FY 2013 WPI Project Progress Report
World Premier International Research Center Initiative (WPI)

Host Institution	University of Tsukuba	Host Institution Head	Kyosuke Nagata
Research Center	International Institute for Integrative Sleep Medicine	Center Director	Masashi Yanagisawa

Summary of center project progress

FY 2013 was an important year for the International Institute for Integrative Sleep Medicine (IIIS) to continue establishing its institutional structure and set research activities on the right track from the initial year of operations:

1. Formation of Institutional Structure
   Under the Director, the Core Research Group (8 labs), University of Tsukuba Collaborative Research Group (5 labs), Japan Satellite (1 lab), Overseas Satellite (4 labs), and Administration form our institutional structure.
2. Recruitment of Principal Investigators (PIs) and Researchers
   Moving towards full-scale research implementation, we have hired 2 professors, 4 visiting professors, 5 associate professors (3 Junior PI), 7 assistant professors (1 Junior PI) and 9 postdoctoral fellows, forming an institution with 13 PIs, 28 non-PI scientists (including non-independent faculty members and postdocs), 9 technicians, and 32 undergrad/graduate students for a total of 82 members (excluding administrative staff members).
3. Sleep Research System
   Out of the three research objectives that we set out to achieve in the plan at the start of the project, "Elucidation of the fundamental mechanisms of sleep/wake regulation" has been divided into two objectives: elucidation of molecular/cellular mechanisms and studies of neural circuits and systems. The research objectives were thus reorganized into the four items listed below.
   1) Elucidation of the substances and genes of sleep/wake regulation
   2) Elucidation of the regulating neural circuits and functions of sleep
   3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases
   4) Development of treatments for sleep disorders
4. Research Results
   In the FY 2013, IIIS published 73 original papers. The results were reported in international journals with high impact factors, including papers in *PNAS*, *Cell*, *Nature Commun*, etc. In addition, 4 PIs received awards for their research achievements.
5. Securing Competitive Research Funding
   The total amount of external funds acquired by IIIS researchers in the 2013 fiscal year was 457,560,000 JPY.
6. Collaborative Institutions
   We have concluded a collaboration research agreement with the Akita University Graduate School of Medicine, forming a satellite with Tetsuo Shimizu as a PI. In addition, we have also concluded collaboration research agreements with the University of Texas Southwestern Medical Center with Qinghua Liu as a PI, having established a satellite laboratory, along with Robert Greene, invited as a visiting professor (PI).
7. Research Support
   Under the leadership of the Administrative Director with the experience of Senior Director of Laboratories in a private sector company, the administration was established with a Vice Administrative Director and four teams (General Affairs, Accounting, Research Strategy & Management, Alliance & Communication), aiming to create a global institutional structure.
8. Outreach / Research Meetings and Activities
   The 2nd Annual IIIS Symposium was held on January 20, 2014 at the Tsukuba International Congress Center. Including the distinguished invited speakers from Japan and overseas, along with the principal investigators from our satellite institutions, about 150 people attended.
   Director Yanagisawa appeared on the television programs "Domoto Koichi no chokotto saiensu" on NHK and "Yume no tobira plus" on TBS to introduce our achievements and commitment to sleep research, receiving a great response.
   We held 15 presentations in which researchers in sleep and neuroscience were invited as lecturers for the WPI-IIIS Seminar Series, which plays a major role in providing opportunities for talent acquisition and the expansion of our social network.
9. General / Environmental Improvements
   The design for the new IIIS research building (6-story building with 8,000 square meters of floor space) was completed in November 2013. In January 2014, the construction company was decided through a bidding process. The preparatory work was done for the groundbreaking in February.
   For our laboratory facilities, we have introduced new state-of-the-art equipment for shared use to develop a world-class research environment: LC-MS with high resolution and sensitivity (Orbitrap Fusion), an *in vivo* micro X-ray CT system, multiphoton-excitation microscope, etc.

1. Summary of center project

<Plan at start of project> Sleep is a remarkably universal phenomenon in the higher animal species, and its disturbances reduce mental and physical wellbeing. However, the function of sleep and the mechanism for sleep regulation still remain unknown; these questions are among the most important challenge in modern neuroscience. We gather globally prominent scientists from multiple research fields contributing to the neurobiology of sleep. They cooperate together to elucidate the fundamental principles of sleep/wake regulation, and develop new strategies to make diagnoses and treat sleep diseases as well as the closely associated metabolic and mental disorders.	<Results/progress/alternations from plan at start of project > No changes to the plan center project. As described below, we are proceeding steadily with setting up the hub of international sleep medicine research. Initiative of invited researchers in Japan: 1.) Leading the medicinal chemistry group, Hiroshi Nagase (IIIS Professor, formerly Kitasato University) was invited to IIIS, having been engaged in collaboration with Yanagisawa prior to the establishment of IIIS; 2.) Yoshihiro Urade (IIIS Professor, formerly Osaka Bioscience Institute - OBI), boasting outstanding achievements in sleep substance research, was invited to IIIS along with researchers from OBI's Department of Molecular Behavioral Biology; 3.) Three bright young researchers were invited as Junior PIs to IIIS for their distinguished research achievements (Masanori Sakaguchi, IIIS Associate Professor, Michael Lazarus, IIIS Associate Professor, and Yu Hayashi, IIIS Assistant Professor). For the initiative of invited researchers from overseas, Robert Greene (Visiting Professor) from the University of Texas Southwestern Medical Center (UTSW) and Kaspar Vogt (IIIS Associate Professor) from the University of Basel were invited to IIIS as PI and Junior PI, respectively, to establish the Greene/Vogt Laboratory. Also, Qinghua Liu was added as a IIIS Professor from UTSW under the newly introduced joint appointment system and created Liu Laboratory at IIIS. Further, it was decided that Yang Dan (Professor, University of California, Berkeley), a brilliant female scientist who participated in IIIS International Symposium held on January 20th, 2014, should be invited as a satellite PI of IIIS. Although it was originally planned for Takeshi Sakurai to move from Kanazawa University to IIIS in order to establish the Sakurai/Sakaguchi Laboratory to collaborate with Associate Professor Masanori Sakaguchi, due to personal reasons and circumstances at Kanazawa University, it was decided to delay the transfer. Negotiations are ongoing to determine a new date of transfer, which is still undecided.

2. Research fields

<Plan at start of project >	<Results/progress/alternations from plan at start of project >
Sleep medicine The research area consists of a fusion of neuroscience, medicine, pharmacology, chemistry and biology. While focusing on sleep, the field is also interdisciplinary with respect to its integral research targets, e.g., studying mood disorders as well as metabolic diseases that are closely associated with pathological variations in sleep/wake states and sleep deficiencies.	No changes to target research fields. As described below we are moving forward steadily with our research activities. Principal investigators with expertise across different fields, including neuroscience, biochemistry, cell biology, pharmacology, chemistry, etc. worked together collaboratively for the implementation of the orexin agonist project, leading to the successful creation of a lead compound (patent filings completed). In addition, we were successful in obtaining a compound with a dramatically improved selectivity for orexin receptor type 2 and two compounds with improved water solubility as a result of structural optimization. We are planning on strengthening the project management across three labs participating in the project. For fundamental research, we have obtained multiple pedigrees with abnormalities in sleep/wake behaviors through molecular genetic research in mice (forward genetics). We were successful in identifying the "*Dreamless*" mutation that results in abnormal REM sleep and the "*Sleepy*" mutation that results in a remarkable reduction of waking hours. We expect the discovery of additional regulatory genes playing a vital role in the sleep/wake neuronal network by proceeding with further analysis of the other pedigrees obtained so far. In addition, the studies of neuronal activity visualization, Designer Receptors Exclusively Activated by Designer Drugs (DREADD) and optogenetics research are progressing well, and we are conducting research toward the elucidation of the cellular network vital to memory and sleep/wake regulation. Recently, we have succeeded in identifying the brain regions that are responsible for the control of REM sleep. Through these studies we successfully established the world's first mouse with REM sleep deprivation by artificial regulation. On top of this, we have discovered that the nucleus accubens of the basal ganglia is an important part of the brain that promotes sleep through activation of the adenosine A2A receptor. Through behavior related to emotion, cognitive function and motivation, the possibility of the stratium being involved in the abdominal region was presented in an unexpected discovery.

3. Research objectives

< Plan at start of project >

The research objectives that we seek to achieve are: 1) elucidation of the fundamental mechanisms of sleep/wake regulation, 2) elucidation of molecular pathogenesis of sleep disorders and related diseases, and 3) development of treatments for sleep disorders.

1.) Elucidation of the fundamental mechanisms of sleep/wake regulation
[Research objectives to be accomplished by the end of the grant period]

- Identification of new gene in sleep/wake regulation
- Elucidation of operating principles of neural networks regulating sleep/wake

Our current knowledge on sleep/wake regulation is actually quite limited when judged under the rigorous standards of today's neuroscience. Among the limited tidbits is the notion that the lateral hypothalamic orexin neurons and the wake-active monoaminergic and cholinergic neurons of the classical ascending activation system, together with the sleep-active GABAergic neurons of the preoptic hypothalamus, likely constitute important parts of the executive circuitry for sleep/wake switching. Orexin neurons are clearly important for the stability of the switch. We know that the sleep-inducing substance adenosine (which is blocked by caffeine) is importantly involved in the regulation of the "depths" of non-REM sleep. We know that these executive systems are powerfully governed by the circadian clock in the suprachiasmatic nucleus and by the presumed "sleep homeostat" somewhere in the brain. Overall, our current level of understanding is rudimentary at best. We will conduct precise neurophysiological analyses of these known components. We will dissect neuronal and molecular mechanisms of sleep regulation by circadian clocks and sleep-inducing substances. At the same time, we will use a completely blind (unbiased) genetic approach in order to identify new and unexpected genes that are importantly involved in the regulation of sleep/wake.

