**. VGAT-Cre mice were injected with AAV-FLEX-hM3Dq-mCherry into the VMP. **D**. Chemogenetic activation of the VMP GABAergic neurons induced slow-wave sleep.

They used an ablation technique based on the viral expression of diphtheria toxin fragment A (DTA) and found that nonspecific or GABAergic neuron-specific ablations in the VMP largely reduced slow-wave sleep and increased wakefulness in mice (**Fig. 10 A, B**). The increased wakefulness was also observed after chemogenetic inhibition of the VMP GABAergic neurons. Pharmacologic tools revealed that this arousal effect was mediated by the dopaminergic systems. Furthermore, chemogenetic activation of the VMP GABAergic neurons strongly induced slow-wave sleep while suppressing wakefulness (**Fig. 10 C, D**). These findings indicate that the VMP GABAergic cells are critical for the control of sleep–wake behavior.

They also found that ablation of inhibitory neurons in the same area exhibits mania-like behaviors such as hyperlocomotion or anti-depressive behaviors, suggesting that multiple types of mood-related behaviors are regulated by this brain area (**21**).

*[10] Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors (Liu/Sakurai Lab)

Innate behaviors are genetically encoded, but their underlying molecular mechanisms remain largely unknown. Predator odor 2,4,5-trimethyl-3-thiazoline (TMT) and its potent analog 2-methyl-2-thiazoline (2MT) are believed to activate specific odorant receptors to elicit innate fear/defensive behaviors in naive mice. We conducted a large-scale recessive genetics screen of ethylnitrosourea (ENU)-mutagenized mice. We found that loss of Trpa1, a pungency/irritancy receptor, diminishes TMT/2MT and snake skin-evoked innate fear/defensive responses (**Fig. 11; 22**). Accordingly,

Trpa1$^{-/-}$ mice fail to effectively activate known fear/stress brain centers upon 2MT exposure, despite their apparent ability to smell and learn to fear 2MT. Moreover, Trpa1 acts as a chemosensor for 2MT/TMT and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. Our results indicate that Trpa1-mediated nociception plays a crucial role in predator odor-evoked innate fear/defensive behaviors. This work establishes the first forward genetics screen to uncover the molecular mechanism of innate fear, a basic emotion and evolutionarily conserved survival mechanism. *This work was performed in collaboration with following labs at IIIS, Yanagisawa/Funato, Sakurai/Hirano, Nagase/Kutsumura.

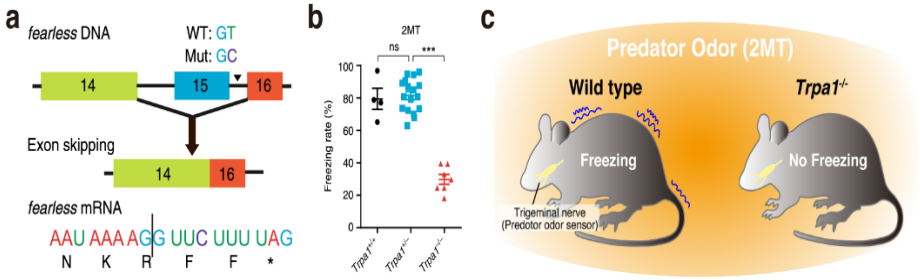

Fig. 11. Trpa1 acts as a chemosensor for predator odor (2MT) and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. **a.** Identification of fearless (Trpa1) mutant mice by forward genetics screening. A schematic of partial exon/intron structure of Trpa1 gene. The 5′ splice site (GT to GC) mutation results in the skipping of exon 15 and introduction of a premature stop codon. **b.** Trpa1 mediates 2MT-evoked innate fear/defensive responses (freezing). *Trpa1*$^{-/-}$ mice show drastically less response to predator odor (2MT) than wild-type mice. **c.** Schematic of innate fear response in wild-type and *Trpa1*$^{-/-}$ mice.

[11] Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia (Liu/Sakurai Lab)

The neural mechanisms of fear-associated thermos-regulation remain unclear. Innate fear odor 2-methyl-2-thiazoline (2MT) elicits rapid hypothermia and elevated tail temperature, indicative of vasodilation-induced heat dissipation, in wild-type mice, but not in mice lacking Trpa1–the chemosensor for 2MT (**Fig. 12**). We found that *Trpa1*$^{-/-}$ mice show diminished 2MT-evoked c-fos expression in the posterior subthalamic nucleus (PSTh), external lateral parabrachial subnucleus (PBel) and nucleus of the solitary tract (NTS). Whereas tetanus toxin light chain-mediated inactivation of NTS-projecting PSTh neurons suppress, optogenetic activation of direct PSTh-rostral NTS pathway induces hypothermia and tail vasodilation. Furthermore, selective opto-stimulation of 2MT-activated, PSTh-projecting PBel neurons by capturing activated neuronal ensembles (CANE) causes hypothermia. Conversely, chemogenetic suppression of vGlut2^{+} neurons in PBel or PSTh, or PSTh-projecting PBel neurons attenuates 2MT-evoked hypothermia and tail vasodilation. In summary, this study identified PSTh as a major thermoregulatory hub that connects PBel to NTS to mediate 2MT-evoked innate fear-associated hypothermia and tail vasodilation (**23**).

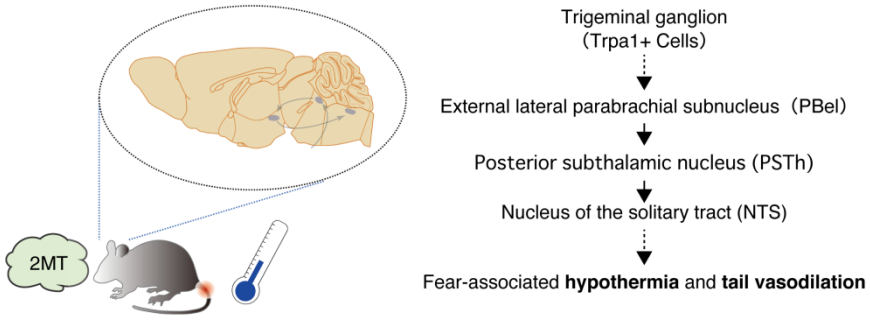

Fig. 12. Uncovering the neural pathway involving innate fear odor induced hypothermia. Neural pathways for 2MT-evoked hypothermia (23)

[12] Intracellular signaling of sleep function (Greene/Vogt Lab)

Neuronal activity and gene expression in response to the loss of sleep provides a window into the enigma of sleep function. Sleep loss is associated with brain differential gene expression, an increase in pyramidal cell mEPSC frequency and amplitude, and a characteristic rebound and resolution of slow wave sleep-slow wave activity (SWS-SWA). MEF2C is a transcription factor that regulates the expression of many target genes, including those involved in negative regulation of synaptic strength. As such this transcription factor is an excellent candidate to signal the down-scaling of synaptic strength during recovery sleep that follows the overall increase of synaptic strength associated with sleep deprivation (**24**).

Our work shows that sleep-loss dephosphorylates MEF2C to activate it, thus facilitating expression

of its gene targets (**Fig. 13**). Furthermore, MEF2C's sleep-loss-induced, transcriptional activity is required for the altered gene expression and the sleep associated down-scaling of synaptic strength (**Fig. 14**), an essential aspect of sleep homeostatic function.

One of MEF2C's most important gene targets is *Arc*. The activity-regulated cytoskeleton-associated protein (*Arc*) gene is a neural immediate early gene that is involved in synaptic downscaling and is robustly induced by prolonged wakefulness in rodent brains, an effect that requires *Mef2c*. Loss of function of Arc results in an attenuated homeostatic response to SD including many of the identified SD-response genes. In wild-type brains, SD increased Arc protein expression in multiple subcellular locations, including the nucleus, cytoplasm, and synapse. This SD-induced localization (especially in the nucleus) is reversed in part by recovery sleep and may be important for the altered SD-induced gene expression. Thus *Arc* is not only a target gene of MEF2C but, like MEF2C, plays an important role in the sleep homeostatic response (**25**).

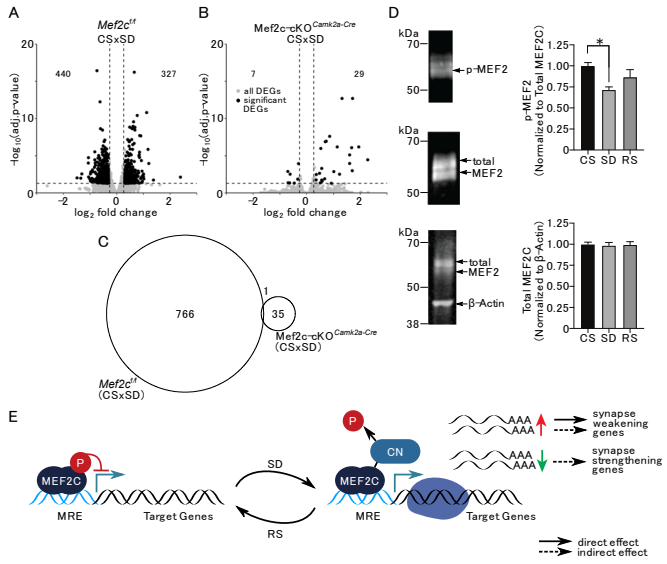

Fig. 13. Sleep need induced transcriptomic changes. (A) Volcano plots showing differentially expressed genes across CS to SD and (B) CS to SD in Mef2c ko mutants. Significantly differentially expressed genes (DEGs) (adj. p-value<=0.05, absolute log2 fold change >= 0.3 indicated by gray dashed lines). (C) A Venn diagram showing overlap between DEGs for Mef2cf/f and Mef2c-cKOCamk2a-Cre between CS and SD sleep states. (D) The ratio of pMEF2C to total Mef2C decreases with SD indicating dephosphorylation with SD. Immunoprecipitation of MEF2C to detect MEF2C, Phospho-S396 MEF2C Phospho-S396 and total MEF2C for each sleep condition. Phospho-MEF2 (top blot) re-labeled from immunoprecipitation of total MEF2C (middle blot) was quantified and normalized to total MEF2C signal for each sample (n = 4 samples/condition). Total MEF2C from total cell lysate was quantified and normalized to B-actin (bottom blot). Data reported as mean +/- SEM. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test (interaction = $p < 0.05$, post-hoc test comparing CS to SD *=$p < 0.05$). (E) Model showing role of de-phosphorylated, activated MEF2C, increased relative to total MEF2C by loss of sleep and leading to synapse remodeling. MRE = Mef2 response element, CN = calcineurin.

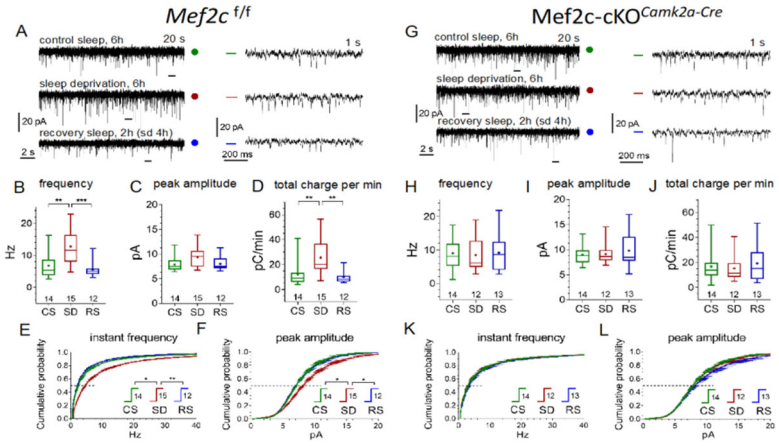

Fig. 14. Conditional Mef2c knock-out in forebrain excitatory neurons eliminates sleep/wake remodeling of synaptic excitatory inputs to pyramidal neurons in anterior cingulate cortex slices. (A, G) Representative recordings of miniature excitatory postsynaptic current (mEPSC) traces (20 s, left panels, and expanded 1 s traces on the right corresponding to time bars underneath original traces) obtained for three different experimental sleep/wake conditions, CS (green), SD (red), and RS (blue), for Mef2cf/f mice (A) and Mef2c-cKOCamk2a-Cre mice (G). (B, C, D) and (H,I,J) illustrate mEPSC functional parameters obtained from Mef2cf/f and Mef2c-cKOCamk2a-Cre (number of cells for each condition shown above X axis condition label).

*[13] Cortical organization in waking and NREM sleep (Greene/Vogt Lab)

Because of the absence of overt behavior, NREM sleep is still widely regarded as a silent brain state. Our investigations have shown that this is not the case. Instead we have found many indicators for increased activity in the cortex during NREM sleep. First, a large number of cortical neurons, mostly those with low wake activity, actually increase their firing rates in NREM sleep (**Fig. 15**). Burst firing is significantly increased in the transition from waking to NREM sleep in excitatory neurons – burst firing is typically associated with much higher functional impact compared to single action potential firing. In-vivo two-photon calcium imaging in naturally waking and sleeping animals corroborated these findings - peak calcium transients, likely driven by burst firing, are increased in NREM sleep. Interestingly, by observing multiple neurons at the same we found more predictable and organized firing patterns in waking and less predictable and ordered firing patterns in NREM

sleep.

To better understand this increased spontaneous cortical activity, we stimulated cortical circuits in a highly controlled manner throughout waking and sleep using optogenetic activation of defined cortical inputs. Cortical responses were strongly and rapidly increased in the transition from waking to NREM sleep. This effect was observed at significant distances from the site of stimulation. The rapid onset and offset of the large responses are best explained by changes in the neuromodulatory environment that are known to occur in the transition between vigilance states.

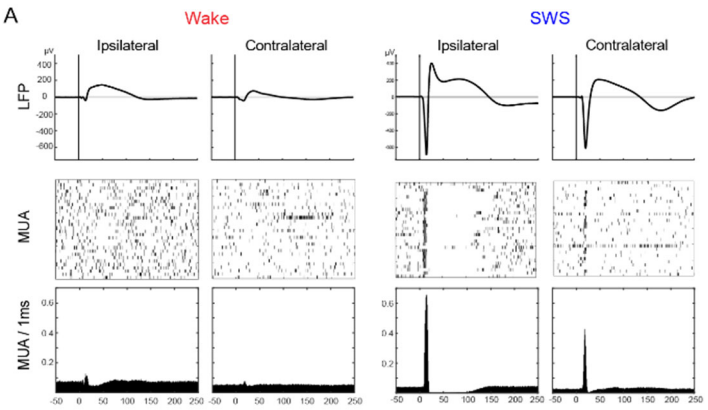

Fig. 15. Evoked cortical field potentials (LFP) and cortical action potentials (MUA) in waking and NREM sleep (SWS)

In summary, NREM sleep is a highly active cortical state and this activity is likely linked to its function (**26, 27**).

[14] Thalamic regulation of wake and sleep (Honjoh Lab)

Thalamic neurons are classified into two subpopulations, core and matrix cells, depending on its cortical projection pattern. Core cells project mainly to layer IV of specific primary cortical areas and play critical roles in relay of sensory information from periphery to cortex. In contrast, matrix cells project diffusely to superficial layers of wide-spread cortex areas and their function remained unknown. This study investigated the thalamic regulation of wake and sleep, focusing on two thalamic nuclei, ventral posteromedial nucleus (VPM) and ventromedial thalamic nucleus (VM), which are comprised of mainly core cells and matrix cells respectively. We showed that the activity of VM matrix cells is high in wake and REM sleep and low in NREM sleep, and increases before cortical and muscle activity and at the sleep-to-wake transition (**Fig. 16**). Optogenetic stimulation of VM matrix cells rapidly awoke mice from NREM sleep, while arousal did not occur from REM sleep. Furthermore, chemogenetic inhibition of VM matrix cells decreased wake duration, showing the physiological role of matrix cells in arousal. In contrast, optogenetic activation of the VPM core cells did not cause arousal from either NREM or REM sleep. Taken together, this study demonstrated that matrix cells, but not core cells, plays a critical role in the transition from NREM sleep to wakefulness (**Fig. 17**) and that the thalamocortical interaction dynamically changes across vigilance states (**28**).

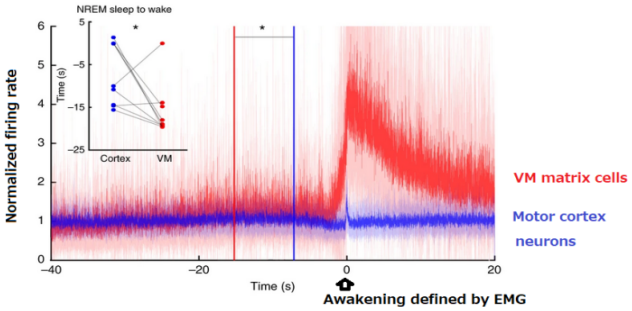

Fig. 16. Thalamic matrix cells promote arousal.

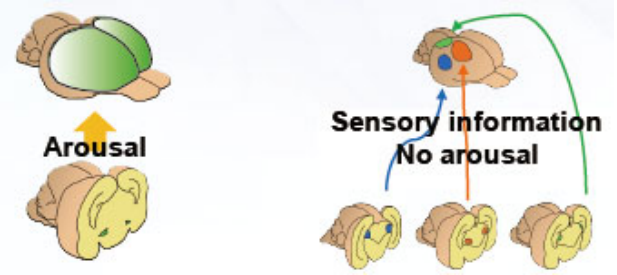

Fig. 17. Cortical and thalamic multi unit activities in transition from NREM sleep to wake.

[15] A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila (Toda Lab)

Drosophila served as a great model system, in which many biological questions including development, immunity and circadian clock system have been answered. Especially, the success of the circadian clock mutants originally isolated from a behavior screen using Drosophila encouraged us to do the same type of screen for sleep research using Drosophila. Indeed, Drosophila sleep paradigm using the behavior criteria for sleep was established two decades ago. Since then, through non-biased loss-of-function screen identified a variety of novel genetic components important to

maintain sleep. However, little is known about the molecules that can induce to sleep. To discover sleep-inducing factor, we carried out genome-wide and non-biased gain-of-function screen and discovered a novel gene, named '*nemuri*'. *nemuri* encodes anti-microbial peptide which kills bacteria. Upon infection, Drosophila sleeps more but *nemuri* mutants showed significant reduction in infection-induced sleep, suggesting that Nemuri is a molecular link between sleep and immunity. We also showed that *nemuri* expression is increased after sleep deprivation and is important for the induction of sleep response after deprivation. Nemuri is a bona fide sleep homeostasis factor that is particularly important under conditions of high sleep need; because these conditions include sickness, our findings provide a link between sleep and immune function (**Fig. 18; 29**).

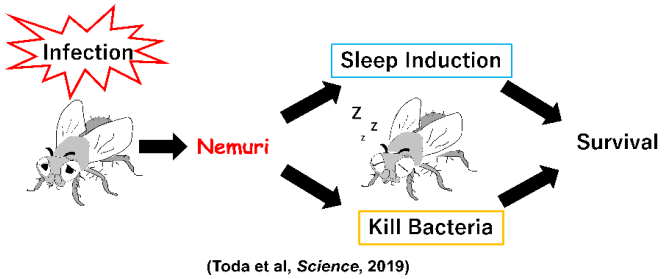

Fig. 18. "Nemuri" links sleep and immunity.

***[16] Novel delta opioid receptor agonists with oxazatricyclodecane structure (Nagase/Kutsumura Lab)**

Commonly available delta opioid receptor (DOR) agonists, SNC-80 derivatives with a piperazine ring were known to analgesic activity, antianxiety and antidepressant activity. In the early 2000s, the many SNC-80 derivatives were tried to develop for analgesic drugs, antianxiety and antidepressant drugs. However, all these derivatives were dropped out at early clinical trials because of severe side effects such as convulsion and catalepsy. On the other hand, Nagase et al succeeded in synthesizing novel DOR agonists with oxazatricyclodecane structure by utilizing novel rearrangement reaction of morphinan derivatives (**Fig. 19; 30**). Among them, *N*-methyl derivative was highly selective and the most effective DOR agonist in functional assays. Subcutaneous administration of the *N*-methyl derivative produced dose-dependent and NTI (selective DOR antagonist)-reversible antinociception without any convulsive behaviors in the mice acetic acid writhing tests. The further modification by Nippon Chemiphar Co., Ltd. Collaborating with Prof. Hiroshi Nagase afforded a candidate compound for development, NC-2800 with the financial support from the Japan Agency for Medical Research and Development (AMED) under the ACT-M program. The resulting NC-2800 showed antianxiety and antidepressant activity without convulsant and catalepsy. Moreover, NC-2800 has been selected by AMED for its Fiscal 2017 Cyclic Innovation for Clinical Empowerment (CiCLE) funding program as "Development of Opioid δ Receptor Agonist Modulating Emotional System". A clinical trial phase I study of NC-2800 will start this year.

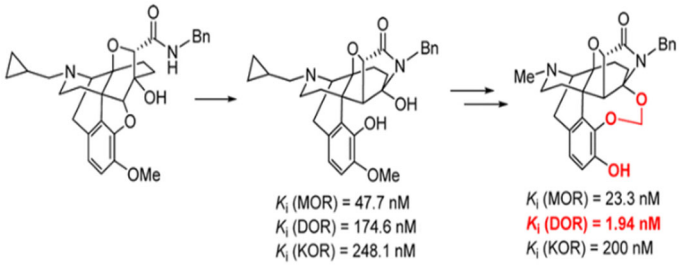

Fig. 19. Design and synthesis of novel DOR agonist.

[17] Novel method for tracking vigilance decrement during sleep deprivation (Abe Lab)

We developed an algorithm that detects multilevel vigilance by integrating several eye-related indices (**31**). Although many systems have been developed to detect impairment of vigilant attention in order to prevent human-error-related accidents, a majority of them only measure severe vigilance impairment that typically involves eye closure while falling asleep. Therefore, we aimed to determine novel markers which can detect intermediate impairment of vigilance when eyes are open and to develop a new technique that could measure such impairment by integrating these novel markers. Sixteen participants performed the widely used assay of vigilance impairment (i.e., the Psychomotor Vigilance Test (PVT)) with simultaneous recording of eye metrics every 2 hours during 38 hours of continuous wakefulness (**Fig.20 A**). According to the results, a novel marker was found that measured vigilant attention (**Fig.20 B**) when the eyes were open—the prevalence of microsaccades (**Fig.20 C**). In addition, a novel algorithm for detecting multilevel vigilant attention was developed, which estimated performance of the PVT by integrating the novel marker with other eye-related indices. The novel algorithm also tracked changes in intermediate vigilance impairment (specific

reaction times in the PVT, i.e., 300–500 ms) during prolonged time-on-task and sleep deprivation, which had not been tracked previously by conventional techniques (i.e., percent of slow eyelid closure; PERCLOS) (**Fig.20 D**). The implication of the findings is that this novel algorithm can be used to reduce human-error-related accidents caused by vigilance impairment even when the deficit level is intermediate.

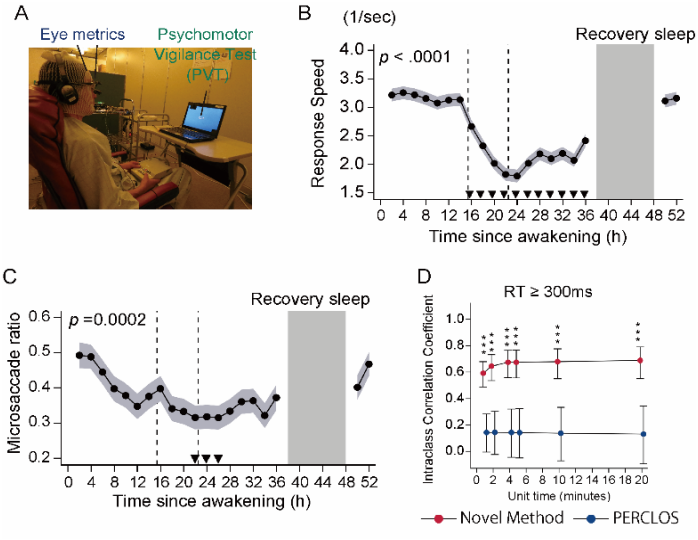

Fig. 20. Novel method that tracks multilevel vigilance by using multiple eye metrics. (A) Simultaneous recording of eye metrics and psychomotor vigilance test (PVT). (B) The effect of time since awakening on PVT response speed. (C) The main effect of time since awakening on microsaccade ratio. (D) Comparisons of the intraclass correlation coefficients between the novel method and percent of slow eyelid closure (PERCLOS) with the actual PVT performance.

*** [18] Energy metabolism during sleep (Tokuyama/Satoh Lab)**

Most people are monophasic sleepers, which imposes an extended duration of fasting as a metabolic consequence. Oxidized substrates during sleep were assumed to progressively shift from carbohydrate to fat, thereby gradually decreasing the respiratory quotient (RQ). Contrary to this assumption, indirect calorimetry using a whole-room indirect calorimetry with improved time resolution revealed that RQ re-ascended prior to awakening suggesting that carbohydrate oxidation began to increase after midnight despite prolonged fasting (**Fig. 21**). We isolated the effects of sleep stage and time after sleep onset on sleeping energy metabolism using a semi-parametric regression analysis. Specifically, a parametric analysis was used for the effect of sleep stage and a non-parametric analysis was applied for the effect of time. Energy expenditure differed significantly between sleep stages: wake after sleep onset (WASO) > stage 2, slow wave sleep (SWS), and REM; stage 1 > stage 2 and SWS; and REM > SWS. Energy expenditure and carbohydrate oxidation decreased during the first half of sleep followed by an increase during the second half of sleep (**32**). Our first study paid little attention to sex differences in sleeping energy metabolism; subjects were mainly men and the data was not analyzed by sex. In a next study, re-ascent of RQ prior to awakening was confirmed in women during follicular and luteal phase (**33**). Our studies revealed characteristic phenotypes of sleeping energy metabolism; sleeping metabolic rate differs between sleep stages, related to time after sleep onset, and affected by menstrual cycle. Importantly, inter-individual difference in RQ become apparent during sleep, and it might serve as a window to gain insight into the early-stage pathogenesis of metabolic inflexibility.

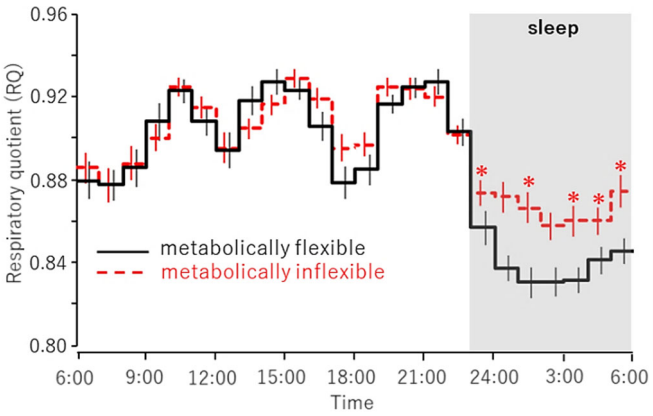

Fig.21. Time course of RQ in metabolically flexible and inflexible subjects. During sleep, difference in substrate oxidation profile between the 2 groups becomes significant.

***[19] Effect of orexin receptor antagonist on sleep, sleeping energy metabolism and physical/cognitive functions (Tokuyama/Satoh Lab)**

Insomnia is a common symptom representing an important health burden. Widely prescribed hypnotic agents enhance the function of γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter. The ability to arouse and respond to unexpected environmental stimuli is a feature of normal sleep, which is crucial when people are faced with urgent situations. There is a general concern that hypnotic agents may impair physical and cognitive functions, eliciting muscle atonia,

ataxia, loss of balance, retrospective amnesia, attention deficits, and slower response time, and patients might become temporarily incapacitated and unable to appropriately respond under the effect of hypnotic agents. When patients need to be awake under the influence of a hypnotic agent, the impairment of physical and cognitive functions might manifest as a fall or serious misjudgment. This double-blind, randomized, placebo-controlled, cross-over study evaluated the side-effect profile of an orexin receptor antagonist (suvorexant, 20 mg) and γ-aminobutyric acid A ($GABA_A$) receptor agonist (brotizolam, 0.25mg) on physical/cognitive functions upon forced awakening (**34**). Fifteen minutes before bedtime, the subjects took a pill or placebo and were forced awake 90 min thereafter. Polysomnographic recordings revealed that the efficacies of the hypnotic agents in prolonging total sleep time (~30 min) and increasing sleep efficiency (~6%) were comparable. When the subjects were allowed to go back to sleep after the forced awakening, the sleep latency was shorter under the influence of hypnotic agents (~2 min) compared to the placebo trial (24 min), and the rapid eye movement latency was significantly shorter under suvorexant (98.8, 81.7, and 48.8 min for placebo, brotizolam, and suvorexant, respectively). Although brotizolam significantly impaired the overall physical/cognitive performance (sum of z score) compared with placebo upon forced awakening, there was no significant difference in the total z score of performance between suvorexant and placebo. Notably, the score for static balance with the eyes open was higher under suvorexant compared to brotizolam administration. The energy expenditure was lower under suvorexant and brotizolam compared with the placebo, suggesting that intervention on sleep also affect energy metabolism during sleep.

[20] Cerebrospinal fluid orexin measurements in various disorders (Kanbayashi Lab)

We measured CSF orexin in various diseases and conducted research to find the relationship between each pathophysiology of diseases and orexin system (**35-37**).

Low levels of orexin are found in patients with narcolepsy type 1 (**Fig. 22**). Even if narcolepsy / hypersomnia is caused by other diseases, we continue to make efforts to approach the pathological condition by measuring the orexin level. The goal is to elucidate the pathophysiology of each disease and to seek clues as to why the orexin nervous system is shed.

The most common symptom- atological hypersomnia is neuromyelitis optica caused by AQP4 antibody. Among hereditary and congenital diseases, low orexin levels are also observed in Niemann-pick type C in parallel with confirming the symptoms of cataplexy, which contributes to the early diagnosis of the disease and the start of treatment (Imanishi et al., 2021).

The measurement of orexin level is a method for clinical diagnosis, but it is also applied to basic research and contributes to the progress of various researches (**35, 37**).

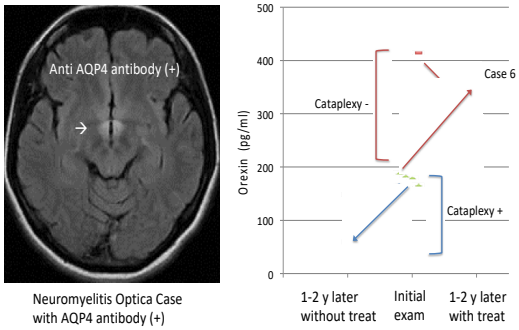

Fig.22. Left: a case of symptomatic narcolepsy with a lesion around the third ventricle (arrow). Right: six cases with cataplexy exhibit low (<110pg/ml) or intermediate (110-200pg/ml) orexin levels, while 4 cases without cataplexy exhibit normal orexin levels. In two cases with cataplexy without miglustat treatment, orexin levels at the onset were intermediate, and became lower in the later period. Among 5 cases with miglustat treatment, cataplexy of case 6 disappeared and orexin level increased (183→351 pg/ml). Three other cases without cataplexy remained normal orexin levels during miglustat treatment.

2-2. Research environment including facilities and equipment

Describe the degree to which the Center has prepared a research environment appropriate for a world premier international research center, including facilities, equipment and support systems, and describe the functionality of that environment.

The construction of the IIIS Building (6-stories with 8,000 m^2 of floor space) was completed in June 2015. The IIIS Building serves as a globally unrivaled venue for conducting interdisciplinary sleep research under one roof, covering 3 research fields of a) basic biology, b) pharmaceutical science and c) experimental medicine. It accommodates 2,600 m^2 of the vivarium exclusive to IIIS on the 5th and 6th floors.

Centering around a vaulted ceiling space with a symbolic spiral staircase linking floors one to four, lab offices, lounges and auditorium are arranged in migratory fashion, so that researchers can naturally meet, interact and influence each other to further boost intelligent inspiration from various disciplines and fields of research and accelerate the fusion research.

Concerning the design of experimental areas from the 2nd to 4th floors, researchers actively participated in it from the initial planning and their feedback was sufficiently taken into account. Functional and efficient experimental environments were successfully created by zoning according to the nature and purpose of experiments. For example, an experimental area on one floor is divided into 3 categories; the "lab" zone with benches exclusive for respective laboratories, the "lab support" zone where state-of-the-art measuring equipment and/or analytical equipment are mostly arranged, and the "share support" zone where common equipment and functions are arranged.

The animal breeding/experimental areas, which require high levels of cleanliness and security, are located on the upper floors (5, 6F). These areas are comprised of clean rooms with state-of-the-art air conditioning system featuring airtight barriers established with room pressures controlled section by section, as well as individually configurable temperature and humidity. The breeding area on the 6th floor includes the cleaning and sterilization room with two large autoclaves and a rack washer, and breeding rooms with an automated water feeder, RO (reverse osmosis membrane) water-purifying apparatus and a device for producing weakly acidic sterilizing water, which can accommodate thousands of IVC cages for mice/rats, capable of breeding tens of thousands the same. Current holding capacity of breeding rooms has grown up to 3,600 cages/18,000 mice. We plan further increase of IVC racks, cementing its role as a leading global research resource.

The experimental area on the 5th floor also includes 7 sleep recording rooms and 6 behavioral labs with the equipment for sleep/behavior analysis such as sleep recording chambers with the simultaneous EEG/EMG/video recording system.

In FY 2017, we launched the Transgenic Core Facility, which provides mouse embryo manipulation services, e.g., generation of gene modified mice by CRISPR microinjection/electroporation, in vitro fertilization, embryo transfer and embryo/sperm freezing, available for researchers of IIIS.

In FY 2018, to expand the experimental medicine in IIIS, we established a facility for human sleep research, Human Sleep Lab in Innovation Medical Research Institute located in Kasuga Campus of the University. This 211 m^2 wide facility near Tsukuba station has 4 beds of sleep recording chambers, a bath room, and 1 bed of human calorimeter chamber (for whole room indirect calorimetry during sleep). It allows sleep measurement from multiple subjects (up to 5) in parallel and improves efficiency of our human sleep research significantly (see more in the section 7-2). It also enables us to conduct intervention tests such as the constant routine protocol. Since the establishment of the new Human Sleep Lab, over 500 sleep measurements had been conducted at IIIS, and tremendous human sleep data had been collected. Further analysis of these data could accelerate our studies on experimental medicine.

In FY 2019, for further expansion of neuroscience study, we have set up a new lab with all the equipment that are required for sleep research using flies. The "fly" facilities include a dark chamber with a precise air-conditioning system with temperature and humidity, over a hundred of Drosophila Activity Monitors (DAM) system, dozens of incubators for fly cultures, and a fully functional lab for molecular genetic manipulations under the dissection microscopes. With such systems, a full range of sleep studies using *Drosophila* such as non-biased genetic screens can be performed.

2-3. Competitive and other funding

Describe the results of the Center's researchers to date in securing competitive and other research funding.
- In Appendix 3-6, describe the transition in acquiring research project funding.

Since most of the PIs in the core group of IIIS were externally recruited to University of Tsukuba, very limited competitive funding was available at the launching stage, except for Yanagisawa's grant of **the Funding Program for World-Leading Innovative R&D on Science and Technology** (**FIRST**) that had started before the inauguration of IIIS. Since then, we made great efforts to raise competitive funding and the amount of our external grants have drastically increased year by year, as shown in Appendix 3-6. The total amount of the funds executed each year had grown from ¥1.5 M in FY 2012 to ¥818 M in FY 2019. In FY 2020 we earned ¥659 M, which is 20% less than annual income from the previous year mainly due to fewer opportunities of joint research and commissioned research projects affected by a pandemic outbreak of COVID-19. Nevertheless, we gained **KAKENHI** from ¥280 M in FY 2019 to ¥285 M in FY 2020, demonstrating our strength in basic sciences.

Towards the end of WPI in FY 2021, we sought large-scale and long-term competitive funding during FY 2020. Eventually our research proposal aiming to solve mysteries of sleep and hibernation that involves all research groups in IIIS was successfully accepted by **AMED as one of Moonshot-type R&D projects**. Award of this support is a tremendous encouragement for us to further develop our research programs without compromising the entirety of IIIS built by the WPI support. In FY2021, the

total amount of the competitive funds will be ¥1,089 M, of which ¥289 M is supplied from the Moonshot. We will continue our efforts to acquire external funding every year to exceed the level of FY 2021.

IIIS hosts a preliminary-stage research project supported by the JST MIRAI program, in which the Yanagisawa and the experimental medicine groups are involved in collaboration with groups in Faculty of Health and Sport Sciences and Center for Computational Sciences, University of Tsukuba. The project will undergo the stage gate review for stepping up to a large-scale project in FY 2021 and the groups involved work hard for succeeding the project.

IIIS PIs and other junior researchers are strongly encouraged to seek KAKENHI and very active to submit proposals to different categories. We are proud that our results opened in April marked over 40% success rate for the FY 2021 screening. Remarkably, one of T. Sakurai's proposals was accepted to the Scientific Research (KIBAN) A category with a possibility to scale up to the S category. Additionally, junior PIs are very active in seeking project grants funded by public agencies such as JST and AMED and private foundations. Furthermore, 6 proposals by our junior researchers including postdocs were funded in the Early-Career Scientists (WAKATE) category out of total 9 proposals submitted from IIIS, which provides evidence of our success in growing young scientists. Overall, IIIS is a very challenging cohort of scientists seeking competitive external funding opportunities to continue our research programs.

2-4. State of joint research

Describe the results of joint research conducted with other research organizations both in and outside Japan.

(1) MSD

From FY 2015 to FY 2018, we performed the joint research with MSD to identify orexin 2 receptor (OX2R) small molecule agonists representing one or more structural series to serve as lead compounds. IIIS is responsible for compound generation and chemical structure optimization. This is the first large-scale joint research project with an overseas company at University of Tsukuba. IIIS supplies a few compounds/quarter, while MSD evaluates PK/PD of the compounds. At the end of the 2-year term collaboration, IIIS discovered one lead candidate, YNT-1757. We filed the patent covering the compound. We are seeking for a patent licensee, and we are further optimizing YNT-1757.

(2) Nishikawa Co.

In FY 2015 we started the joint research with Nishikawa to validate the effect of body-pressure dispersion of a mattress on sleep. We found that better body-pressure dispersion improved sleep quality (*Sleep Med Res* 10(2): 1-5, 2019). In this study we realized that a mattress with better body-pressure dispersion might help us to keep the lateral positions for a longer time and reduce durations at the supine position.

Another method of decreasing time spent in sleeping at the supine position would be a body pillow. Obstructive sleep apnea (OSA) is a common sleep disorder that is associated with significant negative health outcomes including cardiovascular disease, daytime sleepiness, and neurocognitive deficits. OSA could be categorized into either positional or non-positional. In order to treat positional OSA, avoidance of supine sleeping position is recommended, and use of body pillow could suppress the occurrence of positional OSA. We are thus conducting a randomized crossover study on use of body pillow as an intervention to treat positional OSA and publishing the positive results (*Sleep Med Res*, 2021 in press).

(3) Kyocera Cooperation

Since FY 2018, we have conducted the collaboration with Kyocera Co. This collaboration is planned to continue for three years step by step with enough research funding for clinical studies in normal subjects testing their device. Nocturnal polysomnographic data were collected from 45 participants in FY 2019. IIIS has a role in examining the dynamics of heart rate variability and blood flow during sleep, while the participants wear the device and sleep. We compared several indices among sleep stages using the conventional frequency analysis. However, we could not detect differences among all sleep stages using this method. We, therefore, examined indices that show differences among sleep stages using non-linear analysis such as detrended fluctuation analysis (DFA). We found that DFA for the heart rate variability shows differences among all sleep stages except between awake and stage N1. Based on this result, we filed the patent jointly, and Kyocera is developing a device for estimating sleep stages using blood flow data.

(4) Toray Chemical Industries, Ltd

Since FY 2014 to FY 2021, we have been performing the ongoing joint research with Toray to study sleep modulating activities of Nalfurafin-related compounds. We discovered a kappa opioid receptor agonist with the morphinan structure, YNT-1612, and Toray estimated its *in vivo* activity under MTA.

YNT-1612 was found to show a potent antinociceptive activity without addiction and aversion. The most striking potential of YNT-1612 is that it exerts no sedation effect. Although the kappa opioid receptor agonist is approved for an antipruritic drug, Nalfurafine has the adverse effect showing severe sedation. That's why it was not applied for postoperative pain. YNT-1612 is expected to be applied for more efficacies than Nalfurafine, and we transfer the patent to Toray after execution of paid contract.