2.) Elucidation of molecular pathogenesis of sleep disorders
[Research objectives to be accomplished by the end of the grant period]

<Results/progress/alternations from plan at start of project >

Out of the three research objectives that we set out to achieve in the plan at the start of the project, 1) Elucidation of the fundamental mechanisms of sleep/wake regulation, 2) Elucidation of molecular pathogenesis of sleep disorders and related diseases, and 3) Development of treatments for sleep disorders, 1) Elucidation of the fundamental mechanisms of sleep/wake regulation has been divided into two objectives: elucidation of molecular/cellular mechanisms and studies of neural circuits and systems. The research objectives were thus reorganized into the four items listed below.

1) Elucidation of the substances and genes of sleep/wake regulation
2) Elucidation of the regulating neural circuits and functions of sleep
3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases
4) Development of treatments for sleep disorders

For each research objective, goals to be accomplished by the end of the grant period are set as listed below:

1) Elucidation of the substances and genes on sleep/wake regulation
- Identification of new genes involved in sleep/wake regulation
- Elucidation of the substances regulating sleep/wake

2) Elucidation of the regulating neural circuits and functions of sleep
- Elucidation of operating principles of the networks regulating sleep
- Elucidation of physiological function of sleep

3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases
- Elucidation of sleep/wake regulation in the brain and in associated peripheral organs
- Elucidation of intracellular events and molecular association of sleep/wake behavior in the body

4) Development of treatments for sleep disorders
- Development of sleep disorder therapy drug-candidate

● Elucidation of sleep/wake regulation in the brain and in associated peripheral organs ● Elucidation of intracellular events and molecular association of sleep/wake behavior in the body Irregular sleep/wake cycle and insomnia are a risk factor for metabolic syndrome as well as for mood disorders. However, the mechanism for the link is unknown. Using genetically engineered mouse models, the possible molecular links between sleep/wake, mood regulation, and metabolic control will be studied. 3.) Development of new treatment methods for sleep disorders [Research objectives to be accomplished by the end of the grant period] ● Development of sleep disorder therapy drug-candidate proceeding to clinical trial stage ● Development of multi-faceted "Good Sleep" program that does not use drugs for the prevention of sleep disorders was based on basic and clinical research We will develop new drug-candidate compounds modulating sleep/wake that are different from existing sleep-inducing agents or psychostimulants in their mechanism of action. We will also develop methods for prevention and early intervention of sleep disorders and related diseases. This includes behavioral modifications to specific aspects of lifestyle, such as sleep, diet, exercise, and stress-coping. It is likely that these new drugs and intervention programs are not only effective for sleep disorders, but also for mood disorders and metabolic diseases. We will utilize such associations in order to elucidate the molecular mechanisms behind the association.	● Development of multi-faceted "Good Sleep" program that does not use drugs for the prevention of sleep disorders For the goals set for 4 objectives, research achievements and progress until the end of FY 2013 and future directions are described in the Supplement.

4. Management

<Plan at start of project >

1) Composition of administrative staff

1. Composition of administrative staff

Under the supervision of the administrative director who is thoroughly knowledgeable in both the research contents of the center and the administrative affairs of the national university corporation, the administrative staff will be composed of the administrative director, assistant administrative director, and the following three sections.

・General affairs section (5 staff members)

General affairs section will be engaged in legal affairs, general affairs, personnel affairs, employment, travel, work management, public relations (outreach activities), symposia, conferences, and international affairs. One full-time University staff member who has a thorough knowledge of general affairs will be assigned to the Center. With regard to the support for a large number of foreign researchers coming to the Center, we will take full advantage of the City of Tsukuba as an international scientific research park, and commission it to the Japan International Science and Technology Exchange Center (JISTEC) as required.

・Accounting section (4 staff members)

Accounting section will be responsible for budget management and execution, procurement, and domestic and overseas transfer of funds and supplies. One full-time University staff member who has a thorough knowledge of budgetary and accounting will be assigned to the Center.

・Research fund section (3 staff members)

The research fund section will be in charge of a wide variety of tasks related to competitive research funds, including information collection, application support, administrative affairs, and support for report preparations. One full-time University staff who is highly experienced in the affairs for securing research funds and knowledgeable of the governmental systems will be assigned to the Center.

<Results/progress/alternations from plan at start of project >

1) Composition of administrative staff

1. Composition of administrative staff

The administration is under the leadership of the new Administrative Director, who has a lot of experience of research management and experience in research strategies at the research division of a pharmaceutical company. Supporting the Administrative Director's aim for a global research institution is the Vice Administrative Director (set to retire at the end of October due to personal reasons) and the four revised teams listed below.

- General Affairs Team (4 staff members)
  This team carried out work related to general affairs, human resources, hiring, business trips, office time management, etc. One full-time university personnel having a long and deep familiarity with general affairs at the university was assigned from the headquarters to the Center. While members with English proficiency from the Research Strategy & Management Team or Alliance & Communication Team provided support for foreign researchers joining the institute, we also took advantage of Tsukuba's location as an international science city to contract the Japan International Science and Technology Exchange Center for additional support.

- Accounting Team (5 staff members)
  This team carried out work relating to budget management and enforcement, supply purchases, movement of funds and goods domestically and internationally, etc. Two full-time university personnel having a long history of familiarity with university budgeting and accounting were assigned to the Center.

- Research Strategy & Management Team (3 staff members)
  This team was charged with a wide range of work relating to budget planning, workforce planning, competitive research funding application support, research support, conclusion of contracts, patent support, report preparation, etc. A doctoral degree holder with experience in contracts and patents at the research division of a pharmaceutical company was selected as the team leader.

2. Use of English as the official language English will be used as an official language at the research center. All assigned staff members will be fluent in spoken and written English, except for the people who have specific skills that cannot be replaced by any other people. Documentation will be in English or bilingual as much as possible, except where it has to be in Japanese for external reasons. *3. Recruitment and development of quality staff members* We will preferentially hire people with overseas experiences and/or with an excellent command in English language. The TOEIC/TOEFL scores and particularly the writing and speaking abilities will be considered as important factors for hiring. English language training sessions will be conducted regularly for the staff members. Once every two years, overseas training sessions will be recommended even to administrative staff members, providing them the opportunities to see the "cultural melting pot" and directly learn from the open-minded attitudes welcoming foreigners. Their experiences from such sessions will be used for creating a positive environment for foreign researchers at the Center. 2) Decision-making system In order to facilitate efficient and flexible administration of the research center, the Center Director will have the sole authority of decision-making related to the personnel and management matters within the Center. The Center Director has the entire authority relating to the general management of the Center except for the removal of himself and the determination of his	• Alliance & Communication Team (3 staff members) This team carried out work relating to public relations (news coverage, press releases and outreach activities), campus seminars, PI meetings, international symposia, report preparation, etc. A doctoral degree holder with research experience and English proficiency was selected as the team leader. *2. Use of English as the official language* English is used as an official language at the research center. Sixty percent of assigned staff members are fluent in spoken and written English, except for the people who have specific skills that cannot be replaced by any other people. Especially, English is used as the official language for official meetings (PI meetings, etc.) and also regularly during Skype meetings with overseas satellite members. Documentations are in English or bilingual as much as possible, except where it has to be in Japanese for external reasons. *3. Recruitment and development of quality staff members* IIIS hired people with overseas experiences and/or with an excellent command in English language. English proficiency, especially the speaking abilities are judged at the interview by native speakers belonging to IIIS. Newly hired members are thus all fluent in English, so that administration office can smoothly communicate with foreign researchers. Many administration staff members regularly attend the PI meeting and vigorously discuss in English. Some staff members also join lab meeting to update the scientific knowledge and share information. These experiences largely improve the language ability of the staff members. 2) Decision-making system For administrative management within the Center, all decision-making was done in accordance to the Center Director's top-down approach. So that the will of the Center Director takes effect quickly, organizational bylaws and other related regulations continue to be revised or enacted.

own salary. He has the authority over recruitment, hiring, contract renewal, salary, research space allocation, evaluation, and promotion regarding all Principal Investigators, visiting researchers, and post-doctoral fellows who are invited to the Center. He also has the right to make decisions on behalf of the Center, related to contracts with its Satellite institutes and the assignment and dismissal of researchers as the Center's Satellite Principal Investigators. In addition, he has the authority over the hiring and contract renewal of the Center's administrative staff members, excluding the full-time University staff members assigned to the Center by the University. An external advisory board will be established to provide the Center Director with advice on the Center management by using video conference. In order to facilitate center-wide discussions of administrative matters and personnel recruitment, the Center Director can create and convene, as needed, various internal committees comprised of the Administrative Director and Principal Investigators. The Administrative Director will supervise the administrative division and provide an environment where researchers can focus on their research. The Principal Investigators can make recommendations to the Center Director regarding the hiring of post-doctoral fellows and technical support staff members in the research laboratory he/she is supervising. Regardless of the position, anyone who is participating in this Center can offer his/her opinions regarding the management or treatment directly to the Center Director.	Having concluded a contract with Katsutoshi Goto, Professor Emeritus of the University of Tsukuba (and former Administrative Director of IIIS) to serve as an external adviser, the Center Director received assorted advice on institutional management during times of decision-making. 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. PI meetings are held once a month with teleconferencing capability to allow satellite PIs outside of Japan to also attend. The functions of the existing steering committee (deliberation on institutional organization/management, research plans, etc.) within IIIS have also been attached to the meetings. Junior PIs are also allowed to participate in the meetings, which gives a degree of motivation to the talented young researchers by granting them a forum for the management of their laboratories as independent researchers. Committed to providing an environment where researchers can devote their full attention to research, the Administrative Director overseas the entire administration, and supports the Center Director by conducting personnel and budget planning based on his policies.
3) Allocation of authority between center director and host institution By positioning the prospective research center as an independent research institute of the University, it is intended to assure a wide range of independent management, including personnel, facility management, and budget execution. As a result, under the strong leadership of the Center Director, a dynamic and prompt organizational management will be enabled. Specifically, whereas the President of the University has the authority to elect or dismiss the Center Director, the Center Director has a wide range of authorities regarding the general management and internal administration of the research center. The Center Director has authorities over hiring, contract renewal, salary, allocation of research space, evaluation, and the promotion of the invited researchers, including Principal Investigators and post-doctoral fellows. He has the authority to hire and renew the administrative staff members, excluding the full-time staff members of the University assigned to the Center. This type of system is widely seen and	3) Allocation of authority between center director and host institution For human resource management within our institute, we established a human resource committee with decision-making authorization capable of the speedy appointment of researchers. Regarding the authority of the host institution to appoint or dismiss the Center Director, the President of University of Tsukuba established a joint appointment system (tentative name) as the Center Director was affiliated as a Howard Hughes Medical Institute Investigator and did not previously have a direct employment arrangement with University of Tsukuba. The formal employment of the Center Director as the professor of University of Tsukuba was confirmed as of April 1, 2014.

most usual in the major universities and research institutes in the U.S., which will make the most of the Center Director's research and administrative experiences in the U.S. Moreover, the Center will establish and maintain an intimate cooperation channel with the office of the President of the University and the Vice President in charge of research. When an important and legitimate issue arises regarding the management of the Center that requires amending or revising the current regulations and codes of the University, the President will earnestly consider doing so through his top-down authority, while incessantly examining the system so that a prompt and flexible response is possible.	

5. Researchers and center staffs

i) "Core" to be established within host institution

Principal investigators

	At beginning	Final goal (Date: month, year)	Results at end of FY 2013	Results at end of April 2014
Researchers from within host institution	7	7	7	7
Foreign researchers invited from abroad	0	4	2	2
Researchers invited from other Japanese institutions	0	4	4	4
Total principal investigators	7	15	13	13

All members

- In the "Researchers" column, put the number and percentage of overseas researchers in the < > brackets and the number and percentage of female researchers in the [ ] brackets.
- In the "Administrative staffs" column, put the number and percentage of bilingual staff members in the ( ) brackets.

	At beginning	Final goal (Date: 3, 2015)	Results at end of FY 2013	Results at end of April 2014
Researchers	41 <1, 2%> [8, 20%]	115 <35, 30%> [35, 30%]	41 <9, 22%> [12, 29%]	41 <10, 24%> [11, 27%]
Principal investigators	7 <1, 14%> [0, 0%]	15 <5, 33%> [1, 7%]	13 <3, 23%> [0, 0%]	13 <3, 23%> [0, 0%]
Other researchers	34 <0, 0%> [8, 24%]	100 <30, 30%> [34, 34%]	28 <6, 21%> [12, 43%]	28 <7, 25%> [11, 39%]
Research support staff members	17	40	9	10
Administrative staff members	14	14	20 (9, 45%)	17 (10, 59%)
Total	72	169	70	68

Students

	Results at end of FY 2013	Results at end of April 2014
Graduate School	12	20
Undergraduate	20	16
Total Students	32	36

ii) Satellites <Plan at start of project > Institution (1) University of Texas Southwestern Medical Center -Role Joint research on the relationship between sleep/wake regulation and circadian rhythm, and the ENU Project (molecular genetic research) -Personnel composition and structure Carla Green, Joseph Takahashi, -Collaborative framework A Satellite site will be installed at the University of Texas Southwestern Medical Center where the prospective Center Director Masashi Yanagisawa has been conducting his research for over 20 years. As Satellite Principal Investigators, two world-leading researchers in the field of circadian rhythm, Joseph Takahashi and Carla Green, will participate. A total of two WPI-funded post-doctoral fellows will be hired for these laboratories. A close collaboration with the Takahashi laboratory has been ongoing for over two years already, concerning the mouse forward genetics project. His continued contributions will be essential in order to carry through the project. The presence of Takahashi and Green will further elevate the global visibility of this WPI Center. Institution (2) Akita University -Role Joint research in translational research -Personnel composition and structure Tetsuo Shimizu, Takashi Kanbayashi	<Results/progress/alternations from plan at start of project> Institution (1) The University of Texas Southwestern Medical Center (UTSW) - Role Joint research on the relationship between sleep/wake regulation and circadian rhythm, and the ENU Project (molecular genetic research) - Personnel composition and structure Qinghua Liu, Robert Greene, Carla Green, Joseph Takahashi - Collaborative framework A satellite site was installed at UTSW where Masashi Yanagisawa spent more than 20 years. Satellite PIs include Joseph Takahashi and Carla Green, active in the field of circadian rhythm, Robert Greene, active in adenosine research, and Qinghua Liu, active in the field of RNA interference. Qinghua Liu was invited from UTSW as a visiting professor in April 2013 and his laboratory was established on campus at IIIS. After that, we entered into a collaboration research agreement once negotiations of about half the year were completed in December. Based on this agreement, we hired a postdoctoral fellow at the laboratory of UTSW. In addition, through the newly introduced joint appointment system by the Division of Human Resources Development, Qinghua Liu will be appointed as a full professor at the University of Tsukuba from April 1, 2014. On the other hand, while Robert Greene was also invited as a visiting professor at the same time as Qinghua Liu, the installation of his lab was delayed slightly until February 2014. We also plan to conclude a collaboration research agreement for Robert Greene to build a more robust collaboration with UTSW. We expect to improve the visibility of IIIS with the presence of Joseph Takahashi, Carla Green, Robert Greene and Qinghua Liu at its satellite in the US. Institution (2) Akita University -Role Collaboration in translational research

-Collaborative framework We will establish a Satellite at the Akita University, which is by far Japan's largest site for patient-based clinical studies on the orexin system. The Satellite Principal Investigator, Tetsuo Shimizu, is a professor of the Department of Neuropsychiatry, and has an extensive network of patients and medical institutions for clinical research of sleep disorders including narcolepsy. In order to facilitate the progress of research based on intimate interactions between the Tsukuba Core and the Satellite sites, we will have internet-based weekly video conferences. Also, these Satellite Principal Investigators and the Center Director will regularly visit each other in person.	-Personnel composition and structure Tetsuo Shimizu, Takashi Kanbayashi -Collaborative framework From this fiscal year, a collaborative research agreement was concluded with Tetsuo Shimizu to establish a IIIS satellite in Akita University. Tetsuo Shimizu is a clinician/professor at Department of Neuropsychiatry, Akita University Graduate School of Medicine. Forming bio-resources comprised of the clinical information and samples taken from patients suffering from sleep disorders, particularly focusing on narcolepsy, we aim to search for abnormal pedigrees for the purpose of molecular genetic research and diagnostic markers. In the future, we plan to conduct the clinical research of orexin agonists being developed in IIIS at Akita University.
iii) Partner institutions < Plan at start of project > Institution (1) RIKEN BioResource Center, Tsukuba -Role Joint research in ENU screening -Personnel composition and structure Shigeharu Wakana -Collaborative framework Shigeharu Wakana of the Technology and Development Team for Mouse Phenotype Analysis, RIKEN BioResource Center, has identified a large number of pathogenic mutations from their systematic ENU-mutagenesis screening in mice. He is also a Japan representative of the International Mouse Phenotyping Consortium (IMPC). We have an ongoing close collaboration with his team in our forward genetic screening and mapping of sleep/wake mutant mice, which is a major pillar of the FIRST project. RIKEN BioResource Center will serve as a partner institution in the present proposal, and function as the local provider of ENU-mutagenized mice, and as the core facility for systematic mouse phenotyping.	<Results/progress/alternations from plan at start of project> Institution (1) RIKEN BioResource Center, Tsukuba -Role Collaboration for ENU screening -Personnel composition and structure Shigeharu Wakana -Collaborative framework The Technology and Development Team for Mouse Phenotype Analysis, RIKEN BioResource Center served as a resource for the analysis of sleep/wake abnormalities at the individual mouse level, i.e. providing ENU-mutagenized mice and services of genome-wide linkage analyses. While the collaborative research institution had been supporting the activities of IIIS, upon completion of the FIRST Program, both sides agreed to end the contract.

Institution (2)	Institution (2) Niigata University -Role Collaboration for the development of genetically modified mice -Personnel composition and structure Kenji Sakimura, Manabu Abe -Collaborative framework We are conducting collaboration with Professor Kenji Sakimura's group at the Brain Research Institute, Niigata University. The collaboration concerns the creation of genetically modified mice for the purpose of validating new sleep/wake regulating genes that have been discovered by forward genetics. Genetically modified mice that express mutated "*Sleepy*" or "*Dreamless*" genes in the presence of Cre are being created in his group. Crossing of the created mice and various Cre-driver mouse lines, phenotypes and functions of the mutated genes will be examined by site-specific and neuron-specific expression.

6. Summary of center's research environment

< Plan at start of project >

1) Environment in which researchers can devote themselves to their research

1. Support by administrative division

We will implement an administrative support system that will reduce the burden of administrative obligations of researchers and allow them to devote themselves exclusively to their research. Our administrative team will promptly respond to the intentions of the Center Director. Under the supervision of the Administrative Director who has a thorough knowledge in both the science research and the management affairs of national universities, the administrative division will function autonomously and independently from the University administration. Specifically, the administrative division will fully and promptly perform any necessary support for the conduct of research, including legal affairs, general affairs, personnel affairs, employment, travel, work management, public relations (outreach activities), symposia, conferences, international affairs, acceptance of overseas personnel, budget management and execution, procurement, domestic and overseas transfer of funds and supplies, as well as the tasks related to competitive research funds, including information collection, application support, administrative affairs, and support for report preparations.

2. Exempting on-campus researchers from non-research institutional duties, while providing support for their affiliated departments

Regarding those top researchers who are recruited to and participate in the Center from within the University of Tsukuba, the Center will ensure that they can further their research without hindrance, by cooperating with their affiliated departments. Those researchers will be exempted from some of their non-research duties at their affiliated departments. In return, we will compensate their affiliated departments by providing relevant personnel costs.