(5) S'UIMIN Inc.

In October 2017, S'UIMIN Inc. was established as a spin-out venture of IIIS. We are performing the collaboration with them on the research and development of a system of sleep measurement at home. In October 2019, we started the new joint research with S'UIMIN and Center for Computational Science, University of Tsukuba (CCS) to develop an automated sleep stage scoring software based on a deep learning model. The deep leaning model is to be built by using, as training data, results of the manual sleep staging of the wearable EEG device data by skilled clinical technologists.

As a long-term objective, we also plan to develop a software for automated assessment/diagnosis of sleep disorders based on deep learning in collaboration with S'UIMIN and CCS, as a part of the new project of Moonshot R&D Program. To this end, IIIS will cooperate with S'UIMIN to build big data of sleep and epidemiology through their sleep measurement/examination services, and S'UIMIN will participate in the project as a satellite member. The construction of the big data would enable us to develop a method to predict risks affected by diseases caused/aggravated by sleep depreciation/debt, in future.

2-5. Appraisal by society and scientific organizations

Describe how society and/or scientific organizations in and outside Japan have recognized the Center's research achievements.

- To substantiate the above evaluation, list the main awards received and invitational/Keynote lectures given by the Center's researchers in Appendix 1-3.

The research achievements yielded by IIIS are highly recognized in and outside Japan. In fact, IIIS researchers have received many awards.

Yanagisawa has received many prestigious academic awards from around the world such as Jokichi Takamine Memorial Award (2013), the Walter B. Cannon Memorial Award (2015), Erwin von Bälz Preis (2017), the Keio Medical Science Prize (2018), and European Narcolepsy Research Award and Takamine Memorial Daiichi Sankyo Prize (2019) for his contribution to revolutionizing vascular biology and sleep medicine by the discovery of endothelin and orexin. Since endothelin and orexin served as drug targets of pulmonary hypertension and insomnia, respectively, the contributions to public health were also appreciated. In recent years, he was also commended for his contribution to the development of culture and society through his promotion of sleep research and received several awards which are well known to general public such as Medal with Purple Ribbon (2016) and the Asahi Prize (2018). He also received Ibaraki Prefecture Honor Award (2019) honoring his contribution to Ibaraki Prefecture through his sleep research, and further was designated as Bunkakorosha (Person of Cultural Merits) in 2019 by the Japanese Government.

T. Sakurai received the Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (2013) and Shiono Prize (2017) for the discovery of orexin and the elucidation of its physiological functions. Nagase has been highly evaluated for his contribution to drug discovery, such as the design and synthesis of the first κ opioid receptor specific agonist, nalfurafine, and received the Okochi Memorial Technology Prize (2013), National Commendation for Invention – the Invention Prize (2013), and Yamazaki Teiichi Prize (2014).

Moreover, other PIs, non-PI faculties, postdocs, and graduate students have also been accredited with their outstanding achievements by various academic organizations, and the number of awards received so far exceeds 60 in total since the establishment of IIIS.

With regard to invitational/keynote lectures, all PIs have been invited as speakers and they have given more than 400 lectures at domestic and international conferences so far. Along with lots of awards given so far, it reflects high acclaim from research communities for IIIS, which further enhances the recognition of IIIS as a world-leading research institute specializing in fundamental sleep research.

Furthermore, Yanagisawa was appointed to the chairman of the 45th Annual Meeting of Japanese Society of Sleep Research (JSSR) held in September 2020, Yokohama. However, it was unfortunately cancelled because of the pandemic of COVID-19. Alternatively, Yanagisawa has been already re-appointed as the chair of the organizing committee for the 48th Annual Meeting of JSSR in 2023.

3. Feeding Research Outcomes Back into Society (within 2 pages)

3-1. Applications of research results

Describe the applications created from research results, their effect in spawning innovation, intellectual properties (IPs) obtained, and joint research activities conducted with corporations, etc.

(1) Patent applications

We aim at contributing to the society by solving the issues of sleep disorders and sleep debt. The development of drugs and early intervention of sleep disorders/debt is one of our major objectives, as discussed in 2-1. To implement and promote broad use of new treatments we develop, alliances with health industries are essential. We thus intensively secure intellectual property rights of our research achievements and seek opportunities of collaborations and licensing to companies. We have filed 80 patent applications since FY2013. These inventions are all commensurate with developing new technologies related to sleep and sleep disorders.

(2) Establishment of S'UIMIN

Tsukuba Global Innovation Promotion Agency (TGI), which is the organization established by University of Tsukuba, Tsukuba City and Ibaraki Prefecture as the hub of alliances among research institutions in Tsukuba, made a proposal to start 2 R&D projects for Regional Innovation Ecosystem Program initiated by MEXT in FY2016, and the proposal was adopted successfully. One of the R&D projects was the development of an in-home sleep measuring system, in which 3 groups, *i.e.,* 1) IIIS, 2) Center for Computational Science (CCS), and 3) Cyberdyne Inc., participated. CCS and Cyberdyne were responsible for the development of an EEG-analyzing algorithm based on machine learning for fully-automated sleep staging and an EEG-measuring device, respectively, while IIIS took charge of their validations and the collection of training data for machine learning.

In FY 2017, the project succeeded in developing the 1st prototype of the device with 3 channels of EEG and the sleep staging model of polysomnography (PSG) data on the basis of deep learning. Based on this achievement, a startup, S'UIMIN Inc. was established as the spin-out of IIIS in October 2017 and succeeded in raising fund of ¥900 M in capital as Series A in December 2018. S'UIMIN has taken over the development of the device from Cyberdyne and also has born responsibility for the development of the system, to provide users with the sleep measuring/examination services. As described in 2-4., IIIS, CCS and S'UIMIN continue the collaboration to develop the EEG-analyzing model based on deep learning, which consists of the core of the system "InSomnograf" as the analysis engine. S'UIMIN started to provide a closed β service of the sleep measurement in September 2020, and will initiate a sleep examination service in the complete check-up in University of Tsukuba Hospital in April 2021. Improving and expanding the services, the in-home sleep measuring system could bring innovation to sleep medicine.

(3) Licensing of research tools/lead compounds through S'UIMIN Inc as IIIS-TLO.

As one of potential measures to ensure the continuous operation of IIIS after the completion of the WPI program, we are considering a system whereby license revenues of the intellectual property rights created by IIIS could be directly used to offset partially the costs of operating the Center. To distribute to IIIS a significant portion of the licensing revenues, a) the TLO function was outsourced to S'UIMIN Inc. in order to maximize the licensing opportunity, and b) it was agreed with the university management that, after deduction of the compensation for inventors and 10% of overhead expenses, licensing revenues received by the University should be distributed to the headquarters and IIIS in proportion to IP cost burdens.

For commercial enterprises, the research tools are to be licensed through S'UIMIN Inc., which will negotiate a license fee with a licensee and obtain commission revenue adequate for its contribution as a private TLO. In addition to research tools, S'UIMIN Inc. is active to promote licensing of the lead compounds created in IIIS to pharmaceutical companies to implement non-clinical and clinical development. So far S'UIMIN Inc. has succeeded in the negotiations for licensing an animal model of narcolepsy to a pharmaceutical company and for transferring the IP right of two opioid δ agonists to other pharmaceutical companies.

3-2. Achievements of Center's outreach activities

* Describe what was accomplished in the center's outreach activities during the period from 2012 through March 2021 and how the activities have contributed to enhancing the center's "globally visibility." In Appendix 5, describe the concrete contents of these outreach activities and media reports or coverage of the activities.

3-2-1. Outreach events aimed at face-to-face communication

In order to enhance the visibility of IIIS, we have organized and took a lead of lots of outreach events. One of the most outstanding events in the past 8.5 years was the 6th WPI Science Symposium which we organized as a host institute in 2017. Setting two venues targeting different ages, we provided a wide variety of activities such as panel discussions, cross talk sessions, science café, comedy shows, and quiz rallies. The event was concluded in great success with more than 800 onsite visitors and 16,000 live broadcast viewers on Nico Nico Live, which was the largest number of participants in the past WPI symposia.

Since 2012, we have held workshops and events of science café every year targeting adults who are interested in sleep or have some troubles with sleep. For example, in 2017, we held a workshop with Yanagisawa sponsored by Hibiya Library in Tokyo. As a guest speaker, we invited a high school student who suffered from narcolepsy and asked him to share his experiences as a patient. Greatly influenced by Yanagisawa, after the workshop he studied very hard and succeeded in passing the entrance examination to a medical school. As a medical student, he reunited with Yanagisawa in March 2019, and this story was reported in the Asahi Shimbun and received a great response.

Moreover, not only organizing the outreach events, we have also participated in many science events such as the Super Science High School (SSH) Annual Research Meeting, AAAS Annual Meeting and **WPI Science Symposium** every year. In FY 2019, we opened a booth in Kagaku-Zammai in Aichi 2019, which was a science event for high school students and teachers in Aichi prefecture. These events provided us with opportunities for open dialogue with general people, and greatly contributed to our mission to disseminate information on sleep and health to the society.

For IIIS researchers and PR staffs, these events have offered great opportunities not only to introduce our research achievement, but also to know feedback regarding the importance of sleep research through direct interactions with general audience.

3-2-2. Online events

At least 6 onsite events, including open house, seminars, visit of school students, and exhibiting in scientific events, were planned at the beginning of FY 2020. However, all of them were cancelled due to the pandemic of COVID-19. Instead of onsite programs, we held 2 seminars, online exchanges with the students of Uto Senior High School, and joined online symposia some WPI centers initiated.

3-2-3. Media Coverages

The appearance of IIIS on the media including newspapers, magazines, books, radio programs, TV shows and web sites has been increasing year by year. In FY 2018, the number of media coverage was the highest on record, i.e., 180, which was about 1.7 times as many as that of FY 2017.

Since the number of media coverage in FY 2018 reached 180, the highest number so far, it appeared that our reputation as one of the world's leading research institute for sleep was well established, and we changed our media strategy toward the next stage. Due to careful screening of collaborating media, the number of media coverage in FY 2019 was 87, which was significantly lower than the previous year. However, it should be noted that several media featured IIIS at unprecedented scale. For example, the featured articles on "Newton" and "Newton Extra Issue," which were both supervised by Yanagisawa, devoted a full of 50 pages to the introduction of cutting-edge sleep science including achievements of IIIS. In FY 2020, the number of media coverage was increased to about 180, which was at the same level in FY 2018, although careful screening of collaborating media has made.

The press releases on the research achievements of IIIS have attracted great interest from the media. Especially, the two achievements published in Nature were reported by more than 100 commercial media in total. Furthermore, the two papers reporting human sleep physiology remarkably received worldwide attention in FY 2020.

Various overseas media such as "National Geographic," "The Atlantic," "Quanta Magazine" and "Nature Index" have also covered IIIS and its achievements. The intra- and inter-national recognition of IIIS has reached a certain level, by which corroborating that IIIS has become the world leading sleep research institute representing University of Tsukuba.

4. Generating Fused Disciplines (within 3 pages)

4-1. State of strategic (or "top-down") undertakings toward creating new interdisciplinary domains

Describe the content of "top-down" measures taken by the Center to advance research by fusing disciplines. For example, measures that facilitate doing joint research by researchers in differing fields.

To achieve our major research objectives, we have to conduct wide-ranging sleep research, covering a scope from basic biology such as neuroscience and molecular genetics to pharmaceutical science and further to experimental medicine, as shown in **Fig. 23**. It is the new interdisciplinary research domain, "sleep science," we aim to create by fusing 3 research fields.

A crucial driving force to create "sleep science" remains the leadership of the Center Director, Yanagisawa. To foster the interdisciplinary research under his leadership, the team of IIIS has been organized by PIs with sufficient expertise and achievements in 3 research fields, basic biology, pharmaceutical science and experimental medicine.

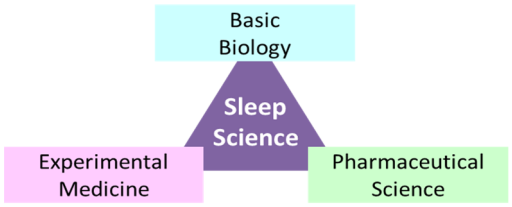

Fig.23. Establishment of "sleep science" by fusing 3 research fields, basic biology, pharmaceutical science and experimental medicine.

4-2. State of "bottom-up" undertakings from the center's researchers toward creating new interdisciplinary domains

Describe the content of "bottom-up" measures taken by the Center to advance research by fusing disciplines. For example, measures that facilitate doing joint research by researchers in differing fields.

Collaborative research among labs in IIIS is crucial to fuse 3 research fields into "sleep science." The internal collaborations have become more active recently, owing to physically and psychologically open atmosphere created/enhanced by 2 factors, i.e., the open structure of the IIIS Building and the open communication through unique activities, e.g., Work in Progress (WIP) and Dojo journal club meetings.

The labs and offices in IIIS Building are designed in a large open space and shared by several PIs' groups. Further, the open-office on each floor is connected with a spiral staircase in the stairwell spanning 1st through 4th floor. This open structure makes closer the physical as well as psychological distance of the scientists.

Holding the WIP and Dojo journal club meetings every week alternately, where all of IIIS members get together at the auditorium and discuss progress of own research or data published in others' articles, facilitates open communication among labs. WIP is a review meeting, in which a presenter assigned from each lab reports progresses and plans of his/her study, sharing them with all faculties, postdocs and graduate students in IIIS. It is a good training for young researchers as well as his/her mentor to obtain positive and negative feedbacks.

4-3. Results of research in fused research fields

Describe the Center's record and results by interdisciplinary research activities yielded by the measures described in 4-1 and 4-2.

- In Appendix 1-2, list up to 20 of the Center's main papers on interdisciplinary research that substantiate the above record of results, and describe their content.

[1] MC-SleepNet: Large-scale Sleep Stage Scoring in Mice by Deep Neural Networks (Biology x Informatics).

High-throughput screening of randomly mutagenized mice has generated a rich accumulation of EEG/EMG data of C57BL/6 mice which is ideal training data for machine learning. In collaboration with Kitagawa Lab (CCS) which is specialized in data science and machine learning, the Yanagisawa/Funato lab developed a deep neural networks-based automated EEG/EMG scoring system, called MC-SleepNet. This scoring system can analyze sleep/wakefulness with high reliability at least a thousand times faster than a human. Successful development has proved the same approach can be expand to sleep/wake analysis of animals other than mice (**1**).

[2] Novel Tools to Analyze Sleep Quality (Biology x Computation Science)

The effect of physical exercise on sleep quality can be difficult to assess, since exercise may cause discomfort that makes it difficult for subjects to judge their sleep quality. Using novel tools to determine the stability of electro-encephalographic oscillations we were able to determine that objective sleep quality increased after physical exercise.

In a collaboration between the Greene/Vogt Lab and the Tokuyama Lab at WPI-IIIS we introduced a novel EEG-based analysis method to the study of sleep and sleep depth. The coefficient of variation of the envelope (CVE) quantifies short-term fluctuations in EEG power. Low CVE values are observed in stable, sinusoidal oscillations, while high CVE values indicate brief, large spikes in power. We have shown that the temporal characteristics measured by CVE are useful to quantify sleep. Our novel analysis tools

have high translational potential, since the underlying EEG recordings are readily obtained in humans and in experimental animals. The collaboration is ongoing and we will continue to develop and apply novel analytical tools (**2**).

[3] Label-free imaging of neurons by multimodal nonlinear optical imaging (Biology x Applied Physics)

In a collaboration with Dr. Hideaki Kano (Department of Chemistry, Kyusyu Univ.), the Hayashi lab applied nonlinear optical microscopy to the nematode *Caenorhabditis elegans* (**Fig.24**). Using coherent anti-Stokes Raman scattering, second harmonic generation, and third harmonic generation, spatial information about various molecular properties was obtained without the usage of any labeling such as genetic or chemical probes. In particular, the group succeeded in visualizing neurons in vivo (**3**). This new method allows the exploration of the molecular changes that accompany changes in external/internal conditions, such as during sleep under label-free conditions. The nonlinear optical microscope is expected to be applicable to other types of biological samples such as mouse brain slices. Thus, it will be a powerful tool for assessing molecular dynamics in the brain.

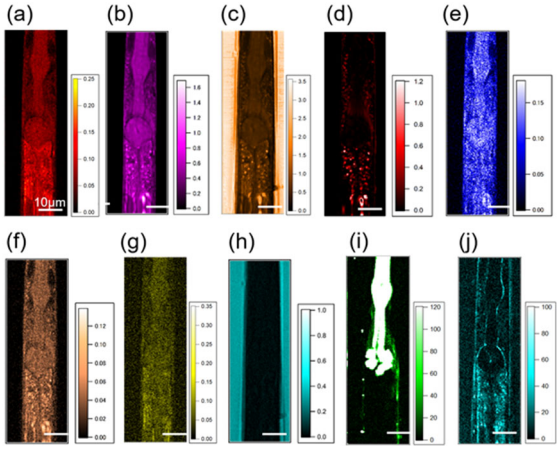

Fig. 24. Imaging of the nematode C. elegans with a nonlinear optical microscope. (a)-(h) CARS spectroscopic imaging, (i) SHG image, (j) THG image.

[4] Technical developments for sleep research (Biology x Instrumentation Engineering)

We made devices and/or research technique to analyze sleep or sleep related function. We made a miniaturized microscope and optrode devices capable of analyzing neuronal activity and manipulating it in freely moving mice (**4, 5**). Furthermore, we developed a method to dampen a fearful memory during sleep by exposing fear-related sensory stimuli (i.e., sound)(**6**). This finding suggests that exposure therapy for PTSD (i.e., flooding) could be enhanced through the use of sound while patients sleep.

[5] Structure-activity relationship between thiol group-trapping ability of morphinan compounds with a Michael acceptor and anti-*Plasmodium falciparum* activities (Biology x Medicinal Chemistry)

In collaboration with the Ōmura/Otoguro/Iwatsuki group (Kitasato Institute for Life Sciences, Kitasato University), the Nagase/Kutsumura Lab reported that BNTX and most of its derivatives showed *in vitro* antimalarial activities against chloroquine-resistant and -sensitive *Plasmodium falciparum* strains. The research showed the thiol group-trapping ability of the BNTX derivatives is expected to become an alternative method for *in vitro* malarial activity and related assays (**7**).

[6] Discovery of attenuation effect of orexin 1 receptor to aversion of nalfurafine and structure-activity relationship between orexin 1 receptor antagonist YNT-707 and orexin receptors (Biology x Medicinal Chemistry)

In collaboration with the Yanagisawa/Funato Lab (IIIS), the Nagase/Kutsumura Lab revealed the essential structures and the key pharmacophores of YNT-707, which is a potent and selective orexin 1 receptor antagonist (**8, 9**). In addition, they showed that the D-nor-nalfurafine derivatives had no affinity for orexin 1 receptor. Through the experimental results, the dual affinities of nalfurafine for orexin 1 receptor and κ opioid receptor led us to elucidate the mechanism by which only nalfurafine showed no aversion but U-50488H (**10**).

[7] Design and synthesis of potent and highly selective orexin 1 receptor antagonists with a morphinan skeleton and their pharmacologies (Biology x Medicinal Chemistry)

In collaboration with the Yanagisawa/Funato Lab (IIIS), the Nagase/Kutsumura Lab discovered kappa-opioid receptor agonist, nalfurafine showed a selective orexin 1 receptor antagonistic activity. Modification of the side chains finally led to YNT-1310 with improvement of the selective orexin 1 receptor antagonistic activity without any detectable affinity for the opioid receptors. The dihydrosulfate salt attenuated the physical dependence of morphine. The YNT-1310 disulfate hydrate is now being sold by FUJIFILM Wako Chemicals (**11**).

[8] Design and Synthesis of Non-Peptide, Selective Orexin Receptor 2 Agonists (Biology x Medicinal Chemistry)

In collaboration with the Yanagisawa/Funato Lab (IIIS), the Nagase/Kutsumura Lab discovered a

potent and orexin 2 receptor selective agonist YNT-185. The *in vivo* assay of the dihydrochloride salt showed a definite wake-promoting effect. The YNT-185 dihydrochloride hydrate is now being sold by FUJIFILM Wako Chemicals. These results would be helpful for further design of orexin 2 receptor selective agonists. We expect that the selective orexin 2 receptor agonists would be useful for the drug therapy of narcolepsy/cataplexy et al. (**12, 13**).

[9] Novel treatment of insomnia by enhancing adenosine A2A receptor signaling (Biology x Medicinal Chemistry x Engineering)

In collaboration with the Nagase/Kutsumura Lab (IIIS Medicinal Chemistry), the Lazarus/Oishi Lab (IIIS Systems Pharmacology) developed the first positive allosteric modulator of adenosine A2A receptors (A2AR), named A2AR PAM-1, that evokes A2AR responses in the brain (e.g., sleep induction) without affecting cardiovascular function, unlike classic A2AR agonists. Sleep disturbances are also common in schizophrenia patients and other psychotic conditions. Given that A2AR are also implicated in psychotic conditions, enhanced A2AR signaling may constitute an important molecular mechanism for sleep regulation and sound mental health (**14**). Furthermore, the Lazarus/Oishi and Nagase/Kutsumura Labs collaborated with the Abe Lab (Hiroshima University, Graduate School of Advanced Science and Engineering) to develop a visible-light (420 nm) photoactivatable ('caged') A2AR PAM-1. Using the opto-version of the A2AR PAM, they, for the first time, optochemically induced sleep in freely behaving mice (Roy K et al., in preparation). In the future, pharmacotherapy may offer the possibility to cure diseases and alleviate symptoms while preventing uncontrolled drug activity in time and space, i.e. the drug is only active when and where it requires its therapeutic effect.

[10] Molecular/neural mechanisms of predator odor-induced innate fear (Genetics x Chemical Biology)

In collaboration with Dr. Bruce Beutler (UT Southwestern Medical Center, USA), Drs. Ko and Reiko Kobayakawa (Kansai Medical University, Japan) and the Yanagisawa/Funato lab (IIIS), the Liu/Sakurai lab conducted the first large-scale recessive forward genetic screen on innate fear and identified Trpa1 mutant mice as deficient for 2MT/TMT and snake skin-evoked innate fear behaviors.

In collaboration with the Nagase/Kutsumura lab (IIIS Medical Chemistry), the Liu/Sakurai lab determined that TRPA1, a well-known receptor for pungent/irritant compounds, is a novel chemosensor for predator odor 2MT/TMT (**15**). Together with the Kobayakawa lab (Kansai Medical University, Japan), the Liu/Sakurai lab showed that TRPA1 is also essential for 2MT-evoked bioprotective effects, including hypothermia, hypometabolism and anti-inflammatory responses (**16**). In another collaboration with Dr. Peng Cao's lab (NIBS, China), the Liu/Sakurai lab identified PSTh as a novel thermoregulatory hub that mediates 2MT-evoked innate fear-associated hypothermia in mice (**17**).

The series of studies described above are fusion among molecular genetics, biochemistry, chemical biology and neuroscience.

[11] Effect of food ingredients on sleep and sleeping energy metabolism (Human Physiology x Food Biotechnology)

Coffee and tea are widely consumed beverages throughout the world, and contain caffeine and polyphenols, which might yield benefits in body weight control but might interfere with sleep. Because insufficient sleep is a risk factor of weight gain, it is important to simultaneously evaluate quality of sleep, when the effect of food ingredients on energy metabolism is assessed. As collaboration with food and biotechnology corporations (chlorogenic acids with Kao Co. and catechins with Suntory Holdings Ltd.), double-blind, randomized, placebo-controlled, cross-over studies revealed that subacute ingestion of chlorogenic acids in coffee (**18**) and catechins in oolong tea (**19**) increased fat oxidation without interfering sleep. Interestingly, the effect of oolong tea to stimulate fat oxidation was salient during sleep. The strong effects of meal consumption on blood glucose and subsequent insulin secretion masked the effect of oolong tea consumption to enhance fat oxidation during the daytime.

[12] Sleep disorder risk factors among athletes (Human Physiology x Sports Science)

In collaboration with Health and Sports Sciences, University of Tsukuba, sleep disorder risk factors among 906 student athletes were surveyed. Sleep disorders among student athletes were related to lifestyle habits such as late bedtime, early wake-up time, late night part-time jobs, and use of smartphones/cellphones after lights out; psychological distress; and competition activities such as morning practices and motivation loss stressors related to competition. It is well known that regular exercise promotes sleep, and good sleep is an important for the athletes. However, in real life, athletes do not sleep long enough due to their training schedule. This study suggests the importance of improving these lifestyle habits, mental health, and competition activities in student athletes (**20**). This study was partly supported by Sports Research Innovation Project of Japan Sports Agency.

5. Realizing an International Research Environment (within 4 pages)

5-1. International Circulation of Best Brains

5-1-1. Center's record of attracting and retaining top-world researchers from abroad

Describe the participation of top-world researchers as PIs and their stays as joint researchers at the Center.

・ In Appendix 3-2, give the number of overseas researchers among all the Center's researchers, and the yearly transition in their numbers. In Appendix 4-2 give the achievements of overseas researchers staying at the center to substantiate this fact.

Liu and Greene, the overseas PIs, have actively participated in research activities at IIIS through The University of Texas Southwestern Medical Center (UTSW) and National Institute of Biological Science, Beijing (NIBS), respectively. While appointed as Satellite PIs, they have established their own labs in the Center's core group since IIIS was launched in FY 2013. Liu stayed at IIIS for 571 days during 39 visits to Japan totally, though he couldn't visit IIIS even once in FY 2020 due to the COVID-19 epidemic unfortunately. In the meantime, Greene stayed at IIIS for 213 days on 21 visits to Japan totally. He was also unable to visit IIIS during the outbreak of COVID-19 in FY 2020. Both Greene and Liu have contributed to the management of IIIS by joining the PI meeting held monthly, even when absent from the institute, via online from UTSW or NIBS and also take an active part in important events including the symposia hosted by IIIS and the annual site visits for over 8 years.

To **WPI-IIIS Symposia**, totally 43 outstanding foreign researchers have been invited from abroad each year in order to present the latest achievements in sleep research or relevant fields. On each following day, we have offered them the post-symposium seminar at IIIS onsite to have researchers in the Tsukuba community share the recent progresses in sleep research.

Consequently, we hosted **168 WPI-IIIS Seminars** where we invited domestic and foreign researchers in sleep/neuroscience fields almost every other week; 68 speakers from overseas gave us lectures and the ratio of foreign researchers was 41% of the total seminar speakers since the inauguration in December 2012 (see more in the section 5-2).

Another thing especially worth mentioning is that the number of foreign researchers visiting IIIS has increased significantly. As **Fig. 25** illustrates, 144 excellent researchers visited IIIS. They all communicated with the Center Director, found some potential for collaboration and enjoyed the visit very much. The increase of visitors from abroad indicates that IIIS has become a globally attractive and well known institution. Regrettably we could invite no researchers from abroad in FY 2020.

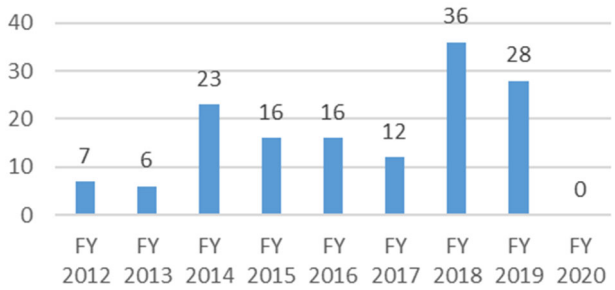

Fig. 25. Visit Records of Researchers from Abroad

5-1-2. Employment of young researchers at the Center and their job placement after leaving the Center

Describe the Center's employment of young researchers, including postdoctoral researchers, and the positions they acquire after leaving the Center.

・ Enter the following to substantiate the facts provided above:
- In Appendix 4-3, describe the Center's state of international recruitment of postdoctoral researchers, the applications received, and selections made.
- In Appendix 3-2, give the percentage of postdoctoral researchers employed from abroad
- In Appendix 4-4, describe the positions that postdoctoral researchers acquire upon leaving the Center.

Recruiting and fostering young researchers is essential for the future development of IIIS, while it plays a crucial role in the perspective of International Circulation of Best Brains. IIIS has actively engaged in international open recruitment by placing job advertisements on websites such as the homepage of IIIS, jREC-IN jobsite and naturejobs.com, etc. We renewed IIIS's website completely in 2017, and since then foreign researchers can easily check job opportunities and apply directly to the labs matching to their interests and expertise. Unfortunately, the number of applicants has decreased since 2019 comparing to the ones before 2018 due to COVID-19 situation as shown in Appendix 4-3.

In addition to posting job advertisements, we have employed some brilliant researchers owing the continued efforts in international recruitment through networks of PIs over past 9 years. We had regularly invited speakers from abroad for the IIIS Seminar series and made use of these opportunities to look for candidates of Junior PIs and postdoctoral researchers.

By the end of FY 2020, we hired 63 postdocs, of which 28 (44%) are overseas postdocs. In recent years, nearly 30 postdoctoral researchers are constantly resident at IIIS to obtain skills and knowledge of sleep research, to advance their career paths.

Noteworthy, the proportion of female researchers has been kept high (30-35%) continuously for last 9 years as shown in Appendix 3-2. It is also worth mentioning that nearly 50 female graduate students on average are carrying out their research in IIIS.

In recent years, prestigiously Qinghua Liu (PI) was appointed as the Senior Investigator at National Institute of Biological Science, Beijing (NIBS), and Yu Hayashi (junior PI) were promoted to a full professor in Human Health Science, Kyoto University.

Ex-IIIS young researchers acquired positions in top-level universities; Tomoya Sugai (a postdoc) was promoted to an assistant professor in Chuo University, Zhiqiang Wang (a postdoc) was appointed to a senior research fellow (PI) at Harbin Institute of Technology, and other researchers, Takato Honda, Chia-Ying Lee, Yan Zhang and Jing Ma obtained postdoc positions in Massachusetts Institute of Technology, Fred Hutchinson Cancer Research Center, Shenzhen University College of Materials, and Harbin Institute of Technology, respectively. Sayaka Ohrui (a Ph.D. student) acquired a position of an assistant professor at Meiji Pharmaceutical University after spending one year as a researcher at Research Foundation Itsuu Laboratory, and Mustafa Korkutata (a Ph.D. student) and Sumire Matsumoto (a Ph.D. student) acquired a position of a postdoctoral fellow in Harvard Medical School and a project assistant professor in Tohoku University, respectively.

As mentioned above, research achievements and experiences at IIIS evidently contribute to the career development. The number of the young researchers acquiring positions outside IIIS has been increasing as shown in Appendix 4-4.

5-1-3. Overseas satellites and other cooperative organizations

・ In Appendix 4-1, describe the state of cooperation with overseas satellites and other cooperative organizations. In Appendix 4-5, describe the state of the Center's agreements concluded with these organizations.

(1) Satellite institutions

University of Texas Southwestern Medical Center (UTSW)

The three Satellite PIs (Joseph Takahashi, Robert Greene, and Carla Green) have conducted research collaboration under the collaborative research agreements and/or the sponsored research agreements since FY 2013. C. Green engages in the sponsored research of RNA-Seq analysis of sleep-deprived mice, and R. Greene covers the sponsored research on sleep homeostasis and the sleep-awakening regulation of adenosine. J. Takahashi conducts the sponsored research on circadian rhythm control of sleep.

National Institute of Biological Sciences (NIBS)

As of January 2019, Q. Liu was appointed to NIBS, Beijing and a new collaborative research agreement was executed for the cross-appointment between NIBS and University of Tsukuba. He serves as Senior Investigator at NIBS and professor at IIIS with relative efforts of 92:8, respectively. Although his primary research commitment is dedicated to his lab at NIBS, it serves as the first overseas satellite of IIIS in Asia.

Akita University Graduate School of Medicine

We have concluded collaborative research agreement with Kazuo Mishima, a Head of the Department of Psychiatry, Akita University Graduate School of Medicine and engaged in collaboration in the pathological study of sleep disorders, clinical study, human genetics, etc.

(2) Partner institutions

From FY 2018, we started a collaboration with Sleep Disorder Centre, Neurology Department, Gui de Chauliac Hospital, Montpellier, France in order to discover human genetic factors of sleep disorders using their biobank of several cohorts suffering from disturbed sleep. IIIS performs exome and whole genome sequences of DNA samples extracted from their clinical samples of the patient cohort such as narcolepsy and idiopathic hypersomnia in Sleep Disorder Centre.

Fig. 26. Representative collaborators

We also perform many collaborative researches under material transfer agreements on animal models or compounds, and published many collaborative papers as the following schematic figure shows (**Fig. 26**).

5-2. Center's record of holding international symposia, workshops, research meetings, training meetings and others

・ In Appendix 4-6, describe the main international research meetings held by the Center.

IIIS hosts **International Symposium** every year since the establishment in 2012. About 200 of researchers and students participate each time, and enjoy lectures by prominent sleep researchers from Japan and abroad and active scientific discussions. Many invited speakers disclose unpublished data of their studies, which inspire new ideas and expand collaboration opportunities.

Since 2014, we have co-hosted the annual symposia with other universities, research institutes and companies. The co-sponsored partners include RIKEN, The University of Tokyo, Wako Pure Chemical Industries, Ltd., MSD K. K., and the team of Grant-in-Aid for Scientific Research on Innovative Area, "Creation and Promotion of WILLDYNAMICS." The joint meetings attracted many participants, regardless of academia or industries, beyond the boundaries of the research fields and largely contributed to form novel networks. In addition, the sponsorship significantly saves our budget for the international meetings. In FY 2019, we co-hosted WPI-IIIS Symposium with Ph.D. Program in Humanics, which was launched in FY 2018 as one of the Doctoral Programs for World-leading Innovative & Smart Education (WISE Programs) of MEXT, and 36th Takamine Conference, which commemorates Yanagisawa's 17th Takamine Memorial Daiichi Sankyo Prize. The joint symposium attracted more than 200 participants from academia as well as industries, beyond the boundaries of the research fields, and largely contributed to inspire new ideas and expanded collaboration opportunities. In addition, on the following day in Tsukuba we held the post-symposium seminar and it was also a great opportunity for all participants to intensively discuss unique research on sleep using nematode as a model.

With regard to the **IIIS Symposium in 2020**, we planned to hold it jointly with "the 45th Annual Meeting of Japanese Society of Sleep Research (JSSR)" and a project of JST's Core Research for Evolutional Science and Technology program (CREST), "OPTBIO." However, we indefinitely postponed it due to the pandemic of COVID-19.

Other than above, we have regularly held the **WPI-IIIS Seminar series**, counting 168 times by now (ca. 3 seminars/month) since the inauguration in December 2012. Invited speakers are all prominent domestic/foreign researchers in sleep/neuroscience fields and interviewed by each PIs individually throughout the visiting day beside their seminar for further discussion to a larger extent. Regrettably however, we can hold only 2 seminars online in FY2020 under the COVID-19 related crisis.

5-3. System for supporting the research activities of overseas researchers

Describe the Center's preparations to provide an environment conducive for overseas researchers to concentrate on their work, including for example living support in various languages or living support for their families.

University of Tsukuba has a department, "University of Tsukuba, Global Commons, International Exchange Support Office" which engages in livelihood support for foreign researchers and their families. They provide information on accommodation for foreigners in and outside the university and daily life in Tsukuba, and offer services including Japanese classes, proxy application for the certificate of eligibility (visa), etc.

IIIS has concluded the agreement on support for foreign researchers with Japan International Science and Technology Exchange Center (JISTEC). They offer services of accompanying foreign researchers to the City Hall for the residence registration, opening a bank account, etc. Further, many IIIS foreign researchers reside in the international accommodations operated by JISTEC.

Global Village and the Global Guest House are available on campus for the accommodation accepting researchers, students, short-term trainees and guests from overseas. The facilities are located in in a highly convenient spots close to the on-campus Shopping Plaza.

Conversely, in IIIS, we translate various forms into English, including documents of regulatory applications and formats/documents related to employment, personnel affairs and general affairs. We also support our foreign researchers by making sure all notices and announcements received from the university administration are translated into English from the original Japanese by the IIIS Administration. In this way, our researchers are able to stay aware of university-wide as well as institutional information.

For researchers themselves, we have introduced "**Buddy System**", assigning a personal mentor, "Buddy" for a newly arrived foreign researcher. Buddy shall be the first contact whenever they have questions or concerns in their daily lives such as shopping and showing the way around the campus, as well as lab activities such as basic rules/manners, handling of lab notebooks and so on.

The Research Strategy Team in the IIIS Administration have charged with a wide range of work relating to budget planning, workforce planning, competitive funding application, conclusion of

contracts, patent application, etc. All the members of the team are fluent in English and are able to support foreign PIs equally.

The General Affairs Team recently introduced the online residency card application procedure for the first time in University of Tsukuba. The online system will eliminate the need for IIIS foreign researchers to go to the Immigration Office in Tokyo that saves a lot of time and costs.

Following the relocation to IIIS Building, we have equally assigned secretaries proficient in English with full of hospitality to all lab members for sufficient supports. We accepted 21 international students/trainees in FY 2020 through various sponsoring systems to let them study in University of Tsukuba. Earlier, we could not obtain any students through the **Tsukuba Short-term Study Program** (**TSSP**), which allows only short-term trainees to use the student dormitory at a nominal fee and yet requires no entrance and tuition fees. However, the bylaw for TSSP was revised in March 2016, in response to our request to extend the longest period of stay from 3 months to 1 year. We planned and took procedures for 5 students from abroad by TSSP at the beginning of FY 2020; regardless, we were not able to receive them due to the influence of COVID-19. Beside, we invited 1 student from University of Bordeaux under **the Campus–In-Campus (CiC) Initiative**, which is aimed to enable sharing of educational and research resources and to contribute to the mutual enhancement of research and education capacities and capabilities between University of Tsukuba and University of Bordeaux. IIIS accepted 3 graduate students through Japanese Government (MEXT) Scholarship Program which offers scholarships to international students that aim to get Master or Ph.D. degree at the Graduate School of Comprehensive Human Sciences (Master's Program in Medical Sciences and Doctoral Program in Biomedical Sciences/Clinical Sciences) of University of Tsukuba. Other than above, IIIS took care of 14 graduate foreign students through Ph.D. programs in University of Tsukuba. Concerning financial support for foreign students, 1 graduate student was approved for Research Assistants to obtain monthly wages in FY 2020. For another graduate student, we approved "**IIIS Scholarship**" which supports a student who would like to go on to a graduate school in University of Tsukuba to continue a dissertation study in IIIS but without financial support as TA. This supporting system was newly established in July 1, 2019, originally by IIIS.

Taking advantage of these acceptance programs and supporting systems, we will broaden up opportunities for training of foreign students.

5-4. Others

Describe the Center's policy for sending Japanese researchers overseas to gain international experience, and give examples of how the Center is working to create career paths for its researchers within a global environment of researcher mobility.

We are appointed as a training site of **the International Sleep Research Training Program (ISRTP)** that is organized by World Sleep Society (WSS) in 2019. The ISRTP program has nominated major academic institutions in the world and aims to enrich the growing field of sleep medicine by training future leaders in basic and clinical sleep research in the world. Trainees submit their research plans and are matched to mentors who are scientists or clinicians with special expertise so that the trainees can acquire maximum experiences in basic sleep research or sleep clinic. Trainees need to be self-funded to travel to and stay at their host institutions during their training year but are invited to two major international sleep meetings supported by the program. This program is expected to be excellent opportunities for us to strengthen our international environment, however due to the CODIV-19 pandemic we have not accommodated any trainees thus far. We will actively engage with the program to host trainees as well as to promote our juniors to be a trainee.

IIIS encourages young researchers to get international experiences. Three researchers attended international meetings with competitive travel grants during FY 2019-20: The Society for Neuroscience Annual Meeting, International Brain Research Organization in Washington D.C., USA, the 12th FENS Forum of Neuroscience, the Japan Neuroscience Society and Federation of European Neuroscience Societies in Glasgow, UK and International Meeting for Biosensors and Actuators for Cellular and Systems Neuroscience in Bordeaux, France.