3. Living support

The University of Tsukuba promotes "Globalization as a matter of daily living" as one of the institutions that have been selected as a core university

<Results/progress/alternations from plan at start of project>

1) Environment in which researchers can devote themselves to their research

1. Support by administrative division

The administration is under the leadership of the new Administrative Director, who has a lot of experience of research management and expertise in research strategies at the research division of a pharmaceutical company. Supporting the Administrative Director's aim for a global institution is the Vice Administrative Director and four teams (General Affairs, Accounting, Research Strategy & Management, Alliance & Communication), with each having fully implemented the necessary support mechanisms for research to be carried out. An additional characteristic of the administration is the level of science background in medicine and medical biology, with 3 staff members, including the Administrative Director, holding doctoral degrees. The IIIS office staff is capable of carrying out independently administrative duties that require knowledge of advanced science, including institution-wide research funding acquisition, internal grant review, various project reports, etc. Led by the administration, international symposia and IIIS seminars, along with various outreach activities were executed smoothly without interfering with our researchers' activities.

2. Exempting on-campus researchers from non-research institutional duties, while providing support for their affiliated departments

An administrative staff member (secretary) is placed in each large-scale laboratory, charged with administrative assistance for the PI in addition to the general administration within the laboratory. Secretary meetings are held regularly aiming for close cooperation with the central administration by sharing information and reporting on developments from the PI meetings.

3. Living support

For foreign researchers and their families, living support is provided on campus (Kasuga Plaza Support Center for International Researchers and

of the "Global 30" sponsored by the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The town of Tsukuba has an advantage of being built an international research park. The Japan International Science and Technology Exchange Center (JISTEC) is located in Tsukuba and provides a wide range of living support for researchers from foreign countries. Partly in collaboration with JISTEC, the Center will provide various supports, including visa application, paperwork such as alien registration, opening bank account, purchasing insurance, and arranging for residence. The University of Tsukuba will offer the university guesthouses and its affiliated housings nearby for the researchers (especially foreigners) and administrative staff members who are recruited to work in this research center. For the researchers who visit the Center to attend seminars or to conduct collaborative research, various accommodation facilities of the University will be available.	Families). The support center engages in the following support services: provision of information and consultation to assist with daily living, provision of off-campus accommodation listings for foreign researchers, administration of basic Japanese classes, explanations regarding requests for Certificates of Eligibility by proxy and the various associated procedures, and assistance in document preparation. IIIS foreign researchers also receive support through these services. Some of our foreign researchers also make use of the highly convenient location of the University of Tsukuba's on-campus dormitories exclusively for foreign researchers. This is one of the support systems in place from the university. In addition, we continue to receive generous support from the Japan International Science and Technology Exchange Center (JISTEC), having renewed the contract for foreign researcher support services.
2) Startup research funding For the independent researchers who are recruited to the Tsukuba Core from other institutions (especially those from overseas institutions), the Center will provide a sufficient amount of startup research funding. The amount of the startup fund will be individually considered and negotiated, but will be similar to startup funds in the U.S. academia under equivalent situations. It will be decided by the Center Director who has plentiful experience in the U.S. academia. When it is time for them to apply for external funding, the administrative division will provide strong administrative support through the entire application process.	2) Startup research funding Research start-up funds were offered to PIs invited from outside of the University of Tsukuba, along with Junior PIs. The provision of these research funds are planned by the Administrative Director and carried out based on the budget plan decided by the Center Director. For the acquisition of competitive research funds, the administration placed a full-time staff member familiar with the process in the office to actively collect applicable funding information and provide application information to researchers. The administration also encouraged researchers to attend relevant informational seminars, etc. In the case of Grants-in-aid for Scientific Research, all eligible researchers were encouraged to apply for them and 34 applications were submitted from the core research groups.
3) Postdoctoral positions through open international solicitations 1. Prominent international journals such as Nature and Science; 2. Personnel database JREC-IN (Japan Research Career Information Network) operated by the Japan Science and Technology Agency; 3. Web sites of academic research societies such as the Japan Neuroscience Society; 4. University of Tsukuba web site (in four languages); 5. Departmental web sites; 6.	3) Postdoctoral positions through open international solicitations Including the website of WPI-IIIS, along with job boards on sites such as Naturejobs, Federation of European Neuroscience, Sleep Research Society, Society for Neuroscience, jREC-IN, American Society for Neurochemistry, etc., we have been actively posting job advertisements internationally. The total number of applicants for the 2013 fiscal year

University of Tsukuba's overseas offices; 7. Our overseas Satellite (public release by the University of Texas Southwestern Medical Center), 8. Personal international networks of the Center Director and Principal Investigators. The University of Tsukuba is equipped with various career and living support systems for the development of young researchers at all levels. By utilizing such systems, we will be aggressively promoting the participation of outstanding post-doctoral fellows, especially foreign researchers and female scientists. The Center Director will strive to create an environment that attracts quality personnel by aggressively outreaching to society, thereby increasing the visibility of the research center. The young researchers working at the Center will strive to achieve research accomplishments sufficiently high so that they will then be recruited by other institutions for the next career stage. This will promote healthy personnel mobility, ultimately helping the Center to sustain its world premiere status.	was 172, with 98% of the applicants from foreign researchers. However, unable to meet the standards set by our institute, the majority of applicants were passed upon. Out of the group of applicants, one was successfully chosen to begin employment during the 2014 fiscal year. In addition, we were also successful in recruiting foreign researchers (1 Swiss/1 German/1 Canadian) through the international networks of our satellite PIs. We also have plans to employ one postdoctoral fellow from our overseas satellite at the University of Texas Southwestern Medical Center in the 2014 fiscal year. Outside of these channels, we will also engage proactively in recruiting on the occasions of international conferences, etc. We regularly invite speakers from outside IIIS for seminars (WPI-IIIS Seminar Series) and make use of the opportunity to look for candidates for PI (especially Junior PI) positions. The Center Director and other PIs have also appeared on television programs delivering science information in which WPI-IIIS and associated research contents and achievements are explained. This had a significant impact in raising awareness about the institute and its activities. Also contributing to awareness about IIIS are the many articles that have appeared in magazine publications.
4) Administrative personnel who can facilitate the use of English in the work process Needless to say, all science will be conducted in English at the Center. In addition, all administrative staff members will be fluent in spoken and written English, except for the people who have specific skills that cannot be replaced by any other people. Documentation will be in English or bilingual as much as possible, except where it has to be in Japanese for external reasons. The TOEIC/TOEFL scores and particularly the writing and speaking abilities will be considered as important factors when appointing administrative personnel. English language training sessions will be conducted regularly for the staff members. Once every two years, overseas training sessions will be recommended even to administrative staff members.	4) Administrative personnel who can facilitate the use of English in the work process The administrative staff has been assembled with the ability to function in English. Specifically, English is used as the official language for official meetings (PI meetings, etc.) and also regularly during Skype meetings with overseas satellite members. In addition, each laboratory meeting, journal club session, WPI-IIIS Seminar Series, IIIS Science Lounge, etc. are also all conducted in English, along with other important meetings. Beginning with experimental plans for various regulatory applications, each type of application form and documents for employment, personnel affairs, and general affairs are all available in English. Other documents are also converted into English if necessary. 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.
5) Rigorous system for evaluating research and system of merit-based compensation The President of the University will decide on the renewal and the salary of the Center Director. The Principal Investigators and other independent researchers will be annually evaluated by the external advisory board, considering publications and their citations, invitations to international meetings, level of external funding, generation of significant intellectual properties, etc. The Center Director decides on the salaries of the Principal Investigators and other independent researchers considering the results of the annual evaluations. The salaries of the other researchers and administrative staff members are decided by the Center Director based on the opinions of the supervising investigator and Administrative Director, respectively. When inviting Principal Investigators and other independent investigators from outside of the host institution, their salaries will be determined according to their research accomplishments and previous salaries.	5) Rigorous system for evaluating research and system of merit-based compensation This fiscal year, we were unable to implement the evaluation system for researchers as we are currently proceeding with the appointment of the candidates for the advisory board. However, as we regard the major role for the advisory board as scientific evaluation of research programs, we will continue to carefully consider how the advisory board's evaluations should be reflected to a system of merit-based compensation. The salaries for the administrative staff were determined by the Center Director, based on the opinion of the Administrative Director. Determinations on salary for researchers invited from outside of IIIS were made based on their past achievements and salary situations.
6) Equipment and facilities, including laboratory space, appropriate to a top world-level research center The Center will be provided with a sufficient amount of floor space that can be favorably compared, on a per-capita basis, with the floor spaces of premier research centers in the U.S. These will include wet-lab spaces, dry-lab/office spaces, and animal housing spaces especially for mice. Generous space for animal housing is absolutely essential considering the Center's target research field. Specifically, the Center will be provided with all floors on the E Building of the University of Tsukuba Hospital, which will be vacated by January, 2013 into the new ward building currently under construction. The Center will continue to use the floors on the Health and Medical Science Innovation Laboratory that are currently occupied by the FIRST Program. Together, the	6) Equipment and facilities, including laboratory space, appropriate to a top world-level research center The design for the new IIIS research building was completed in November 2013. In January 2014, the construction company was decided through a bidding process. The preparatory work was done for the groundbreaking in February. As a result, the "International Institute for Integrative Sleep Medicine Building Construction Liaison Committee" was established at IIIS for the purpose of construction supervision on the new building with construction related vendors, planning and design firms and the Department of Facilities, University of Tsukuba. This Liaison Committee was also formed to promote communication and coordination with the practical handling of the overall management and budget. In addition, a smoothly functioning communication system has been developed to work towards the targeted completion date of March

Center will be provided with more than 5,000 m^2 of research floor space. The Hospital E Building is located in close physical proximities to both the Innovation Laboratory and the Laboratory Animal Resource Center, which should be highly advantageous. During the required renovation of the E Building, the Center will be provided with temporary floors on the Laboratory of Advanced Research D.

The building that houses the current FIRST Program, the Health and Medical Science Innovation Building, was newly constructed in 2011, incorporating the recent design trend of overseas research laboratories. It has a coffee-break area on each floor, which can be a place for casual communications between researchers. On the top floor is a large, 200-people conference room. The Center's own seminar series will be held there, providing opportunities for exchanges among the Center members and with other researchers on campus.