University of Tsukuba aims to foster human resources with a global view by promoting international exchange to improve academic standards. To achieve this the university has established agreements with overseas universities and the United Nations University Institute of Advanced Studies and offer a variety of activities such as delegating students and faculties abroad, transferring/exchanging credits with the universities and accepting faculties from abroad. In Graduate School of Comprehensive Human Sciences Majors of Medical Sciences, the partners for education and research exchanges include University of Edinburgh, University of Bordeaux, University of Bonn, National Taiwan University and Vietnam National University. IIIS encourages graduate students and young researchers to participate actively in these university's programs.

6. Making Organizational Reforms (within 3 pages)

6-1. Decision–making system in the center

Describe the strong leadership that the director is giving on the Center's operation and its effect, and the division of roles and authority between the Center and its host institution.
- In Appendix 3-3, draw a concrete diagram of the Center's management system.

Decision-making policy

For important matters concerning the operation of IIIS, all decision-making was done by the Center Director's top-down approach in accordance to the WPI policy. So that the intention of the Center Director takes effect quickly, organizational bylaws and other related regulations continue to be revised or enacted. By positioning IIIS as an independent research center in University of Tsukuba, wide-ranging autonomous operations, including personnel affairs, finances and facilities are secured.

A briefing session between Vice President for Research and Administrative Director is held every month to share progresses in the Center and take counsel policies or personnel matters between the University headquarters and IIIS. The discussion may be extended further to include Vice President for Human Resources or Vice President for Finance and Facilities as necessary.

Principal Investigators' meeting (PI meeting)

Spearheaded by the Administration, PI meetings were established to provide a periodic opportunity for PIs to openly discuss their opinions and concerns with the Center Director and to form a consensus as to important matters concerning IIIS. In this meeting, the Director acts as the chairman, and participation by all PIs is mandatory. PI meetings are held once a month with video conferencing capability to allow participation of all PIs under the COVID-19 situation. The functions of the steering committee of IIIS have also been attached to the PI meetings.

6-2. Arrangement of administrative support staff and effectiveness of support system

Describe the assignment of the Center's administrative support staff who have English language and other specialized skills, effort made in establishing the support system, and the system's effectiveness.

IIIS Administration provided support services so that researchers could entirely focus on research, under the leadership of the Administrative Director. Having served as Senior Director of the research institute of a pharmaceutical company, the Administrative Director has high expertise. He was assisted by 2 Vice Administrative Directors and the following four teams: the General Affairs (3 persons), the Accounting (3 persons), the Research strategy & Management (4 persons) and the Alliance & Communication (2 persons).

The first Vice Administrative Director, who used to be a section chief at the University of Tsukuba headquarters, concurrently serves as the leader of the General Affairs Team and the Accounting Team, striving to resolve various problems that required coordination with the university headquarters. The other Vice Administrative Director, a Ph.D. (professor) who is a long-term expert of sleep research abroad, was appointed in the previous fiscal year to supervise the Alliance & Communication (public relations) Team and the Research Strategy & Management Team led by another Ph.D. (associate professor) who has real comprehension of research details and a good knowledge of contract and patent matters in his former career at a pharmaceutical company. The team received a URA assigned by the university headquarter to provide services of pre- and post-awards management. With all the teams working together, IIIS Administration has taken a driving role in promotion of research projects within IIIS and acquisition of funding including great success in **the Moonshot R&D Program by AMED**.

Another point to note is that the use of English is strongly encouraged as the official language of IIIS. Approximately 70 percent of the administrative staff members are bilingual and enable to communicate smoothly with foreign researchers. Within IIIS, documents and papers are prepared in English or both in Japanese and English, in principle.

6-3. System reforms advanced by WPI program and their ripple effects

Concisely itemize the system reforms made to the Center's research operation and administrative organization, and describe their background and results. Describe the ripple effects that these reforms have on the host institution. (If any describe the ripple effects on other institutions.)

Concept of organization/operation to be learned from major US universities' "departments"

The basic concept of the organization and the operation of IIIS involves creating a new style of research center at the University by learning from the merits and virtues in the organization of "departments" in major US universities. The strong leadership of the "Department Head" would be ideal, and we thus assigned similar authority to the Center Director Yanagisawa, who had served as a professor/PI for 24 years at The University of Texas Southwestern Medical Center (UTSW). Other characteristics of this "department-style" organizational operation we adopted include:

・Flexible and timely appointment of PIs at the discretion of the Department Head within the budget,
・Appointment of independent PIs regardless of their age and career stage with a startup package,
・A flexible and dynamic allocation of the floor space for each lab considering the lab's scale of funding, number of personnel and facility requirements, and
・Sharing of large facilities and capital equipment among labs.

Indeed, all of these characteristics are perfectly realized in the organization and operation of IIIS.

Introducing a system to evaluate research results and ability-linked salary system

In FY 2017, IIIS introduced a simple system to evaluate achievements of faculties and researchers. In the new system, they are requested to update their own CV and provide it to their appraisers, PI and the Center Director with their self-evaluation. Then, PI and the Center Director make assessment of their achievements including publications, acquired grant funding, external journal/grant reviewing, outreach activities, and further contribution to IIIS activities. We consider the salary-raise based on this appraisal to build a system of merit-based compensation.

Authority over personnel matters and simplification of the appointment system

IIIS Personnel Committee was established in FY2012 and distinctive authority over personnel matters was assigned. Among quite a few research centers in the University, only 3 research centers, *i.e.,* IIIS, TARA and CCS, are allowed to propose candidate nomination. In particular, the appointment system of IIIS is simplified to be comprised of two steps, namely the intensive deliberation at IIIS Personnel Committee followed by the approval at Headquarters Personnel Council of the University, allowing speedy judgment and appointment under the leadership of the Center Director.

Joint appointment system

With the purpose of enabling Yanagisawa to hold concurrent posts at University of Tsukuba and UTSW, the joint appointment system was newly introduced to University of Tsukuba in March 2014. At the same time, a collaborative research agreement was concluded between University of Tsukuba and UTSW to determine terms and conditions of the research alliances accompanied with the joint appointment. In response to the execution of the agreement, University of Tsukuba made the tenure appointment of Yanagisawa as of April 1, 2014. Subsequently, Liu was also employed from FY 2014 under the joint appointment system. Since then, the cross-appointment has been rapidly increased among the University.

Tsukuba Short-term Study Program (TSSP)

As for the short-term stay for training, many trainees have been accepted as described in Appendix 5-1. To invite them, we have employed the system of Tsukuba Short-term Study Program (TSSP) of the University, which allows even short-term trainees to use the student dormitory at a nominal fee and requires no entrance and tuition fees. Initially the program limited their stay only for 3 months. However, after IIIS consulted the Vice President in charge of student affairs, the bylaw for TSSP was revised. Since March 2016, the internship training for up to one year became possible.

6-4. Support by Host Institution

The following two items concern the support that the host institution provides the Center. Describe the measures that the host institution has taken to sustain and advance the Center's project. That include those items of support that it committed to at the time of the initial project proposal submittal or in its revised commitment following the project's interim evaluation.

6-4-1. Record of host institution support and its effects

・In Appendix 6-1, describe the concrete measures being taken by the host institution.

University of Tsukuba has provided IIIS with various resources as operational and financial supports. The provided supports were equal to or greater than the supports planned in the Center Plan proposed in the application for the WPI program as follows;

1. The University established the Organization for the Support and Development of Strategic Initiatives, and IIIS receives ¥10 M for management expenses as the support from the Organization every year.
2. The Department of Research Promotion, as a counterpart in the University headquarters to IIIS, supports various office procedures including the applications for competitive funding.
3. After resigning from Howard Hughes Medical Institute in March 2014 to serve 95% of his efforts for IIIS, tenure was granted to the Centre Director.
4. By using the strategic positions secured by the Faculty of Medicine, T. Sakurai and A. Hirano were appointed as Vice Center Director and PI, respectively. Their personnel expenses were borne by the Faculty of Medicine and the University headquarters.
5. The University delegates 3 university personnel to the administrative positions, including Vice Administrative Director, the key liaison of general affairs and accounting. A university research administrator (URA) has been also assigned to the Research Planning and Management Team.

6. It costed ¥3,800 M for IIIS to construct the IIIS Building (total floor area of 8,000 m^2) and its interior, to landscape the exterior including the parking lot, to develop infrastructure and facilities including animal experimental facility, and to move labs to the building. MEXT generously covered ¥2,000 M by the supplementary budget, while the University bore ¥1,800 M. It was agreed that the University own a part of the IIIS Building (2,000 m^2), which is rent out to IIIS for a fee.
7. IIIS rents for ¥70 M/year the part of the IIIS Building owned by the University, while the University bore more than ¥88 M of utility costs of IIIS Building.
8. From April 2019, the University let IIIS use the research spaces (211 m^2) in Innovation Medical Research Institute at a minimal cost to build up the Human Sleep Lab.

6-4-2. Position of the Center within the host institution's mid-term plan

・ To Appendix 6-2, excerpt the places, in the host institution's "Mid-term objectives" and/or "Mid-term plan" that clearly show the positioning of the WPI center within its organization.

During the third mid-term plan of University of Tsukuba starting from FY2017, the University aims to develop a globally unrivaled frontier research of 2 objectives, i.e., research to deeply seek truth and research for application contributing to society, in wide-ranging academic disciplines. To realize this objective, the University will make plans of reorganization/restructuring/ merger of all research centers and implement it during the period of the 3rd mid-term plan. IIIS is positioned as a pioneering model of the forefront research organization the mid-term plan targets.

Based on this strategy the research centers have been classified by function into the Advanced Research Centers and the Research Support Centers. The former has been further classified as R1 (World-class Research Center), R2 (National-class Research Center), R3 (Developing Research Center), and R4 (Research Unit) to facilitate strategic resource allocation. Center for Computational Science (CCS) and Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA) are classified as the R1 status of World-class Research Center in physics and bioscience, respectively. The WPI center, IIIS is also accredited as R1, together with CCS and TARA, to represent the top-tier of the research centers in the University.

6-5. Others

Describe efforts advanced to foster young researchers (e.g., start-up funding, autonomous research environment) and to enlist female researchers.

・ In Appendix 3-1, 3-2, give the transition in the number and ratio of female researchers.

Internal grant system

This system is mainly intended for rescuing researchers who failed to acquire competitive research funding such as Grants-in-Aid for Scientific Research. The researchers can apply in April, and four members holding Ph.D. in the Administration serve as reviewers and perform a face-to-face interview with every applicant to examine the proposals and prioritize them.

IIIS Research Assistantship System and Scholarship

We have continued the IIIS Research Assistantship (RA) system to encourage students to go on to Ph.D. courses since FY2017. Graduate students in the doctoral program can apply for the IIIS RA only if he/she applied for the JSPS Research Fellowship for Young Scientists (DC1/DC2) but unsuccessful. Graduate students in the master's program are also eligible to apply for the IIIS RA only if he/she is committed to go to the Ph.D. program. We have another supporting system under IIIS Scholarship Program for undergraduate students who plan to go on to a graduate school in University of Tsukuba with a dissertation study in IIIS. Selection of applications have been conducted through a peer review process by the Center Director and the Admin staff members

Mental Care Programs in IIIS

We support students not only financially but also mentally. The mental care counseling for students has been conducted once a year since FY 2017. When a student in trouble is found, we offer additional counseling by the Admin staff including the Administrative Director to solve the trouble.

Online Residence Application Procedures

IIIS accepts many foreign researchers, and we recently introduced the online residency card application procedure for the first time in University of Tsukuba. The international office in the university headquarters is quite conservative, and they have no plan to apply for this online system. Since it was possible for an individual department or center to use this system via the corporate ID of the University, we applied for the registration as the user at Mito Branch Office, Tokyo Regional Immigration Bureau. This online system will eliminate the need for IIIS foreign researchers (and possibly foreign students) to go to the Immigration Office in Tokyo and secure their research time. This is just one example of our "system reforms," to be spread over the entire university in future.

7. Others

In addition to the above 1.-6. evaluation items, note any of the Center's leading activities, distinctive features or other important points that denote its status as an "internationally visible research center."

7-1. Characteristic outreach activities

7-1-1. Development of new approach for outreach: Nico Nico Cho-Kaigi

As events to introduce our studies and facility to general public, we broadcasted Nico Nico Live programs.

In FY 2017, lots of researchers including Yanagisawa, T. Sakurai, other PIs and students at IIIS introduced cutting-edge sleep science and their own studies, interacting with viewers through real-time comments online. Furthermore, we held virtual tours of IIIS Building to show our labs and research facilities, which had not been generally open to the public. The total numbers of viewers in the programs were about 20,000 (2 hour-program on January 29, 2018) and 116,000 (36 hour-program on May 28-29, 2018). In the both programs, more than 95% of the viewers answered "enjoyed" in the questionnaire taken at the ending. The programs showed a great potential of the new interactive outreach activities via internet broadcasting.

In FY 2019, we joined Nico Nico Cho-Kaigi (Super Meeting), an on-site event, which was held at Makuhari Messe on April 27 and 28. In order to introduce the cutting-edge sleep science, we had a panel exhibit at Cho-Suimin (Super Sleep) Booth and broadcasted two Nico Nico Live programs with Yanagisawa and T. Sakurai. In the two day event, about 1,000 people visited the booth and 40,000 people watched the live broadcasts. They all asked out of their curiosity about sleep and enjoyed talking with Yanagisawa and Sakurai. This event was a great opportunity for the effective outreach, taking advantage of both onsite and online.

7-1-2. Education programs for junior/senior high school and college students

To raise interest in sleep science and explore the next generation of scientists, we have accepted visitors from junior/senior high schools and provided various kinds of educational programs such as lectures, demonstration of experiments, round-table talks with researchers, lab tours, and so on.

The number of domestic and foreign schools delegating students and teachers to IIIS was more than 60, and we offered various kinds of the educational program such as lectures, demonstration of experiments, round-table talks with researchers, workshops about scientific literacy, and lab tours. Our educational programs are highly appreciated; at present 7 schools visit IIIS on a regular basis.

Importantly, "**Sleep Science Challenge**" with Kumamoto prefectural Uto Senior High School, which has annually held since FY 2013, has resulted in the recruitment of students engaging sleep research at IIIS, while in the high school the students began voluntary efforts to improve their sleep habits. Hibiki Okamura, who had visited IIIS in 2014 and 2015 as a student of Uto Senior High School, entered University of Tsukuba in 2019 aiming to join sleep research at IIIS and began her undergraduate dissertation study at Toda lab.

Although the number of visitors from junior/senior high schools had been increasing, we cannot accept visitors in FY 2020 due to concern of COVID-19. Instead of face to face programs, we provided an online lecture and Q & A interactions between IIIS researchers and the students of Uto Senior High School.

7-1-3. Events for IIIS members and the university staff

We have conducted the internal events in order to encourage the open communication and exchange of ideas possibly leading to collaborations amongst IIIS labs. We have held **Brie & Bordeaux (B&B)** sessions 20 times so far. It is a casual meeting with nice drinks and foods, and people enjoy short research presentations and discussions under a relaxed atmosphere in-house.

In addition to this, we planned "IIIS Retreat" in FY 2019, which is an excursion for all IIIS members to enjoy lectures by PIs, scientific discussion, and refreshing sightseeing. The executive committee was organized by students from several labs, and it organized the whole event with the support by the Admin, but unfortunately we had to postpone it due to the concern of COVID-19.

Moreover, in order to promote better understanding of IIIS and its activities, we held "**IIIS Open House**" for administrative staffs in the headquarters of University of Tsukuba. This event has led to enhanced cooperation with various departments in the university and effective PR activities of IIIS. We also believe that we can contribute to the system reform throughout the university by sharing our management and operation know-how.

7-1-4. Crowdfunding

From March 14 to May 31 2018, we launched the crowdfunding for the purpose of improving publicity and acquiring research budgets for a large-scale survey of the relationship between sleep and mental health. It was covered by various media such as the Asahi Shimbun, the Yomiuri Shimbun and the Mainichi Shimbun. However, the amount of donations reached only 50% at the end of April. We thus conducted "Nico-Nico" broadcast for 36 hours. Finally, we successfully reached the target amount of ¥3,000,000 (total: ¥3,566,000). As thank-you gifts, we offered the sleep analysis to reconsider their own lifestyle and sleep habit. We also gave, to the donors who made a large contribution, lectures by IIIS researchers, IIIS lab tour, etc.

Our new attempt attracted the attention of media and was reported in a news program "Keyaki Hills" in Abema TV, an internet broadcast, on September 12, 2018.

7-2. Human Sleep Lab and FC Bus

Human Sleep Lab (HSL) was established in University of Tsukuba, Innovation Medical Research Institute building (COI Building) in April 2019 (**Fig. 27**).

In HSL there is 4-bed sleep laboratory in which temperature, humidity and illuminance can be controlled, and polysomnography (PSG) can be performed in 4 measuring chambers simultaneously. The bed in each chamber is equipped with reclining function enabling a wide variety of experimental protocols. In addition, the facility for energy metabolism measurement, "Human Calorimeter," with the world's highest level of time resolution has been relocated to HSL from IIIS Building. HSL is one of the leading facilities for human sleep research in Japan.

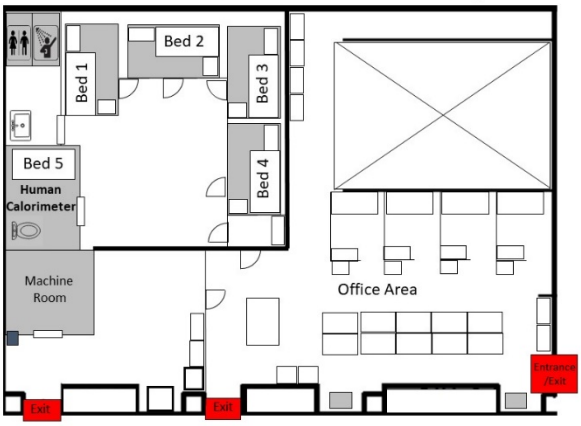

Fig. 27. Human Sleep Lab (HSL) (211m^2) in Innovation Medical Research Institute Building

Another significant development is a mobile sleep experiment facility. We converted a fuel cell bus, SORA, donated by Toyota Motor Corporation into a mobile sleep experiment facility in the collaborative research of sleep measurement with F-MIRAI, R&D Center for Frontiers of MIRAI in Policy and Technology. Inside the Mobile Sleep Lab (MSL, **Fig. 28**), there are 2 beds for sleep recording chambers, a monitoring room, a toilet and a wash sink. We performed a validation study to confirm the quality of sleep recordings by a PSG system in MSL in FY2020. We examined whether it can measure normal sleep without disturbing the subject in MSL comparing with sleep recorded in a HSL. As a result, MSL has been shown to function as sleep laboratories similar to HSL.

Fig. 28. Mobile Sleep Lab (MSL) converted a fuel cell bus, SORA (Nature digest, 2020)

This will make it possible for a sleep experiment facility itself to become transferable and eliminates location constrains for accurate sleep measurement. It is expected to be a device that can drastically change both aspects of basic sleep research and clinical sleep medicine in the future.

7-3. Research ethics

To avoid research misconduct, we have launched educational campaigns for research ethics since FY 2015, and held 5 seminars in the series of Research Ethics Seminar as follows;

1. The first seminar entitled "Research ethics and risk management: Lessons learnt from the STAP incident" by Ms. Momoko Suda on September 29, 2015.
2. The second seminar entitled "Research Misconduct: The Beginning of the End What Makes a Scientist 'Dr. Con Artist'?" by Dr. Susumu Inamoto on October 4, 2016.
3. The third seminar entitled "Research misconduct - lessons learnt from case studies" by Dr. Toshio Kuroki on March 22, 2017.
4. The 4th seminar entitled "Professional ethics for scientists and journalists" by Mr. Takao Fujiyoshi on September 25, 2017.

5. The 5th seminar entitled "Anti-Vax Campaign Using Pseudoscience and Lawsuits" by Dr. Riko Muranaka on February 13, 2018.

In addition, we introduced the official laboratory notebook of IIIS to formalize and let everyone use a common hard-covered laboratory notebook for better data management and prevention of research misconduct. We prepared the standard operating procedures (SOP) on purchasing, distribution, weekly check by mentors, storage, return, archiving, etc. One important key issue on SOP is that the laboratory notebook shall be kept under the supervision of the responsible PI. The responsible PI or a faculty member/postdoc appointed by the responsible PI, shall periodically (once a week is suggested) check the laboratory notebook.

7-4. Ph.D. Program in Humanics

In FY 2018, under the leadership of the Center Director and in close cooperation with key members of the Faculty of Medicine, University of Tsukuba, including the Dean and Associate Dean of the Faculty, we applied for the Doctoral Program for World-leading Innovative & Smart Education (WISE Program) of MEXT, and presented a proposal to create the **Ph.D. Program in Humanics**. Fortunately, our application was adopted in October 2018. The Ph.D. Program in Humanics aims to create a new academic discipline called "Humanics," which merges high levels of expertise in a) biomedical sciences and in b) physical sciences/engineering/informatics.

The Ph.D. Program in Humanics aims to train a new generation of leaders who have knowledge and skills at the doctoral level and sufficient scientific expertise to merge two disciplines, and who have the ability to apply the expertise to make contributions to society. For this purpose, the program features the bi-disciplinary education system (double major) in which each student receives guidance from two mentors from the fields of a) and b), respectively.

One well-known successful example of Humanics is the HAL robot suit by CYBERDYNE Inc., a startup company originating from University of Tsukuba, which was developed by combining neuroscience and robotics engineering. The program envisions cross-disciplinary research, such as analysis of big data on sleep through the combination of sleep medicine and artificial intelligence technology, and this is expected to offer greater opportunities for interdisciplinary studies at IIIS and other fields. Moreover, we aim to attract excellent students through this program. For students who have already conducted research in sleep science, the program will provide more options to widen their study subjects, raising expectations that it will facilitate the training of young researchers.

17 out of the 41 students enrolled in FY2020 have selected PIs in IIIS as one of their dual-mentors, and a total of seven students conduct their dissertation studies in IIIS, including the three students enrolled last year. Many of these students have backgrounds in sleep research, but a student with the background of informatics enrolled in the Ph.D. program aiming to conduct the fusion research in IIIS.

The WISE Program's budget allows us to employ some faculty members contributing to the Ph.D. program and improve research facilities to be used for dissertation studies of the students, enhancing the education and research systems of IIIS and the university. Actually, personnel expenses of two young faculty members in IIIS are born by the Ph.D. program, and more than ¥200 M has been allocated for purchasing equipment tor set-up/improve the lab environment requisite for dissertation studies, which contributes to IIIS significantly.

Enabling the sustainable development of this degree program requires acquisition of financial resources after the WISE Program that finishes in only 7 years. Therefore, we set up the Collaboration Council as a portal for academic-industrial alliance and we had executed the first collaborative research agreement in FY2020 with pharmaceutical company on the research enrolled by members of society.

7-5. Art project

Incorporated into the construction design of the IIIS Building, five formative art works created by the joint project with artists in Faculty of Art and Design, University of Tsukuba are arranged/exhibited at each location to stimulate the intellectual curiosity of researchers and symbolize the fusion.

Another exciting collaboration project with the School of Art and Design in the university, called "Art Street Satellite Gallery" for which we provide several spaces in IIIS Building to exhibit a part of the collection of prize-winning artworks created by the students of the School of Art, and four of their artworks have been displayed. In addition, Ms. Atsuko Tsurumi, an artist who continues to express "dreams" throughout her life, donated four paintings, and they are also displayed on the wall of the building.

8. Center's Response to Results of FY 2020 Follow-up (including Site Visit Results)

* Describe the Center's response to results of FY 2020 follow-up. Note: If you have already provided this information, please indicate where in the report.

(1) *As for sleep regulation by the SIK3 pathway, where and how this pathway acts still remain unknown. IIIs should try to solve these issues so as to acquire a better understanding sleep regulation.*

For intracellular signaling cascade involving the SIK3 protein kinase that regulates sleep need, we have already identified direct upstream regulator and downstream substrate of SIK3 and the findings will be published.

To identify cell populations responsible for sleep homeostasis under the SIK3 pathway, we have been monitoring and analyzing sleep/wakefulness behavior of mice in which Slp-type SIK3 is expressed in a selected cell population. So far, we found that the altered SIK3 pathway in postnatal neurons leads to increased sleep need（Iwasaki et al. JNS 2021). Having continued to narrow down neuronal groups responsible for sleep need change we discovered that alterations of the SIK3 pathway in excitatory neurons, not inhibitory neurons, within the ventral hypothalamus (VH) cause long NREM sleep time. Based on this observation we concluded that the SIK3 pathway in the excitatory neurons located at VH is responsible for sleep need and homeostasis.

(2) *Toward giving qualified PIs tenured appointments, University of Tsukuba has proposed resource redistribution and made draft budget requests to MEXT for the appointment of a few qualified PIs in IIIS. However, only three tenure positions including director and vice director are currently secured by the university. IIIS has proposed acquiring governmental grants and using them to cover PI labor costs for the time being, but that is not deemed appropriate. University of Tsukuba should consider resource redistribution to provide IIIS with more than ten tenured positions. Otherwise, some PIs, especially young and talented PIs, will leave IIIS before the WPI program term ends.*

To make the foundation of IIIS sustainable, the President of University of Tsukuba has repeatedly stated at the WPI Program Committee that PIs with a proven track record of achievement should be promoted to receive the status of 'tenure.' The Center Director and the Deputy Director have already acquired this status, and in FY 2018, with the cooperation of the Faculty of Medicine, a female PI (A. Hirano) was appointed to a tenure track assistant professor by using the strategic position secured by the University. Recently, President Nagata has led the strategic process of initiating the tenure reviews of 4 PIs whose terms of employment contracts are approaching to the renewal limitation stipulated by the Labor Contract Act. By the termination of the supporting period of the WPI program on March 31, 2022, 7 PIs of IIIS will have received the status of tenure, pending successful reviews. Accordingly in the next fiscal year, the Center Director will nominate additional PIs for the tenure review to the Personnel Committee under the approval by Vice President for Human Resources.

(3) *As for organization reform, a ripple effect on other departments of the host institution is not great so cannot be highly appraised, as described above. University of Tsukuba should promote the organizational reform of other departments by actively sharing the experience, knowledge, and know-how that IIIS has accumulated.*

We feel that IIIS has given a considerable impact on the reform of our university systems. During the third mid-term plan starting from FY 2017, the University aims to pursue the globally unrivaled frontier research of 2 objectives, i.e., the research for quest for truth, and the research for innovation contributing to society, in wide-ranging disciplines and research fields. To realize these objectives, the University planed reorganization/restructuring/merger of all research centers and is implementing it during the period of the 3rd mid-term plan. In this framework IIIS is expected to contribute by actively sharing of our accumulated experience, knowledge and know-how. For example, IIIS was first in the University to initiate **the Cross-Appointment System** for faculty members with international affiliations back in 2014; cross-appointments have now spread across the University, including 56 faculty members at present within eight Departments/Centers out of twelve.

In FY 2020 University of Tsukuba was selected as one of **the Designated National University Corporations** defined under the directions for the fourth mid-term targets set by MEXT. During the screening, the University actively involved IIIS in the site-visit evaluation by the referees and MEXT

officers so that we presented summary of our research and visions and conducted a guide tour in our building. Upon nomination the University has published a document introducing IIIS as one of the world class research centers representing University of Tsukuba.

(4) *It will be important for quality and branding purposes to find ways to involve industry more, although the percentage of industry-funded (collaborative) projects may remain modest. Even before the end of the WPI term, they will need to show more active involvement with industry, e.g. as partners (and funders) in collaborative research or in "affiliate program" membership frameworks.*

Basic biology research groups, our majority, focus on the major questions in sleep regulation by studying fundamental molecular and neural mechanisms. Their current research outcome is likely to be foundation for future innovations in human health and medicine but is presently not very relevant to industrial applications. Conversely, the group studying pharmaceutical science, namely Nagase-Kutsumura lab, has had multiple collaborative research projects with pharmaceutical and chemical companies, one of which has currently been ongoing under the AMED Cyclic Innovation for Clinical Empowerment (CiCLE) program. Similarly, experimental medicine groups have a number of collaborations with private companies that aim to develop products that assist sound sleep. It should be emphasized that **S'UIMIN Inc.,** the spin-out venture of IIIS, has been attracting a variety of companies and organizations in both commercial and public sectors involving shiftworkers since its launch of commercial in-home EEG service in September 2020. Additionally, we have proposed private companies that are interested in developing health care products improving sleep quality and approached IIIS to start collaborative projects with S'UIMIN Inc. Some of their products have been experimentally assessed using the in-home EEG device. Success of their business will certainly increase publicity of the IIIS. Overall, we believe that we are very well prepared to seek, start and maintain collaborations with industry or any kind.

(5) *The plan for post-WPI funding is still a bit weak. The overall mix of post-WPI funding sources seems like a realistic projection, although it is rather highly leveraged on large competitive projects (e.g. the Moonshots). They need to be working more at the present time to increase funding from collaborative projects. Relationships that produce funding through collaboration take a long time to build up.*

Our success in obtaining a long-term and large-scale research funding support by **AMED Moonshot** makes us continue our current research program. However, we agree with the reviewer's comment that we need to seek even stronger funding support for our future development. IIIS is a unique research institute that focuses on sleep medicine and most if not all of us are running at the leading edge of the field, we situate at a very advantageous position to make internal collaboration. In fact, IIIS internal collaborations are funded by the frameworks of JST CREST and JST MIRAI. Each PI of IIIS, especially young ones, actively seeks solo project funding programs sponsored by public agencies, but we should be always ready to look for more collaborative opportunities when any calls for relevant areas are open.

Appendix 1-1 List of Papers Underscoring Each Research Achievement

* List papers underscoring each research achievement [1] ~ [20] listed in the item 2-1 "Research results to date" of 2. "Advancing Research of the Highest Global Level" (up to 40 papers) and provide a description of the significance of each (within 10 lines).
* For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is the same. If a paper has many authors, underline those affiliated with the Center.
* If a paper has many authors (say, more than 10), all of their names do not need to be listed.
* Place an asterisk (*) in front of those results that could only have been achieved by a WPI center.

* [1] Identification of novel sleep-regulating genes

*1. Park M, Miyoshi C, Fujiyama T, Kakizaki M, Ikkyu A, Honda T, Choi J, Asano F, Mizuno S, Takahashi S, Yanagisawa M, Funato H (2020) Loss of the conserved PKA sites of SIK1 and SIK2 increases sleep need. *Sci Rep* **10**: 8676. Doi:10.1038/s41598-020-65647-0

Our previous study showed that SIK3 is crucial for the regulation of sleep need and that well-conserved protein kinase A (PKA) phosphorylation site, S551, is involved in this regulation. Given that S551 of SIK3 is conserved in other members of the SIK family, such as SIK1 (S577) and SIK2 (S587), it is possible that the PKA-phosphorylation sites of SIK1 and SIK2 are also involved in sleep/wake regulation. This study clearly showed that alanine substitution of PKA-phosphorylation site led to higher NREM delta density and/or longer NREMS time compared to wild-type mice. Thus, this study broadened and confirmed the hypothesis that PKA-SIK signaling constitutes an intracellular signaling pathway regulating sleep/wakefulness, especially sleep need, as was originally conceived based on findings in Sik3 mutant mice.

2. Miyoshi C, Kim SJ, Ezaki T, Ikkyu A, Hotta-Hirashima N, Kanno S, Kakizaki M, Yamada M, Wakana S, Yanagisawa M, Funato H (2019) Methodology and theoretical basis of forward genetic screening for sleep/wakefulness in mice. *P Natl Acad Sci USA* **116**(32): 16062-16067. Doi:10.1073/pnas.1906774116

Forward genetics is a powerful approach to elucidate important biological phenomena such as sleep/wakefulness in which one cannot predict sleep-regulating genes base on the function of known genes. Although we have previously reported successful identification of novel sleep-regulating genes through forward genetic approach, methodological and theoretical basis has not been made public. This study showed statistical and physiological ground for successful large-scale screening of sleep, which can be expand to animal behaviors other than sleep. This study also reported a long sleep pedigree, *Drowsy*, which has a loss-of-function mutation of a voltage-gated calcium channel, Canca1a. Thus, this study has showcased the whole range of forward genetics study and proved that novel sleep-regulating genes remains to be identified through this approach.

*3. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, Ma J, Nakane S, Tomita J, Ikkyu A, Kakizaki M, Hotta-Hirashima N, Kanno S, Komiya H, Asano F, Honda T, Kim SJ, Harano K, Muramoto H, Yonezawa T, Mizuno S, Miyazaki S, Connor L, Kumar V, Miura I, Suzuki T, Watanabe A, Abe M, Sugiyama F, Takahashi S, Sakimura K, Hayashi Y, Liu Q, Kume K, Wakana S, Takahashi JS, Yanagisawa M. 2016 Forward-genetics analysis of sleep in randomly mutagenized mice. *Nature* **539**: 378-383. Doi:10.1038/nature20142

This is the first genuine forward genetic EEG/EMG-based screening for sleep using randomly mutagenized mammals. This study identified two novel sleep-regulating molecules: SIK3 and NALCN. A splicing mutation in the Sik3 protein kinase gene causes a profound decrease in total wake time, owing to an increase in inherent sleep need. Sik3 orthologues also regulate sleep in fruitflies and roundworms. A missense, gain-of-function mutation in the sodium leak channel NALCN reduces the total amount and episode duration of REMS, by increasing the excitability of REMS-inhibiting neurons. This study substantiates the use of a forward genetics approach for studying sleep behaviours in mice and demonstrate the role of SIK3 and NALCN in regulating the amount of NREMS and REMS, respectively.

* [2] Elucidation of an intracellular signaling that regulates sleep need

*1. Park M, Miyoshi C, Fujiyama T, Kakizaki M, Ikkyu A, Honda T, Choi J, Asano F, Mizuno S, Takahashi S, Yanagisawa M, Funato H (2020) Loss of the conserved PKA sites of SIK1 and SIK2 increases sleep need. *Sci Rep* **10**: 8676. Doi:10.1038/s41598-020-65647-0

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*3. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, Ma J, Nakane S, Tomita J, Ikkyu A, Kakizaki M, Hotta-Hirashima N, Kanno S, Komiya H, Asano F, Honda T, Kim SJ, Harano K, Muramoto H, Yonezawa T, Mizuno S, Miyazaki S, Connor L, Kumar V, Miura I, Suzuki T, Watanabe A, Abe M, Sugiyama F, Takahashi S, Sakimura K, Hayashi Y, Liu Q, Kume K, Wakana S, Takahashi JS, Yanagisawa M. 2016 Forward-genetics analysis of sleep in randomly mutagenized mice. *Nature* **539**: 378-383. Doi:10.1038/nature20142

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*4. Iwasaki K, Fujiyama T, Nakata S, Park M, Miyoshi C, Hotta-Hirashima N, Ikkyu A, Kakizaki M, Sugiyama F, Mizuno S, Abe M, Sakimura K, Takahashi S, Funato H, Yanagisawa M (2021) Induction of mutant Sik3Sleepy allele in neurons in late infancy increases sleep need. *J Neurosci* **41**(12): 2733-2746. Doi:10.1523/JNEUROSCI.1004-20.2020.

The propensity to accumulate sleep need during wakefulness and to dissipate it during sleep underlies the homeostatic regulation of sleep. However, little is known about the developmental stage and cell types involved in determining the homeostatic regulation of sleep. This study has shown that the induction of exon13-skipped Sik3 in mature neurons in late infancy is sufficient to increase NREM sleep amount and EEG delta power during NREM sleep. SIK3 signaling in neurons constitutes an intracellular mechanism to increase sleep. This also suggest that we are able to narrow down a group of neurons that crucially determine sleep need. This study reported a valuable mouse line, Synapsin I-CreERT2, which enables neuron-specific, inducible regulation of target genes.

*5. Wang Z, Ma J, Miyoshi C, Yuxin Li, Sato M, Ogawa Y, Lou T, Ma C, Gao X, Lee C, Fujiyama T, Yang X, Zhou S, Hotta-Hirashima N, Klewe-Nebenius D, Ikkyu A, Kakizaki M, Kanno S, Cao L, Takahashi S, Peng J, Yu Y, Funato H, Yanagisawa M, Liu Q (2018) Quantitative phosphoproteomic analysis of the molecular substrates of sleep need. *Nature* **558**: 435-439. Doi:10.1038/s41586-018-0218-8

We challenged a very simple question: what is the molecular basis of sleep need? Using quantitative phosphoproteomic analysis of the sleep deprived and Sik3 mutant mouse models of increased sleep need. Comparison of the two models identifies 80 mostly synaptic sleep-need-index phosphoproteins (SNIPPs), and most of SNIPPs are associated with the structure and function of synapses. This finding may support the synaptic homeostasis hypothesis that waking encodes memories by potentiating synapses, whereas sleep consolidates memories and restores synaptic homeostasis by global downscaling of synaptic strength. This study led to hypothesize that the phosphorylation–dephosphorylation cycle of SNIPPs represent a major regulatory mechanism that underlies both synaptic homeostasis and sleep–wake homeostasis to maximize cognitive functions of the brain.

6. Honda T, Fujiyama T, Miyoshi C, Ikkyu A, Hotta-Hirashima N, Kanno S, Mizuno S, Sugiyama F, Takahashi S, Funato H, Yanagisawa M (2018) A single phosphorylation site of SIK3 regulates daily sleep amounts and sleep need in mice. *P Natl Acad Sci USA* **115**: 10458-10463. Doi:10.1073/pnas.1810823115

The neural substrate for sleep need, as well as the molecular mechanisms determining daily sleep amounts, remain mysterious. Previously we identified a protein kinase, SIK3, as a novel sleep-regulating gene and showed that the lack of 52 amino acids resulted in increased sleep need. This study found that a single amino acid, S551, of the protein kinase SIK3 has a crucial role in the regulation of sleep need and daily NREM sleep amounts. Importantly, S551 in SIK3 is evolutionally conserved as a PKA phosphorylation site from nematodes and fruit flies to mice and humans. These findings implicate SIK3 in the molecular basis of homeostatic sleep/wake regulation.

*** [3] Molecular mechanisms of homeostatic sleep regulation**

*3. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, Ma J, Nakane S, Tomita J, Ikkyu A, Kakizaki M, Hotta-Hirashima N, Kanno S, Komiya H, Asano F, Honda T, Kim SJ, Harano K, Muramoto H, Yonezawa T, Mizuno S, Miyazaki S, Connor L, Kumar V, Miura I, Suzuki T, Watanabe A, Abe M, Sugiyama F, Takahashi S, Sakimura K, Hayashi Y, Liu Q, Kume K, Wakana S, Takahashi JS, Yanagisawa M. 2016 Forward-genetics analysis of sleep in randomly mutagenized mice. *Nature* **539**: 378-383. Doi:10.1038/nature20142
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*5. Wang Z, Ma J, Miyoshi C, Yuxin Li, Sato M, Ogawa Y, Lou T, Ma C, Gao X, Lee C, Fujiyama T, Yang X, Zhou S, Hotta-Hirashima N, Klewe-Nebenius D, Ikkyu A, Kakizaki M, Kanno S, Cao L, Takahashi S, Peng J, Yu Y, Funato H, Yanagisawa M, Liu Q (2018) Quantitative phosphoproteomic analysis of the molecular substrates of sleep need. *Nature* **558**:435-439. Doi:10.1038/s41586-018-0218-8
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*** [4] A discrete neuronal circuit induces a hibernation-like state in rodents**

*7. Takahashi TM, Sunagawa GA, Soya S, Abe M, Sakurai K, Ishikawa K, Yanagisawa M, Hama H, Hasegawa E, Miyawaki A, Sakimura K, Takahashi M, Sakurai T (2020) A discrete neuronal circuit induces a hibernation-like state in rodents. *Nature* **583**: 109–114. Doi:10.1038/s41586-020-2163-6

We found that chemogenetic excitatory manipulation of a neuronal population, Q neurons, in the anteroventricular periventricular hypothalamus, that express neuropeptide Qrfp induces a long-lasting hypothermic/hypometabolic state similar to hibernation (Q neurons-induced hypometabolic state, QIH). Q neurons act mainly on the dorsomedial hypothalamus to induce the QIH. We also found that glutamatergic

neurotransmission in Q neurons is important for inducing QIH. In the QIH, ability to regulate metabolism and behavior was conserved, showing a stark contrast to hypothermic states induced by anesthesia. No obvious tissue/organ damage or abnormalities in behavior were observed after recovery. This finding opens the door to the development of induction of a hibernation-like state, which would have potential applications in non-hibernating mammalian species including humans.

[5] Orexin modulates behavioral fear expression through the locus coeruleus

8. Soya S, Takahashi MT, McHugh T, Maejima T, Herlitz S, Abe M, Sakimura K, Sakurai T (2017) Orexin modulates behavioral fear expression through the locus coeruleus. *Nat Commun* **8**(1): 1606. Doi:10.1038/s41467-017-01782-z

We demonstrate the circuit involving orexin, noradrenalin neurons in the locus coeruleus and lateral amygdala neurons mediates fear-related behavior and suggests inappropriate excitation of this pathway may cause fear generalization sometimes seen in psychiatric disorders, such as PTSD. Pharmacogenetic or optogenetic silencing of this circuit inhibited sustained expression of fear responses, as did acute pharmacological blockade of the orexin receptor-1 by an antagonist administered just before the test session. In contrast, optogenetic stimulation of this circuit altered fear conditioning-induced freezing behavior in a similar but distinct context. Upregulating orexinergic tone by fasting also enhanced freezing behavior in neutral context. These findings demonstrate that the neural circuit that involves orexin, noradorenergic neurons and the lateral amygdala plays an important role in the regulation of fear-related behavior in response to environmental stimuli, and dysfunction of this circuit may cause inappropriate generalization of fear.

*** [6] Identification of a neural circuit that regulates REM and non-REM sleep**

9. Hayashi Y, Kashiwagi M, Yasuda K, Ando R, Kanuka M, Sakai K, Itohara S (2015) Cells of a common developmental origin regulate REM/non-REM sleep and wakefulness in mice. *Science* **350**: 957-61. Doi:10.1126/science.aad1023

The mechanisms and functions of REM sleep were poorly understood. Previous studies have shown the involvement of neurons in the pontine tegmental area. However, due to the complexity and heterogeneity of the neurons that comprise this area, the precise identity of the neurons regulating REM sleep was unknown. Here, based on an approach to profile neurons based on developmental lineage, glutamatergic neurons that temporally express Atoh1 during embryonic development were shown to negatively regulate REM sleep. Chemogenetic manipulation of the identified circuit allowed to either strongly decrease or increase REM sleep, respectively. The group also found that manipulating REM sleep affects slow wave activity during subsequent non-REM sleep, suggesting that one function of REM sleep is to regulate the quality of non-REM sleep. Slow wave activity contributes to synaptic plasticity and memory consolidation.

*10. Kashiwagi M, Kanuka M, Tatsuzawa C, Suzuki H, Morita M, Tanaka K, Kawano T, Shin JW, Suzuki H, Itohara S, Yanagisawa M, Hayashi Y. (2020) Widely distributed neurotensinergic neurons in the brainstem regulate NREM sleep in mice. *Curr Biol* **30**:1002-1010. Doi:10.1016/j.cub.2020.01.047

To further identify neurons that regulate REM sleep, the Hayashi lab profiled neurons within the pontine tegmental area based on gene expression. As a result, neurons here that express the neuropeptide Neurotensin were shown to negatively regulate REM sleep. Moreover, it was revealed that Neurotensin itself is involved in regulating REM sleep. Interestingly, the neurons positive for Neurotensin projected to various neurons within the brainstem which also express Neurotensin, and these neurons were also involved in regulating REM and non-REM sleep.

11. Liu CY, Tsai CJ, Yasugaki S, Nagata N, Morita M, Isotani A, Yanagisawa M, Hayashi Y (2021) Copine-7 is required for REM sleep regulation following cage change or water immersion and restraint stress in mice. *Neurosci Res* **165**: 14-25. Doi: 10.1016/j.neures.2020.04.002

To further identify neurons that regulate REM sleep, the Hayashi lab profiled neurons within the pontine tegmental area based on gene expression. As a result, neurons here that express Copine7 were shown to negatively regulate REM sleep. Moreover, it was revealed that mice lacking Copine7 tend to enter REM sleep more frequently under stressed conditions or in a novel environment, demonstrating that Copine7 itself is involved in regulating REM sleep.

*12. Maezono SEB, Kanuka M, Tatsuzawa C, Morita M, Kawano T, Kashiwagi M, Nondhalee P, Sakaguchi M, Saito T, Saido TC, Hayashi Y (2020) Progressive Changes in Sleep and Its Relations to Amyloid-β Distribution and Learning in Single App Knock-In Mice. *eNeuro* **7**(2): ENEURO.0093-20.2020. Doi:10.1523/ENEURO.0093-20.2020

Alzheimer's disease (AD) patients often suffer from sleep disturbances. To accurately characterize the sleep

impairments accompanying AD and their underlying mechanisms using animal models, it is crucial to use models in which brain areas are affected in a manner similar to that observed in the actual patients. Here, the Hayashi lab focused on AppNL-G-F mice, in which expression levels and patterns of mutated amyloid precursor protein (APP) follow the endogenous patterns. It was revealed that these mice exhibit reduced REM sleep from early stages. Moreover, at later stages where learning impairments were detected, the amount of REM sleep, but not non-REM sleep, was strongly and positively correlated with the learning abilities. These findings support the notion that changes in REMS are an early marker of AD and provide a starting point to address the mechanism of sleep deficits in AD and the effects on cognition.

*** [7] Role of sleep in functional brain regeneration**

13. Koyanagi I, Sonomura K, Naoi T, Ohnishi T, Kaneko N, Sawamoto K, Sato T, Sakaguchi M (2021) Metabolic fingerprints of fear memory consolidation during sleep. *Mol Brain* **14**: 30. Doi:10.1186/s13041-021-00733-6

Metabolites underlying brain function and pathology are not as well understood as genes. We analyzed real-time changes in metabolites in the dentate gyrus in different sleep-wake states in mice. Metabolite profiles changed dramactically upon sleep-wake state transitions, leading to a clear separation of phenotypes between wakefulness and sleep. By contrast, contextual fear memory consolidation induced less obvious metabolite phenotypes. However, changes in purine metabolites were observed upon both sleep-wake state transitions and contextual fear memory consolidation. These results point toward the importance of purine metabolism in fear memory processing during sleep.

*14. Kumar D, Koyanagi I, Carrier-Ruiz A, Vergara P, Srinivasan S, Sugaya Y, Kasuya M, Yu T, Vogt KE, Muratani M, Ohnishi T, Singh S, Teixeira CM, Chérasse Y, Naoi T, Wang S, Nondhalee P, Osman BAH, Kaneko N, Sawamoto K, Kernie SG, Sakurai T, McHugh TJ, Kano M, Yanagisawa M, Sakaguchi M (2020) Sparse activity of hippocampal adult-born neurons during REM sleep is necessary for memory consolidation. *Neuron* **107**: 552-65. Doi:10.1016/j.neuron.2020.05.008

The mechanism of memory consolidation during sleep remains largely unexplained. The applicant found that newborn neurons activated during learning are reactivated during subsequent REM sleep. When the activity of these neurons was manipulated by optogenetics, it was found that memory consolidation was impaired. This indicates that newborn neurons regulate memory fixation through precisely controlled reactivation during REM sleep. We found that the synaptic plasticity of newborn neurons plays an important role in this mechanism. These results suggest that sleep plays critical roles for the functional integration of adult-born neurons in the adult-brain, futher underscoing the importance of sleep in brain regeneration.

15. Purple R, Sakurai T, Sakaguchi M (2017) Auditory conditioned stimulus presentation during NREM sleep impairs fear memory in mice. *Sci Rep* **7**: 46247. Doi:10.1038/srep46247

Long-term exposure therapy is an effective treatment for post-traumatic stress disorder (PTSD), but it places a heavy psychological burden on patients when they repeatedly recall traumatic memories. In this study, we used a mouse model and showed that playing a sound associated with a memory during unconscious sleep can promote memory erasure while the patient is asleep. This study demonstrates the potential of sound stimulation during sleep as an adjunctive treatment for PTSD.

*** [8] The gating of sleep homeostasis by motivation**

*16. Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, Luo YJ, Chérasse Y, Schiffmann SN, de Kerchove d'Exaerde A, Urade Y, Qu WM, Huang ZL, Lazarus M (2017) Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. *Nat Commun* **8**(1): 734. Doi:10.1038/s41467-017-00781-4

By using chemo-genetic and optical techniques to remotely control the activities of adenosine A_{2A} receptors (A_{2A}R)-expressing nucleus accumbens (NAc) neurons and the behaviors they mediate, the Lazarus/Oishi Lab discovered that nucleus accumbens neurons have an extremely strong ability to induce sleep that is indistinguishable from the major component of natural sleep, known as slow-wave sleep, as it is characterized by slow and high-voltage brain waves. Homoeostatic sleep pressure produced by sleep deprivation did not change the neuronal activity of NAc A_{2A}R neurons, whereas motivational stimuli attenuated the activity of these neurons and reduced sleep amount. The paper revealed a sleep-inducing role of the NAc that is regulated by motivational factors.

*17. Oishi Y, Suzuki Y, Takahashi K, Yonezawa T, Kanda T, Takata Y, Chérasse Y, Lazarus M (2017) Activation of ventral tegmental area dopamine neurons produces wakefulness through dopamine D2–like receptors in mice. *Brain Struct Funct* **222**: 2907-2915. Doi:10.1007/s00429-017-1365-7

A growing body of evidence suggests that dopamine plays a role in sleep–wake regulation, but the

dopamine-producing brain areas that control sleep–wake states have been unclear. By using chemo-genetic techniques to remotely control the activities of ventral tegmental area (VTA) dopaminergic neurons, the Lazarus/Oishi Lab found that these neurons strongly induce and consolidate wakefulness. The increased wakefulness evoked by VTA activation was completely abolished by pretreatment with the dopamine D2/D3 receptor antagonist raclopride, but not by the D1 receptor antagonist SCH23390. These findings indicate that the activation of VTA dopamine neurons promotes wakefulness via D2/D3 receptors.

*18. Zhou X, Oishi Y, Chérasse Y, Korkutata M, Fujii S, Lee CY, Lazarus M (2019) Extracellular adenosine and slow-wave sleep are increased after ablation of nucleus accumbens core astrocytes and neurons in mice. *Neurochem Int* **124**: 256-263. Doi:10.1016/j.neuint.2019.01.020

The cellular and molecular processes underlying the build-up of sleepiness and maintenance of sleep are unknown. Glia are far from being merely support cells of the brain. They may just be as dynamic as neurons and actively guide brain function and behavior. One of the intriguing possibility of this is sleep. The nucleus accumbens (NAc), a new sleep-regulating area through the integration of motivational stimuli provides an excellent opportunity to study the regulation of sleep by glia–neuron interactions. The Lazarus/Oishi Lab revealed that elevated adenosine levels caused by ablation of NAc astrocytes promote sleep via A2AR. These findings may indicate that sleep control is an essential physiological function of astrocytes and provide the first evidence that adenosine is an endogenous candidate for activating NAc A2AR neurons that have the ability to induce slow-wave sleep.

19. Lazarus M, Oishi Y, Bjorness TE, Greene RW (2019) Gating and the need for sleep: dissociable effects of adenosine A1 and A2A receptors. *Front Neurosci* **13**: 740. Doi:10.3389/fnins.2019.00740

Currently, there is no model that places the regulation of arousal and sleep homeostasis in a unified conceptual framework. Adenosine is well known as a somnogenic substance that affects normal sleep-wake patterns through several mechanisms in various brain locations via A1 or A2A receptors (A1Rs or A2ARs). Many cells and processes appear to play a role in modulating the extracellular concentration of adenosine at neuronal A1R or A2AR sites. Emerging evidence suggests that A1Rs and A2ARs have different roles in the regulation of sleep. IIIS investigators Michael Lazarus, Yo Oishi and Robert Greene propose a model in which A2ARs allow the brain to sleep, i.e., these receptors provide sleep gating, whereas A1Rs modulate the function of sleep, i.e., these receptors are essential for the expression and resolution of sleep need. In this model, sleep is considered a brain state established in the absence of arousing inputs.

*** [9] Neural circuits controlling sleep and mania-like behaviors**

*20. Takata Y, Oishi Y, Zhou XZ, Hasegawa E, Takahashi K, Chérasse Y, Sakurai T, Lazarus M (2018) Sleep and wakefulness are controlled by ventral medial midbrain/pons GABAergic neurons in mice. *J Neurosci* **38**(47): 10080-10092. Doi: 10.1523/JNEUROSCI.0598-18.2018

Current understanding of the neuronal mechanisms and populations that regulate sleep–wake behavior is incomplete. Here, we identified a GABAergic ventral midbrain/pons area that is necessary for controlling the daily amount of sleep and wakefulness in mice. We also found that these inhibitory neurons control wakefulness by suppressing dopaminergic systems. Surprisingly, activation of these neurons strongly induced slow-wave sleep while suppressing wakefulness. Our study reveals a new brain mechanism critical for sleep–wake regulation

21. Honda T, Takata Y, Chérasse Y, Mizuno S, Sugiyama F, Takahashi S, Funato H, Yanagisawa M, Lazarus M, Oishi Y (2020) Ablation of Ventral Midbrain/Pons GABA Neurons Induces Mania-like Behaviors with Altered Sleep Homeostasis and Dopamine D2R-mediated Sleep Reduction. *iScience* **23**(6): 101240. Doi:10.1016/j.isci.2020.101240

Individuals with the neuropsychiatric disorder mania exhibit hyperactivity, elevated mood, and a decreased need for sleep. The brain areas and neuronal populations involved in mania-like behaviors, however, have not been elucidated. In this study, we found that ablating the ventral medial midbrain/pons (VMP) GABAergic neurons induced mania-like behaviors in mice, including hyperactivity, anti-depressive behaviors, reduced anxiety, increased risk-taking behaviors, distractibility, and an extremely shortened sleep time. Strikingly, these mice also showed no rebound sleep after sleep deprivation, suggesting abnormal sleep homeostatic regulation. Dopamine D2 receptor deficiency largely abolished the sleep reduction induced by ablating the VMP GABAergic neurons without affecting the hyperactivity and anti-depressive behaviors. Our data demonstrate that VMP GABAergic neurons are involved in the expression of mania-like behaviors, which can be segregated to the short-sleep and other phenotypes on the basis of the dopamine D2 receptors.

*** [10] Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors**

22. Wang Y, Cao L, Lee CY, Matsuo T, Wu K, Asher G, Tang L, Saitoh T, Russell J, Klewe-Nebenius D, Wang L, Soya S, Hasegawa E, Chérasse Y, Zhou J, Li Y, Wang T, Zhan X, Miyoshi C, Irukayama Y, Cao J, Meeks JP, Gautron L, Wang Z, Sakurai K, Funato H, Sakurai T, Yanagisawa M, Nagase H, Kobayakawa R, Kobayakawa K, Beutler B, Liu Q (2018) Large-scale forward genetics screening identifies Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors. *Nat Commun* **9**(1): 2041. Doi:10.1038/s41467-018-04324-3

Innate behaviors are genetically encoded, but their underlying molecular mechanisms remain largely unknown. We conducted a large-scale recessive genetics screen of ethylnitrosourea (ENU)-mutagenized mice. We found that loss of Trpa1, a pungency/irritancy receptor, diminishes fear/defensive behavior evoking predator odors and snake skin-evoked innate fear/defensive responses. Accordingly, *Trpa1*$^{-/-}$ mice fail to effectively activate known fear/stress brain centers upon 2MT exposure, despite their apparent ability to smell and learn to fear 2MT. Moreover, Trpa1 acts as a chemosensor for 2MT/TMT and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. Our results indicate that Trpa1-mediated nociception plays a crucial role in predator odor-evoked innate fear/defensive behaviors. This work establishes the first forward genetics screen to uncover the molecular mechanism of innate fear, a basic emotion and evolutionarily conserved survival mechanism.

*** [11] Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia**

*23. Liu C, Lee CY, Asher G, Cao L, Terakoshi Y, Cao P, Kobayakawa R, Kobayakawa K, Sakurai K, Liu Q (2021) Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia. *Nat Portfolio*. In press. Doi:10.21203/rs.3.rs-112564/v1

The neural mechanisms of fear-associated thermoregulation remain unclear. Innate fear odor 2-methyl-2-thiazoline (2MT) elicits rapid hypothermia and elevated tail temperature, indicative of vasodilation-induced heat dissipation, in wild-type mice, but not in mice lacking Trpa1–the chemosensor for 2MT. We found that *Trpa1*$^{-/-}$ mice show diminished 2MT-evoked c-fos expression in the posterior subthalamic nucleus (PSTh), external lateral parabrachial subnucleus (PBel) and nucleus of the solitary tract (NTS). Whereas inactivation of NTS-projecting PSTh neurons suppress, activation of direct PSTh-rostral NTS pathway induces hypothermia and tail vasodilation. Furthermore, selective stimulation of 2MT-activated, PSTh-projecting PBel neurons causes hypothermia. Conversely, suppression of PBel or PSTh, or PSTh-projecting PBel neurons attenuates 2MT-evoked hypothermia and tail vasodilation. This study identified PSTh as a major thermoregulatory hub connecting PBel to NTS to mediate 2MT-evoked innate fear-associated hypothermia and tail vasodilation.

[12] Intracellular signaling of sleep function

24. Bjorness TE, Kulkarni A, Rybalchenko V, Suzuki A, Bridges C, Harrington AJ, Cowan C, Takahashi J, Konopka G, Greene RW (2020). An essential role for MEF2C in the cortical response to loss of sleep in mice. *eLife* **9**. Doi:10.7554/eLife.58331

In this study, we examined neurobiological changes in the mouse frontal cortex in response to sleep loss, involving transcriptomic architecture, regulation of synaptic strength in excitatory cortical neurons and SWS-SWA, correlated with sleep need. As changes in neuronal activity may be observed across sleep-wake states, we investigated the possible role of MEF2C as a key regulator of sleep-related control of gene expression, sleep-related modulation of synaptic function and sleep-need correlated SWS-SWA buildup and resolution.

*25. Suzuki A, Yanagisawa M, Greene RW (2020) Loss of Arc attenuates the behavioral and molecular responses for sleep homeostasis in mice. *P Natl Acad Sci USA* **117**(19): 10547-10553. Doi:10.1073/pnas.1906840117

In this study, to investigate the mechanisms underlying the role of Arc in sleep homeostasis, we first evaluated the sleep phenotype in constitutive Arc knockout (KO) mice under baseline conditions, as well as in response to continuous 4-h total sleep deprivation (SD) and selective REM deprivation. Subsequently, we assessed Arc involvement in the associated molecular events that occur in response to SD: SD-induced sleep-related gene expression, synaptic GluA1 expression, and Arc nuclear distribution within the context of the homeostatic sleep response. We report that, in the absence of Arc, all of the sleep homeostatic responses were disrupted at both the behavioral and molecular levels.

*** [13] Cortical organization in waking and NREM sleep**

*26. Ohyama K, Kanda T, Miyazaki T, Tsujino N, Ishii R, Ishikawa Y, Muramoto H, Grenier F, Makino Y, McHugh TJ, Yanagisawa M, Greene RW, Vogt KE (2020) Structure of cortical network activity across natural wake and sleep states in mice. *PLOS One* **15**(5): e0233561. Doi:10.1371/journal.pone.0233561

NREM sleep is typically considered to be a low-activity cortical state, mainly used for energy preservation. We studied neural activity in somatosensory and motor cortex in freely behaving mice using tetrode recordings. We found that many neurons increase their overall activity in the transition from waking to NREM sleep.

Excitatory neurons also showed an increase in burst firing, which is considered particularly impactful. In-vivo two-photon imaging revealed larger peak calcium transients in NREM sleep compared to waking. Taken together this indicates an increased rather than decreased cortical activity in NREM sleep. When we analyzed the activity of multiple neurons recorded together, we found more predictable and organized patterns in waking, whereas in sleep the patterns were less organization and predictable. This shows that cortical activity in NREM does not simply recapitulate wake activity.
Our findings reveal that contrary to expectations, cortex is highly active during NREM sleep and that this activity is not merely replaying wake activity. Underscoring the urgent need for novel explanations of the role of cortical firing in NREM sleep.

*27. Matsumoto S, Ohyama K, Díaz J, Yanagisawa M, Greene RW, Vogt KE (2020) Enhanced cortical responsiveness during natural sleep in freely behaving mice. *Sci Rep* **10**: 2278. Doi:10.1038/s41598-020-59151-8

Most overt behavior is absent during NREM sleep. Cortical activity transitions from ongoing irregular action potential firing to rhythmic synchronized activity driven by rapid transitions between a hyperpolarized OFF state and a depolarized active ON state. We applied brief optogenetic stimuli to defined cortical inputs to determine the impact of these ON-OFF oscillations on cortical reactivity. In particular, it has been hypothesized that OFF states are due to opening of calcium dependent potassium channels, effectively silencing cortical neurons. Surprisingly we found much larger responses to optogenetic stimuli during NREM sleep compared to waking. The rapid change in response strength following state transitions is best explained by the change in the neuromodulatory environment that occurs with vigilance state transitions.
Stimuli that fell on OFF states generated even larger responses than stimuli that occurred during ON states – indicating that neurons can easily generate action potentials during OFF states.
Natural NREM sleep is accompanied by a significant functional rearrangement of cortical circuits, with strengthened functional connectivity. ON-OFF transitions do not affect the excitability of cortical neurons per-se and must therefore be explained by other mechanisms.

[14] Thalamic regulation of wake and sleep
28. Honjoh S, Sasai S, Schiereck SS, Nagai H, Tononi G, Cirelli C (2018) Regulation of cortical activity and arousal by the matrix cells of the ventromedial thalamic nucleus. *Nat Commun* **9**: 2100. Doi:10.1038/s41467-018-04497-x

The thalamus relays sensory information received from peripheral nerves to the cerebral cortex, where higher-order information processing is executed. Therefore, the thalamus has long been proposed to be involved in sleep/wake control. For example, the "passive sleep theory" proposed that the "fatigue" of the thalamic neurons due to frequent firing during wakefulness reduces the transmission of sensory information to the cerebral cortex, resulting in sleep. This study demonstrated that a specific neural subpopulation, matrix cells, plays an important role in the transition from non-REM sleep to arousal. In contrast, another neural subpopulation that plays critical roles in relay of sensory information from periphery, core cells, did not involve sleep/wake regulation. Therefore, this study addressed the long-standing issue in the field and provided the experimental evidence that a thalamic neural subpopulation promotes wakefulness. It also showed that reduced sensory inputs by itself can not account for sleep.

[15] A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila
29. Toda H, Williams JA, Gulledge M, Sehgal A (2019) A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila. *Science* **363**: 509-515. Doi:10.1126/science.aat1650

Drosophila served as a great model system by which many biological questions including development, immunity and circadian clock system have been answered. Two decades ago, Drosophila was proposed to be used for sleep research by measuring their behavior. Since then, using non-biased loss-of-function screen identified a variety of novel genetic components important to maintain sleep. However, little is known about the molecules instructive to sleep. To discover such molecules, authors carried out genome-wide and non-biased gain-of-function screen and discovered a novel gene, named '*nemuri*'. *nemuri* encodes anti-microbial peptide which kills bacteria. Upon infection, Drosophila sleep more but *nemuri* mutants showed significant reduction of infection-induced sleep, suggesting that Nemuri is a molecular link between sleep and immunity.

[16] Novel delta opioid receptor agonists with oxazatricyclodecane structure
30. Fujii H, Hayashida K, Saitoh A, Yokoyama A, Hirayama S, Iwai T, Nakata E, Nemoto T, Sudo Y, Uezono Y, Yamada M, Nagase H (2014) Novel delta opioid receptor agonists with oxazatricyclodecane structure. *ACS Med Chem Lett* **5**: 368–372. Doi:10.1021/ml400491k

The δ opioid receptor (DOR) is one of the opioid receptors and activation of the DOR is associated with various pharmacological effects such as antinociceptive, antidepressive, anxiolytic, and cardioprotective

effects. Nagase et al. synthesized the compounds containing the oxazatricyclodecane structure from a novel rearrangement reaction product. Those compounds exhibited full agonistic activities for the DOR. Among them, the *N*-methyl derivative was highly selective and the most effective DOR agonist in functional assays. Subcutaneous administration of the *N*-methyl derivative produced dose-dependent and NTI (selective DOR antagonist)-reversible antinociception without any convulsive behaviors in the mice acetic acid writhing tests. The *N*-methyl derivative is expected to be a promising lead compound for selective DOR agonists with a novel chemotype.

[17] Novel method for tracking vigilance decrement during sleep deprivation

31. Abe T, Mishima K, Kitamura S, Hida A, Inoue Y, Mizuno K, Kaida K, Nakazaki K, Motomura Y, Maruo K, Ohta T, Furukawa S, Dinges DF, Ogata K (2020) Tracking Intermediate Performance of Vigilant Attention Using Multiple Eye Metrics. *Sleep* **43**, zsz219. Doi:10.1093/sleep/zsz219.

Current techniques to detect vigilance impairment measure only the most severe level evident in eyelid closure and falling asleep, which is often too late to avoid an accident or error. Here, we show that the prevalence of microsaccades can be an early biometric of waning vigilance impairment when the eyes are open. Additionally, we developed an algorithm that detects multilevel vigilance by integrating the novel biometric with other eye-related indices. The new algorithm tracked a range of deficits in vigilant attention, including intermediate performance (i.e. 300–500 ms reaction times in the Psychomotor Vigilance Test), during prolonged time-on-task and sleep deprivation. The novel algorithm can be used to reduce human-error-related accidents caused by vigilance impairment even when its level is intermediate.

*** [18] Energy metabolism during sleep**

*32. Kayaba M, Park I, Iwayama K, Seya Y, Ogata H, Yajima K, Satoh M, Tokuyama K (2017) Energy metabolism differs between sleep stages and begins to increase prior to awakening. *Metabolis* **69**: 14–23. Doi:10.1016/j.metabol.2016.12.016.

Human sleep is generally consolidated into a single prolonged period, and its metabolic consequence is to impose an extended period of fasting. Changes in sleep stage and homeostatic sleep drive following sleep onset may also affect sleeping metabolic rate. The purpose of this study was to isolate the effects of sleep stage and time after sleep onset on sleeping metabolic rate. The effects of sleep stage and time after sleep onset on sleeping metabolic rate were evaluated using a semi-parametric regression analysis. Energy expenditure differed significantly between sleep stages: wake after sleep onset (WASO) > stage 2, slow wave sleep (SWS), and REM; stage 1 > stage 2 and SWS; and REM > SWS. Similarly, carbohydrate oxidation differed significantly between sleep stages: WASO > stage 2 and SWS; and stage 1 > SWS. Energy expenditure and carbohydrate oxidation decreased during the first half of sleep followed by an increase during the second half of sleep.

33. Zhang S, Osumi H, Uchizawa A, Hamada H, Park I, Suzuki Y, Tanaka Y, Ishihara A, Yajima K, Seol J, Satoh M, Omi N, Tokuyama K (2020) Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiol Rep* **8**: e14353. Doi:10.14814/phy2.14353.

Women with ovulatory menstrual cycles show an increase in body temperature in the luteal phase, compared with follicular phase, particularly during the night. Q_{10} of biological reactions lies between 2.0 and 3.0, predicting a 7-12% increase in energy expenditure when body temperature rises by 1℃. In this study, temperature dependence of energy expenditure was assessed by comparing changes in sleeping energy expenditure and thermoregulation with menstrual cycle in 9 young females. In the luteal phase, a significant increase in core body temperature (+0.27℃) and energy expenditure (+6.9%) were observed. The 6.9% increase in metabolic rate would require a Q_{10} of 12.4 to be attributable solely to temperature (+0.27℃), suggesting that energy expenditure in the luteal phase is enhanced through the mechanism, dependent and independent of luteal phase rise in body temperature presumably reflects other effects of the sex hormones.

*** [19] Effect of orexin receptor antagonist on sleep, sleeping energy metabolism and physical/cognitive functions**

*34. Seol J, Fujii Y, Park I, Suzuki Y, Kawana F, Yajima K, Fukusumi S, Okura T, Satoh M, Tokuyama K, Kokubo T, Yanagisawa M (2019) Distinct effects of orexin receptor antagonist and GABAA agonist on sleep and physical/cognitive functions after forced awakening. *P Natl Acad Sci USA* **116**: 24353-24358. Doi:10.1073/pnas.1907354116

Insomnia is a common symptom representing an important health burden. Widely prescribed hypnotic agents enhance the function of γ-aminobutyric acid (GAB_A), a major inhibitory neurotransmitter. The ability to arouse and respond to unexpected stimuli is a feature of normal sleep, and one of the concerns of this class of hypnotic agents is that patients may become physically and/or cognitively impaired while the drug is

in effect. As a new approach for the treatment of insomnia, orexin receptor antagonists have been recently approved, which specifically inhibit the orexin-mediated wake-promoting system, supposedly without affecting the whole brain. This double-blind, randomized, placebo-controlled, cross-over study found that, compared with the $GABA_A$ receptor agonist brotizolam, the orexin receptor antagonist suvorexant induced less impairment in body balance after taking the medicine.

[20] Cerebrospinal fluid orexin measurements in various disorders

*35. Kaushik MK, Aritake K, Chérasse Y, Imanishi A, Kanbayashi T, Urade Y, Yanagisawa M (2021) Induction of narcolepsy-like symptoms by orexin receptorantagonists in mice. *Sleep*: zsab043. Doi:10.1093/sleep/zsab043.

The orexin receptor antagonist suvorexant promotes sleep by blocking both OX1R and OX2R. Whereas suvorexant has been clinically approved for the treatment of insomnia because it is well tolerated in experimental animals as well as in human patients, a logical question remains as to why orexin receptor antagonists do not induce overt narcolepsy-like symptoms. Here we show that acute and chronic suvorexant promotes both REM and NREM sleep without inducing cataplexy in mice. When mice are chronically treated with suvorexant and then re-challenged with the antagonist after a 1-week washout, cataplexy and sleep-onset REM (SOREM) are observed, which are exacerbated by chocolate administration. These results suggest that suvorexant can inhibit orexin synthesis resulting in susceptibility to narcolepsy-like symptoms in mice under certain conditions.

36. Imanishi A, Kawazoe T, Hamada Y, Kumagai T, Tsutsui K, Sakai N, Eto K, Noguchi A, Shimizu T, Takahashi T, Han G, Mishima K, Kanbayashi T, Kondo H (2020) Early detection of Niemann-pick disease type C with cataplexy and orexin levels: continuous observation with and without Miglustat. *Orphanet J Rare Dis* **15**(1): 269. Doi:10.1186/s13023-020-01531-4

Niemann-Pick type C (NPC) is a hereditary disorder that causes symptomatic narcolepsy and is attributed to impaired cholesterol metabolism. Although there was no effective treatment, miglustat has been approved as a treatment in recent years. In this report, narcolepsy causes cataplexy at a low value of 110 pg / ml or less, but NPCs have seizures even at a decrease of 200 pg / ml or less (about 300 pg / ml in healthy subjects). In addition, orexin gradually decreased when untreated, but recovery was observed when miglustat was used, and improvement of cataplexy was also observed. Early diagnosis is important for a good prognosis, but if cataplexy develops in early childhood between the ages of 2 and 10, NPCs are likely, so suspect cataplexy and measure orexin. It is important to use it together to lead to early diagnosis.

*37. Kaushik MK, Aritake K, Imanishi A, Kanbayashi T, Ichikawa T, Shimizu T, Urade Y, Yanagisawa M (2018) Continuous intrathecal orexin delivery inhibits cataplexy in a murine model of narcolepsy. *P Natl Acad Sci USA* **115**(23): 6046-6051. Doi:10.1073/pnas.1722686115.

Human narcolepsy is treated by providing symptomatic therapies, which can be associated with an array of side effects. Although peripherally administered orexin does not efficiently penetrate the blood-brain barrier, centrally delivered orexin can effectively alleviate narcoleptic symptoms in animal models. Chronic intrathecal drug infusion through an implantable pump is a clinically available strategy to treat a number of neurological diseases. Here we demonstrate that the narcoleptic symptoms of orexin knockout mice can be reversed by lumbar-level intrathecal orexin delivery. Intrathecally delivered orexin was detected in the brain by radioimmunoassay at levels comparable to endogenous orexin levels. Cataplexy and sleep-onset REM sleep were significantly decreased in orexin knockout mice during and long after slow infusion of orexin. Sleep/wake states remained unchanged both quantitatively as well as qualitatively. This study supports the concept of intrathecal orexin delivery as a potential therapy for narcolepsy-cataplexy to improve the well-being of patients.

Appendix 1-2 List of Papers of Representative of Interdisciplinary Research Activities

* List **up to 20 papers** underscoring each interdisciplinary research activity and give brief accounts (within 10 lines).
* For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is the same. If a paper has many authors, underline those affiliated with the Center.
* If a paper has many authors (say, more than 10), all of their names do not need to be listed.

1. Yamabe M, Horie K, Shiokawa H, Funato H, Yanagisawa M, Kitagawa H (2019) MC-SleepNet: Large-scale sleep stage scoring in mice by deep neural networks. *Sci Rep* **9**: 15793. Doi:10.1038/s41598-019-51269-8

Automated sleep stage scoring for mice is in high demand for sleep research, since manual scoring requires considerable human expertise and efforts. The existing automated scoring methods do not provide the scoring accuracy required for practical use. This research proposes a novel automated scoring method named "MC-SleepNet", which combines two types of deep neural networks. The experimental results show that MC-SleepNet can automatically score sleep stages with an accuracy of 96.6%. We confirm that the scoring accuracy does not significantly decrease even if the target biological signals are noisy. These results suggest that MC-SleepNet is very robust against individual differences and noise. To the best of our knowledge, evaluations using such a large-scale dataset and high scoring accuracy have not been reported in previous related studies

2. Park I, Díaz J, Matsumoto S, Iwayama K, Nabekura Y, Ogata H, Kayaba M, Aoyagi A, Yajima K, Satoh M, Tokuyama K, Vogt KE (2021) Exercise improves the quality of slow-wave sleep by increasing slow-wave stability. *Sci Rep* **11**: 4410. Doi:10.1038/s41598-021-83817-6

The impact of exercise on sleep is still debated. Some studies found increased sleep quality after exercise, others found detrimental effects. This controversy is in part due to the divergent methodology. We used subjective and objective measures to assess sleep quality and found that subjective assessment was not positive, whereas objective EEG-derived parameters showed an improved sleep, especially in the early phases. We used a novel analytical tool to measure both the power of oscillations in the delta (0.5-4 Hz) band and its temporal stability. Stability was assessed using the coefficient of variation of the envelope (CVE). Low CVE values are found with stable sinusoidal oscillations, high values indicate large, sharp transients in in EEG power. Such large transients are found during instable sleep phases e.g. in the transition between sleep states. Exercise before sleep significantly reduced CVE values, indicating a more stable EEG. Together with higher delta power this shows that exercise can improve objective measures of sleep that may not be revealed in the subjective assessment of sleep quality.

3. Miyazaki S, Leproux P, Couderc V, Hayashi Y, Kano H (2020) Multimodal nonlinear optical imaging of Caenorhabditis elegans with multiplex coherent anti-Stokes Raman scattering, third-harmonic generation, second-harmonic generation, and two-photon excitation fluorescence. *Appl Phys Express* **13**:072002-1-5. Doi:10.35848/1882-0786/ab9711

In a collaboration led by Dr. Hideaki Kano (Department of Chemistry, Kyusyu Univ.), the Hayashi lab applied nonlinear optical microscopy to the nematode Caenorhabditis elegans. Using coherent anti-Stokes Raman scattering, second harmonic generation, and third harmonic generation, information on molecular properties was obtained without the usage of any labels such as genetic or chemical probes. In particular, the group succeeded in visualizing neurons in vivo. This new method allows the exploration of the molecular changes that accompany changes in external or internal conditions, such as during sleep in label-free conditions. The nonlinear optical microscope is expected to be applicable to other types of biological samples such as mouse brain slices. Thus, it will be a powerful tool for assessing molecular dynamics in the brain.

4. Srinivasan S, Hosokawa T, Vergara P, Chérasse Y, Naoi T, Sakurai T, Sakaguchi M (2019) Miniaturized microscope with flexible light source input for neuronal imaging and manipulation in

freely behaving animals. *Biochem Bioph Res Co* **517**:520-4. Doi:10.1016/j.bbrc.2019.07.082

Simultaneous imaging and manipulation of a genetically defined neuronal population can provide a causal link between its activity and function. Here, we designed a miniaturized microscope (or 'miniscope') that allows fluorescence imaging and optogenetic manipulation at the cellular level in freely behaving animals. This miniscope has an integrated optical connector that accepts any combination of external light sources, allowing flexibility in the choice of sensors and manipulators. Using this miniscope, we demonstrate the optogenetic silencing of hippocampal CA1 neurons using two laser light sources—one stimulating a calcium sensor and the other serving as an optogenetic silencer. This new miniscope can contribute to efforts to determine causal relationships between neuronal network dynamics and animal behavior.

5. Sakaguchi M, Kim K, Yu LMY, Hashikawa Y, Sekine Y, Okumura Y, Kawano M, Hayashi M, Kumar D, Boyden ES, McHugh TJ, Hayashi Y (2015) Inhibiting the activity of CA1 hippocampal neurons prevents the recall of contextual fear memory in inducible ArchT transgenic mice. *Plos ONE* **10**(6): e0130163. Doi:10.1371/journal.pone.0130163

The optogenetic manipulation of light-activated ion-channels/pumps (i.e., opsins) can reversibly activate or suppress neuronal activity with precise temporal control. Therefore, optogenetic techniques hold great potential to establish causal relationships between specific neuronal circuits and their function in freely moving animals. We established a transgenic mouse line in which tetracycline trans-activator induces ArchT expression. By crossing this line with a CaMKIIα-tTA transgenic line, the delivery of light via an implanted optrode inhibits the activity of excitatory CA1 neurons. We found that light delivery to the hippocampus inhibited the recall of a contextual fear memory. Our results demonstrate that this optogenetic mouse line can be used to investigate the neuronal circuits underlying behavior.

6. Purple R, Sakurai T, Sakaguchi M (2017) Auditory conditioned stimulus presentation during NREM sleep impairs fear memory in mice. *Sci Rep* **7**:46247. DOI:10.1038/srep46247

Externally manipulating memories by presenting a memory-associated sound during sleep is a new approach to investigating memory processing during sleep. However, whether presenting the sound during REM or NREM sleep enhances or extinguishes fear memory has not been clearly delineated. In this study, mice underwent trace fear conditioning consisting of a sound paired with a foot shock, and the sound was re-presented during subsequent REM or NREM sleep. Mice that received the sound stimulation during NREM but not REM sleep showed impaired fear memory upon later presentation of the sound. These findings have implications for the use of cueing during sleep and advance our understanding of the role of REM and NREM sleep in memory consolidation.

7. Kutsumura N, Koyama Y, Saitoh T, Yamamoto N, Nagumo Y, Miyata Y, Hokari R, Ishiyama A, Iwatsuki N, Otoguro K, Ōmura S, Nagase H (2020) Structure-Activity Relationship between Thiol Group-Trapping Ability of Morphinan Compounds with a Michael Acceptor and Anti-Plasmodium falciparum Activities. *Molecules* **25**(5): 1112. Doi:10.3390/molecules25051112.

7-Benzylidenenaltrexone (BNTX) and most of its derivatives showed *in vitro* antimalarial activities against chloroquine-resistant and -sensitive *Plasmodium falciparum* strains. In addition, the time-dependent changes of the addition reactions of the BNTX derivatives with 1-propanethiol were examined by ^{1}H-NMR experiments to estimate their thiol group-trapping ability. The relative chemical reactivity of the BNTX derivatives to trap the thiol group of 1-propanethiol was correlated highly with the antimalarial activity. Therefore, the measurements of the thiol group-trapping ability of the BNTX derivatives with a Michael acceptor is expected to become an alternative method for *in vitro* malarial activity and related assays.

8. Yamamoto N, Ohrui S, Okada T, Saitoh T, Kutsumura N, Nagumo Y, Irukayama-Tomobe Y, Ogawa Y, Ishikawa Y, Watanabe Y, Hayakawa D, Gouda H, Yanagisawa M, Nagase H (2019) Essential

structure of orexin 1 receptor antagonist YNT-707, part III: Role of the 14-hydroxy and the 3-methoxy groups in antagonistic activity toward the orexin 1 receptor in YNT-707 derivatives lacking the 4,5-epoxy ring. *Bioorg Med Chem* **27**(8): 1747-1758. Doi:10.1016/j.bmc.2019.03.010.