Regular intra-laboratory and Inter-laboratory research meetings will be held by using internet-based video conferencing services (such as Skype), so that the meeting is attended by the Principal Investigators and researchers at the Satellite sites. The prospective Center Director has been managing his two laboratories across the pacific (Tsukuba, Japan and Dallas, Texas) for over two years now. These spaces for communication will continue to function as the key facility of the WPI center after its establishment.

The existing research facility for the FIRST program is equipped with shared capital devices such as a system for large-scale mouse EEG/EMG recording and analysis, a fiber-optic fluorescence confocal endo-microscope, a two-photon microscope with electrophysiology rigs, multiple sets of slice and cellular patch-clamp station, and an ultra-low-power, wide-field confocal microscope. Cutting-edge shared devices will be systematically acquired in the proposed center, according to the requirements of its laboratories.

Additional capital equipment will be available to the Center through the Open Facility function of the University, which is scheduled to start sometime this fiscal year. This includes such equipment as cutting-edge mass spectroscopy, super-high resolution ultrasound echo sonography for mice, and *in vivo* luminescence/fluorescence imaging system for mice. This function will be expanded in stages, and will make possible the use of the pioneering research facilities in the Tsukuba area.

2015 with weekly meetings to ensure that onsite progress and related work for the new building is proceeding as planned.

For our laboratory facilities, we have introduced new state-of-the-art equipment for shared use, e.g. LC-MS with high resolution and sensitivity (Orbitrap Fusion), an *in vivo* micro X-ray CT system (R_mCT2-SP), an *in vivo* imaging system for bioluminescence and fluorescence (IVIS), a Two-Photon/Confocal microscope (Zeiss Axio), FACS (BD 4LS) and a slide scanner (NanoZoomer).

While we are still making use of the Health and Medical Science Innovation Laboratory as the central base of operations until the new research building is complete, our activities are also being expanded by using the labs at TARA Center, the Project Research Building and Bldg. E in the University of Tsukuba Hospital.

7) International research conferences or symposiums held regularly to bring world's leading researchers together The prospective Center Director served as the organizer of the international symposium "Frontiers in Behavioral Brian Science ~ Solving the Mystery of Sleep" through the 2011 Funding Program for World-Leading Innovative R&D on Science and Technology (open seminars of the FIRST program). We could gather a total of 16 prominent researchers in the field as invited speakers, including a Nobel Laureate (9 from the U.S., 3 from Europe, and 4 from Japan). The symposium was conducted in English including all oral and poster presentations. In order to create a "globally visible research center", we will regularly hold similar symposia once a year, and invited seminar series twice a month. Moreover, we will hold a retreat once a year for the development of students and young researchers, promotion of collaborative researches, and also an increased sense of a family-like unity of the Center. Providing workshops at overseas Satellites will also increase the visibility of the research center overseas.	7) International research conferences or symposiums held regularly to bring world's leading researchers together 1. International Symposium The 2nd Annual IIIS Symposium ~Solving the mystery of sleep~ was held on January 20, 2014 at the Tsukuba International Congress Center. Including the distinguished invited speakers from Japan and overseas, along with the principal investigators from our satellite institutions, about 150 people attended, making the symposium a success. 2. Public Seminars We held 28 presentations for the WPI-IIIS Seminar Series, which plays a major role in providing opportunities for talent acquisition and the expansion of our social network.
8) Other measures, if any The University Research Administrator (URA) office of the University of Tsukuba (director: vice president in charge of research), which has been established as a part of the "Global 30" initiative by the MEXT, will provide the Center with additional know-how on development strategies, international cooperation, and compliance. Graduate students conducting thesis studies at the Center will be, as a rule, all hired as research assistants (RA). Based on the objectives of the 3rd and 4th Science and Technology Basic Plans, the level of their salaries will be the amount equivalent to living expenses. By providing reasonable compensations, the graduate students will concentrate on their research activities as their professional work. The prospective Center Director discovered endothelin when he was a graduate student. In that spirit, the Center will promote creative researches through casual but intensive discussions with young graduate students who have flexible and free ideas.	8) Other measures, if any Together with the collaborative principal investigators at IIIS, we received a grant from the Ministry of Education, Culture, Sports, Science and Technology "Grants for Excellent Graduate Schools" program. This funding source was used for our new RA system to support graduate students at the doctoral level. Aside from this, we also offered financial support for short-term employment of students in Faculty of Medicine using FIRST research funding. Also as part of career support, we held a career development seminar for students and postdocs, inviting an editor from the journal Science.

7. Criteria and methods used to evaluate center's global standing

< Plan at start of project >

1. As for the prospective Center Director's work, the number of citations for the article that reported the discovery of orexins is 2668 and for the article describing narcolepsy episodes in orexin-deficient mice, the number of citations are 1660. These high numbers of citations suggest that these articles are remarkable reports that greatly affected research activities of other researchers in the field.

2. The existing FIRST research core is so new and this way of evaluation is impossible at this point. However, speaking of the research laboratory of the prospective Center Director, Masashi Yanagisawa, many researchers who received trainings as post-doctoral fellows have become professors and assistant professors of domestic and overseas universities, working in responsible positions in research institutions and corporate settings. The value of such personnel network is very immense. Such real examples will attract excellent graduate students and post-doctoral fellows to this research center.

3. Masashi Yanagisawa, the prospective Center Director, has attained domestic funding worth 1.8 billion yen (US $22,500,000) over five years as the Core Researcher of the FIRST project. In addition, he secured an average of US$1,260,556 per year of competitive funding in the U.S. in the last 5 years.

<Current assessment>

1) Status of research paper publications

In the 2013 fiscal year (until the end of March 2014), IIIS published 73 original papers. It is a noteworthy point that numerous research results were reported in international journals with high impact factors, including papers from Suzuki *et al.* in the *PNAS* (2013, **110**(25):10288-93), Ikeda *et al.* in *Cell* (2013, **155**(6):1323-36), Hamada *et al.* in *Nature Communications* (2014, **5**:3147), etc. In addition, the achievements from the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST, 1.8 billion JPY/5 years) have begun to pile up, presenting us with extremely important knowledge regarding the molecular genetic basis for sleep/wake regulation. When these results are published, a breakthrough can be expected that will lead to a paradigm shift, serving as a major step forward on a global level in elucidating the fundamental mechanism of sleep/wakefulness.

2) Main awards

The numerous achievements of PIs were recognized with the following awards in fiscal year 2013:
Masashi Yanagisawa: 17th Jokichi Takamine Memorial Award
Hiroshi Nagase: National Commendation for Invention – the Invention Prize
Takeshi Sakurai: The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology (Prizes for Science and Technology - Research Category)
Junichi Hayashi: 24th Tsukuba Award

3) Rise in institutional awareness

For the 2014 fiscal year symposium (required to be held once a year as a WPI center), we received an offer from Professor Hiroki Ueda, RIKEN Center for Developmental Biology (CDB) / The University of Tokyo, and Professor Joseph Bass, Northwestern University, to hold it jointly with each of their respective institutions on the topic of sleep, the circadian

	clock, and appetite/obesity. This clearly shows that IIIS is recognized as a top runner in sleep research. We have been actively involved from the planning stage, with the secretariat for the symposium also placed within IIIS. 4) Status of applicants for Junior PI and postdoctoral fellow positions In the 2013 fiscal year, we received 15 applications for Junior PI and 160 applications for postdoctoral fellow positions. From this data, we can infer that IIIS appears to be attractive for many young researchers. While we did not make any new hires in FY2013 due to the slate of candidates not meeting our entry standards, we will continue to search out qualified candidates and secure human resources for our institute. 5) Efforts to secure research funding With the FIRST Program funding that was acquired by Center Director Yanagisawa ending this fiscal year, acquisition of a successor program is urgently needed by IIIS. For this reason, applications have been coordinated between researchers and the administration with the aim to acquire large grants, such as CREST, ImPACT, COI Stream, etc. In addition to this, 34 applications for Grants-in-aid for Scientific Research were filed mainly by our young researchers. The total includes almost all of those qualified to submit applications.

8. Securing competitive research funding

<Plan at start of project >	<Results/progress/alternations from plan at start of project>
The total amount of competitive research funding raised by the prospective Principal Investigators in the past five years is $44,316,704 averaging at $8,863,341/year.	The total amount of external funds acquired by IIIS researchers in the 2013 fiscal year was 457,560,000 JPY. We aim to further acquire competitive funding from the 2014 fiscal year.