Morphinan derivatives lacking the 4,5-epoxy ring were synthesized to examine the participation of the 14-OH group, the 3-OMe group, and the aromaticity of the A-ring in the activity and selectivity for the OX_1R. The assay results and the conformational analyses of the 14-dehydrated and 14-H derivatives suggested that the orientations of the 6-amide side chain and the 17-benzenesulfonyl group would play important roles in the activity for OX_1R. In the 6β-derivatives, removal of the 3-OMe group and the reduction of the A-ring significantly decreased the activity toward the OX_1R, but these changes did not affect the 6α-derivatives. These results indicate that the 3-OMe group and the A-ring would be essential structural moieties for the 6β-derivatives.

9. Ohrui S, Yamamoto N, Saitoh T, Kutsumura N, Nagumo Y, Irukayama-Tomobe Y, Ogawa Y, Ishikawa Y, Watanabe Y, Hayakawa D, Gouda H, Yanagisawa M, Nagase H (2018) Essential structure of orexin 1 receptor antagonist YNT-707, Part II: Drastic effect of the 14-hydroxy group on the orexin 1 receptor antagonistic activity. *Bioorg Med Chem Lett* **28**(4): 774-777. Doi:10.1016/j.bmcl.2017.12.069.

The 14-dehydration- and 14-H derivatives of OX_1R antagonist YNT-707 were synthesized. The obtained derivatives showed higher affinities for OX_1R than the corresponding 14-hydroxy derivatives. The conformational analysis suggested that the 17-sulfonamide groups in the derivatives without the 14-hydroxy group have a greater tendency to be oriented toward the upper side of the D-ring compared with the 14-hydroxy derivatives. Additionally, the 14-dehydration-derivative with 6α-amide side chain showed significantly higher affinity than the 14-hydroxy derivative, while the corresponding 14-H derivative showed only slightly higher affinity. Thus, the 14-hydroxy group strongly affects the affinity of the antagonist for the OX_1R.

10. Nagumo Y, Katoh K, Saitoh T, Kutsumura N, Yamamoto N, Ishikawa Y, Irukayama-Tomobe Y, Ogawa Y, Tanimura R, Yanagisawa M, Nagase H (2020) Discovery of attenuation effect of orexin 1 receptor to aversion of nalfurafine: Synthesis and evaluation of D-nor-nalfurafine derivatives and analyses of the three active conformations of nalfurafine. *Bioorg Med Chem Lett* **30**(17): 127360. Doi:10.1016/j.bmcl.2020.127360

Nagase et al. reported that nalfurafine, κ opioid receptor (KOR) agonist showed a selective antagonist activity toward the OX_1R (K_i = 250 nM) in 2017. However, the D-nor-nalfurafine derivatives, which were synthesized by contraction of the six-membered D-ring in nalfurafine, had no affinity for OX_1R. The 17*N*-lone electron pair in nalfurafine oriented toward the axial direction, while that of D-nor-derivatives was directed in the equatorial configuration. The axial lone pair can form a hydrogen bond with the 14-hydroxy group, which could push the 6-amide side chain toward the downward direction with respect to the C-ring. Nagase et al. reported that the resulting conformation would be an active conformation for binding with OX_1R.

11. Nagase H, Yamamoto N, Yata M, Ohrui S, Okada T, Saitoh T, Kutsumura N, Nagumo Y, Irukayama-Tomobe Y, Ishikawa Y, Ogawa Y, Hirayama S, Kuroda D, Watanabe Y, Gouda H, Yanagisawa M (2017) Design and Synthesis of Potent and Highly Selective Orexin 1 Receptor Antagonists with a Morphinan Skeleton and Their Pharmacologies. *J Med Chem* **60**(3): 1018-1040. Doi:10.1021/acs.jmedchem.6b01418.

Nalfurafine, a κ-selective opioid receptor (KOR) agonist, unexpectedly showed a selective antagonist activity toward the OX_1R (K_i = 250 nM). Modification of the 17-amino side chain of the opioid ligand to an arylsulfonyl group and the 6-furan acrylamide chain to 2-pyridyl acrylamide led to a morphinan derivative YNT-1310 with improvement of the antagonist activity (OX_1R, K_i = 1.36 nM; OX_2R, not active) without any detectable affinity for the opioid receptor. The dihydrosulfate salt of YNT-1310, freely soluble in water, attenuated the physical dependence of morphine. Furthermore, all of the active

nalfurafine derivatives in this study had almost no activity for OX_2R, which led to high OX_1R selectivity. These results suggest that nalfurafine derivatives could be a useful series of lead compounds to develop highly selective OX_1R antagonists.

12. Irukayama-Tomobe Y, Ogawa Y, Tominaga H, Ishikawa Y, Hosokawa N, Ambai S, Kawabe Y, Uchida S, Nakajima R, Saitoh T, Kanda T, Vogt K, Sakurai T, Nagase H, Yanagisawa M (2017) Nonpeptide orexin type-2receptor agonist ameliorates narcolepsy-cataplexy symptoms in mouse models. *P Natl Acad Sci USA* **114**(22): 5731-5736. Doi:10.1073/pnas.1700499114.

Narcolepsy-cataplexy is a debilitating disorder characterized by excessive daytime sleepiness (sleep attacks) and cataplexy, a sudden bilateral loss of muscle tone often triggered by emotion. The disease is caused by a selective loss of hypothalamic neurons producing the neuropeptide orexin. Currently, only symptomatic therapies are available for narcolepsy. Here, we examine the pharmacological effect of YNT-185, a nonpeptide, selective agonist for the orexin type-2 receptor in mouse models of narcolepsy-cataplexy. We show that peripheral administration of YNT-185 significantly ameliorates the narcolepsy symptoms in model mice, providing a proof-of-concept for the mechanistic treatment of narcolepsy with orexin receptor agonists. YNT-185 also promotes wakefulness in wild-type mice, suggesting that orexin receptor agonists may be useful for treating sleepiness due to other causes.

13. Nagahara T, Saitoh T, Kutsumura N, Irukayama-Tomobe Y, Ogawa Y, Kuroda D, Gouda H, Kumagai H, Fujii H, Yanagisawa M, Nagase H (2015) Design and Synthesis of Non-Peptide, Selective Orexin Receptor 2 Agonists. *J Med Chem* **58**(20), 7931-7937. Doi:10.1032/acs.jmedchem.5b00988.

Orexins are a family of neuropeptides that regulate sleep/wakefulness, acting on two G-protein-coupled receptors, OX_1R and OX_2R. Genetic and pharmacologic evidence suggests that orexin receptor agonists, especially OX_2R agonist, will be useful for mechanistic therapy of the sleep disorder narcolepsy/cataplexy. We herein report the discovery of a potent (EC_{50} on OX_2R is 0.023 μM) and OX_2R-selective (OX_1R/OX_2R EC_{50} ratio is 70) agonist, 4′-methoxy-*N*,*N*-dimethyl-3′-[*N*-(3-{[2-(3-methylbenzamido)ethyl]amino}phenyl)sulfamoyl]-(1,1′-biphenyl)-3-carboxamide, YNT-185. This paper was selected as "Featured Article" in 2015 and also selected as "Highly Read Article of 2015 (J. Med. Chem.)" in 2017.

14. Korkutata M, Saitoh T, Cherasse Y, Ioka S, Duo F, Qin R, Murakoshi N, Fujii S, Zhou XZ, Sugiyama F, Chen JF, Kumagai H, Nagase H, Lazarus M (2019) Enhancing endogenous adenosine A2A receptor signaling induces slow-wave sleep without affecting body temperature and cardiovascular function. *Neuropharmacology* **144**:122-132. Doi:10.1016/j.neuropharm.2018.10.022

Insomnia is one of the major sleep problems with an estimated prevalence of 10% to 15% in the general population. Moreover, insomnia frequently co-occurs with a wide range of psychiatric disorders, including depression and anorexia. The most widely prescribed agents for the treatment of insomnia are benzodiazepines and non-benzodiazepines, which are plagued by a wide range of adverse effects. In recent years, remarkable progress has been made in the discovery of ligands that act at allosteric sites to regulate receptor function with more selectivity. On the basis of highly collaborative research, the Lazarus/Oishi lab (IIIS Systems Pharmacology) and Nagase/Kutsumura lab (IIIS Medicinal Chemistry) succeeded in identifying the first positive allosteric modulator for adenosine A_{2A} receptors ($A_{2A}R$) that induces natural sleep. Allosterically enhancing $A_{2A}R$ signaling may provide a new therapeutic avenue for treating insomnia. This possibility is now vigorously pursued by the Lazarus/Oishi and Nagase/Kutsumura labs.

15. Wang Y, Cao L, Lee CY, Matsuo T, Wu K, Asher G, Tang L, Saitoh T, Russell J, Klewe-Nebenius D, Wang L, Soya S, Hasegawa E, Chérasse Y, Zhou J, Li Y, Wang T, Zhan X, Miyoshi C, Irukayama Y, Cao J, Meeks JP, Gautron L, Wang Z, Sakurai K, Funato H, Sakurai T, Yanagisawa M, Nagase H, Kobayakawa R, Kobayakawa K, Beutler B, Liu Q (2018) Large-scale forward genetics screening identifies Trpa1 as a chemosensor for predator odor-evoked innate fear behaviors. *Nat Commun*

9:2041. Doi:10.1038/s41467-018-04324-3

Innate behaviors are genetically encoded, but their underlying molecular mechanisms remain largely unknown. Predator odor 2,4,5-trimethyl-3-thiazoline (TMT) and its potent analog 2-methyl-2-thiazoline (2MT) are believed to activate specific odorant receptors to elicit innate fear/defensive behaviors in naive mice. Liu/Sakurai lab conducted a large-scale recessive genetics screen of ethylnitrosourea (ENU)-mutagenized mice and identified that loss of Trpa1, a pungency/irritancy receptor, diminished TMT/2MT and snake skin-evoked innate fear/defensive responses. Accordingly, *Trpa1* knockout mice failed to effectively activate known fear/stress brain centers upon 2MT exposure, despite their apparent ability to smell and learn to fear 2MT. Moreover, Trpa1 could act as a chemosensor for 2MT/TMT and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. These results indicate that Trpa1-mediated nociception plays a crucial role in predator odor-evoked innate fear/defensive behaviors. The work establishes the first forward genetics screen to uncover the molecular mechanism of innate fear, a basic emotion and evolutionarily conserved survival mechanism.

16. Matsuo T, Isosaka T, Hayashi Y, Tang L, Doi A, Yasuda A, Hayashi M, Lee CY, Cao L, Kutsuna N, Matsunaga S, Matsuda T, Yao I, Setou M, Kanagawa D, Higasa K, Ikawa M, Liu Q, Kobayakawa R, Kobayakawa K (2021) TRPA1 commands intrinsic bioprotective effects of innate fear odors. *Nat Commun* **12**: 2074. Doi:10.1038/s41467-021-22205-0

Innate behaviors are genetically encoded, but their underlying molecular mechanisms remain largely unknown. We conducted a large-scale recessive genetics screen of ethylnitrosourea (ENU)-mutagenized mice. We found that loss of Trpa1, a pungency/irritancy receptor, diminishes fear/defensive behavior evoking predator odors and snake skin-evoked innate fear/defensive responses. Accordingly, Trpa1−/− mice fail to effectively activate known fear/stress brain centers upon 2MT exposure, despite their apparent ability to smell and learn to fear 2MT. Moreover, Trpa1 acts as a chemosensor for 2MT/TMT and Trpa1-expressing trigeminal ganglion neurons contribute critically to 2MT-evoked freezing. Our results indicate that Trpa1-mediated nociception plays a crucial role in predator odor-evoked innate fear/defensive behaviors. This work establishes the first forward genetics screen to uncover the molecular mechanism of innate fear, a basic emotion and evolutionarily conserved survival mechanism.

17. Liu C, Lee CY, Asher G, Cao L, Terakoshi Y, Cao P, Kobayakawa R, Kobayakawa K, Sakurai K, Liu Q (2021) Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia. *Nat Portfolio*. In press. Doi:10.21203/rs.3.rs-112564/v1

The neural mechanisms of fear-associated thermoregulation remain unclear. Innate fear odor 2-methyl-2-thiazoline (2MT) elicits rapid hypothermia and elevated tail temperature, indicative of vasodilation-induced heat dissipation, in wild-type mice, but not in mice lacking Trpa1–the chemosensor for 2MT. We found that Trpa1-/- mice show diminished 2MT-evoked c-fos expression in the posterior subthalamic nucleus (PSTh), external lateral parabrachial subnucleus (PBel) and nucleus of the solitary tract (NTS). Whereas inactivation of NTS-projecting PSTh neurons suppress, activation of direct PSTh-rostral NTS pathway induces hypothermia and tail vasodilation. Furthermore, selective stimulation of 2MT-activated, PSTh-projecting PBel neurons causes hypothermia. Conversely, suppression of PBel or PSTh, or PSTh-projecting PBel neurons attenuates 2MT-evoked hypothermia and tail vasodilation. This study identified PSTh as a major thermoregulatory hub connecting PBel to NTS to mediate 2MT-evoked innate fear-associated hypothermia and tail vasodilation.

18. Park I, Ochiai R, Ogata H, Kayaba M, Hari S, Hibi M, Katsuragi Y, Satoh M, Tokuyama K (2017) Effects of subacute ingestion of chlorogenic acids on sleep architecture and energy metabolism through activity of the autonomic nervous system: a randomised, placebo-controlled, double-blinded crossover trial. *Brit J Nutr* **117**: 979-984. Doi:10.1017/S0007114517000587

Chlorogenic acids (CGA) are the most abundant polyphenols in coffee. Continuous consumption of CGA reduces body fat and body weight. Since energy metabolism and sleep are controlled by common regulatory factors, consumption of CGA might modulate sleep. Nine healthy subjects completed a

placebo-controlled, double-blinded, cross-over intervention study, during which subjects consumed a test beverage containing 0 or 600 mg of CGA for 5 days. On the fifth night, indirect calorimetry and polysomnographic sleep recording were performed. A period of 5 days CGA consumption significantly increased fat oxidation during sleep, suggesting that beverages containing CGA may be beneficial to reduce body fat and prevent obesity. Consumption of CGA shortened sleep latency and did not adversely affect sleep quality.

19. Zhang S, Takano J, Murayama N, Tominaga M, Abe T, Park I, Seol J, Ishihara A, Tanaka Y, Yajima K, Suzuki Y, Suzuki C, Fukusumi S, Yanagisawa M, Kokubo T, Tokuyama K (2020) Subacute ingestion of caffeine and oolong tea increases fat oxidation without affecting energy expenditure and sleep architecture: a randomized, placebo-controlled, double-blinded cross-over trial. *Nutrients* **12**(12):3671. Doi: 10.3390/nu12123671

Ingesting oolong tea or caffeine acutely increases energy expenditure, and oolong tea, but not caffeine, stimulates fat oxidation. The acute effects of caffeine, such as increased heart rate and interference with sleep, diminish over 1–4 days, known as caffeine tolerance. During each 14-day session of the present study, 12 subjects consumed oolong tea (100 mg caffeine, 21.4 mg gallic acid, 97 mg catechins and 125 mg polymerized polyphenol), caffeine (100 mg), or placebo. On day 14 of each session, 24-h indirect calorimetry and polysomnographic sleep recording were performed. Caffeine or oolong tea ingestion increased fat oxidation without interfering sleep. The effects of subacute ingestion of caffeine and oolong tea differed from the acute effects, which is a particularly important consideration regarding habitual tea consumption.

20. Monma T, Ando A, Asanuma T, Yoshitake Y, Yoshida G, Miyazawa T, Ebine N, Takeda S, Omi N, Satoh M, Tokuyama K, Takeda F (2018) Sleep disorder risk factors among student athletes. *Sleep Med* **44**:76-81. Doi:10.1016/j.sleep.2017.11.1130

To clarify sleep disorder risk factors among student athletes, this study examined the relationship between lifestyle habits, competition activities, psychological distress, and sleep disorders in 906 student athletes. Survey items were attributes (age, gender, and body mass index), sleep disorders (Pittsburgh Sleep Quality Index), lifestyle habits (bedtime, wake-up time, smoking, drinking alcohol, meals, part-time jobs, and use of electronics after lights out), competition activities (activity contents and competition stressors), and psychological distress. Multivariate logistic regression analysis showed that "bedtime," "wake-up time," "psychological distress," "part-time jobs," "smartphone/cellphone use after lights out," "morning practices," and "motivation loss stressors," were risk factors that were independently related to sleep disorders. This study suggests the importance of improving these lifestyle habits, mental health, and competition activities.

Appendix 1-3
Major Awards, Invited Lectures, Plenary Addresses (etc.) (within 2 pages)

*Prepare the information below during the period from the start of the center through March 2021.

1. Major Awards

*List main internationally-acclaimed awards received/unofficially announced in order from the most recent.
*For each, write the recipient's name, the name of award, and the date issued.
In case of multiple recipients, underline those affiliated with the center.

Date	Recipient's name	Name of award
Feb 22, 2021	Arisa Hirano	The Young Scientists' Award (Ministry of Education, Culture, Sports, Science and Technology)
Jan 28, 2021	Toru Takahashi	JSPS Ikushi Prize (Japan Society for the Promotion of Science)
Dec 11, 2020	Emi Morita	Japan Wood Design Award 2020 (Forestry Agency)
Oct 15, 2020	Yasutaka Niwa	Healthy Longevity Global Grand Challenge Catalyst Award (US National Academy of Medicine)
Oct 15, 2020	Yu Hayashi	Healthy Longevity Global Grand Challenge Catalyst Award (US National Academy of Medicine)
Apr 7, 2020	Sakiko Honjoh	The Young Scientists' Award (Ministry of Education, Culture, Sports, Science and Technology)
Nov 6, 2019	Masashi Yanagisawa	Ibaraki Prefecture Honor Award (Ibaraki Prefecture)
Oct 29, 2019	Masashi Yanagisawa	Person of Cultural Merit (Ministry of Education, Culture, Sports, Science and Technology)
Aug 2, 2019	Yu Hayashi	Special Prize, The Frontier Salon Nagase Prize (The Frontier Salon Foundation)
Jun 21, 2019	Masashi Yanagisawa	Takamine Memorial Daiichi Sankyo Prize (Daiichi Sankyo Foundation of Life Science)
May 27, 2019	Yuki Saito	Encouraging Prize (Japanese Society of Sleep Research)
May 18, 2019	Masashi Yanagisawa	Research Award (European Narcolepsy Network)
Sep 13, 2018	Masashi Yanagisawa	The Keio Medical Science Prize (Keio University Medical Science Fund)
May 30, 2018	Yo Oishi	The Encouraging Prize (Japanese Society of Sleep Research)
Jan 4, 2018	Masashi Yanagisawa	The Asahi Prize (The Asahi Shimbun Company)
Nov 30,2017	Masashi Yanagisawa, Hiromasa Funato	Erwin von Bälz Preis (Boehringer Ingelheim Japan, Inc.)
Jul 10, 2017	Takeshi Sakurai	The Shiono Prize (The Cell Science Research Foundation)
Apr 11, 2017	Yu Hayashi	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology The Young Scientists' Prize (Ministry of Education, Culture, Sports, Science and Technology)
Nov 22, 2016	Yu Hayashi	The Tsukuba Encouragement Prize (The Science and Technology Promotion Foundation of Ibaraki)
Oct 25, 2016	Joseph Takahashi	Peter C. Farrell Prize in Sleep Medicine (Harvard Medical School Division of Sleep Medicine)
May 13, 2016	Yu Hayashi	The Encouraging Prize (Japanese Society of Sleep Research)
Apr 28, 2016	Masashi Yanagisawa	Medal with Purple Ribbon (Cabinet Office, Government of Japan)
Oct 6, 2015	Akiyoshi Fukamizu	Jokichi Takamine Memorial Award (The Society of Cardiovascular Endocrinology and Metabolism)
Mar 28, 2015	Masashi Yanagisawa	The Walter B. Cannon Memorial Award (American Physiological Society)
Sep 24, 2014	Hiroshi Nagase	Yamazaki Teiichi Prize (Foundation for Promotion of Material Science and Technology of Japan)
Nov 26, 2013	Junichi Hayashi	Tsukuba Award (The Science and Technology Promotion Foundation of Ibaraki)
Nov 22, 2013	Masashi Yanagisawa	Jokichi Takamine Memorial Award (The Society of Cardiovascular Endocrinology and Metabolism)
Jun 18, 2013	Hiroshi Nagase	National Commendation for Invention –the Invention Prize (Japan Institute of Invention and Innovation)
Mar 22, 2013	Hiroshi Nagase	Okochi Memorial Technology Prize (Okochi Memorial Foundation)
Apr 1, 2013	Takeshi Sakurai	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (Ministry of Education, Culture, Sports, Science and Technology)

2. Invited Lectures, Plenary Addresses (etc.) at International Conferences and International Research Meetings

*List up to 20 main presentations in order from most recent.
*For each, write the lecturer/presenter's name, presentation title, conference name and date(s)

Date(s)	Lecturer/Presenter's name	Presentation title	Conference name
Feb 10, 2021	Takeshi Sakurai	A novel neuronal circuit that induces hibernation-like state in rodents	The 7th Neuroscience Network, Diversity of Brain Science
Nov 20, 2020	Yanagisawa Masashi	Toward the Mystery of Sleep	The Inaugural International Symposium on Aging Research [ISAR]-Achieving Productive Aging
Oct 26-29, 2020	Takeshi Sakurai	A neuronal circuit that induces regulated hypometabolism in mice	virtual 8th International Conference on the Physiology and Pharmacology of Temperature Regulation (vPPTR 2020)
Sep 22-24, 2020	Qinghua Liu	Molecular Substrates of Homeostatic Sleep Regulation	25th Congress of the European Sleep Research Society (ESRS) virtual meeting
Dec 4, 2019	Takeshi Sakurai	Keynote Lecture: Neural circuits that link the limbic system and systems that regulate arousal	IRC* Decoding Sleep Symposium
Oct 23, 2019	Hiroshi Nagase	Opening Lecture: Design and synthesis of orexin receptor selective ligands and their pharmacology	Novel Pain Therapeutics: From Basic Research to Clinical Translation and Rehabilitation
Sep 24, 2019	Masashi Yanagisawa	Keynote Lecture: Toward the mysteries of sleep	World Sleep 2019
Sep 14, 2019	Takeshi Sakurai	Keynote Lecture: The neuronal circuitry of narcolepsy/ cataplexy	Workshop on Sleep Regulation and Circadian Rhythms
Aug 26, 2019	Yu Hayashi	Identifications of neurons regulating REM sleep & insights to the mechanisms of REM sleep behavior disorder	XVI Congress of the European Biological Rhythms Society
May 19, 2019	Masashi Yanagisawa	Keynote Lecture: Toward the mysteries of sleep	10th European Narcolepsy Day
Nov 10, 2018	Masashi Yanagisawa	Keynote Lecture: "Toward the Mysteries of Sleep/ What makes you sleepy? Elusive molecular substrates for homeostatic sleep need"	The 3rd Japan-US Science Forum in Boston 2018/Sleep Grand Rounds
Sep 28, 2018	Qinghua Liu	"Quantitative phosphoproteomic analysis of the molecular substrates of sleep need"	The 24th Congress of the European Sleep Research Society
Sep 11, 2018	Masashi Yanagisawa	Keynote Lecture: "Orexin agonism as a potential mechanistic therapy for narcolepsy/ cataplexy"	7th International Narcolepsy Symposium
Jul 7 11, 2018	Michael Lazarus	"The link between REM sleep loss and the desire for junk food"	11th FENS Forum of Neuroscience
Jul 7 11, 2018	Yu Hayashi	"The mystery of rapid eye movement sleep: New circuits and insights"	11th FENS Forum of Neuroscience
Mar 18-23, 2018	Qinghua Liu	Plenary Lecture: "Cumulative Phosphorylation of SNIPPs as a Function of Sleep Need"	Gordon Research Conference on Sleep Regulation and Function
Oct 4-7, 2017	Masashi Yanagisawa	Special Lecture: "Highlights of ET-15"	15th International Conference on Endothelin (Prague, Czech)
May 19-21, 2017	Takeshi Sakurai	Plenary Speaker: "The Mechanism of Narcolepsy: what it tells on clinical perspectives?"	The 2nd International Taiwanese Congress of Neurology
Aug 30, 2016	Hiroshi Nagase	"The science and the development of non-addictive opioid receptor agonists"	76th FIP World Congress of Pharmacy and Pharmaceutical Sciences 2016
Jun 8, 2015	Masashi Yanagisawa	Plenary Session: "Toward solving the mystery of sleep: From reverse genetics to forward genetics in mice"	Sleep2015

Appendix 1-4 2020 List of Center's Research Results

Refereed Papers

- List only the Center's papers published in 2020. (Note: The list should be for the calendar year, not the fiscal year.)

(1) Divide the papers into two categories, A and B.
   A. WPI papers
      List papers whose author(s) can be identified as affiliated with the WPI program (e.g., that state "WPI" and the name of the WPI center (WPI-center name)). (Not including papers in which the names of persons affiliated with the WPI program are contained only in acknowledgements.)
   B. WPI-related papers
      List papers related to the WPI program but whose authors are not noted in the institutional affiliations as WPI affiliated. (Including papers whose acknowledgements contain the names of researchers affiliated with the WPI program.)

      Note: On 14 December 2011, the Basic Research Promotion Division in MEXT's Research Promotion Bureau circulated an instruction requiring paper authors to include the name or abbreviation of their WPI center among their institutional affiliations. From 2012, the authors' affiliations must be clearly noted.

(2) Method of listing paper
   - List only refereed papers. Divide them into categories (e.g., original articles, reviews, proceedings).
   - For each, write the author name(s); year of publication; journal name, volume, page(s), and article title. Any listing order may be used as long as format is consistent. (The names of the center researchers do not need to be underlined.)
   - If a paper has many authors (say, more than 20), all of their names do not need to be listed.
   - Assign a serial number to each paper to be used to identify it throughout the report.
   - If the papers are written in languages other than English, underline their serial numbers.
   - Order of Listing
      A. WPI papers
         1. Original articles
         2. Review articles
         3. Proceedings
         4. Other English articles

      B. WPI-related papers
         1. Original articles
         2. Review articles
         3. Proceedings
         4. Other English articles

(3) Submission of electronic data
   - In addition to the above, provide a .csv file output from the Web of Science (e.g.) or other database giving the paper's raw data including Document ID. (Note: the Document ID is assigned by paper database.)
   - These files do not need to be divided into paper categories.

(4) Use in assessments
   - The lists of papers will be used in assessing the state of WPI project's progress.
   - They will be used as reference in analyzing the trends and whole states of research in the said WPI center, not to evaluate individual researcher performance.
   - The special characteristics of each research domain will be considered when conducting assessments.

(5) Additional documents
   - After all documents, including these paper listings, showing the state of research progress have been submitted, additional documents may be requested.

A. WPI papers

1. Original articles

1. Abe T, Mishima K, Kitamura S, Hida A, Inoue Y, Mizuno K, Kaida K, Nakazaki K, Motomura Y, Maruo K, Ohta T, Furukawa S, Dinges DF, Ogata K (2020) Tracking intermediate performance of vigilant attention using multiple eye metrics. *Sleep* **43**(3). Doi:10.1093/sleep/zsz219

2. Bjorness TE, Greene RW (2020) Sleep Deprivation Enhances Cocaine Conditioned Place Preference in an orexin Receptor-Modulated Manner. *Eneuro* **7**(6). Doi:10.1523/ENEURO.0283-20.2020

3. Bjorness TE, Kulkarni A, Rybalchenko V, Suzuki A, Bridges C, Harrington AJ, Cowan CW, Takahashi JS, Konopka G, Greene RW (2020) An essential role for MEF2C in the cortical response to loss of sleep in mice. *eLife* **9**. doi:10.7554/eLife.58331

4. Chang YH, Katoh MC, Abdellatif AM, Xiafukaiti G, Elzeftawy A, Ojima M, Mizuno S, Kuno A, Takahashi S (2020) Uncovering the role of MAFB in glucagon production and secretion in pancreatic alpha-cells using a new alpha-cell-specific Mafb conditional knockout mouse model. *Exp Anim Tokyo* **69**(2): 178-188. Doi:10.1538/expanim.19-0105

5. Choi D, Sato T, Ando T, Abe T, Akamatsu M, Kitazaki S (2020) Effects of cognitive and visual loads on driving performance after take-over request (TOR) in automated driving. *Appl Ergon* **85**.

Doi:10.1016/j.apergo.2020.103074

6. Daida K, Ogaki K, Hayashida A, Ando M, Yokoyama K, Noda K, Kanbayashi T, Hattori N, Okuma Y (2020) Somnolence Preceded the Development of a Subthalamic Lesion in Neuromyelitis Optica Spectrum Disorder. *Internal Med* **59**(4): 577-579. Doi:10.2169/internalmedicine.2947-19

7. Deng ZB, Matsumoto Y, Kuno A, Ojima M, Xiafukaiti G, Takahashi S (2020) An Inducible Diabetes Mellitus Murine Model Based on MafB Conditional Knockout under MafA-Deficient Condition. *Int J Mol Sci* **21**(16). Doi:10.3390/ijms21165606

8. Disha SI, Furlani B, Drevensek G, Plut A, Yanagisawa M, Hudoklin S, Zitnik IP, Marc J, Drevensek M (2020) The role of endothelin B receptor in bone modelling during orthodontic tooth movement: a study on-ETB knockout rats. *Sci Rep* **10**(1). Doi:10.1038/s41598-020-71159-8

9. Enomoto M, Kitamura S, Tachimori H, Takeshima M, Mishima K (2020) Long-term use of hypnotics: Analysis of trends and risk factors. *Gen Hosp Psychiat* **62**: 49-55. Doi:10.1016/j.genhosppsych.2019.11.008

10. Fujino M, Tagami A, Ojima M, Mizuno S, Abdellatif AM, Kuno A, Takahashi S (2020) c-MAF deletion in adult C57BL/6J mice induces cataract formation and abnormal differentiation of lens fiber cells. *Exp Anim Tokyo* **69**(2): 242-249. Doi:10.1538/expanim.19-0137

11. Fukuda K, Shibata Y, Sato H, Okabe S (2020) How the large-scale blackout following the 2018 Hokkaido Eastern Iburi earthquake impacted adolescents' sleep patterns. *Sleep Biol Rhythms* **18**(4): 351-354. Doi:10.1007/s41105-020-00278-6

12. Hashimoto M, Kumabe A, Kim JD, Murata K, Sekizar S, Williams A, Lu WZ, Ishida J, Nakagawa T, Endo M, Minami Y, Fukamizu A (2020) Loss of PRMT1 in the central nervous system (CNS) induces reactive astrocytes and microglia during postnatal brain development. *J Neurochem*. Doi:10.1111/jnc.15149

13. Hirose Y, Kitazono T, Sezaki M, Abe M, Sakimura K, Funato H, Handa H, Vogt KE, Yanagisawa M (2020) Hypnotic effect of thalidomide is independent of teratogenic ubiquitin/proteasome pathway. *P Natl Acad Sci USA* **117**(37): 23106-23112. Doi:10.1073/pnas.1917701117

14. Honda T, Takata Y, Cherasse Y, Mizuno S, Sugiyama F, Takahashi S, Funato H, Yanagisawa M, Lazarus M, Oishi Y (2020) Ablation of Ventral Midbrain/Pons GABA Neurons Induces Mania-like Behaviors with Altered Sleep Homeostasis and Dopamine D2R-mediated Sleep Reduction. *Iscience* **23**(6). Doi:10.1016/j.isci.2020.101240

15. Hori D, Sasahara S, Doki S, Oi Y, Matsuzaki I (2020) Prefrontal activation while listening to a letter of gratitude read aloud by a coworker face-to-face: A NIRS study. *Plos One* **15**(9). Doi:10.1371/journal.pone.0238715

16. Hori D, Sasahara S, Oi Y, Doki S, Andrea CS, Takahashi T, Shiraki N, Ikeda T, Ikeda Y, Kambayashi T, Aoki E, Matsuzaki I (2020) Relationships between insomnia, long working hours, and long commuting time among public school teachers in Japan: a nationwide cross-sectional diary study. *Sleep Med* **75**: 62-72. Doi:10.1016/j.sleep.2019.09.017

17. Ikeda T, Hori D, Ikeda Y, Takahashi T, Shiraki N, Andrea CS, Ohtaki Y, Doki S, Oi Y, Sasahara S, Saito T, Matsuzaki I (2020) School Ijime (Bullying) Experience Is a Possible Risk Factor for Current Psychological Distress among Science City Workers: A Cross-Sectional Study in Tsukuba, Japan. *Tohoku J Exp Med* **250**(4): 223-231. Doi:10.1620/tjem.250.223

18. Imanishi A, Kawazoe T, Hamada Y, Kumagai T, Tsutsui K, Sakai N, Eto K, Noguchi A, Shimizu T, Takahashi T, Han G, Mishima K, Kanbayashi T, Kondo H (2020) Early detection of Niemann-pick disease type C with cataplexy and orexin levels: continuous observation with and without Miglustat. *Orphanet J Rare Dis* **15**(1). Doi:10.1186/s13023-020-01531-4

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Appendix 2　FY 2020 List of Principal Investigators

NOTE:

*Underline names of principal investigators who belong to an overseas research institution.

*In the case of researcher(s) not listed in the latest report, attach a "Biographical Sketch of a New Principal Investigator"(Appendix 2a).

*Enter the host institution name and the center name in the footer.

<Results at the end of FY2020> **Principal Investigators Total: 28**

Name	Age	Affiliation (Position title, department, organization)	Academic degree, Specialty	Effort (%)*	Starting date of project participation	Status of project participation (Describe in concrete terms)	Contributions by PIs from overseas research institutions
Center director Masashi Yanagisawa	60	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D. ; Neuroscience, Pharmacology	95	December 2012	Usually stays at the center	
Takeshi Sakurai	56	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Professor, Faculty of Medicine, University of Tsukuba	M.D., Ph.D.; Neuroscience	80	April 2013	Usually stays at the center	
Hiromasa Funato	51	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba Professor, Toho University	M.D., Ph.D.; Neuroscience	40	December 2012	Usually stays at the center three times a week	

Robert Greene	70	Professor, Department of Psychiatry, University of Texas Southwestern Medical Center	M.D., Ph.D.; Neuroscience	10	December 2013	a) visits center 3X/yr for ~2 weeks /visit b) Skype meeting with lab 1X/week c) attends (by Zoom) PI meeting 1X/month d) participates in person with the annual IIIS symposium e) participates in person in annual Site Visit	Collaboration of ongoing research project at UTSW investigating the transriptomic changes induced by homeostatic sleep
Qinghua Liu	49	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba, Japan; Investigator, National Institute of Biological Sciences (NIBS), Tsinghua University, China	Ph.D. ; Genetics, Molecular Biology, Biochemistry	10	April 2013	a) Usually stays at the satellite center b) Joins a videoconference from abroad >once /2 weeks c) attends (by Skype) PI meeting once /month	Collaboration of ongoing research project at NIBS investigating the neural mechanisms of innate fear-associated hypothermia
Hiroshi Nagase	73	Specially Appointed Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Medicinal Chemistry, Organic Chemistry	65	April 2013	Usually stays at the center	
Noriki Kutsumura	43	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Organic Chemistry, Medicinal Chemistry	80	April 2013	Usually stays at the center	
Makoto Satoh	65	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D.; Sleep Medicine	65	April 2015	Usually stays at the center	
Kumpei Tokuyama	67	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D., ; Energy Metabolims	80	April 2015	Usually stays at the center	

Takashi Kanbayashi	57	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Physician, Ibaraki Prefectural Medical Center of Psychiatry	M.D., Ph.D.; Sleep Medicine and Psychiatry	80	April 2019	Stays 2 days at IIIS and 3 days at the satellite center	
Ichiyo Matsuzaki	61	Professor, Faculty of Medicine, University of Tsukuba	M.D., Ph.D. ; Occupational Psychiatric Medicine, Space	10	March 2013	About 10% of effort. The remaining is allocated for Faculty of Medicine.	
Hitoshi Shimano	61	Professor, Faculty of Medicine, University of Tsukuba	M.D., Ph.D. ; Endocrinology, Metabolism	15	March 2013	Usually stays at Faculty of Medicine	
Akiyoshi Fukamizu	61	Professor, Tsukuba Advanced Research Alliance, University of Tsukuba	Ph.D.,; Molecular Biology	2	March 2013	Usually stays at the satellite center Started the collaboration with Chika Miyoshi (Yanagisawa/Funato Lab.).	
Satoru Takahashi	59	Professor, Laboratory Animal Resource Center, Department of Anatomy and Embryology, Faculty of Medicine, University of Tsukuba	M.D., Ph.D.; Developmental biology	20	March 2013	Participates in generation of genetically modified mice by using CRISPR/Cas9 system at Laboratory Animal Resource Center	
Joseph Takahashi	69	Professor, Department of Neuroscience, University of Texas Southwestern Medical Center Investigator, Howard Hughes Medical Institute	Ph.D.; Neuroscience	5	December 2012	Usually stays at the satellite center	Collaboration. Available to accept young scientists from WPI for collaborative projects.

Carla Green	58	Professor, Department of Neuroscience, University of Texas Southwestern Medical Center	Ph.D.; Molecular Biology, Biochemistry, Circadian rhythms	5	March 2013	Usually stays at the satellite center	Collaboration. Available to accept young scientists from WPI for collaborative projects.
Yang Dan	53	Professor, Department of Molecular and Cell Biology, University of California, Berkeley	Ph.D.; Neurobiology	5	April 2014	Usually stays at the satellite center	
Kazuo Mishima	58	Professor, Department of Neuropsychiatry, Akita University Graduate School of Medicine	M.D., Ph.D.; Medical Science	5	October 2018	Usually stays at the satellite center	
Kaspar Vogt	54	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D.; Physiology, Pharmacology, Neurobiology	100	February 2014	Usually stays at the center	
Michael Lazarus	51	Associate Professor International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	April 2013	Usually stays at the center	
Masanori Sakaguchi	44	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D.; Neuroscience	100	January 2013	Usually stays at the center	

Yu Hayashi	40	Professor (WPI-IIIS), International Institute for Integrative Sleep Medicine, University of Tsukuba Professor, Graduate School of Medicine, Kyoto University	Ph.D.; Neuroscience	20	April 2013	Stays at the center once a week	
Takashi Abe	41	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Behavioral Science Psychophysiology	100	November 2017	Usually stays at the center	
Sakiko Honjoh	40	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Molecular biology, Genetics, Neuroscience	100	September 2017	Usually stays at the center	
Yo Oishi	40	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	April 2013	Usually stays at the center	
Katsuyasu Sakurai	42	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	July 2017	Usually stays at the center	
Arisa Hirano	35	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Assistant Professor, Faculty of Medicine, University of Tsukuba	Ph.D.; Molecular biology, Genetics, Neuroscience	80	April 2019	Usually stays at the center	

Hirofumi Toda	42	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Genetics	100	July 2019	Usually stay at the center	

*Percentage of time that the principal investigator devotes to his/her work for the center vis-à-vis his/her total working hours.

Principal investigators unable to participate in project in FY 2020

Name	Affiliation (Position title, department, organization)	Starting date of project participation	Reasons	Measures taken
Hitoshi Okamura	Professor, Graduate School of Pharmaceutical Sciences, Kyoto University	July 2015	Retired at the end of March in 2019	

Appendix 3-1 FY 2020 Records of Center Activities

1. Researchers and other center staffs, satellites, partner institutions

1-1. Number of researchers and other center staffs

* Fill in the number of researchers and other center staffs in the table blow.