9. Other important measures taken to create a world premier international research center

<Plan at start of project >	<Results/progress/alternations from plan at start of project>
Center Director Yanagisawa intends to retire from HHMI promptly after the application is funded.	After careful consideration, we have come to a resolution in response to the letter that we received from the Ministry of Education, Culture, Sports, Science and Technology on November 11, 2013, "Instructions for Enforcement of the Grant for World Premier International Research Center Initiative", in regards to the plan for Center Director Yanagisawa's retirement from HHMI noted on the left-hand side. We took seriously the request from the WPI Program Committee in light of the results from the hearing on October 30, 2013. Under the leadership of Yasuo Miake, Vice President, University of Tsukuba (in charge of research), a task force was formed on behalf of the university which includes a patent attorney and a lawyer in order to solve the intellectual property rights issues surrounding Center Director Yanagisawa. From November, 10 meetings were conducted and then in December, a team composed of the Vice President, the Administrative Director of IIIS and the Technology Transfer Manager in the Liaison Center visited UTSW to conduct negotiation with the Vice President of UTSW. We made efforts to proceed in a highly transparent manner so that the progress and conclusion thereof from these discussions could be disclosed as needed. Negotiations were held in earnest with UTSW in order to determine the degree of contribution assigned to University of Tsukuba for intellectual property rights generated from collaboration between University of Tsukuba and UTSW, whereby 1) intellectual property rights would be assigned to University of Tsukuba from inventions by Masashi Yanagisawa, and 2) regardless of whether Masashi Yanagisawa is the inventor or not the rights would be assigned to University of Tsukuba. UTSW recognized the great significance in furthering collaboration with University of Tsukuba for the continued development of both universities, and expressed understanding that problems relating to the attribution of intellectual property rights could inhibit this process. Therefore the vice presidents of both universities came to a basic agreement to cooperate and resolve this problem. However, the answer was difficult, specifically in regards to the attribution of Masashi Yanagisawa's intellectual property rights to University of Tsukuba due to the rules of HHMI and UTSW, the Bayh Dole Act (Patent and Trademark Act Amendments of 1980) and employment contract limitations. There was a

	suggestion for the appropriate distribution of revenue through licensing of patents depending on the contribution of University of Tsukuba as an alternative solution. An agreement was reached in principle after evaluating the proposal as it was a practical solution to allow licensing revenue allocation according to the contribution of both universities regardless of the intellectual property rights. On the other hand, for one of the collaborative research arrangements between UTSW and University of Tsukuba in which Masashi Yanagisawa is not directly involved (collaboration with Qinghua Liu), a collaboration research agreement was already concluded that defines University of Tsukuba's intellectual property rights depending on the degree of contribution when intellectual property rights arise from research results. We expect to conclude collaboration research agreements with Masashi Yanagisawa and other satellite PIs (Robert Greene, Carla Green) sequentially. For the timing of Masashi Yanagisawa's retirement from HHMI, an extensive discussion was carried out under the leadership of Vice President Miake with support of the Department of Research Promotion. After details for the transition were finalized with UTSW by Yanagisawa, his retirement as of March 31, 2014, and employment at the University of Tsukuba, starting from April 1, 2014, was agreed upon by President Nagata. In order for Yanagisawa to continue his partial employment relationship with UTSW after retirement from HHMI, using a joint appointment system newly introduced to University of Tsukuba, it was agreed that efforts at UTSW and University of Tsukuba should be 5% and 95%, respectively.

10. Host institution's commitment

<Plan at start of project > -Provision in host institution's mid-to-long-term plan	<Results/progress/alternations from plan at start of project> -Provision in host institution's mid-to-long-term plan
In University of Tsukuba's Midterm Goals (for the period of April 2010 to March 2016), it is proclaimed that the University shall "strive for deeply specialized expertise, open up new cross-disciplinary fields, and achieve research results which are outstanding by international standards over a wide range of academic fields." In the University's Midterm Plan to reach these goals, it is stated that the University shall "promote high quality fundamental research taking a long term view of academic progress and ambitiously open up new cross-disciplinary fields," and "under the leadership of the President carry out research intensively in fields where the University has distinctive capabilities, in particular those which are internationally recognized to have great potential and budding new fields which invite the interaction of existing fields." Therefore, the World Premier International Research Center Initiative (WPI) is in complete agreement with the University's Midterm Goals and Midterm Plan. Further, as the strategy to achieve the University's goals regarding systems to carry out research, the Midterm Plan stipulates that "research groups and organizations which are expected to achieve outstanding research results will be singled out for appropriate support, including allocation of research resources, hiring of supporting personnel, and reforming of organizations, and will be aggressively promoted as international centers of research." In accordance with this Midterm Plan, these research centers will be given the highest priority in the University's development efforts. In particular, under the leadership of the President, a deliberative committee preparing for the establishment of the University of Tsukuba International Institute for Integrative Sleep Medicine (provisional name) has been launched. The whole University will get involved to make the level of work at this international research center on par with any center in the world. The first of the Midterm Goals is to make this an "open university" in every	We will continue university-wide efforts, described on the left, aimed at forming a world premier international research center.

aspect, with a "flexible educational and research organization" that is not tied down by past conceptions and that will take the lead in promoting "new ventures for the university" that are called for by tomorrow's society. Our candidate for center director, Dr. Masashi Yanagisawa, has been a professor at the University of Texas for over 20 years, and will use this experience in managing this center. By incessantly supporting his efforts, the University will realize the "new ventures for the university" that are demanded by tomorrow's society.	
-Concrete Measures (1) Competitive grants obtained by researchers participating in the project and in-kind contributions, etc. In order to manage this center and carry out research activities there, the University will give support as indicated below which will amount to at least as much as the support supplied by the WPI program. Further, even after the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) ends its "Molecular Mechanism and Control of Complex Behaviors" project which formed the basis of this center, personnel and laboratory resources will be maintained at the same level through outside funding to support the participating researchers.	-Concrete Measures (1) Competitive grants obtained by researchers participating in the project and in-kind contributions, etc. The contents of the support that University of Tsukuba provides for the research and administrative activities of IIIS are shown below. Provided support from the university is equal to or greater than the amount of support provided from this program (including FIRST Program funding).
1) The University of Tsukuba Organization for the Support and Development of Strategic Initiatives (formed by the University President and Vice-President) shall continue to give intensive support to make this an international center pioneering new fields, as one of the Organization's strategic initiatives. Specifically, the Organization will support the center with ¥10 million per year to cover expenses of center activities. 2) Competitive research funds obtained by researchers participating in the center Competitive research funds obtained by researchers participating in the center amounted to an average of US$8.86 million in the past 5 years, and it is anticipated that an equivalent amount will be gained. Even after the completion in FY2014 of the FIRST program that founded this center, we anticipate that the center will have about 115 researchers who will gain at least the same level of funds as the previous year. The University will be providing full support for their applications for competitive	1) The Organization for the Support and Development of Strategic Initiatives was established, and in addition to the funding from the FIRST Program, we received 10 million JPY in management expenses grants as support from the initiative. 2) Support was launched for research funding and also with applications for competitive funding. 3) Measures were taken to provide personnel costs for research to 7 researchers and 7 technicians, totaling 14 persons. 4) Measures were taken to provide personnel costs for administration with 7 positions of contract staff and 3 positions of university personnel (13 in total) for the key areas of general affairs, accounting and research strategy. 5) 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. Although less than 5,000 square meters is divided between the 4 locations, a new research building listed in section (5) below is scheduled for completion in the

external and internal research funds. 3) Support through handling of personnel expenses The personnel expenses of University faculty participating in this center will be treated as University personnel expenses for researchers. University staff (approximately 3 persons) will be assigned to carry out the chief administrative work of the center such as general affairs, accounting, and research funding. The expenses for this staff will be handled by considering them to be administrative staff participating in an administrative support division. 4) Support through supply of research space The University will use its good offices to facilitate the building of research facilities (more than 5,000m^2) according to a plan that will give the center an outstanding research environment and distinctive tangible advantages. 5) Support for the use of research facilities Various core facilities will be offered for use (see paragraph (5) below).	next fiscal year with 8,000 square meters that will bring everyone together. 6) The site location was planned for convenient use of other research equipment and the provided support for research equipment is listed also in section (5).
(2) System under which the center's director is able to make substantive personnel and budget allocation decisions This center will be established as an independent research organization separate from other research bodies, and also as a special institute for the purpose of making the level of research at the center as high as any in the world. In order for the center director to be able to fully exercise his/her leadership, a system will be instituted in which the director has managerial powers over key aspects of the center, including the power to decide upon personnel and the budget. In order to implement this system, the University will take necessary measures such as amending relevant regulations if needed. Further, the director will be supported by the administrative division established within this center which includes Professor Emeritus Katsutoshi Goto who has rich administrative experience such as the director of the Center for Tsukuba Advanced Research Alliance, administrative personnel dispatched from the University for support, and personnel	(2) System under which the center's director is able to make substantive personnel and budget allocation decisions We established the HR Committee in IIIS to develop a system to appoint faculties. The appointment system is different from the previous personnel system with the step of the examination accelerated through intensive deliberations (2 stages: HR Committee and Administration Center Appointment Committee). Thus, rapid determination and appointment under the leadership of the Center Director are possible. So far, we have appointed 4 young researchers as Junior Principal Investigators (1 assistant professor and 3 associate professors). For budget execution, we are working to operate an efficient management system that is adequate to support researchers by reviewing the system of administrative organization under the leadership of the Administrative Director.

recruited from outside the University. These personnel will supply the director with the information needed for him/her to hand down decisions, and keep the director's work load from becoming excessive.	
(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	(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
The Deliberative Committee to Prepare for Establishment of the University of Tsukuba International Institute for Integrative Sleep Medicine (provisional name) has been established with the President as the head and persons in charge of research, administration, finances, and facilities, University Vice Presidents and Division Directors with international responsibilities, and related institute directors participating. Necessary adjustments will be made within the University to support design of the organization of this center. Further, after the establishment of this center, if persons from other institutes in the University assemble in this center as principal investigators, thorough adjustment will be made with those institutes in keeping with this center's status as a target for the Organization for the Support and Development of Strategic Initiatives. Specifically, adjustment and support will be carried out so that there is no impediment to the research and education carried out by the institute in question; these support includes securing replacement personnel and taking measures to reduce administrative and educational workloads. Further, a place for interchanges with the researchers at this center will be created, so as to aid in the nurturing of outstanding new personnel.	Eight members including PIs in IIIS (Masashi Yanagisawa, Hiroshi Nagase, Noriki Kutsumura, Masanori Sakaguchi, Michael Lazarus, Hiromasa Funato, Qinghua Liu and Yoshihiro Urade) were admitted as instructors in the Graduate School of Comprehensive Human Sciences Majors of Medical Sciences, so that they will be able to work as mentors for graduate students on research guidance in the future. So far we are composing syllabi which include lectures, seminars and experimental classes. And, 5 core PIs (Yanagisawa, Urade, Liu, Sakaguchi and Lazarus) were admitted as instructors in the Ph.D. Program in Human Biology, 4 PIs (Nagase, Funato, Lazarus and Kutsumura) were admitted in the Master's Program in Graduate School of Comprehensive Human Science, Medical Sciences. In addition, we are preparing the application for admission into the Graduate School of Pure and Applied Sciences, which is in the field of science and engineering. We are thus fixing the education system steadily and aiming to implement initiatives for developing human resources capable of working in a global environment in the future.
(4) Revamping host institution's internal systems to allow introducing of new management methods (e.g., English-language environment, merit-based pay, top-down decision making) unfettered by conventional modes of operation	(4) Revamping host institution's internal systems to allow introducing of new management methods (e.g., English-language environment, merit-based pay, top-down decision making) unfettered by conventional modes of operation
This center shall be made an independent research organization reporting directly to the University President and kept separate from other research centers. University systems will be executed flexibly, making amendments	All official meetings (such as PI meeting) are held in English only, and a system was established to use Skype videoconferencing with satellite institutions abroad regularly. Other meetings, such as lab-wide meetings to