* Describe the final goals for achieving these numbers and dates when they will be achieved described in the last "center project."

a) Principal Investigators

(full professors, associate professors or other researchers of comparable standing)

(number of persons)

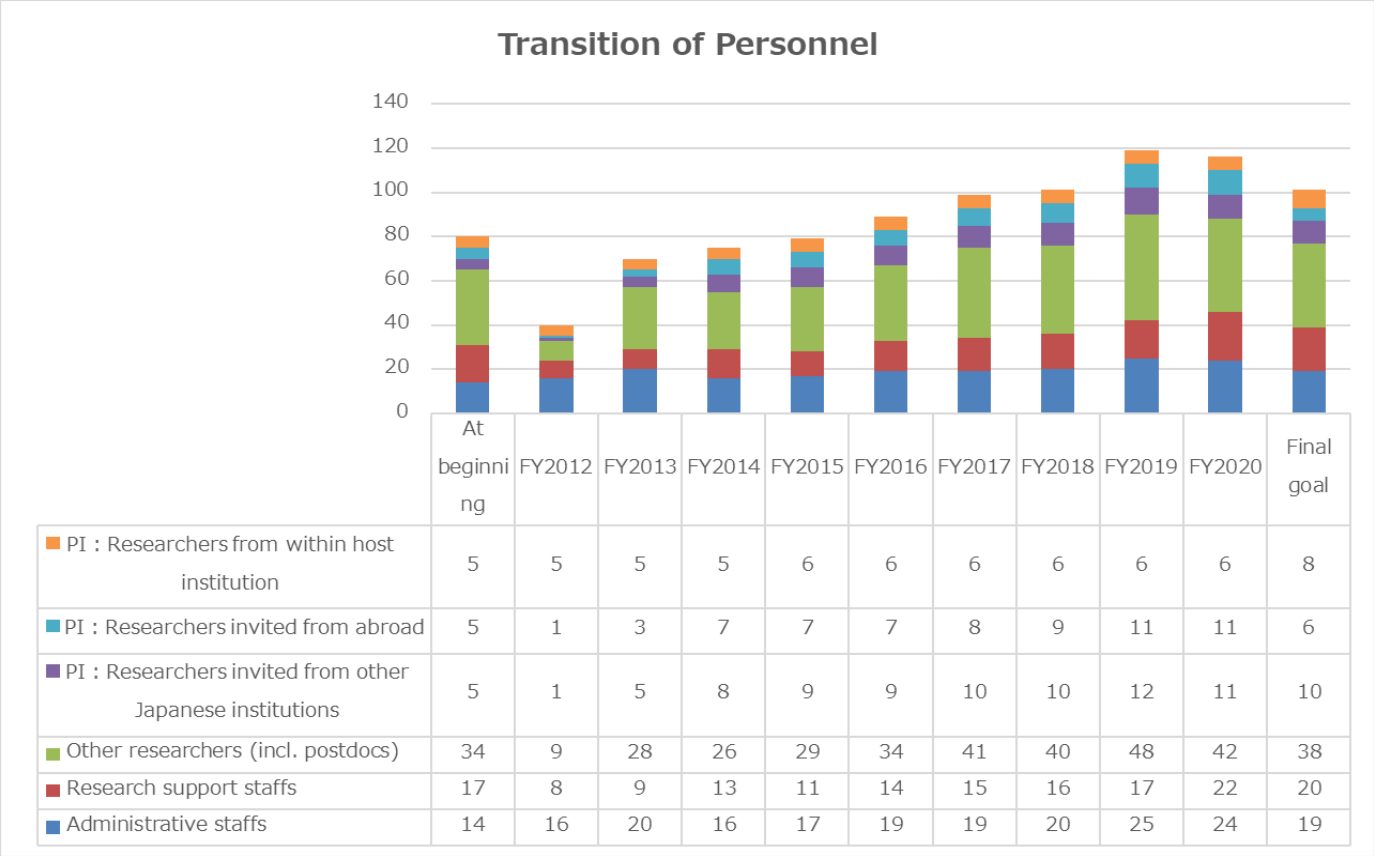
	At the beginning of project	At the end of FY 2020	Final goal (Date: March, 2022)
Researchers from within the host institution	5	6	8
Researchers invited from abroad	1	11	6
Researchers invited from other Japanese institutions	1	11	10
Total principal investigators	7	28	24

b) Total members

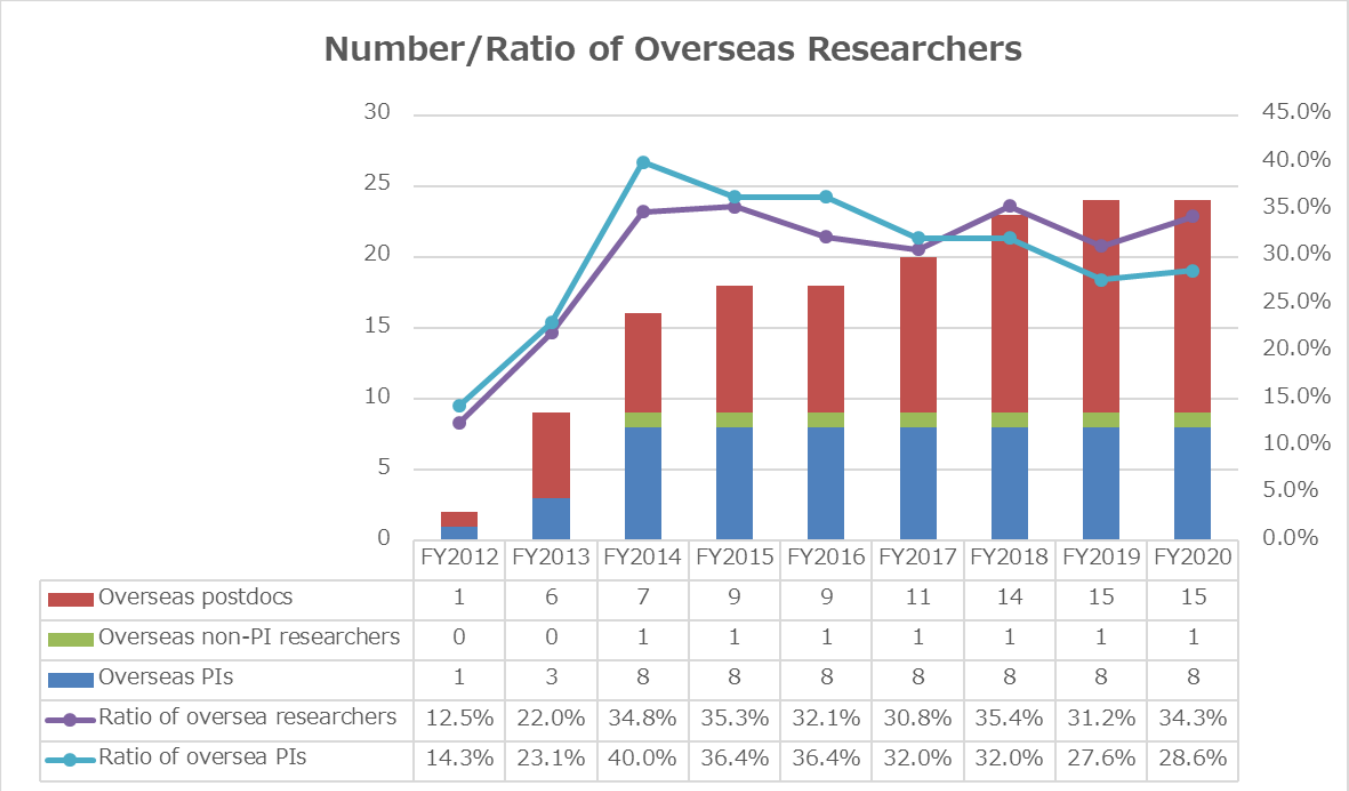
	At the beginning of project		At the end of FY 2020		Final goal (Date: March, 2022)	
	Number of persons	%	Number of persons	%	Number of persons	%
Researchers	41		70		62	
Overseas researchers	1	2	24	34	21	34
Female researchers	8	20	25	36	22	35
Principal investigators	7		28		24	
Overseas PIs	1	14	8	29	8	33
Female PIs	0	0	4	14	4	17
Other researchers	0		12		15	
Overseas researchers	0	0	1	8	1	7
Female researchers	0	0	4	33	4	27
Postdocs	34		30		23	
Overseas postdocs	0	0	15	50	12	52
Female postdocs	8	24	17	57	14	61
Research support staffs	17		22		20	
Graduate students	4		65		68	
Administrative staffs	14		24		19	
Total number of people who form the "core" of the research center	76		181		169	

Appendix 3-2　Annual Transition in the Number of Center Personnel

*Make a graph of the annual transition in the number of center personnel since the start of project.

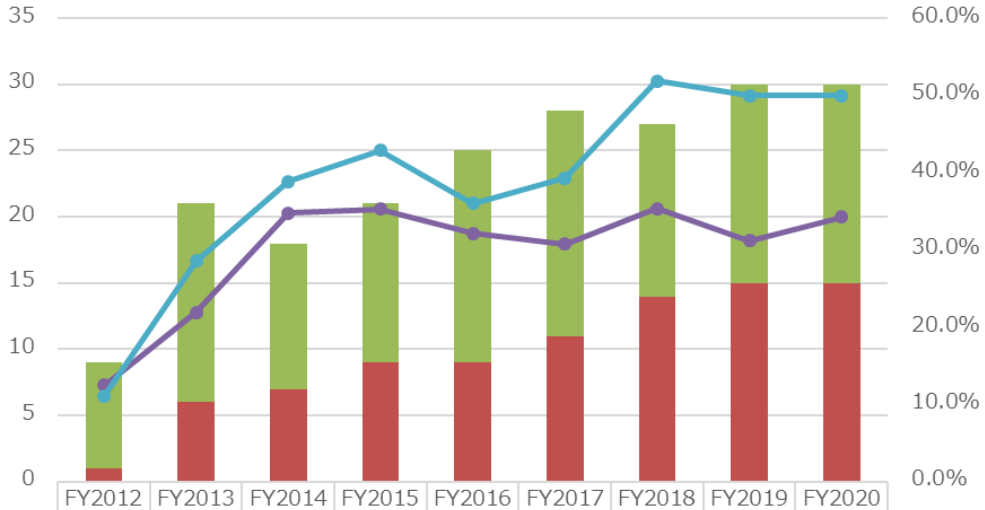


	At beginning	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020	Final goal
PI : Researchers from within host institution	5	5	5	5	6	6	6	6	6	6	8
PI : Researchers invited from abroad	5	1	3	7	7	7	8	9	11	11	6
PI : Researchers invited from other Japanese institutions	5	1	5	8	9	9	10	10	12	11	10
Other researchers (incl. postdocs)	34	9	28	26	29	34	41	40	48	42	38
Research support staffs	17	8	9	13	11	14	15	16	17	22	20
Administrative staffs	14	16	20	16	17	19	19	20	25	24	19



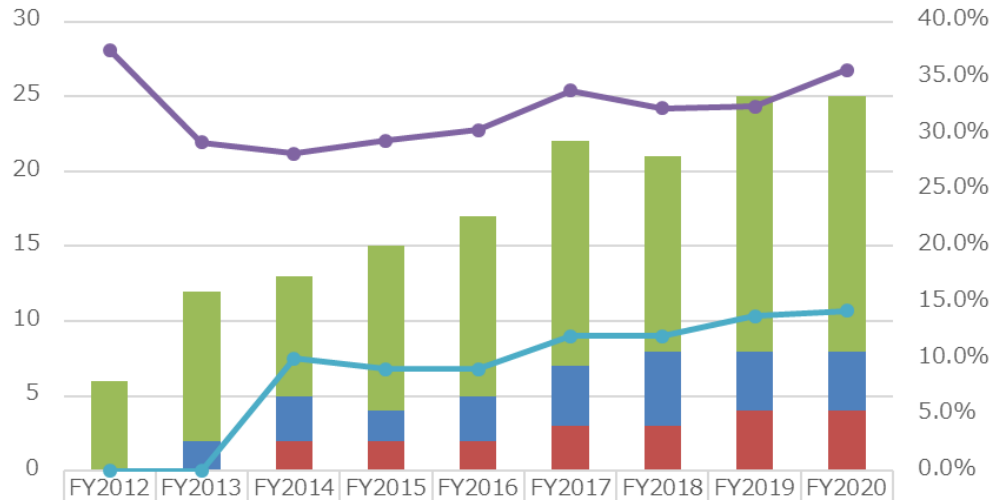
	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020
Overseas postdocs	1	6	7	9	9	11	14	15	15
Overseas non-PI researchers	0	0	1	1	1	1	1	1	1
Overseas PIs	1	3	8	8	8	8	8	8	8
Ratio of oversea researchers	12.5%	22.0%	34.8%	35.3%	32.1%	30.8%	35.4%	31.2%	34.3%
Ratio of oversea PIs	14.3%	23.1%	40.0%	36.4%	36.4%	32.0%	32.0%	27.6%	28.6%

Number/Ratio of Overseas Postdoc



	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020
Japanese postdocs	8	15	11	12	16	17	13	15	15
Overseas postdocs	1	6	7	9	9	11	14	15	15
Ratio of oversea researchers	12.5%	22.0%	34.8%	35.3%	32.1%	30.8%	35.4%	31.2%	34.3%
Ratio of overseas postdocs	11.1%	28.6%	38.9%	42.9%	36.0%	39.3%	51.9%	50.0%	50.0%

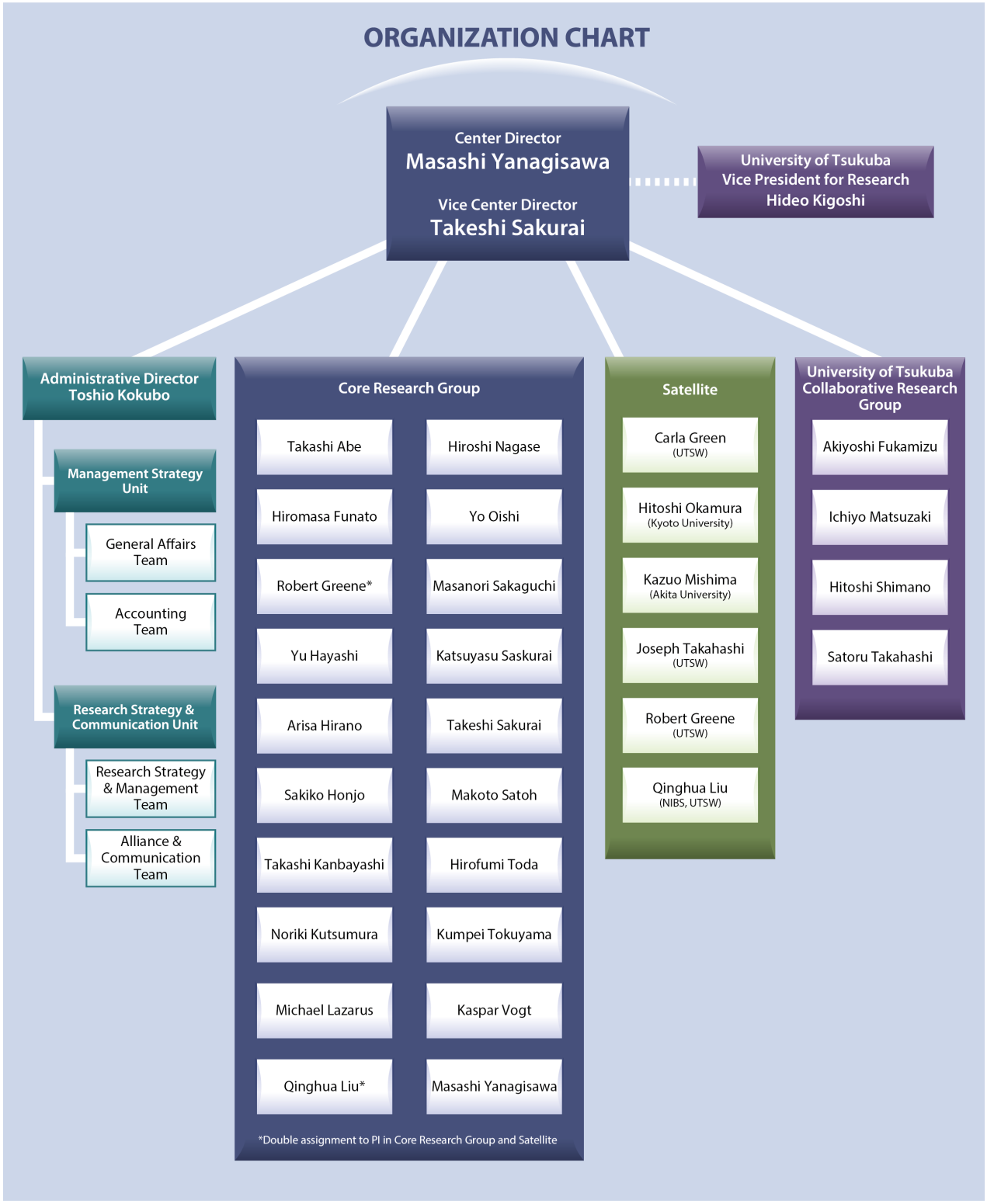
Number/Ratio of Female Researchers



	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020
Female postdocs	6	10	8	11	12	15	13	17	17
Female non-PI researchers	0	2	3	2	3	4	5	4	4
Female PIs	0	0	2	2	2	3	3	4	4
Ratio of female researchers	37.5%	29.3%	28.3%	29.4%	30.4%	33.8%	32.3%	32.5%	35.7%
Ratio of female PIs	0.0%	0.0%	10.0%	9.1%	9.1%	12.0%	12.0%	13.8%	14.3%

Appendix 3-3 Diagram of Management System

- Diagram the center's management system and its position within the host institution in an easily understood manner.
- If any changes have been made in the management system from that in the latest "center project" last year, describe them. Especially describe any important changes made in such as the center director, administrative director, head of host institution, and officer(s) in charge at the host institution (e.g., executive vice president for research).



Appendix 3-4　Campus Map

- Draw a simple map of the campus showing where the main office and principal investigator(s) are located.

IIIS Building

5/6F Animal Facility (ARC Satellite)

4F


Nagase


Kutsumura

F-MIRAI


Sankai

2/3F


Sakurai T.


Hirano


Sakaguchi


Greene


Vogt


Lazarus


Oishi


Yanagisawa


Funato


Liu

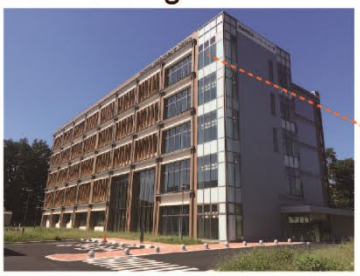

Sakurai K.


Hayashi


Honjoh


Toda

COI Building



1F Admin Office


Kokubo

S'UIMIN


Fujiwara

Human Sleep Lab

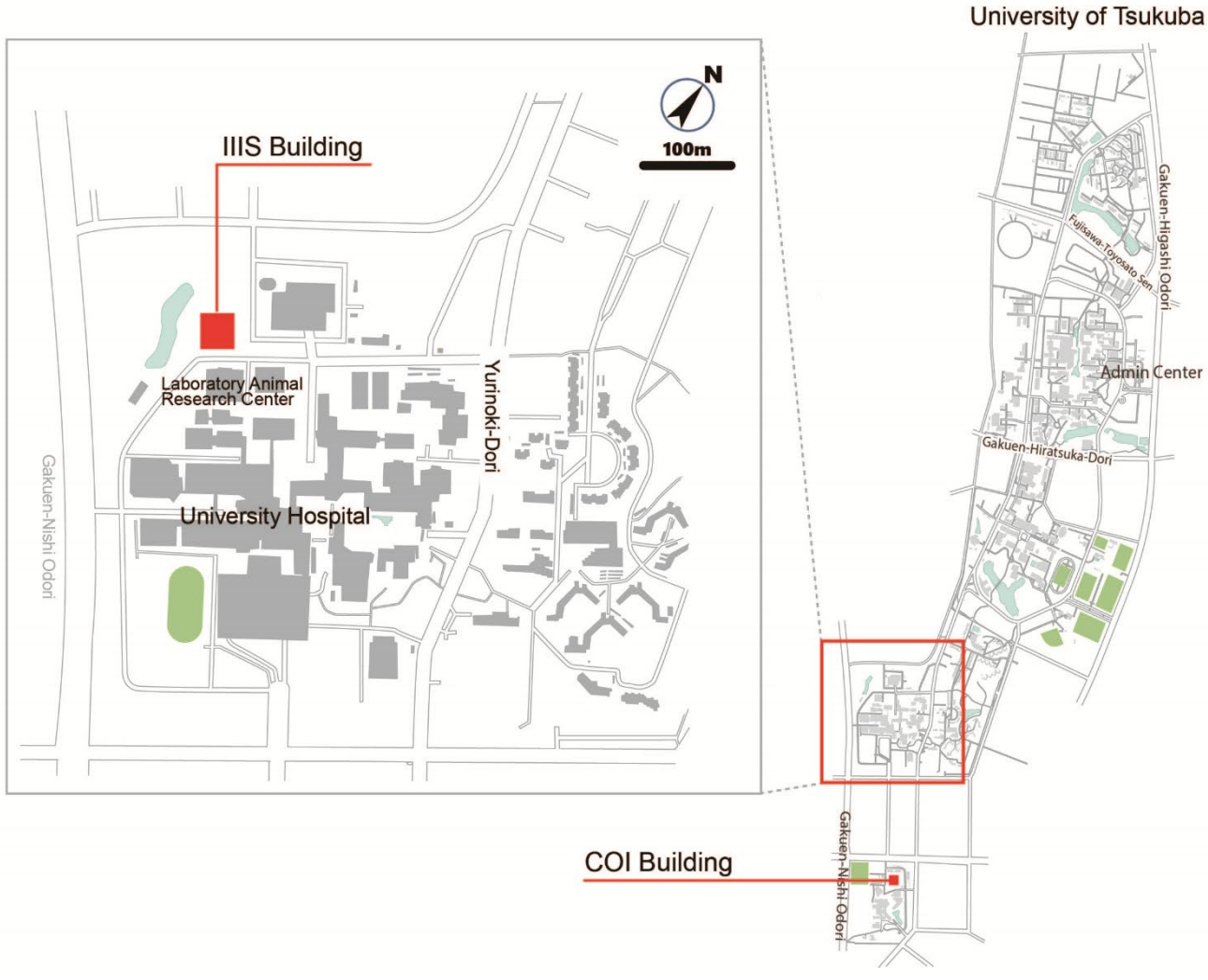

Satoh


Tokuyama


Abe


Kanbayashi

Campus Map



Appendix 3-5 Project Expenditures in FY2020

1) Overall project funding

* In the "Total costs" column, enter the total amount of funding required to implement the project, without dividing it into funding sources.
* In the "Amount covered by WPI funding" column, enter the amount covered by WPI within the total amount.
* In the "Personnel," "Project activities," "Travel," and "Equipment" blocks, the items of the "Details" culumn may be changed to coincide with the project's actual content.

(Million yens)

Cost items	Details (For Personnel - Equipment please fill in the breakdown of fiscal expenditure, and the income breakdown for Research projects.)	Total costs	Amount covered by WPI funding
Personnel	Center director and administrative director	64	50
	Principal investigators (no. of persons):18	148	85
	Other researchers (no. of persons):38	209	174
	Research support staffs (no. of persons):16	50	22
	Administrative staffs (no. of persons):20	73	62
	Subtotal	544	393
Project activities	Gratuities and honoraria paid to invited principal investigators (no. of persons):OO		
	Cost of dispatching scientists (no. of persons):OO		
	Research startup cost (no. of persons):22	26	26
	Cost of satellite organizations (no. of satellite organizations):2	16	16
	Cost of international symposiums (no. of symposiums):OO	0	0
	Rental fees for facilities	72	59
	Cost of consumables	15	6
	Cost of utilities	68	0
	Other costs	27	27
	Subtotal	224	134
Travel	Domestic travel costs		
	Overseas travel costs		
	Travel and accommodations cost for invited scientists (no. of domestic scientists):OO (no. of overseas scientists):OO		
	Travel cost for scientists on transfer (no. of domestic scientists):OO (no. of overseas scientists):OO		
	Subtotal	0	0
Equipment	Depreciation of buildings		
	Depreciation of equipment	809	469
	Subtotal	809	469
Research projects (Detail items must be fixed)	Project supported by other government subsidies, etc. *1		
	KAKENHI	184	
	Commissioned research projects, etc.	17	
	Joint research projects	182	
	Ohers (donations, etc.)	105	
	Subtotal	488	0
Total		2065	996

Costs (Million yens)

WPI grant in FY 2020	551
Costs of establishing and maintaining facilities	0
Establishing new facilities (Number of facilities: , OO m²)	0
Repairing facilities (Number of facilities: , OO m²)	0
Others	0
Costs of equipment procured	3.2
Fear extinction apparatus for mice (Number of units:1)	2.6
Central monitoring PC system (Number of units:1)	0.6
Others	0

*1. Funding sources that include government subsidies (including Enhancements promotion expenses (機能強化促進経費), National university reform reinforcement promotion subsidy (国立大学改革強化推進補助金) etc.), indirect funding, and allocations from the university's own resources.
*2 When personnel, travel, equipment (etc.) expenses are covered by KAKENHI or under commissioned research projects or joint research projects, the amounts should be entered in the "Research projects" block.

2) Costs of satellites

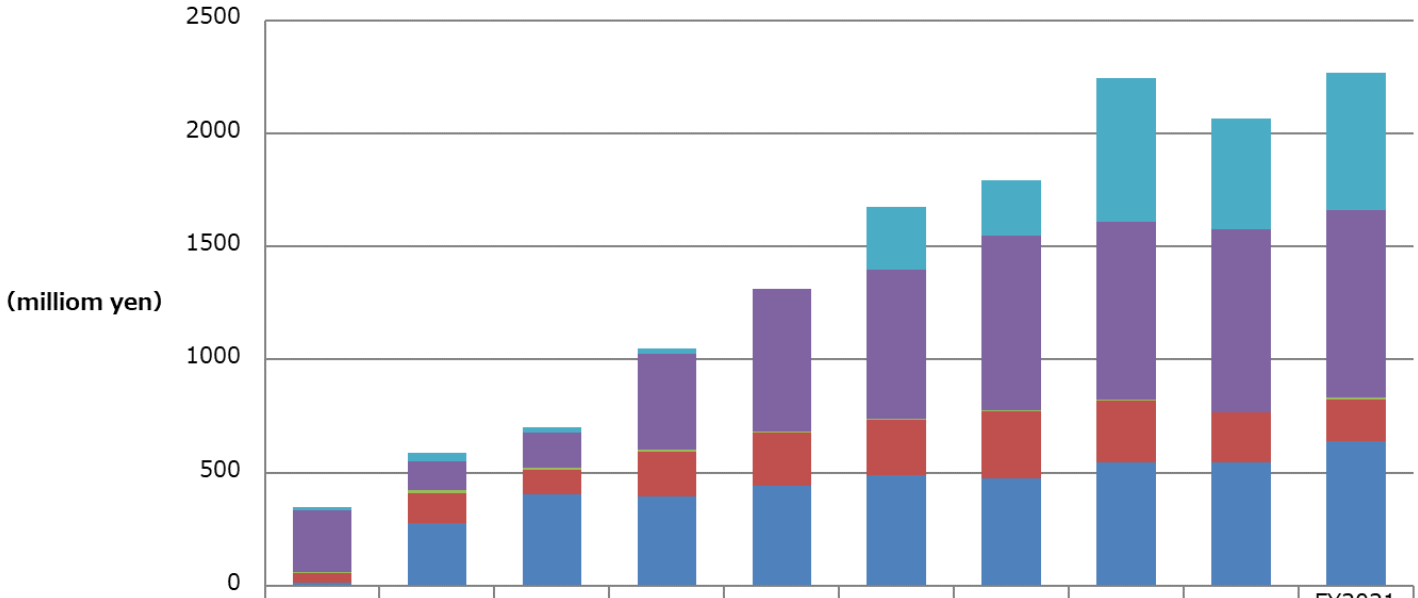
(Million yens)

Cost items	Details	Total costs	Amount covered by WPI funding
Personnel	Principal investigators (no. of persons):2		
	Other researchers (no. of persons):3		
	Research support staffs (no. of persons):OO		
	Administrative staffs (no. of persons):OO		
	Subtotal	16	16
Project activities	Subtotal		
Travel	Subtotal		
Equipment	Subtotal		
Research projects	Subtotal		
Total		16	16

Appendix 3-6　Annual Transition in the Amounts of Project Funding

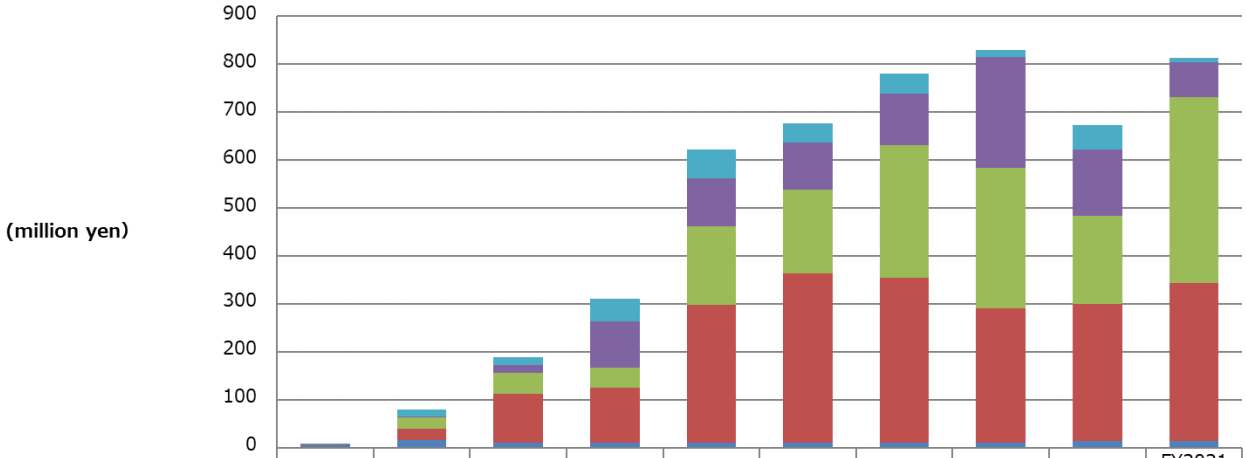
*Make a graph of the transition in the number of overall project funding.

Transition of Project Expenditures



	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020	FY2021 *
Research Projects	12	40	27	24	0	278	245	636	488	609
Equipment	276	125	155	425	628	659	771	789	809	827
Travel	4	15	10	8	6	8	5	4	0	9
Project Activities	39	134	105	200	235	243	300	270	224	183
Personnel	16	276	406	394	443	489	473	547	544	642

Transition of Research Project Expenditures



	FY2012	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020	FY2021 *
Ohers (donations, etc.)	1	14	17	48	59	41	43	14	52	10
Joint research projects	0	2	16	96	100	99	107	230	138	72
Commissioned research projects, etc.	0	24	43	40	165	174	276	294	184	388
KAKENHI	1	23	102	116	287	353	344	280	285	329
Project supported by other government subsidies, etc. *1	5	15	10	10	10	10	10	10	14	14

The number of FY2021* are the projection at the end of April 2021.

Appendix 4-1 FY 2020 Status of Collaboration with Overseas Satellites

- If satellite and partner institutions have been established, fill in required items of the form below.

1. Satellites and partner institutions

- List the satellite and partner institutions in the table below (including the domestic satellite institutes).
- Indicate newly added and deleted institutions in the "Notes" column.

<Satellite institutions>

Institution name	Principal Investigator(s), if any	Notes
University of Texas Southwestern Medical Center	Joseph Takahashi	
University of Texas Southwestern Medical Center	Robert Greene	
University of Texas Southwestern Medical Center	Carla Green	
National Institute of Biological Sciences	Qinghua Liu	
University of California, Berkeley	Yang Dan	
Akita University Graduate School of Medicine	Kazuo Mishima	
Graduate School of Pharmaceutical Sciences, Kyoto University	Hitoshi Okamura	Retired because of the age

< Partner institutions>

Institution name	Principal Investigator(s), if any	Notes
Gui de Chauliac Hospital	Yves Dauvilliers	
RIKEN	Genshiro Sunagawa	

- If overseas satellite institutions have been established, fill in required items on the form below. If overseas satellite institutions have not been established, it is not necessary to complete the form.

2. Coauthored Papers

- List the refereed papers published in FY 2020 that were coauthored between the center's researcher(s) in domestic institution(s) (include satellite institutions) and overseas satellite institution(s). List them by overseas satellite institution in the below blocks.
- Transcribe data in same format as in Appendix 1-4. Italicize the names of authors affiliated with overseas satellite institutions.
- For reference write the Appendix 1-4 item number in parentheses after the item number in the blocks below. Let it free, if the paper is published in between Jan.-Mar. 2021 and not described in Appendix 1-4.

Overseas Satellite 1 University of Texas Southwestern Medical Center (Total: 1 paper)

1) Suzuki A, Yanagisawa M, Greene RW (2020) Proceedings Of The National Academy Of Sciences Of The United States Of America 117(19): 10547-10553. doi:10.1073/pnas.1906840117

Overseas Satellite 2 National Institute of Biological Sciences, Beijing (Total: 0 papers)

Overseas Satellite 3 University of California, Berkeley (Total: 0 papers)

3. Status of Researcher Exchanges

- Using the below tables, indicate the number and length of researcher exchanges in FY 2020. Enter by institution and length of exchange.

- Write the number of principal investigator visits in the top of each space and the number of other researchers in the bottom.

Overseas Satellite 1: University of Texas Southwestern Medical Center

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

Overseas Satellite 2: National Institute of Biological Sciences, Beijing

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

Overseas Satellite 3: University of California, Berkeley

<To satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

<From satellite>

	Under 1 week	From 1 week to 1 month	From 1 month to 3 months	3 months or longer	Total
FY2020	0	0	0	0	0
	0	0	0	0	0

Appendix 4-2 FY 2020 Visit Records of Researchers from Abroad

* If researchers have visited/ stayed at the Center, provide information on them in the below table.
* Enter the host institution name and the center name in the footer.

Total: O

	Name	Age	Affiliation		Academic degree, specialty	Record of research activities (Awards record, etc.)	Time, duration	Summary of activities during stay at center (e.g., participation as principal investigator; short-term stay for joint research; participation in symposium)
			Position title, department, organization	Country				
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Appendix4-3 Postdoctoral Positions through Open International Solicitations

* In the column of number of applications and number of selection, put the total number (upper), the number and percentage of overseas researchers in the < > brackets (lower).

Fiscal year	number of applications	number of selection
FY 2012	3	3
	< 1, 33%>	< 1,33 %>
FY 2013	169	12
	< 158, 93%>	< 4,33 %>
FY 2014	144	9
	< 123, 85%>	< 3, 33%>
FY 2015	98	4
	< 97, 99%>	< 3, 75%>
FY 2016	29	0
	< 29, 100%>	< 0, 0%>
FY 2017	45	4
	< 45, 100%>	< 2, 50%>
FY 2018	33	4
	< 13, 39%>	< 2, 50%>
FY 2019	4	4
	< 4, 100%>	< 4, 100%>
FY 2020	12	4
	< 4, 33%>	< 3, 75%>

Appendix 4-4 Status of Employment of Postdoctoral Researchers

Enter the information below during the period from the start of the center through the end of FY 2020.

- For each person, fill in the spaces to the right. More spaces may be added.
- Leave "Position as of April 2021" blank if unknown.
- Enter the host institution name and the center name in the footer.

Japanese Postdocs

Employment period	Position before employed at WPI center		Next position after WPI center		Position as of April 2021*	
	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located
Apr 1, 2013~Nov 30, 2013	Chief Researcher, Greensogna, Inc.	Japan	Researcher, Tsukuba Primate Research Center, National Institutes of Biomedical Innovation, Health and Nutrition	Japan	Deputy Director, Biotherapy Institute of Japan Inc.	Japan
Jul 1, 2013~Jun 30, 2014	Assistant Professor, University of Texas Southwestern Medical Center	USA	Assistant Professor, The University of Tokyo	Japan	Specially Apointed Assistant Professor, Department of Cardiovascular Medicine, The University of Tokyo Hospital	Japan
Apr 1, 2014~Mar 31, 2015	Researcher, Molecular Mechanism and Control of Complex Behaviors, University of Tsukuba	Japan	Researcher, Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Japan
Apr 1, 2014~Mar 31, 2017	Researcher, Molecular Mechanism and Control of Complex Behaviors, University of Tsukuba	Japan	Researcher, Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan	Researcher(RPD), Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan
Apr 1, 2015~Mar 31, 2017	Researcher, Faculty of Health and Sport Sciences, University of Tsukuba	Japan	Researcher, Institute of Neurology Research	Japan	Lecturer, Department of Somnology, Tokyo Medical University	Japan
Jun 16, 2014~May 31, 2017	Postdoctoral Researcher, University of Texas Southwestern Medical Center	USA	Director CTO, Neurospace Corporation	Japan	Director CTO, Neurospace Corporation	Japan
Oct 1, 2013~Dec 31, 2017	Special Project Researcher, Osaka Bioscience Institute	Japan	Researcher, The University of Tokyo	Japan	Assistant Professor, Department of Animal Radiology, The University of Tokyo	Japan
Apr 1, 2014~Mar 31, 2018	Researcher, Molecular Mechanism and Control of Complex Behaviors, University of Tsukuba	Japan	Researcher, National Agriculture and Food Research Organization	Japan	Senior Researcher, National Agriculture and Food Research Organization	Japan
Apr 1, 2014~Mar 31, 2018	Researcher, Molecular Mechanism and Control of Complex Behaviors, University of Tsukuba	Japan	Visiting Researcher, Department of Life Scitence, Imperial College London	UK	Researcher, National Institute for Integrative Sleep Medicine, University of Tsukuba	Japan
Apr 1, 2017~Mar 31, 2018	PH.D. Student, Graduate School of Medical and Pharmaceutical Sciences , Chiba University	Japan	Researcher, Graduate School of Life Dentistry at Niigata, Nippon Dental University	Japan		
Feb 1, 2017~May 31,2018	Science Communicators, National Museum of Emerging Science and Innovation	Japan	Busuness Strategy and Development Leader, S'UIMIN Inc.	Japan	Director, S'UIMIN Inc.	Japan
Apr 1, 2017~Sep 30, 2018	Ph.D. Student, Keio University	Japan	Researcher, NARD institute Ltd.	Japan		
Apr 1, 2018~Dec 19, 2018	Ph.D. Program student, University of Tsukuba	Japan	Postdoctoral Fellow, Massachusetts Institute of Technology	USA	Postdoctoral Fellow, Massachusetts Institute of Technology	USA
Sep 1, 2017~Mar 31, 2019	Researcher, Kyoto University	Japan	Researcher, Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan	Researcher (RPD), Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan
Apr 1, 2018~Mar 31, 2019	Doctoral course student, Keio University	Japan	Assistant Professor, Chuo University	Japan	Assistant Professor, Faculity of Science and Engineering, Chuo University	Japan

Apr 1, 2013~Mar 31, 2020	Lecturer, Collage of Medical Sciences, University of Tsukuba	Japan	Lecturer, Institute of Natural Medecine, Toyama University	Japan	Lecturer, Institute of Natural Medecine, Toyama University	Japan
Apr 1, 2013~Mar 31, 2020	Researcher, Institute of Physical and Chemical Research	Japan	Professor, Graduate School of Medical and Faculity of Medicine, Kyoto University	Japan	Professor, Graduate School of Medical and Faculity of Medicine, Kyoto University	Japan
Sep 1, 2017~Mar 31, 2019	Researcher, Kyoto University	Japan				
Apr 1, 2010~Mar 31, 2021	Lecturer, Graduate School of Comprehensive Human Sciences, University of Tsukuba	Japan	Specially Lecturer, Medical Mycology Research Center, Chiba University	Japan	Specially Lecturer, Medical Mycology Research Center, Chiba University	Japan
Jan 1, 2018~Mar 1, 2021	Postdoctoral Researcher, Faculty of Science, Kyushu University	Japan	Researcher(RPD), Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan	Researcher(RPD), Research Fellowship for Young Scientists, Japan Society for the Promotion of Science	Japan

Overseas Postdocs

Employment period	Position before employed at WPI center		Next position after WPI center		Position as of April 2021*		Nationality
	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	Position title, organization	Country where the organization is located	
Apr 1, 2013~Dec 31, 2013	Special Project Researcher, Osaka Bioscience Institute	Japan	Postdoctoral Researcher, Department of Pharmacology and Toiology, Indiana University School of Medicine South Bend	USA	Researcher, International Institute for Integrative Sleep Medicine, University of Tsukuba	Japan	India
Jul 28, 2014~Nov 27, 2016	Lecturer, Department of Psychology, MacEwan University	Canada	Assistant Professor, Wasda University	Japan	Wasda University, Associate Professor	Japan	Canada
Sep 16, 2014~Sep 30, 2015	Postdoctoral Researcher, Max Planck Institute of Psychiatry	Germany	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	Researcher, International Institute for Integrative Sleep Medicine, University of Tsukuba	Japan	India
Mar 1, 2014~Mar 31, 2017	Postdoctoral Researcher, Friedrich Miescher Institute for Biomedical Research	Swiss	Reseacher, Faculty of Health and Sport Sciences, University of Tsukuba	Japan	Reseacher, Faculty of Health and Sport Sciences, University of Tsukuba	Japan	Canada
Apr 1, 2015~Mar 31, 2017	Assistant Professor, School of Integrative and Global Majors, University of Tsukuba	Japan	Researcher, Graduate School of Pure and Applied Sciences, University of Tsukuba	Japan	Specially Lecturer, Center for Research and Development of High Education, The University of Tokyo	Japan	UK
Apr 23, 2015~Apr 23, 2016	Postdoctoral Resseacher, Strasbourg University	France	Reseacher, Harvard Medical School, Harvard University	USA	Reseacher, Harvard Medical School, Harvard University	USA	Morroco
Jan 11, 2017~Jan 10, 2018	Postdoctoral Fellow, Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur	India	Reseacher, Southwest University	China			India
Jan 1, 2018~Mar 31, 2018	Research Fellow, Leiden University Medical Center	Nederland	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	University of Tsukuba, Visiting Reseacher	Japan	France
May 1, 2015~Aug 31, 2019	Postdoctoral Researcher, The University of Texas Southwestern Medical Center	USA	Senior Research Fellow, Harbin Institute of Technology	China	Principal Investigator, Harbin Institute of Technology	China	China
Aug 1, 2015~Dec 26, 2019	Postdoctoral Researcher, The University of Texas Southwestern Medical Center	USA	Postdoctoral Researcher, Harbin Institute of Technology	China	Postdoctoral Researcher, Harbin Institute of Technology	China	China
Apr 1, 2019~Dec 31, 2019	Ph.D. Student, Graduate School of Comprehensive Human Sciences, University of Tsukuba	Japan	Postdoctoral Researcher, Shenzhen University Collage of Materials	China	Postdoctoral Researcher, Shenzhen University Collage of Materials	China	China
Jul 1, 2019~Mar 31, 2020	Technical Official, International Institute for Integrative Sleep Medicine, University of Tsukuba	Japan	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	China
Aug 1, 2020~Mar 31, 2021	Researcher, International Institute for Integrative Sleep Medicine, University of Tsukuba	Japan	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	Researcher, Postdoctoral Fellowships for Research, Japan Society for the Promotion of Science	Japan	Taiwan

Appendix4-5 List of the Cooperative Research Agreements with Overseas Institutions

*Prepare the information below during the period from the beginning of the Center through March 2021.

1. Name of an Agreement: Collaboration Research Agreement
   Dates of an Agreement: November, 2013
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW)
   Summary of an Agreement: Dr. Qinghua Liu engaged in research into intracellular signal transduction of sleep control nerve cells by analyzing phosphoprotein using mass spectrometric techniques and joint research into the molecular control mechanism of essential terror by forward genetics using an ENU mutant mouse. He, concurrently serves as Professor at the University of Tsukuba and UTSW as a joint appointment (35:65) between both universities and intellectual property rights belonging to the Institute Director go to both universities based on this joint appointment.

2. Name of an Agreement: Collaboration Research Agreement
   Dates of an Agreement: April, 2014
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW)
   Summary of an Agreement: The UTSW has been the research center of Institute Director, Masashi Yanagisawa, for more than 20 years and has nurtured a close relationship as an IIIS satellite. Institute Director, Masashi Yanagisawa, concurrently serves as Professor at the University of Tsukuba and UTSW as a joint appointment (95:5) between both universities and intellectual property rights belonging to the Institute Director go to both universities based on this joint appointment.

3. Name of an Agreement : Collaborative Research Agreement
   Counterpart of an Agreement: Merck Sharp and Dome (MSD)
   Dates of an Agreement : August, 2015
   Summary of an Agreement : We performed a joint research with MSD with the purpose of "optimizing orexin receptor type 2 selective low molecular agonists." The ultimate objective is to deliver groundbreaking eradicative medicine to patients suffering from narcolepsy by accelerating the structural optimization of low molecular agonists found in the institute through cooperation between the synthesis team of Nagase Lab and the pharmacology team of Yanagisawa Lab, supported by MSD.

4. Name of an Agreement: Sponsored Research agreement
   Dates of an Agreement: October, 2015
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW)
   Summary of an Agreement: Dr. Robert Greene engaged sponsored research on sleep homeostasis and the sleep-awakening control of adenosine.

5. Name of an Agreement: Sponsored Research agreement
   Dates of an Agreement: October, 2015
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW)
   Summary of an Agreement: Dr. Carla Green engaged in sponsored research into RNA analysis of sleep-deprived mice.

6. Name of an Agreement: Collaborative Research Agreement
   Dates of an Agreement: April, 2017
   Counterpart of an Agreement: Gui de Chauliac Hospital
   Summary of an Agreement: We performed a joint research with Gui de Chauliac Hospital for Exome and whole genome sequences of DNA extracted from sleep disorder patients such as narcolepsy and idiopathic hypersomnia.

7. Name of an Agreement: Sponsored Research agreement
   Dates of an Agreement: April, 2018
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW)

Summary of an Agreement: Dr. Joe Takahashi engaged in sponsored research into sleep using Sleepy and Dreamless mice.

8. Name of an Agreement: Collaboration Research Agreement
   Dates of an Agreement: January, 2019
   Counterpart of an Agreement: University of Texas Southwestern Medical Center (UTSW) and National institute of Biological Sciences (NIBS)
   Summary of an Agreement: Dr. Qinghua Liu engaged in both fear and sleep research. He, concurrently serves as Professor at the University of Tsukuba, NIBS and UTSW as a joint appointment (20:25:55) between both universities and intellectual property rights belonging to him go to both universities based on this joint appointment.

9. Name of an Agreement: Collaborative Research Agreement
   Dates of an Agreement: January,2019
   Counterpart of an Agreement: Wenzhou Medical University
   Summary of an Agreement: We performed a joint research with Wenzhou Medical University entitled screening for negative A2AR allosteric modulator. We will use the $mA_{2A}R$-CHO cells (established in Dr. Lazarus's lab) to screen low-molecular-weight compounds (synthesized in Dr. Hiroshi Nagase's laboratory) for allosteric effects at $A_{2A}R$. Dr. Chen's lab at Wenzhou Medical University will send a postdoctoral fellow with the experience in adenosine receptor biology and neuroscience to work at Dr. Lazarus's lab.

10. Name of an Agreement: Collaboration Research Agreement
    Dates of an Agreement: July, 2019
    Counterpart of an Agreement: National institute of Biological Sciences (NIBS)
    Summary of an Agreement: Dr. Qinghua Liu engaged in both fear and sleep research. He retires UTSW and concurrently serves as Professor at the University of Tsukuba and NIBS as a joint appointment (8:92) between both universities and intellectual property rights belonging to him go to both universities based on this joint appointment.

Appendix4-6 Holding International Research Meetings

* Indicate up to twenty of most representative international research conferences or symposiums held from the start of the center through March 2021 and give the number of participants using the table below.

Date	Meeting title and Place held	Number of participants
Nov 26, 2019	Title: The 8th Annual IIIS Symposium Venue: Tokyo Conference Center Shinagawa	From domestic institutions: 203 From overseas institutions: 5
Dec 20, 2018	Title: The 7th Annual IIIS Symposium Venue: Tokyo Conference Center Shinagawa	From domestic institutions: 201 From overseas institutions: 13
Dec 14, 2017	Title: The 6th Annual IIIS Symposium Venue: Tokyo Conference Center Shinagawa	From domestic institutions: 185 From overseas institutions: 11
Dec 12, 2016	Title: The 5th Annual IIIS Symposium Venue: Tokyo Conference Center Shinagawa	From domestic institutions: 170 From overseas institutions: 7
Feb 26, 2016	Title: The 4th Annual IIIS Symposium Venue: IIIS Building, University of Tsukuba	From domestic institutions: 126 From overseas institutions: 56
Sep 28-30, 2014	Aging Sciences: Tsukuba Global Science Week Held at University of Tsukuba	From domestic institutions: 649 From overseas institutions: 15
Sep 25, 2014	Title: The 68th Fujihara Seminar Venue: IBM Amagi Homestead	From domestic institutions: 39 From overseas institutions: 24
Sep 24, 2014	Title: "Homeodynamics in Clock, Sleep and Metabolism" (The 3rd Annual IIIS Symposium) Venue: The University of Tokyo	From domestic institutions: 215 From overseas institutions: 17
Jan 20, 2014	Title: The 2nd Annual IIIS Symposium Venue: International Congress Center, Tsukuba	From domestic institutions: 121 From overseas institutions: 22
Mar 27, 2013	Title: The 1st Annual IIIS Symposium Venue: International Congress Center, Tsukuba	From domestic institutions: 169 From overseas institutions: 30

Appendix 5 List of Achievements of Center's Outreach Activities between FY 2012 – 2020

* Using the table below, show the achievements of the Center's outreach activities from FY2012 through FY2020 (number of activities, times held).

*If there are any rows on activities the center didn't implement, delete that (those) row(s). If you have any activities other than the items stated below, fill in the space between parentheses after "Others" on the bottom with the name of those activities and state the numbers of activities and times held in the space on the right. A row of "Others" can be added, if needed.

Activities	FY2012 (number of activities, times held)	FY2013	FY2014	FY2015	FY2016	FY2017	FY2018	FY2019	FY2020
PR brochure, pamphlet	1	2	2	2	1	1	2	2	2
Lectures, seminars for the general public	0	8	5	12	20	24	24	42	2
Teaching, experiments, training for elementary, secondary and high school students	0	4	9	10	12	15	17	20	1
Science cafe	1	2	2	0	1	2	4	0	0
Open house	1	0	10	1	0	1	5	1	0
Participating, exhibiting in events	0	4	6	3	3	3	3	6	1
Press releases	1	4	3	6	8	25	17	17	16
Publications of the popular science books	2	0	0	1	0	3	2	1	4
Others （Internet Broadcasting）	0	0	0	0	0	0	0	2	0

Appendix 5 List of Media Coverage of Projects Carried out between FY 2012 – 2020

* Select main items of press releases, media coverage, and reports for FY 2012-2020 (especially by overseas media)

1) Japan

No.	Date	Type of the media (e.g., newspaper, magazine, television)	Description
1	Oct 31, 2012	**Newspaper** The Asahi Shimbun, The Yomiuri Shimbun, The Mainichi, The Nikkei, Ibaraki Shimbun, Nikkei Business Daily	Establishment of International Institute for Integrative Sleep Medicine, University of Tsukuba
2	Apr 26 - May 24, May 4 -25, 2013	**Newspaper** Chugoku Shimbun Kobe Shimbun	Introduction of Yanagisawa's research history and current research works (weekly) (Yanagisawa)
3	May 14, 2013	**Newspaper** Fukui Shimbun	Solving the mystery of sleep (Yanagisawa)
4	Jun 18, 2013 Jun 20, 2013	**Newspaper** The Yomiuri Shimbun, The Mainichi, The Nikkei,Ibaraki Shimbun, Tokyo Shimbun, Nikkei Sangyo Shimbun The Asahi Shimbun	Scientists revealed that the quality of prior waking experience greatly influence sleepiness (Yanagisawa)
5	Aug 9, 2013	**Magazine** Beikoku Seiyaku Gyokai Shuho (U.S. Pharmaceutical Industry Weekly Report) Vol. 481	Masashi Yanagisawa's vision – challenges in medicinal chemistry to develop orexin agonist from academia in Japan (Yanagisawa)
6	Sep 1, 2013	**Magazine** Medical Asahi Vol. 502	Challenging the mystery of sleep / wakefulness (Yanagisawa)
7	Sep 17 - 18, 2013	**Newspaper** Nikkei Sangyo Shimbun	Innovators in Japan (1)(2) Scientists discovered that a neuropeptide is involved in the mystery of sleep (Yanagisawa)
8	Sep 25, 2013	**Newspaper** The Nikkei	Discovery of gene adjusting length of sleep (Yanagisawa)
9	Sep 30, 2013	**Magazine** AERA Mook	Introduction of Yanagisawa's research history and current achievements (Yanagisawa)
10	Nov 29, 2013	**Magazine** Nikkei Science Janary 2014	"The Front Runner" Solving the mystery of sleep by genetics and neuroscience (Yanagisawa)
11	Dec 6, 2013 Dec 12, 2013	**Newspaper** The Nikkei, Nikkei Sangyo Shimbun Mainichi Shimbun	Discovery of unknown hormone secretion control mechanism responsible for anxiety in the adrenal cortex (Yanagisawa)
12	Jan 14, 2014	**Newspaper** Mainichi Shimbun	Revealing the mechanisms of the sleep disorder narcolepsy (Sakurai)
13	Jan 24, 2014	**Television** Science Zero, NHK	Development of novel drugs based on protein crystallization technique under a low-gravity environment in the International Space Station (Urade)
14	Jan 31, 2014	**Television** Koichi Dohmoto's a little bit of science, NHK	Discovery of "Sleep Gene"? (Yanagisawa)
15	Feb 2, 2014	**Television** Koji Kato's interview "Koji's Soul," BS4	Discovery of orexin involved in appetite and sleep (Sakurai)
16	Mar 2, 2014	**Television** Yume no tobira plus, TBS	Why do we sleep? Challenges to solve the mystery of sleep (Yanagisawa)
17	May 5, 2014	**Magazine** President, Issue 2014 5.5	Will new drugs controlling sleep/wake regulation be developed? (Yanagisawa)
18	May 25, 2014	**Television** Galileo X, BS Fuji	Mystery of sleep/wakefulness: exploring sleepiness from genetic point of view (Yanagisawa)
19	Jun 10, 2014	**Newspaper** The Nikkei	Scientists elucidate the molecular mechanisms underlying itch and scratching behavior (Nagase)
20	Aug 10, 2014	**Magazine** Nikkei Business Associe Issue 2014.9	How to sleep well for business persons (Yanagisawa)
21	Sep 1, 2014	**Magazine** Ushio September Issue	Interview by Soichiro Tahara: innovator of Japan (Yanagisawa)
22	Sep 20, 2014	**Newspaper** The Yomiuri Shimbun	Top scientist describes the mystery of sleep: Yomiuri Techno Forum (Yanagisawa)
23	Sep 25, 2014 Sep 26, 2014	**Newspaper** Yomiuri Shimbun Nikkan Kogyo Shimbun	Eight scientists were awarded Teiichi Yamazaki Award: Foundation for Promotion of Material Science and Technology of Japan (Nagase)
24	Nov 13, 2014	**Telvision** World Business Satellite, TV Tokyo	Feature: new drug saves sleepless people in Japan (Yanagisawa)
25	Nov 15, 2014	**Radio** Prime Factor, J-WAVE	World-leading scientist explains the importance of sleep (Yanagisawa)
26	Nov 23, 2014	**Television** Galileo X, BS Fuji	Development of active chemical compounds that can penetrate into blood brain barrier (Yanagisawa, Nagase)
27	Dec 3, 2014	**Magazine** The Nikkei Biotech Online	Interview of Teiichi Yamazaki Award laureate (Nagase)
28	Dec 3, 2014	**Magazine** Medical Tribune MTPro	Nalfurafine may improve the quality of sleep (Nagase)
29	Dec 4, 2014	**Newspaper** The Yomiuri Shimbun	Development of the new drug that may cure insomnia by directly acting on sleep center in brain (Yanagisawa)

30	Dec 13, 2014	**Magazine** Igaku No Ayumi vol. 251 No. 11	Synthesis of nalfurafine hydrochloride that may become a remedy for generalized pruritus (Nagase)
31	Feb 24, 2015	**Magazine** GQ Japan	Your Internal Clock (Yanagisawa)
32	Mar 2, 2015	**Book** Yume No Tobira Plus	Rescuing people suffering from sleep disorders (Yanagisawa)
33	Mar 5, 2015	**Television** NHK News	Scientists identified cells responsible for regulating circadian rhythms (Yanagisawa)
34	Mar 13, 2015	**Newspaper** The Mainichi	Warning to 'sleepless country'; Yanagisawa emphasizes the importance of sleep (Yanagisawa)
35	Mar 19, 2015	**Newspaper** The Asahi Shimbun	Scientists identified cells responsible for regulating circadian rhythms (Yanagisawa)
36	May 2015	**Magazine** Medical Asahi, May 2015 issue	Series: Drugs born in Japan volume 10 "Bozentan" (Yanagisawa)
37	Jun 2015	**Magazine** Medical Asahi, June 2015 issue	Series: Drugs born in Japan volume 11 "Suvorexant" (Yanagisawa)
38	Aug 10, 2015	**Radio** Radio Nikkei 1, "Byoyaku Hour"	Regulation of sleep/wakefulness; orexin system as a drug target (radio lecture by Yanagisawa)
39	Aug 13, 2015	**Newspaper** The Mainichi	Towards the mystery of sleep: challenges of IIIS (Yanagisawa)
40	Sep 29 - Oct 1, 2015	**Newspaper** The Daily Engineering & Consruction News, The Yomiuri Shimbun **Web** Medical Tribune Aging Style	International Institute for Integrative Sleep Medicine, University of Tsukuba: Construction of IIIS Building completed and the inauguration ceremony was held
41	Oct 1, 2015	**Newspaper** The Mainichi	Q & A: How to maintain good quality of sleep in the refuge (Yanagisawa answered an interview by newspaper)
42	Oct 11, 2015	**Television** NHK	Science ZERO "Mystery of Sleep: The frontier of sleep medicine" (Yanagisawa)
43	Oct 22 – 27, 2015	**Newspaper/Web** The Asahi Shimbun, The Yomiuri Shimbun, The Mainichi, The Nikkei Biglobe News, MyNavi News *And other 22 coverages	New insights into the role and function of REM sleep (Hayashi)
44	Oct 23, 2015	**Television** NHK News	New insights into the role and function of REM sleep (Hayashi, interview by telephone)
45	Dec 3, 2015	**Newspaper** The Asahi Shimbun	Discovery of a novel compound regulating wakefulness (Nagase)
46	Dec 27, 2015	**Television** TBS	Yume-no-tobira plus "Dream compounds making us sleep well" (Urade)
47	Jan 8, 2016	**Magazine** Bungei Shunju Feb issue	Feature article: Qualification for the leaders making breakthroughs (Yanagisawa interview)
48	Jan 25 - Feb 4, 2016	**Newspaper** The Asahi Shimbun, The Joyo Shimbun **Web** Aging Style	Timing is important for the care of PTSD (Sakaguchi)
49	Feb 4, 2016	**Newspaper** The Yomiuri Shimbun	Feature article: orexin and sleep (Yanagisawa)
50	Feb 26, 2016	**Television** BS Fuji	Kakushin-no-ism (Yanagisawa)
51	Mar 14, 2016	**Newspaper** Nikkei Sangyo Shimbun	Innovative studies on substances regulating sleep (Yanagisawa)
52	Apr 16, 2016	**Television** NHK E-tele	"Why do animals sleep -Sleep Science-" (Sakurai)
53	Apr 28, 2016	**Television** NHK NEWS **Newspaper** The Ibaraki Shimbun, The Asahi Shimbun, The Mainichi, The Yomiuri Shimbun, The Sankei Shimbun, Tokyo Shimbun	Yanagisawa received the Medal with Purple Ribbon (Yanagisawa)
54	Jun 16, 2016 Sep 1, 2016	**Television** NHK E-tele **Newspaper** The Nikkan Kogyo Shimbun	A new device to stop snoring (Satoh)
55	Aug 10, 2016	**Newspaper** Nikkei Shimbun	Introduction of highly internationalized institute, IIIS (Yanagisawa, Vogt, etc.)
56	Sep 6 - 8, 2016	**Newspaper** The Yomiuri Shimbun, The Nikkan Kogyo Shimbun, Nikkei Sangyo Shimbun, The Asahi Shimbun	Hayashi received the 26th Tsukuba Encouragement Prize (Hayashi)
57	Sep 8, 2016	**Magazine** Tarzan	Sleep associated with body and brain condition (Sakurai)
58	Oct 20, 2016	**Radio** Radio NIKKEI	Mechanisms of sleep/wake regulation and the discovery of a novel neurotransmitter (Yanagisawa)
59	Oct 23, 2016	**Television** BS JAPAN	Mirai Eyes (Introduction of research activities in IIIS)

60	Nov 3, 2016 - Jan 13, 2017	**Television/Newspaper/Web** ANN NEWS, NHK News, Kyodo News, The Asahi Shimbun, GIZMODO *And other 48 coverages	Genetic analysis identifies proteins controlling sleep in mice (Yanagisawa, Funato)
61	Nov, 2016	**Magazine** Rikejo	"What is good sleep?" (Hayashi)
62	Dec 26, 2016	**Magazine** Magazine	Mechnisms in the brain controlling appetite (Sakurai)
63	Jan 10, 2017	**Magazine** Tsukuba Style	Healthy lunch boxes of scientists (Vogt etc.)
64	Jan 16 - Feb 2, 2017	**TelevisionWeb** NHK News **Newspaper/Web** The Asahi Shimbun	Direct link between REM sleep loss and the desire for sugary and fatty foods (Lazarus)
65	Feb 16, 2017 Feb 23, 2017	**Web** The Asahi Shimbun Degital, YOMIURI ONLINE	Orexin as a potential drug for treating septic shock (Yanagisawa, Hayashi)
66	Feb 23, 2017	**Television** NHK Sogo TV	TV coverage of a IIIS faculty member
67	Mar 1 - 22, 2017	**Newspaper/Web** The Nikkan Kogyo Shimbun, The Nikkey Sangyo Shimbun, YOMIURI ONLINE	Gene therapy for the Schizophrenia model mice (Hayashi)
68	Apr 7, 2017	**Magazine** CREA Bungeishunju	Interview (Sakurai)
69	Apr 11, 2017 Sep 15, 2017 Oct 19, 2017	**Web** Mynavi News, AERA dot. **Magazine** junior AERA	Does loss of sleep cause obesity? (Lazarus)
70	Apr 14 - Apr 27, July 26, 2017	**Web** Hazard Lab, NEWS SALT, University Journal Online, The Mainichi Shimbun, academist Journal **Newspaper** The Ibaraki Shimbun	Memory can be manipulated using auditory stimuli during sleep (Sakaguchi)
71	Apr 19, 2017	**Television** NHK WORLD TV	Sleep Science (Yanagisawa)
72	May 16 - Jun 19, 2017	**Newspaper** The Nihon Keizai Shimbun, The Ibaraki Shimbun, The Mainichi Shimbun, The Nihon Keizai Shimbun, The Asahi Shimbun, The Nikkan Kogyo Shimbun **Web** Medial News QLifePro **Radio** CBC RADIO	Wake-promoting compound validated—the first step to deliver a magic bullet for curing narcolepsy (Yanagisawa)
73	July 2, 2017	**Web** Medial News QLifePro	The secret connection between anxiety and sleep (Sakurai)
74	Sep 27, 2017 Oct 3, 2017	**Web** Mynavi News **Newspaper** Zaikei Shimbun	Cannabis, "Spice" – better think twice (Urade)
75	Oct 2, 2017	**Television** Asaichi	Interview: "Possible to gain beauty and health?" (Sakurai)
76	Oct 19 - Dec 18, 2017	**Web** University Journal Online, academist Journal, Nature Asia **Newspaper** The Nikkan Kogyo Shimbun	Why do we fall asleep when bored? (Lazarus)
77	Nov 22, 2017	**Web** anannews	Interview: "No worries if you are a night preson! A professional advices you suibable ways for sleep" (Sakurai)
78	Nov 23, 2017	**Magazine** Tarzan	Interview: "7 keys to improve your sleep" (Sakurai)
79	Nov 23, 2017	**Web** anannews	Interview: "Can we talk to a sleep-talker? Sleep Q&A" (Sakurai)
80	Nov 24, 2017 Nov 30, 2017	**Web** Medial News QLifePro, University Journal Online	A neuropeptide that regulates behavior: a key to ease excessive fear (Sakurai)
81	Nov 25, 2017	**Web** GIZMODO JAPAN	Interview: A mastermind of sleep/awake? Secret power of Orexin which controls sleep (Yanagisawa)
82	Nov 25, 2017	**Web** ananweb	Interview: "Prime time" is a lie!? New common sense of sleep you should know (Sakurai)
83	Nov 28, 2017	**Magazine** anan	Interview: "Have a best sleep with the very latest science"(Sakurai)
84	Dec 2, 2017	**Web** Nikkei Health	Interview: Recommendation of mouth tape for better sleep (Satoh)
85	Dec 3, 2017 Mar 11, 2018	**Television** Mainichi Broadcasting System	Interview: Behavoir while sleeping (Sakurai)

86	Jan 1 - 22, 2018	**Web** JIJI PRESS, Sankei News **Newspaper** The Asahi Shimbun	The Asahi Prize 2017 (Yanagisawa)
87	Mar 3 - May 18, 2018	**Newspaper** The Yomiuri Shimbun, The Asahi Shimbun, The Mainichi Shimbun **Web** Healthy Living, SEKAI SUIMIN KAIGI, JB press	Crowdfunding project of sleep study on human (Yanagisawa, Satoh, etc.)
88	Apr 3, 2018 Jun 28, 2018	**Newspaper** The Mainichi Shimbun **Web** My Carat	Sleep disorder risk factors among student athletes (Tokuyama, Sato, etc.)
89	May 2, 2018	**Web** NIKKEI BP	Interview about Orexin (Sakurai)
90	May 7 - Jun 13, 2018	**Newspaper** The Asahi Shimbun, The Yomiuri Shimbun ONLINE	An unexpected chemosensor pathway for innate fear behavior against predator odor (Liu, Yanagisawa, etc.)
91	Jun 13 - Nov 27, 2018	**Newspaper/Web/Magazine** Nikkei Shimbun, Kyodo News, The Asahi Shimbun, SEKAI SUIMIN KAIGI, Newton, NIKKEI SCIENCE, WIRED *And other 50 coverages	Reversible Changes to Neural Proteins May Explain Sleep Need (Liu, Yanagisawa, Funato)
92	Jun 26, 2018	**Web** My Carat	The secret connection between anxiety and sleep (Sakurai)
93	July 7, 2018	**Magazine** Newton	Interview: Solving the mystery of sleep (Yanagisawa)
94	July 20, 2018 July 26, 2018	**Newspaper** THE SCIENCE NEWS **Web** Medical Tribune	How the mammalian brain sexually differentiates – hypothalamic Ptf1a confers the competence (Fujiyama)
95	Aug, 2018	**Magazine** National Geographic	Want to Fall Asleep? Read This Story. (Sakurai, Human Sleep Lab, etc.)
96	Sep 6, 2018 Sep 12, 2018	**Television/Web** Abema News	Interview about IIIS and human sleep lab (Yanagisawa, Morita, etc.)
97	Sep 14 - 15, 2018	**Newspaper** The Yomiuri Shimbun, The Mainichi Shimbun, The Nikkan Kogyo Shimbun, Ibarakinews, Nikkei Shimbun	The Keio Medical Science Prize (Yanagisawa)
98	Oct 10, 2018	**Web** Tarzan web	Why do people get sleepy? What is a sleeping debt? Approaching the mystery of sleep from three keywords (Sakurai)
99	Oct 22, 2018	**Newspaper/Web** Nikkei Shimbun	Interview about Mobile Sleep Laboratory (Yanagisawa, F-MIRAI)
100	Nov 19, 2018	**Newspaper/Web** Nikkei Shimbun, Nikkei Biotechnology & Business	S'UIMIN Received ¥700 Million in Capital from Mirai Creation Fund (S'UIMIN, Yanagisawa)
101	Nov 30, 2018 Feb 28, 2019	**Web** TEIJIN	Interview (Yanagisawa)
102	Dec 5, 2018	**Magazine** Newton	Interview about his sleep research and SNIPPs (Yanagisawa)
103	Jan 6, 2019	**Newspaper** Nikkei Shimbun	Interview "My story" (Yanagisawa)
104	Feb 8, 2019	**Television** NHK "Tohoku-kokokara"	Interview about Human Calorimeter (Tokuyama, Ishihara)
105	Mar 26, 2019	**Web** Kodansha BLUE BACKS WEB	Interview about link between sleep and PTSD treatment (Sakaguchi, Soya)
106	Mar 26, 2019	**Magazine** Newton	Interview about adrenaline (Sakurai)
107	Mar 30, 2019	**Newspaper** Weekly Toyo Keizai	Interview: Why do people sleep? Driving the world in sleep research (Yanagisawa)
108	Jun 12,2020	**Television** NHK	Busines Project of Sleep
109	Jun 11,2020	**Television** NHK	Identify the neural circuit inducing hibernation artifisially
110	Jun 12,2020	**Television** NHK	Identify the neural circuit inducing hibernation artifisially
111	Jul 21,2020	**Radio** TBS	Sekaikan Ozaki, who suffer from insomnia, ask sleep medicine researcher how to sleep well
112	Oct 2,2020	**Radio** J-WAVE	Fearure Focus

113	Dec 14,2020 Jan 1,2021	**Web** HORIEMON.COM	Prof. Takeshi Sakurai, who identified the neural circuit inducing hibernation for the first time in the world, talks "hibernation", "space travel", "emergency medical treatment"
114	Aug 20,2020	**Magazine** Chemistry Today	Ask Prof. Takeshi Sakurai about the neuron inducing hibernation -the first step to artifisial hibernation-
115	Jan 18,2021	**Magazine** Weekly Play Boy	Are human able to hibernate?
116	Jul 19,2020	**Newspaper** The Chunichi Shimbun	<Meet STEAM> Dr. Masashi Yanagisawa, sleep medicine scientist, a professor of University of Tsukuba
117	Oct 13,2020	**Newspaper** The Asahi Shimbun	S'UIMIN, venture company derived from university of Tsukuba. Measuring the personal sleep in home with AI
118	Mar 18,2021	**Newspaper** Nikkei Shimbun	Smart Work "Immersed in the reserch of sleep"

2) Overseas

No.	Date	Type of the media (e.g., newspaper, magazine, television)	Description
1	May 16, 2014	**Web** YouTube channel: Science 200 Miles in the Sky (Boeing)	ISS Discovery: Fighting Duchenne's Muscular Dystrophy (Urade)
2	July 1, 2015	**Web** NASA Official Website	ISS Benefits For Humanity: Hope Crystallizes (Urade)
3	Sep 8, 2015	**Television** NHK World	"Medical Frontiers" Volume 5, Sleep (Yanagisawa)
4	Oct 26, 2015	**Web** nippon. com	Solving the Riddle of Slumber: Cutting-Edge Research Hub in Tsukuba Focuses on Sleep (Yanagisawa) *Translaed in 8 languages
5	Nov 2 - 8, 2016	**Television/Newspaper/Web** NBCDFW.com, Science Daily, ResearchGate *And other 15 coverages	Genetic analysis identifies proteins controlling sleep in mice (Yanagisawa, Funato)
6	May 17, 2017	**Web** Asian Scientist Magazine	Wake-promoting compound validated—the first step to deliver a magic bullet for curing narcolepsy (Yanagisawa)
7	May 30, 2017	**Web** Medical Xpress	Wake-promoting compound validated—the first step to deliver a magic bullet for curing narcolepsy (Yanagisawa)
8	May 30, 2017	**Web** CLUSTER SALUD	Hope: Solid first steps to develop a cure for narcolepsy (Yanagisawa) *in Spanish
9	July 1, 2017	**Web** Science Daily	The secret connection between anxiety and sleep (Sakurai)
10	July 6, 2017	**Web** Asian Scientist Magazine	The secret connection between anxiety and sleep (Sakurai)
11	July 7, 2017	**Web** Sleep Review	The secret connection between anxiety and sleep (Sakurai)
12	Sep 5, 2017	**Web** Science Daily	Cannot sleep due to stress? Here is the cure (Urade)
13	Sep 5, 2017	**Web** Sleep Review	Sugarcane Active Component Restores Stress-affected Sleep (Urade)
14	Sep 10, 2017	**Web** Medical News Today	Sugarcane extract may relieve stress-induced insomnia (Urade)
15	Sep 21, 2017	**Web** Science Daily	Cannabis, 'spice' – better think twice (Urade)
16	Sep 29, 2017	**Web** Science Daily	Why do we fall asleep when bored? (Lazarus)
17	Sep 30, 2017	**Web** Financial Express	Why do we fall asleep when bored? (Lazarus)
18	Oct 4, 2017	**Web** Seeker	Why do we fall asleep when bored? (Lazarus)
19	Oct 24, 2017	**Web** ReliaWire	Why We Still Don't Understand Sleep, And Why It Matters (Yanagisawa)
20	Dec 11, 2017	**Web** Genetic Literacy Project	Chasing a cure for narcolepsy—and why it should be a priority (Yanagisawa)
21	Jan 1, 2018	**Web** The Atlantic	Why do we need to sleep? (Yanagisawa)
22	Mar 20, 2018	**Web** Intellectuals	Sleep Needs and Sleep Mystery (Yanagisawa) in Chinese
23	May 21, 2018	**Web** EurekAlert!, Alpha Galieo	An unexpected chemosensor pathway for innate fear behavior against predator odor (Liu, Yanagisawa, etc.)
24	Jun 1, 2018	**Web** Courrier International	Sciences.Why are we sleeping? (Yanagisawa) *in French

25	Jun 13 - Aug 25, 2018	**Web** EurekAlert!, NNR, Technology Networks, Science Daily, ZME Science, DALLAS NEWS, Sleep Review, Forbes, Quanta Magazine, WIRED US, infosalus.com	Reversible Changes to Neural Proteins May Explain Sleep Need (Liu, Yanagisawa, Funato)
26	Aug, 2018	**Magazine** National Geographic	Want to Fall Asleep? Read This Story. (Sakurai, Human Sleep Lab, etc.)
27	Sep 24 - 26, 2018	**Web** EurekAlert!, Science Daily, Biosicence Technology, Laboratory Equipment, BAZAAR	Never Enough Sleep? Mouse Mutation Shown to Increase Daily Amounts and Need for Sleep (Honda, Yanagisawa)
28	Dec 23, 2018	**Web** Noti-America	The journalist with narcolepsy that can teach you to sleep better (Yanagisawa) *in Spanish
29	Mar 15, 2019	**Web** Rossiya Segodnya	World Sleep Day (Yanagisawa) *in Russian
30	Mar 23, 2019	**Magazine** nature INDEX 2019 JAPAN	Interview (Yanagisawa, Cherasse)
32	Jun 4, 2020	**Web** Medical Xpress	Adult neurogenesis essential for sleep-induced memory consolidation in mice (Sakaguchi)
35	Jun 11, 2020	**Web** Science et Avenue	It is possible to hibernate mice and rats, and potentially other mammals (T. Sakurai)
36	Jun 13, 2020	**Web** International Business Times	Mice Successfully Placed In Hibernation By Triggering Specific Cells In Their Brain. Humans next? (T. Sakurai)
34	Jan 12, 2021	**Web** Yahoo! news	Drinking oolong tea 'could help you burn fat in your sleep' (Tokuyama)
33	Feb 2, 2021	**Web** Power of Positivuty	Japanese Scientists Link Healthy Gut Microbes to Better Sleep (Yanagisawa)
31	Mar 27, 2021	**Web** earth com	Exercise improves sleep quality in undetectable ways (Vogt)

Appendix6-1 Host Institution's Commitment (Fund, Personnel)

1. Contributions from host institution

(1) Fund, Personnel

* Regarding "Fund" entry, describe with reference to the items in the Progress Report (Jisseki-hokoku-sho) based on Article 12 of the Grant Guidelines (Kofu-yoko).

* Don't include competitive funding obtained by researchers (used as research project funding)

(FY 2012-2020)

<Fund> **(million yen)**

Fiscal Year	2012	2013	2014	2015	2016	2017	2018	2019	2020
Personnel	22	22	22	36	36	36	36	43	65
Faculty members	0	0	0	14	14	14	14	21	43
Full-time	0	0	0	0	0	0	0	7	43
Concurrent	0	0	0	14	14	14	14	14	0
Postdocs	0	0	0	0	0	0	0	0	0
RA etc.	0	0	0	0	0	0	0	0	0
Research support staffs	0	0	0	0	0	0	0	0	0
Administrative staffs	22	22	22	22	22	22	22	22	22
Full-time	16	16	16	16	16	16	16	16	16
Concurrent	6	6	6	6	6	6	6	6	6
Project activities	10	10	10	10	10	10	10	10	14
Travel	0	0	0	0	0	0	0	0	0
Equipment	0	0	0	0	0	0	0	0	0
Research projects	0	0	0	0	0	0	0	0	0
Total	32	32	32	46	46	46	46	53	79

<Personnel> **(person)**

Fiscal Year	2012	2013	2014	2015	2016	2017	2018	2019	2020
Personnel	3	3	3	3	4	4	4	5	8
Faculty members	0	0	0	0	1	1	1	2	5
Full-time	0	0	0	0	0	0	0	1	4
Concurrent	0	0	0	0	1	1	1	1	0
Postdocs	0	0	0	0	0	0	0	0	0
RA etc.	0	0	0	0	0	0	0	0	0
Research support staffs	0	0	0	0	0	0	0	0	0
Administrative staffs	3	3	3	3	3	3	3	3	3
Full-time	2	2	2	2	2	2	2	2	2
Concurrent	1	1	1	1	1	1	1	1	1

Appendix6-1 Host Institution's Commitment

Contributions from host institution

University of Tsukuba has provided IIIS with various resources as support. The provided support is equal to or greater than the support planned in the application for the WPI program.

1. Provision of land and/or building(s), lab space, etc.

Resources provided by University of Tsukuba as support for IIIS

1) From FY2015 University support more than 80 million yen as the expenses for utility of the IIIS building from the indirect cost though university collected 70 million yen as the rental fee for own funding area of 2,000 m^2.

2) Support was provided for the research spaces of the Health and Medical Science Innovation Laboratory, University of Tsukuba Hospital E Building, Project Research Building and TARA Center, providing 5,000 square meters divided among the listed venues until the completion of relocation to the new research building in August 2015.

3) From April 2019, University supports the research spaces at Innovation Medical Research Institute to expand IIIS activity especially for the human physiology laboratory.

2. System under which the center's director is able to make substantive personnel and budget allocation decisions

Division of authority

All key matters concerning the operation in the institute have been decided by a top-down system of the Director as shown in 6-1.

Principal Investigators' meeting (PI meeting)

According to the establishment of IIIS, led by the administrative department, a PI meeting was established, during which the PI regularly submits opinions to the Center Director to determine important matters concerning the IIIS as shown in 6-1.

3. Support for the center director in coordinating with other departments at host institution when recruiting researchers, while giving reasonable regard to the educational and research activities of those departments

Teaching qualification

12 with Comprehensive Human Science Biomedical Sciences major (doctoral programs), 11 with Comprehensive Human Science Medical Sciences major (master's programs), 6 with Comprehensive Human Science Kansei, Behavioral and Brain Sciences major (doctoral programs), 10 with Ph. D. Program in Human Biology (HBP), 2 with Ph.D. Program in Life Science Innovation（T-LSI), one with Pure and Applied Sciences Chemistry major, and 12 with Ph. D. Program in Humanics have a teaching qualification as shown in 6-4.

4. Revamping host institution's internal systems to allow introducing of new management methods

(e.g., English-language environment, merit-based pay, cross appointment, top-down decision making unfettered by conventional modes of operation)

Constitution of the administrative department

Administrative department is operated by the following four teams (General affairs, Accounting, Research strategy & management and Alliance & Communication) led by the Administrative Director as shown in 6-2.

Use of English as official language and employment of bilingual staff

English is used as an official language at IIIS. A bilingual environment has been implemented, with 70% of administrative staff members fluent in spoken and written English as shown in 6-2.

A dispensation of distributing license revenues to research centers/departments

University of Tsukuba agree that, after deduction of the compensation for inventors and 10% of overhead expenses, licensing revenues received by the University should be distributed to the headquarters and IIIS in proportion to IP cost burdens (refer 3-1 for details).

5. Utilities and other infrastructure support provided by host institution

(*In addition to those listed in the item 1. "Contributions from host institution")

Cost of constructing a new research building

The Sleep Medicine Research Building costed approximately 3.8 billion yen and the expenses beyond those covered by the facility development subsidies of 2 billion yen kindly provided by MEXT were supported by in-house funding.