and adjustments as necessary. Specifically, English shall be the official language of the center, with the administrative tasks carried out in English and administrative documents made bilingual. Persons with high English proficiency will be recruited from within and outside the University, and further training will be provided to those persons after entering the center. Under the management of the director, systems for determining salaries based on merit, for annual salaries, for evaluating researcher's achievements, for determining salaries based on evaluations of the work, and for renewal of contracts will be introduced. Also, to deal with requests for flexible execution, improvement, or adjustment of systems in the center, the director will exercise the functions established by the Organization for the Support and Development of Strategic Initiatives for dealing with research groups to take appropriate measures, subject to amendment through negotiation with the University President and related Vice Presidents and administrative divisions.	share information and present research progress, IIIS Seminar Series that invites outstanding scientists from outside of IIIS and IIIS Science Lounge in which members of IIIS share information in an informal circumstances, are also held only in English.
(5) Accommodation of center's requirements for infrastructural support (facilities, e.g., laboratory space; equipment; land, etc.) In order to assemble many leading researchers by making the center the one place where they want to work, the University will use its good offices to the greatest extent to facilitate the building of research facilities according to a plan that will give the center an outstanding research environment and distinctive tangible advantages so that it can claim with confidence to be a place for research of the highest level. Specifically, the center will be provided with all floors on the E Building of the University Hospital, which will be vacated by January, 2013 into the new ward building currently under construction. The center will continue to use the floors on the Health and Medical Science Innovation Laboratory that are currently occupied by the FIRST Program. Together, the center will be provided with more than 5,000 m^2 of research floor space. The Hospital E Building is located in close proximities to both the Innovation Building and the Laboratory Animal Resource Center, which should be highly advantageous for the center to facilitate its research. During the required	(5) Accommodation of center's requirements for infrastructural support (facilities, e.g., laboratory space; equipment; land, etc.) Upon the enticement of our research groups, the university conditioned an experimental facility for medicinal chemistry in TARA Center, and facilities for animal experiments / recombinant DNA experiments both in the E building of University of Tsukuba Hospital and Project Research Building. The design of the new research building was completed in November 2013. However, due to rises in material, labor and construction costs from the impact of the earthquake and reconstruction as part of Abenomics, the construction budget was revealed to exceed about 600 million JPY from the original plan. Those problems were solved as the Department of Finance and Accounting of the university loaned us the shortage, thanks to the leadership of the university president and the vice president in charge of finance and facilities management. A company in charge of construction was determined by bidding in January 2014, and preparatory construction was initiated from February.

renovation of the E Building, the center will be provided with temporary floors on the Laboratory of Advanced Research D. The Research Facility Center for Science and Technology will assist persons wishing to use facilities in and outside the University, through the Open Facility function which is scheduled to start being offered sometime this fiscal year. Among these facilities are those for cutting-edge mass spectroscopy, super-high resolution ultrasound echo sonography for mice, and *in vivo* luminescence/fluorescence imaging system for mice. This function will be expanded in stages, and will make possible the use of the pioneering research facilities in the Tsukuba area. Further, University of Tsukuba will provide university housing or satisfactory housing close to the University to researchers and administrative staff participating in this center, including foreign researchers. The University will also make its housing facilities available to foreign and Japanese researchers visiting this center so that they can hold seminars and carry out joint research.	Along with the groundbreaking, the liaison committee for the construction of "IIIS Research Building (tentative name)" was organized by construction workers, designers, the Department of Facilities and IIIS, to supervise the progress of construction. Sub-committee of the liaison was also organized to manage the budget and ensure smooth communication. Towards the completion in March 2015, those meetings will be carried out weekly to build a world premier research facility for sleep medicine.
(6) Support for other types of assistance As a concrete policy for raising the level of research to the highest international level, a goal proclaimed in the University's Midterm Plan, it is stated in this Plan that the University shall "promote high quality fundamental research taking a long term view of academic progress" and that "research groups and organizations which are expected to achieve outstanding research results will be singled out for appropriate support, including allocation of research resources, hiring of supporting personnel, and reforming of organizations, and will be aggressively promoted as international centers of research." The measures necessary to make this one of the world's leading centers, of which the University may be proud, shall be taken.	(6) Support for other types of assistance In response to the directions regarding execution of "Instructions for Enforcement of the Grant for World Premier International Research Center Initiative" as of November 11, 2013 by MEXT, University of Tsukuba formed a task force to deal with problems in Masashi Yanagisawa's resignation from Howard Hughes Medical Institute as well as the attribution of intellectual properties, and solved those problems accordingly.

11. Efforts to improve points indicated as requiring improvement in application review and results of such efforts	
- Major points to be improved	- Efforts to improve them and results
1) Item 1 Although next-generation sequencing and vizualization are noted as start-of-the-art technology, it seems the basic techniques from the technology are only used in sleep research. Is it not necessary to develop technology to lead to a breakthrough?	1) Measure 1 We at IIIS surely understand the importance of originality in developing our own technology, but so far we consider more important discoveries of novel biological principles or novel compounds, and elucidation of unknown mechanisms, by taking advantage of available cutting-edge technologies, than developing new technologies. We utilize currently available methodologies such as next generation DNA sequencing, live-imaging of neural activities, quantitative mass spectrometry, and novel fluorescent probes (although those technologies are not originally developed by us) for sleep research. We regard the following ways to utilize the live-visualization device for neural activities in free-moving mice (especially under natural sleep) as cutting-edge and they are operational in the world only in IIIS; (a) Live-imaging of neural activities using fiber-optic microendoscope (b) Live-imaging of neural activities in mice in sleep/wakefulness states using multi-photon excitation microscope One of the world's smallest, portable electroencephalographs for sleep analysis, developed in collaboration with industry (such as SONY Co. Ltd), is a good example of our truly original technology, and we will continue developing the device to be wireless.
2) Item 2 Is early establishment of a bioinformatics research core combined with informatics and next-generation sequencing not necessary?	2) Measure 2 Since we have already formulated software and database systems to automatically analyze enormous amounts of EEG/EMG data obtained from large screening of mice with ENU mutagenesis, the bioinformatics approach has already been implemented. The novel, effective methodology for mouse exome analysis is currently under formulation. To establish the equivalent function of the Next-generation DNA Sequencing Core of UTSW, we are seeking collaborative institutions such as Toyohashi University of Technology (visited by Masashi Yanagisawa in November 2013), and the feasibility study is underway to confirm the accuracy of sequencing data. An excellent researcher, who completed his Ph.D. in UTSW, will be hired from June 2014 to mainly work on

	informatics and sequencing data analyses. Furthermore, we are seeking the possibility to establish an in-house core for next-generation DNA sequencing in University of Tsukuba and sent a budget request for the upcoming 2015 fiscal year.
	3) Measure 3
3) Item 3 Are collaborations with clinical investigators not necessary for the results of animal experiments carried out at IIIS to be used in humans?	The on-campus collaborative PIs (Matsuzaki and Shimano) are clinical doctors and we communicate with each other on a daily basis. The contract for collaboration research was executed with a clinical doctor, Tetsuo Shimizu at Department of Neuropsychiatry, Akita University, Graduate School of Medicine. Establishing bio-resources comprised of the clinical information and samples from patients suffering from sleep disorders, we aim to search for abnormal pedigrees for the molecular genetics and diagnostic markers. In the future, we are eager to start collaborative clinical research of orexin agonists, which is currently investigated within IIIS. In collaboration with Ichiyo Matsuzaki, we will start collaborative activities, such as clinical studies of sleep-inducing drugs that are able to be administered to astronauts, with JAXA from FY2014.
	4) Measure 4
4) Item 4 The location of RIKEN Brain Science Institute (BSI) is close to Tsukuba and the director, Susumu Tonegawa, operates in a U.S. leadership style. With a field also close to sleep and brain research, should the collaboration not be deepened further?	Two junior PIs in IIIS used to work for RIKEN Brain Science Institute and they keep close relationships with the organization, so that they will bridge IIIS and RIKEN. We invited two researchers in RIKEN BSI (Drs. Thomas J. McHugh and Toru Takumi) to our 2nd Annual IIIS Symposium, held on January 20, 2014. Using this momentum, we began collaborative research with McHugh, sending a postdoctoral fellow from Yanagisawa/Funato Laboratory to BSI for four months. We are currently continuing collaborative research.
	5) Measure 5
5) Item 5 Using the connection with UTSW so far, you have been able to promote the formation of IIIS, but it is not discussed well how you plan to employ excellent researchers in the future.	IIIS always seeks high-quality human resources (especially international), and it has hired researchers from Canada, France, Germany, China and the United States. Advertisements have been posted on 10 free websites and paid ones (Federation of European Neuroscience, Society for Neuroscience Neurojobs) for recruiting

	scientists. As a result of these efforts, 172 young scientists applied for postdoc / junior PI positions, but none of them met the qualification criteria set by IIIS. Other than posting advertisements, we eagerly seek out candidates at international conferences, as well as inviting prospective IIIS scientists to internal seminars (WPI-IIIS Seminar Series).
6) Item 6 In order to build a truly world-class research center, you should hire a female PI to the core research group.	6) Measure 6 IIIS understands the importance of hiring female PIs and always tries to seek them out, but only a very small number of excellent female researchers exist in the field of sleep science. We should not compromise the level of qualification. As stated above, we have already invited many female researchers to the IIIS Seminar Series, and the door is always open. Dr. Yang Dan of the University of California, Berkeley, who delivered a talk at the 2nd Annual IIIS Symposium held in January 20th, 2014, accepted our offer of future collaborations as a satellite PI. She is even interested in opening a secondary laboratory at IIIS.
7) Item 7 How about employing researchers with a focus on neuroscience system in particular, such as 1 or 2 senior neuroscientists for further development of the institute. They don't have to be researchers specializing in sleep, but you may want to consider having them apply knowledge of neuroscience systems to the research results of existing researchers.	7) Measure 7 IIIS has been working actively to employ researchers of excellence in the field of systems neuroscience. Some candidates were invited to the WPI-IIIS Seminar Series, and we will keep searching for good candidates.
8) Item 8 Communication with the satellites is important and should be improved with teleconferencing.	8) Measure 8 IIIS and satellite institutions have been communicating appropriately by using Skype and other teleconferencing. To strengthen the alliance more, the time for PI meetings was adjusted so that all PIs (including ones in the United States) were able to attend the meeting.
9) Item 9	9) Measure 9 Long-term stay programs for international students are under consideration, along with short-term programs (such as workshops) in