6. Support for other types of assistance

Expansion of activities of the professor specially appointed

The president of University of Tsukuba decided to appoint one of PIs in IIIS, Dr. H. Nagase became 70 years old in FY2017 and essential for the success IIIS to "Tokumei-kyoju," the professor specially appointed by the President.

Appendix6-2 The Host Institution's Mid-term Plan

* Excerpt the places in the host institution's "Mid-term objectives" and/or "Mid-term plan" that clearly show the positioning of the WPI center within its organization.

IIIS has given a major impact on the reform of University of Tsukuba. During the third mid-term plan starting from FY 2016, the University aims to pursue the globally unrivaled frontier research of 2 objectives, *i.e.,* research for the quest for truth and research for innovation contributing to society, in wide-ranging disciplines and research fields. To realize these objectives, the University has made a plan of reorganization/restructuring/merger of all research centers and is implementing it during the period of the 3rd mid-term plan. Based on the plan the research centers have been classified by function into the Advanced Research Centers and the Research Support Centers. The former has been further classified as R1 (World-class Research Center), R2 (National-class Research Center), R3 (Developing Research Center), and R4 (Research Unit) to facilitate strategic resource allocation. Center for Computational Science (CCS) and Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA) are classified as the R1 status of World-class Research Center in physics and bioscience, respectively, while the status of all research centers shall be reviewed every five years.

In addition to the research center's reorganization and classification, the University decided to establish Organization for Development of Global Research Centers in order to aid the development of the global research centers through the comprehensive support provided by the creation of an 'On-campus Special Zone for Research Strategy' (provisional name) and strategic allocation of the University's research resources.

IIIS and R1-accredited CCS and TARA, are the initial centers to be supported by the Organization, which aims to expand horizontally among these centers the tasks/achievements of promoting the advanced and interdisciplinary researches, internationalization, and reforming systems thus far headed by the IIIS. Specifically, with Vice President responsible for research as the Organization's Director, an office of strategic planning is to be established, along with an academic coordinator for strategic collaboration and a support division.

As of March 26, 2020, University of Tsukuba officially established Organization for Development of Global Research Centers underpinning the activity of CCS, TARA, and IIIS. Much is expected of IIIS to contribute for the success of the Organization by the active sharing of experience, knowledge, and know-how accumulated at IIIS.

World Premier International Research Center Initiative (WPI)
Progress Plan (For Final Evaluation)

Host Institution	University of Tsukuba	Host Institution Head	NAGATA Kyosuke
Research Center	International Institute for Integrative Sleep Medicine (IIIS)		
Center Director	YANAGISAWA Masashi	Administrative Director	KIMURA Mayumi

* Write your report **within 6 pages**.
* Use yen (¥) when writing monetary amounts in the report. If an exchange rate is used to calculate the yen amount, give the rate.

1. Mid- to Long-term Research Objectives and Strategies Based on the Center's Results during Funded Period

Describe new challenges in the Center's research objectives and plans after the funding period ends. If major adjustments will be made in the Center's operation, such as newly set research themes/objectives or a change in the director, describe the strategic background to the adjustments.

Long-term objectives we aim to achieve in 2040

A. To realize a society where various issues of sleep are well managed to prevent diseases caused/worsened by severe sleep debts

Sound sleep is essential for maintaining physical and mental health; losing sleep chronically poses immense medical and social problems. We thus aim to overcome sleep disorders and resulting sleep debts from which more than 20% of people in developed countries suffer, in order to let them enjoy the lives with relief and release from health concerns until 100 years old. To achieve this vision for 2040, we set out the **long-term objective A** as shown above.

B. To realize a society where innovative emergency medical care based on hibernation is implemented to save lives even in disasters

Last year, the Vice Center Director of IIIS discovered a group of neurons (Q-neurons) in the hypothalamus whose forced-activation induces a hibernation-like state in mice. Besides sleep, hibernation is another behavior that is characterized as regulated hypomobility. The hypomobile behavior adapted to the rotation of the earth is sleep, while another hypomobile behavior accommodated to the revolution of the globe is hibernation. Revealing similarities and differences between sleep and hibernation would lead us to better understanding of both hypomobile behaviors. The characteristics of hibernation, hypometabolism reduces systemic oxygen demand drastically and could offer an effective critical care to avoid tissue injuries and necrosis under hypoxia/anoxia. We thus set the 2nd **long-term objective B** as indicated above.

Expanding our research subject from sleep to hypomobile behaviors (sleep and hibernation), we aim for ensuring to achieve our vision, having people enjoy their lives with relief and release from health concerns until 100 years old.

Five specific goals we aim to reach in 2040

To achieve our vision and 2 long-term objectives mentioned above, we broke down them into 5 specific goals as described below.

1. To develop methods to control homeostasis of sleep to save more time to improve QOL
2. To develop preventive measures against diseases caused/worsened by severe sleep debts
3. To develop methods to predict risks to be suffered from diseases caused/worsened by sleep debts
4. To build a model of the medical network offering sufficient cares for sleep disorders to everyone in the world
5. To develop innovative emergency medical care using the hibernation technology to save lives even in disasters

Goal 1: To develop methods to control homeostasis of sleep to save more time to improve QOL

For many people losing sleep chronically due to hectic schedules of business/schoolwork or a long commuting time, it is not so easy to spare more time for sleep. As a paradigm shift of the measure against

sleep debt, we would like to develop methods to control homeostasis of sleep. Each species of the vertebrates has its inherent time for sleep, which would be determined by a set point under the control of homeostasis. Revealing the biochemical and genetic mechanisms of the set point, we would like to develop a method to change it by pharmacological, epigenetic or genetic approaches. This goal appears to be feasible, since there are short sleepers active and healthy with limited sleeping hours, although in very rare cases.

Major milestones to reach the goal are as follows.

- To identify critical neural circuits where the SIK3 pathway regulate sleepiness or the set point of sleeping time
- To find other pathways regulating sleep homeostasis or determining the set point
- To select a promising molecular target of the drug adjusting the set point
- To identify a lead compound of the drug adjusting the set point of sleeping time

Goal 2: To develop preventive measures against diseases caused/worsened by severe sleep debts

Severe sleep debts due to chronical insomnia and/or depression could not be resolved by the methods to control sleep homeostasis mentioned above, and it is essential to develop methods to prevent diseases caused/worsened by severe sleep debts so as to relief and release from health concerns until 100 years old. We plan to elucidate 1) neural functions of sleep, 2) neural and molecular mechanisms of REM sleep regulation, and 3) regulatory mechanisms of sleep by circadian rhythm and motivation/emotion. Further, at molecular levels, we study cross-talks between the central nervous system and peripheral organs including the immune system, by using sleep deprivation as perturbation of the interaction. Through these studies we will develop preventive drugs/methods against disease caused/worsened by severe sleep debts.

Major milestones to reach the goal are as follows.

- To identify a molecular target specific to the neurons inducing REM sleep
- To elucidate neural and molecular mechanisms of memory consolidation in sleep
- To clarify molecular pathology of representative sleep disorders
- To elucidate neural and molecular mechanisms of sleep regulation by circadian rhythm motivation/emotion.
- To reveal molecular mechanisms of the cross-talks between the CNS and immune systems
- To achieve POC of an intervention through the molecular target to modulate REM sleep
- To elucidate novel molecular mechanisms of neural functions of sleep
- To discover a lead compound of the modulating drug of REM sleep
- To find a novel molecular target of interventions to prevent diseases caused/worsened by severe sleep debts

Goal 3: To develop methods to predict risks to be suffered from diseases caused/worsened by sleep debt

The unconsciousness during sleep make it impossible for us to recognize quality and quantity of own sleep correctly. We thus have been developing an IoT system enabling everyone to visualize status/habit of sleep in home in order to encourage better sleep behaviors/customs spontaneously. Developing the sleep IoT technology further, we aim at building big data of sleep and epidemiology from a million of subjects. The big data should be comprised of EEGs during sleep, hypnograms and epidemiological/receipt (medical bill) data. Analysing the big data by using artificial intelligence (AI) over 5-10 years, we undertake developing methods to predict risks to be suffered from diseases caused/worsened by sleep debt.

Major milestones to reach the goal are as follows.

- To obtain medical device certificates of the in-home EEG device and the cloud analysis system using AI under development by S'UIMIN Inc.
- To promote the sleep measurement using the sleep IoT technology and establish a route through which we access all the data and information necessary to build up the big data
- To build the big data comprised of 100,000 subjects as a platform for developing a method to predict risks of diseases caused/worsened by sleep debt
- To expand the big data to include 500,000 subjects and verify the risk prediction of the diseases
- To expand the big data further to include 1,000,000 subjects and complete the development of the method to predict the risks

Goal 4: To build a model of the medical network offering sufficient cares for sleep

disorders to everyone in the world

There are big gaps in sleep medicine between medical networks in metropolitan areas and those in the provinces. Recently many areas in Japan suffered disaster from the earthquake or flood, and there are no medical networks that can cope with sleep issues frequently caused in disasters, *i.e.,* acute and chronic insomnia at shelters, PTSD of disaster victims as well as rescuers, and delirum of elder victims. We will thus construct a medical network of sleep medicine as a model in Ibaraki Prefecture where there are less than 10 specialists in sleep medicine now. We will then improve it to accommodate the sleep issues in disasters and expand it throughout Japan in collaboration with Japanese Society of Sleep Research.

Major milestones to reach the goal are as follows.

- To develop a Mobile Sleep Lab based on a prototype we built in 2020 by renovation of a fuel cell bus manufactured by Toyota Motor Corporation
- To build an operation system of the Mobile Sleep Lab based on an initial network of sleep medicine in Ibaraki Prefecture, which consists of several core hospitals
- To begin trial operation of the Mobile Sleep Lab in the selected areas
- To establish the network of sleep medicine covering the entire prefecture, which let everyone access always to necessary medical cares for sleep disorders

Goal 5: To develop innovative emergency medical care using the hibernation technology to save lives even in disasters

To achieve the 2nd long-term objective, we will elucidate neural and molecular mechanisms of Q-neuron-induced hypothermia and hypometabolism (QIH) and identify by single-cell gene expression analysis (scRNA-seq) a good molecular target to develop a good intervention specifically activating Q-neurons. Understanding upper/lower reaches of the neural circuit involving Q-neurons, we will identify neurons functioning as an effector at the interface between CNS and peripheral nervous system (or endocrine system) as well as signaling molecules mediating the trigger signal of hypothermia/hypometabolism to peripheral tissues/cells.

Major milestones to reach the goal are as follows.

- To elucidate neural and molecular mechanisms of QIH
- To understand interaction between hibernation and sleep through studying the need of sleep during hibernation
- To elucidate mechanisms of a) the cold tolerance of peripheral tissues/cells and b) the metabolic control of peripheral tissues/cells by CNS and/or endocrine systems
- To achieve QIH in a non-human primate (cynomolgus monkeys) by an invasive method similar to that in rodents
- To identify a molecular target suitable for drug discovery and/or physical stimulation
- To find a lead compound of the drug inducing QIH
- To develop a minimally invasive method to induce QIH in cynomolgus monkeys

Outcomes and impacts on society of the vision and 2 long-term objectives

According to RAND Europe, sleep disorders such as insomnia are causing Japan an estimated annual economic loss of 15.2 trillion yen or 2.92% of the GDP, the largest such loss reported among developed nations. If the complete control of human sleep becomes possible and sleep-related problems are resolved, these economic losses could be eliminated, and numerous diseases caused/worsened by sleep disorders could also be prevented. The resulting economic effect would exceed 30 trillion yen per year including reductions in healthcare costs.

Furthermore, with the development of the artificial hibernation technology, hypothermic/hypometabolic treatments for fatal diseases such as myocardial infarction, cerebral infarction, cerebral trauma, severe infection such as COVID-19 etc., could be developed, and in long-term interplanetary flights, the issues of limited supplies of water, food, and energy could be resolved.

2. Management System of the Research Organization

2-1. Describe the Center's Research Organizational Management System that will Execute the Research Strategy and Plan Described above.

- In Appendix 1-1, list the PIs who will ensure that the Center's project is sustained and advanced after the funding period ends.
- In Appendix 1-2, enter the number of Center personnel (researchers, research-support staff, and administrative staff) in FY 2022
- In Appendix 2, diagram the Center's organizational management system.

To implement the research strategy and plans outlined above, as of April 1, 2021, we will organize a

new project team, consisting of all the PIs in Core Group of the Center (IIIS), and PIs of new Collaborative Groups in University of Tsukuba and new Satellites, as shown in Table 1 (refer to Appendix 1-1 for the details). The new project shall be financed mainly by **Moonshot R&D Program** operated by Japan Agency for Medical Research and Development (AMED). The Center Director, Masashi Yanagisawa stays in the position as it is and will also serve as the project manager (PM) of Moonshot R&D Program.

Table 1. Members of the new project

	Core Group of IIIS	Collaborative Groups in University of Tsukuba	Satellites
Neuro-biology	M. Yanagisawa, H. Funato, T. Sakurai, A. Hirano, Y. Hayashi, M. Lazarus, Y. Oishi, M. Sakaguchi, K. Vogt, S. Honjo, K. Sakurai, H. Toda	M. Matsumoto (Faculty of Medicine)	C. B. Saper (Harvard Univ.), V. Vyazovskiy (Univ. of Oxford), Q. Liu (NIBS), R. Greene (UTSW), A. Yamanaka (Nagoya Univ.), G. Sunagawa (RIKEN)
Pharmaceu-tical science	H. Nagase, N. Kutsumura, T. Saitoh†		
Human physiology	K. Tokuyama, T. Abe, T. Kanbayashi		M. Fujiwara (S'UIMIN Inc.)
Systems biology/ mathemati-cal analysis	H. Kitagawa*	H. Ando (Faculty of Engineering, Information and Systems), H. Ozaki (Faculty of Medicine), T. Amagasa (Faculty of Engineering, Information and Systems)	M. Kurino (Keio Univ.)

*to be appointed for PI as of April 1, 2021; †to be appointed for PI on the retirement of H. Nagase

Basically there would be no changes in the composition of the Core Group, consisting of 3 subgroups, *i.e.,* neurosciences/molecular genetics, pharmaceutical science, and human physiology, to promote the interdisciplinary research of hypomobile behaviors (sleep and hibernation). The subgroup of neurosciences/molecular genetics focuses on solving the mysteries of sleep/hibernation. The subgroups of pharmaceutical science and human physiology continue to focus on sleep studies for a while, and will expand the study subjects to cover hibernation gradually. Currently, the Core Group is formed of 13 Labs led by 17 PIs/CoPIs. As of April 1, 2021, we plan to appoint a new PI in the Core Group, H. Kitagawa, who will move from Center for Computational Science (CCS) in University of Tsukuba and engage in studies of big data and artificial intelligence (AI).

To strengthen our capability of systems biology/mathematical analyses further, we invite to the project 3 more computational scientists in University of Tsukuba as new members of the Collaborative Groups. We nominate also a specialist of non-human primate in University of Tsukuba as a new member of the Collaborative Group for the hibernation study, while we keep good relations with the PIs of the current Collaborative Groups and continue on-going collaborations with them.

Among current Satellite PIs, K. Mishima in Akita University; R. Greene, C. Green and J. Takahashi in University of Texas South Western Medical Center (UTSW); and Q. Liu in National Institute of Biological Sciences, Beijing (NIBS), R. Greene and Q. Liu will join the new project. Besides them, we have invited 6 PIs as new Satellite PIs to reinforce capabilities of the Core Group complementally, as shown in Table 1.

Regarding the Administration, T. Kokubo retired from Administrative Director as of March 31, 2021, and the current Vice Administrative Director, M. Kimura will take over the position on April 1, 2021. To support the PM of Moonshot R&D Program, a Project Coordinator shall be posted to secure smooth operation and expected achievements of the project. Besides the change of Administrative Director and the newly established Project Coordinator, there will be no major changes in the Administration, which is comprised of 1) the management strategy team in charge of general affairs and accounting and 2) the research strategy team responsible for research planning and public relations.

2-2. Initiatives and Plans that will Impel System Reforms

Describe the Center's action plan that embodies the basic policies of the University Reform Plan, and the Center's plan and strategies that lead to host institution reforms either directly or via ripple effects (also to other institutions, if applicable). Describe also the Center's strategies for fostering and securing the next generation of researchers (e.g., introduction of tenure tracks), and the system reform for enhancing the Center's organizational management, such as the implementation/verification PDCA system.

As will be explained in 3-1, University of Tsukuba established Organization for Development of Global Research Centers in March 2020 in order to develop/support world-class research centers in the University. As the first research centers to be supported by the Organization, IIIS and 2 world-class research centers, CCS and Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA) have been selected. Through the Organization, much is expected of IIIS to share experiences and knowledge/know-how on the operation of the World Premier International Research Center such as internationalization, system reforms, interdisciplinary research, and strategies of intellectual property right and alliance.

As previously reported in 7-4 (Progress Report), as means of fostering and securing the next generation of researchers, IIIS played an active role in the application to Doctoral Program for World-Leading and Smart Education (WISE) program started in FY2018 by Ministry of Education, Culture, Sports and Technology (MEXT). The Center Director led the effort, in close cooperation with the deans and associate deans of Faculty of Medicine as well as Faculty of Pure and Applied Science and Faculty of System and Information Engineering to propose the launch of the "Ph.D. Program in Humanics." This program aims to foster future leaders with doctorate level training in both a) biomedical science and b) mathematics, physics, chemistry, engineering or informatics. They are expected to develop scientific expertise to integrate the knowledge in two disciplines organically, and apply their knowledge to make contributions to society. A distinguishing feature of this program is the mentorship system in which one faculty member from the biomedical sciences and another from mathematics, physics, chemistry, engineering or informatics are assigned as double mentors to a graduate student. Fortunately, the Ph.D. Program in Humanics, was adopted in October 2018. The Center Director, M. Yanagisawa serves as the de facto leader of the program, Program Coordinator, and several PIs including him functioned as mentors of 17 graduate students in FY 2020. The unique and original program contributes to the reform of the graduate schools of the University as well as the promotion of the interdisciplinary research significantly.

Besides the Humanics Program, the Core Group of the Center accepted 65 graduate students for dissertation studies in FY2020 from the faculties such as School of Medicine and College of Medical Sciences, and the graduate schools including Master's and Doctoral Programs in Medical Sciences, Master's and Doctoral Program in Neuroscience, and Degree Programs in Pure and Applied Sciences. We also accepted 22 interns/research students even from outside of the University to promote the collaboration or to encourage them to go to the graduate schools of the University.

As another measures for fostering and securing the next generation of researchers, we have introduced 3 new supporting systems, *i.e.,* IIIS Internal Grant System, IIIS RA System, IIIS Scholarship, for 3 levels of trainees, *i.e.,* postdocs, graduate students, and undergraduate students/interns, respectively. We would like to encourage CCS and TARA to share especially the supporting systems as well as the structure of the Administration as ripple effects under the Organization for Development of Global Research Centers.

3. Center's Position within Host Institution and Measures to Provide It with Resources

Describe the Center's future plans with regard to the following points after the funding period ends.

3-1. From a Mid- to Long-term Perspective, the Position of the Center within the Organization of the Host Institution

Describe where the Center will be placed within the host institution's overall organizational strategy under the leadership of the institution's head.

- In Appendix 3, diagram the Center's position within the organization of the host institution, and describe that positioning using excerpts from the institution's mid- to long-term plan. If the plan has not been established yet, describe the consideration being given to the Center's positioning.

IIIS has given a major impact on the reform of University of Tsukuba. During the third mid-term plan starting from FY 2017, the University aims to pursue the globally unrivaled frontier research of 2 objectives, *i.e.,* the research for quest for truth, and the research for innovation contributing to society, in wide-ranging disciplines and research fields. To realize these objectives, the University has made a plan of reorganization/restructuring/merger of all research centers and is implementing it during the period of the 3rd mid-term plan. Based on the plan the research centers have been classified by function

into the Advanced Research Centers and the Research Support Centers. The former has been further classified as R1 (World-class Research Center), R2 (National-class Research Center), R3 (Developing Research Center), and R4 (Research Unit) to facilitate strategic resource allocation. CCS and TARA are classified as the R1 status of World-class Research Center in physics and bioscience, respectively. The status of all research centers shall be reviewed every five years.

In addition to the research center's reorganization and classification, the University established Organization for Development of Global Research Centers in March 2020 to implement its objectives through the comprehensive support provided by the creation of an 'On-campus Special Zone for Research Strategy' and strategic allocation of the University's research resources. IIIS and R1-accredited CCS and TARA, are the initial group of the research centers to be supported by the Organization, which aims to expand horizontally among these centers the tasks/achievements of promoting advanced/interdisciplinary researches, internationalization, and reforming systems thus far headed by IIIS. Much is expected of IIIS to contribute to the initiative by the active sharing of experience, knowledge, and know-how accumulated at IIIS.

3-2. Host Institution's Action Plan for Sustaining and Advancing the Center as a World Premier International Research Center (e.g., Positioning, Financial Resources)

- In Appendix4, describe the host institution's resource allocation plans for the Center, including the allocation of posts (in both its research and administrative divisions).

One of the salient features of IIIS is that, with the exception of 3 PIs, the Core Group is comprised of PIs who were all invited from other institutions. While K. Tokuyama had been engaged in studies of human sleep and metabolism in the University since quite a while ago, M. Yanagisawa and H. Funato were invited in FY2009 to start the project of Funding Program for World Leading Innovative R&D Science and Technology (FIRST) sponsored by the Cabinet office. What made this possible was the leadership of the Center Director, the understanding and support of the President, and the liberal academic philosophy and policies of the University. As a result, it became possible within a span of only several years to establish a world-class research center specializing in sleep science, we believe. We think that IIIS could serve as a role model for the WPI program.

To make the foundation of IIIS sustainable, the President of University of Tsukuba has repeatedly stated at the WPI Program Committee that PIs with a proven track record of achievement should be promoted to receive the status of 'tenure.' The Center Director and the Deputy Director have already acquired this status, and in FY 2018, with the cooperation of the Faculty of Medicine, a female PI (A. Hirano) was appointed to a tenure track assistant professor by using the strategic position secured by the University. President Nagata has led the strategic process of initiating the tenure reviews of 4 PIs whose terms of employment contracts are approaching to the renewal limitation stipulated by the Labor Contract Act. By the termination of the supporting period of the WPI program on March 31, 2022, 7 PIs of IIIS will have received the status of tenure, pending successful reviews. Accordingly in the next fiscal year, the Center Director will nominate additional PIs for the tenure review to the Personnel Committee under the approval by Vice President for Human Resources.

Appendix 1 List of Principal Investigators (for Progress Plan)

* If the number of principal investigators exceeds 10, add rows as appropriate.
* Give age as of 1 April 2022
* For investigators who cannot participate in the center project from FY 2022, indicate the time that their participation will start in the "Notes" column.
* Enter the host institution name and the center name in the footer.

	Name	Age	Current affiliation (position title, organization, department)	Academic degree and current specialties	Effort(%)*	Notes (Enter "new" or "ongoing")
1	Masashi Yanagisawa	61	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D. ; Neuroscience, Pharmacology	95	Ongoing
2	Takeshi Sakurai	56	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Professor, Faculty of Medicine, University of Tsukuba	M.D., Ph.D.; Neuroscience	80	Ongoing
3	Hiromasa Funato	52	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba Professor, Toho University	M.D., Ph.D.; Neuroscience	40	Ongoing
4	Robert Greene	71	Professor, Department of Psychiatry, University of Texas Southwestern Medical Center	M.D., Ph.D.; Neuroscience	10	Ongoing
5	Qinghua Liu	50	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba, Japan; Investigator, National Institute of Biological Sciences (NIBS), Tsinghua University, China	Ph.D. ; Genetics, Molecular Biology, Biochemistry	10	Ongoing
6	Noriki Kutsumura	44	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Organic Chemistry, Medicinal Chemistry	80	Ongoing
7	Hiroyuki Kitagawa	66	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Computer Science	100	New (Started participating from April 1, 2021)
8	Kumpei Tokuyama	68	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D., ; Energy Metabolims	80	Ongoing (Leaving at the end of FY2023)
9	Takashi Kanbayashi	58	Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Physician, Ibaraki Prefectural Medical Center of Psychiatry	M.D., Ph.D.; Sleep Medicine and Psychiatry	80	Ongoing
10	Kaspar Vogt	55	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D.; Physiology, Pharmacology, Neurobiology	100	Ongoing
11	Michael Lazarus	52	Associate Professor International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	Ongoing
12	Masanori Sakaguchi	45	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	M.D., Ph.D.; Neuroscience	100	Ongoing
13	Yu Hayashi	41	Professor (WPI-IIIS), International Institute for Integrative Sleep Medicine, University of Tsukuba Professor, Graduate School of Medicine, Kyoto University	Ph.D.; Neuroscience	20	Ongoing
14	Takashi Abe	42	Associate Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Behavioral Science Psychophysiology	100	Ongoing
15	Sakiko Honjoh	41	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Molecular biology, Genetics, Neuroscience	100	Ongoing
16	Yo Oishi	41	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	Ongoing
17	Katsuyasu Sakurai	43	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Neuroscience	100	Ongoing
18	Arisa Hirano	36	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba; Assistant Professor, Faculty of Medicine, University of Tsukuba	Ph.D.; Molecular biology, Genetics, Neuroscience	80	Ongoing
19	Hirofumi Toda	43	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Genetics	100	Ongoing
20	Tsuyoshi Saito	37	Assistant Professor, International Institute for Integrative Sleep Medicine, University of Tsukuba	Ph.D.; Organic Chemistry, Medicinal Chemistry	80	New (Start participating from April 1, 2022)
21	Masayuki Matsumoto	45	Professor, Faculty of Medicine, University of Tsukuba	Ph.D.; Neuroscience	10	New (Start participating from April 1, 2022)
22	Hiroyasu Ando	43	Associate Professor, Faculty of Engineering, Information and Systems, University of Tsukuba	Ph.D.; Informatics	10	New (Start participating from April 1, 2022)
23	Haruka Ozaki	34	Associate Professor, Faculty of Medicine, University of Tsukuba	Ph.D.; Bioinformatics	10	New (Start participating from April 1, 2022)
24	Toshiyuki Amagasa	51	Professor, Faculty of Engineering, Information and Systems, University of Tsukuba	Ph.D.; Computer Science	10	New (Start participating from April 1, 2022)
25	Clifford B. Saper	70	James Jackson Putnam Professor of Neurology and Neuroscience, Harvard Medical School, and Chair, Department of Neurology, Beth Israel Deaconess Medical Center	M.D., Ph.D.; Neuroscience	2	New (Start participating from April 1, 2022)
26	Vladyslav Vyazofskiy	46	Associate Professor,Neuroscience, University of Oxford	Ph.D.; Neuroscience	5	New (Start participating from April 1, 2022)
27	Akihiro Yamanaka	50	Professor, Research Institute of Environmental Medicine Division of Stress Recognition and Response, Nagoya University	M.D., Ph.D.; Neuroscience	20	New (Start participating from April 1, 2022)
28	Genshiro Sunagawa	45	Senior Research Scientist, RIKEN Center for Biosystems Dynamics Research, Laboratory for Retinal Regeneration	M.D., Ph.D.; Neuroscience	20	New (Start participating from April 1, 2022)
29	Morimitsu Kurino	48	Professor, Economics, Keio University	Ph.D.; Economics	10	New (Start participating from April 1, 2022)
30	Masaaki Fujiwara	61	President, S'UIMIN Inc.	DVM, Prseident of S'UIMIN Inc.	10	New (Start participating from April 1, 2022)

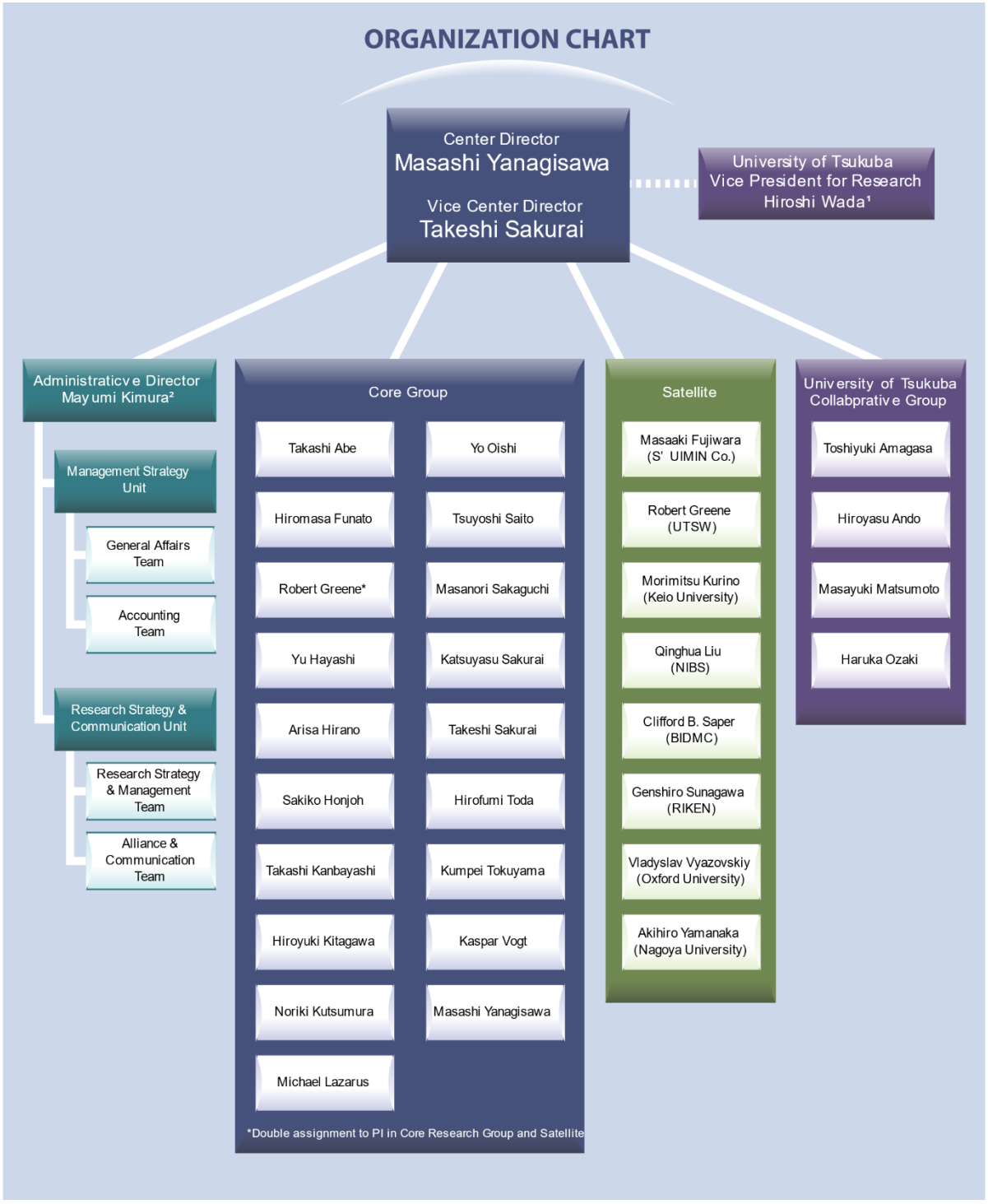
*Percentage of time that the principal investigator devotes to working for the center vis-à-vis his/her total working hours.

Number of Center Personnel

			FY2022	
			Number of persons	%
Researchers			55	
	Overseas researchers		16	29
	Female researchers		15	27
	Principal investigators (PIs)		19	
		Overseas PIs	4	21
		Female PIs	2	11
	Other researchers		12	
		Overseas researchers	2	17
		Female researchers	4	33
	Postdocs		24	
		Overseas Postdocs	10	42
		Female Postdocs	9	38
Research support staffs			6	
Administrative staffs			10	
TOTAL			71	

Appendix 2 Diagram of Center Management System

- Diagram management system after the funding period ends in an easily understood manner.
- If you are planning to change your organization management system and/or its position within the host institution in or after FY 2022 compared to their description in Appendix 3-1 of Activities report, show the changes in the diagram. Especially describe any important changes being planned in such as the center director, administrative director, head of host institution, and officer(s) in charge at the host institution (e.g., executive vice president for research).



[1]Hiroshi Wada has replaced Hideo Kigoshi as Vice President for Research since April 2021.
[2]Mayumi Kimura has replaced Toshio Kokubo as Administrative Director since April 2021.

Appendix 3 Position of the Center within Host Institution

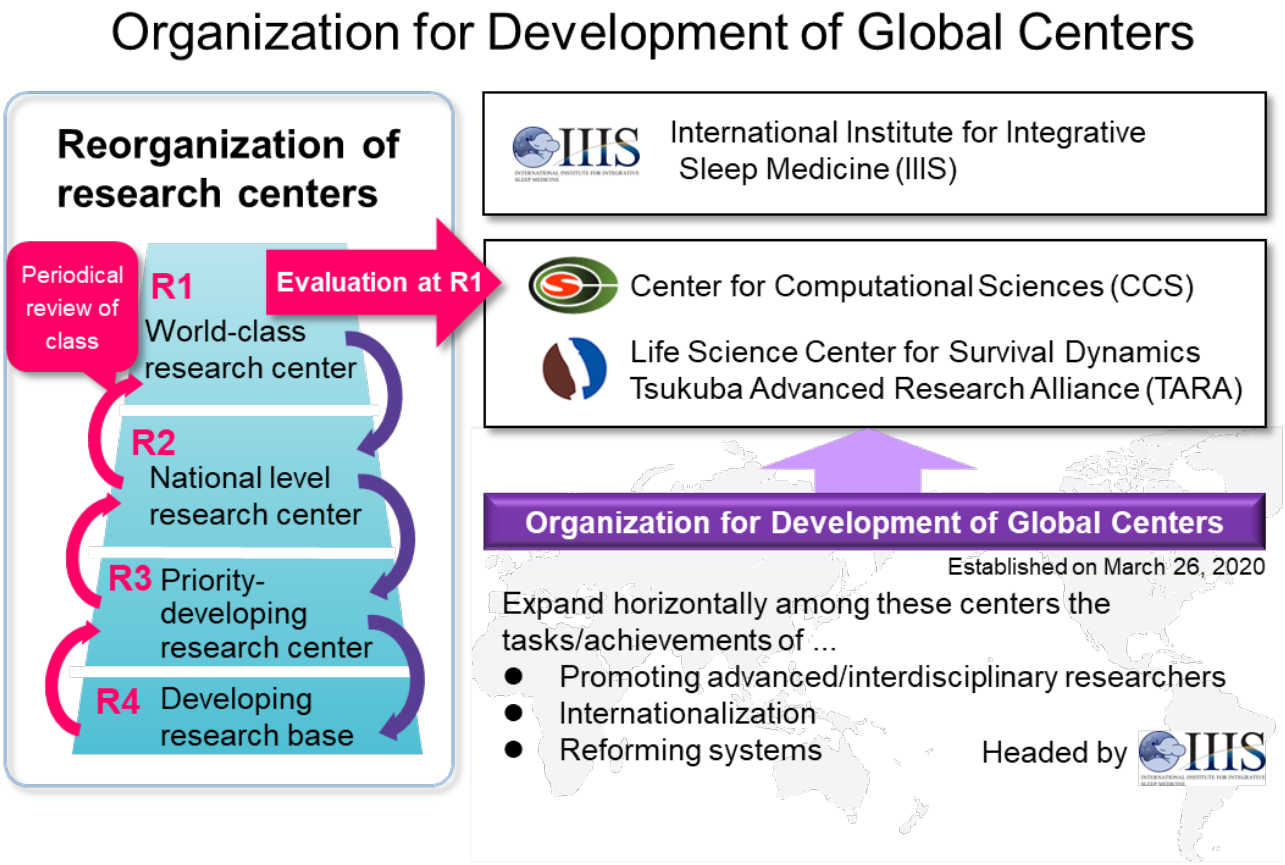
* Diagram the Center's position within the organization of the host institution, and describe that positioning using excerpts from the institution's mid- to long-term plan. If the plan has not been established yet, describe the consideration being given to the Center's positioning.

The University established Organization for Development of Global Research Centers in March 2020, in order to aid the development of the global research centers through the comprehensive support provided by the creation of an 'On-campus Special Zone for Research Strategy' (provisional name) and strategic allocation of the University's research resources.

To facilitate the strategic resource allocation, research centers in the University have been classified as R1 (World-class research center), R2 (National-class research center), R3 (Developing research center) and R4 (Research unit). Center for Computational Sciences (CCS) and Life Science Center for Survival Dynamics Tsukuba Advanced Research Alliance (TARA) are classified as the R1 status in physics and biosciences, respectively.

IIIS and R1-accredited CCS and TARA, are the first research centers to be supported by the Organization, which aims to expand horizontally among these centers the tasks/achievements of promoting the advanced and interdisciplinary researches, internationalization, and reforming systems thus far headed by the IIIS. Much is expected of IIIS to contribute for the success of the Organization by the active sharing of experience, knowledge, and know-how accumulated at IIIS.

Taking advantage of the Organization, the University Administration and IIIS are cooperating mutually to establish details of the positioning of IIIS after the funded period of WPI.



Appendix 4. Resource Allocation Plan for Sustaining and Advancing the WPI Center

Annual Plans (FY 2022 – FY 2026)					
<Fund >					**(million Yen)**
Fiscal Year	**2022**	**2023**	**2024**	**2025**	**2026**
- Funding from host institution	**210**	**210**	**210**	**210**	**210**
(details)					
Personnel	**200**	**200**	**200**	**200**	**200**
Project activities	**10**	**10**	**10**	**10**	**10**
Travel	**0**	**0**	**0**	**0**	**0**
Equipment	**0**	**0**	**0**	**0**	**0**
Other research projects	**0**	**0**	**0**	**0**	**0**
Costs of Satellites	**0**	**0**	**0**	**0**	**0**
- Funding from external sources	**1300**	**1300**	**1300**	**1300**	**1300**
Total	**1510**	**1510**	**1510**	**1510**	**1510**
<Personnel> **					**(person)**
Fiscal Year	**2022**	**2023**	**2024**	**2025**	**2026**
Total number of Personnel	**71**	**71**	**71**	**71**	**71**
- PIs	**19**	**19**	**19**	**19**	**19**
Full-time	**15**	**15**	**15**	**15**	**15**
Concurrent	**4**	**4**	**4**	**4**	**4**
- Other researchers	**12**	**12**	**12**	**12**	**12**
- Postdocs	**24**	**24**	**24**	**24**	**24**
- Research support staffs	**6**	**6**	**6**	**6**	**6**
RAs etc.					
- Administrative staffs	**10**	**10**	**10**	**10**	**10**

- Use yen (¥) when writing monetary amounts. If an exchange rate is used to calculate the yen amount, give the rate.
- When entering amounts, round down numbers to the first decimal.
- When funding is stated in a range between two amounts, explain the reason for the lower and upper amounts and fluctuations between them.

** When the host institution covers the expense, enter the amount in parentheses.

< Measures to be implemented from FY 2022>

– Strategy and action plan for allocating personnel (posts), space, and others measures required for the Centers' Progress.

To make the foundation of IIIS sustainable, the President of University of Tsukuba has committed at the meeting of the WPI Program Committee that PIs with a proven track record of achievements should receive the status of 'tenure.'

To grant tenure to PIs, the University is securing positions and funds by 1) requesting a quota for new faculties needed to establish Organization for Development of Global Centers and 2) strategic reallocation of internal resources through restructuring/reorganizing the research centers. The University has started discussion on the tenure reviews of 4 PIs, whose terms of employment contracts are approaching to the renewal limitation stipulated by the Labor Contract Act. Further, additional PIs qualified for tenure in the Core Group, except for the elder ones expecting the retirement in a few years, shall be subjected to tenure reviews in and after FY2022.

The University established Organization for Development of Global Centers in March 2020 to support IIIS as well as other world-class research centers in the University. Taking advantage of the Organization, the University Administration and IIIS are cooperating mutually to establish details of the positioning of IIIS after the funded period.

The Center Director, M. Yanagisawa was appointed to the Project Manager of Moonshot R&D Program

operated by Japan Agency for Medical Research and Development (AMED) in March 2021. The R&D budget of Moonshot, which is comparable to the WPI subsidy provided to IIIS, will make a significant contribution to research expenses and operating costs of IIIS in and after FY2022 and let IIIS maintain the scale of activities and organization.