Is it not necessary to create a path toward short-term programs for young researchers in order to enhance the existing value of the institute?	collaboration with RIKEN and others. We will initiate the creation of workshop programs to develop the careers of young scientists, widen networking in sleep research and find opportunities of collaboration.

12. FY 2013 funding

(the exchange rate used:)

i) Overall project funding

1 million yen

Cost Items	Details	Costs (1 million yen)
Personnel	Center director and Administrative director	25
	Principal investigators (12 persons):	67
	Other researchers (21 persons):	102
	Research support staffs (9 persons):	42
	Administrative staffs (9 persons):	40
	Total	276
Project activities	Gratuities and honoraria paid to invited principal investigators	1
	Cost of dispatching scientists	6
	Research startup cost	63
	Cost of satellite organizations	12
	Cost of international symposiums	5
	Rental fees for facilities	3
	Cost of consumables	15
	Cost of utilities	4
	Other costs	25
	Total	134
Travel	Domestic travel costs	2
	Overseas travel costs	6
	Travel cost for scientists on secondment	7
	Total	15
Equipment	Depreciation of buildings	34
	Depreciation of equipment	91
	Total	125
Other research projects	Projects supported by donation, etc.	40
	Total	40
Total		590

WPI grant for FY 2013

Costs of establishing and maintaining facilities in FY 2013	34
Repairing facilities (Bldg.E in the university hospital: 253 m²)	34
Cost of equipment procured in FY 2013	91
Base maintenance cost (Common thoughout an institute)	58
Slide Scanner	21
Mouse breeding apparatus	12

ii) Costs of Satellites and Partner institutions

Cost Items	Details	Costs (1million yen)
Personnel	Postdoc researcher (1 person):	
	Total	7
Project activities		1
Travel		0
Equipment		4
Other research projects		0
Total		12

Research objectives/results/progress/alternations from plan at start of project

1. Research objectives

Out of the three research objectives that we set out to achieve in the plan at the start of the project, 1) Elucidation of the fundamental mechanisms of sleep/wake regulation, 2) Elucidation of molecular pathogenesis of sleep disorders and related diseases, and 3) Development of treatments for sleep disorders, 1) Elucidation of the fundamental mechanisms of sleep/wake regulation has been divided into two objectives: elucidation of molecular/cellular mechanisms and studies of neural circuits and systems. The research objectives were thus reorganized into the four items listed below.

1) Elucidation of the substances and genes of sleep/wake regulation
2) Elucidation of the regulating neural circuits and functions of sleep
3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases
4) Development of treatments for sleep disorders

2. Goals

Goals have been set to each research objectives as below.

1) Elucidation of the substances and genes of sleep/wake regulation
- Identification of new genes involved in sleep/wake regulation
- Elucidation of the substances regulating sleep/wake

2) Elucidation of regulating neural circuits and functions of sleep
- Elucidation of operating principles of the networks regulating sleep/wake
- Elucidation of physiological functions of sleep

3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases
- Elucidation of sleep/wake regulation in the brain and in associated peripheral organs
- Elucidation of intracellular events and molecular association of sleep/wake behavior in the body

4) Development of treatments for sleep disorders
- Development of sleep disorder therapy drug-candidate
- Development of multi-faceted "Good Sleep" program that does not use drugs for the prevention of sleep disorders

3. Results and progress until end of FY2013 and future directions

For each goal set for 4 objectives, results and progress until end of FY2013 and future directions are described as follows.

1) Elucidation of the substances and genes of sleep/wake regulation

- **Identification of new genes involved in sleep/wake regulation**

a) Identification of novel genes to control sleep/wakefulness

(Yanagisawa/Funato lab)

We obtained 10 mouse families showing sleep/wakefulness abnormality by forward genetics approach monitoring EEG and EMG with ENU mutagenesis.

We identified the *Sleepy* mutation which brings a short awakening time and the *Dreamless* mutation which brings REM sleep abnormality through gene mapping and exome analysis.

[Future directions]

The discovery of additional important regulator genes in the sleep/wakefulness network is expected by pushing forward with the analysis of mutant pedigrees that have been obtained so far.

b) Identification of the putative mutation in the *Sleepy* mutant pedigree (Yanagisawa/Funato lab)

Heterozygous *Sleepy* mutant mice showed approximately a 20-30% reduction in 24-h wake time and 30% reduction of dark-phase wake time. Decreased wake time could be due to decreased arousal response or increased sleep need. Our preliminary results showed normal arousal response of *Sleepy* mutants to external stimuli, suggesting the possibility that *Sleepy* mutant mice have increased sleep need.

To map the chromosomal region responsible for the sleep phenotype of *Sleepy* mutant mice, we performed linkage analysis of N2 mice, obtained by backcrossing the mutant C57BL/6J male to C57BL/6N female mice for two generations. A QTL analysis based on 24-h wake time revealed a single peak with a LOD score of more than 20. Whole exome sequencing of mutants and wildtype littermates from the *Sleepy* pedigree identified a single nucleotide change specific to *Sleepy* mutant mice within the mapped chromosomal region. The mutation destroys a splice donor site of a gene, which we termed the *Sleepy* gene. RT-PCR analysis of brain and liver mRNA found a short variant of the *Sleepy* mRNA specific to the mutant mice (Figure 1). Direct sequencing of the short RT-PCR product confirmed exon skipping in the *Sleepy* mutant mRNA due to the splice mutation. The deletion of this in-frame exon is predicted to produce a Sleepy

protein lacking approximately 50 amino acid residues in the middle of the protein. We then made antibodies against a synthetic peptide within the deleted region. The produced antibody specifically recognized the wildtype but not the mutant form of the

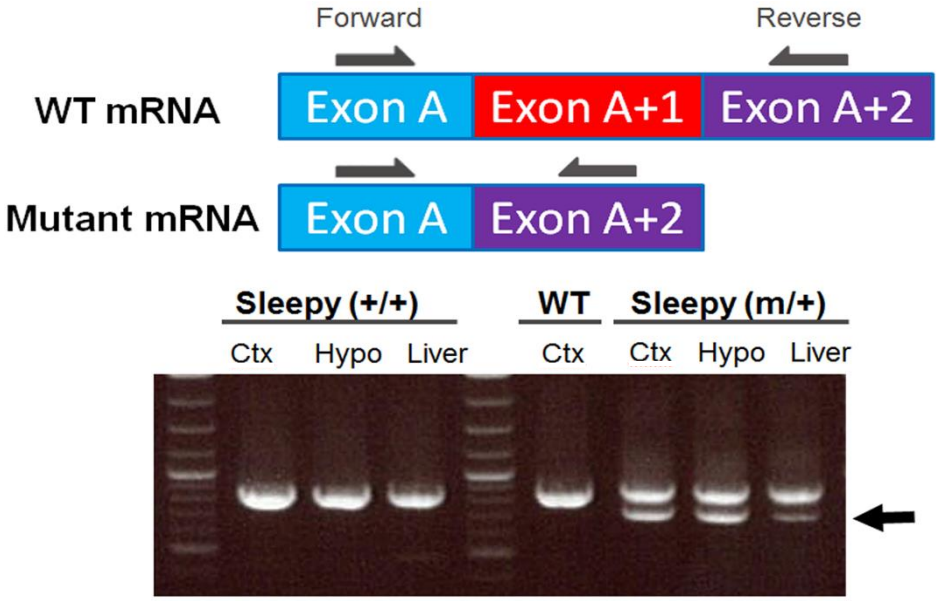

Figure 1. RT-PCR of *Sleepy* mRNA
Shorter PCR products (indicated by an arrow) are specific to *Sleepy* heterozygous mouse. The size difference between the two bands corresponds to the size of EzonA+1. Ctx: Cortex, Hypo: Hypothalamus.

Sleepy protein. To prove the causal role of this mutation in the *Sleepy* phenotype, the *Sleepy* mutation is being replicated through the ZFN and CRISPR technologies.

[Future directions]

Elucidation of the mechanism for sleep needs a setpoint through the *Sleepy* gene.

Although so far we have examined only heterozygous *Sleepy* mutant mice, we will examine the sleep/wakefulness behavior of homozygous *Sleepy* mutants to see whether the total sleep time of homozygous mice are further shortened. The brains of homozygous *Sleepy* mutants will be a valuable source for proteomic and genomic analyses in order to examine protein and mRNA changes.

c) Identification of the putative mutation in the *Dreamless* mutant pedigree (Yanagisawa/Funato lab)

Our high-throughput screening of ENU-mutagenized mice also identified a pedigree which exhibits short REM sleep episode duration and short total REM sleep time (Figure 2). We named the pedigree *Dreamless*. Linkage analysis of N2 mice of the *Dreamless* pedigree revealed a single QTL peak with a LOD score of more than 10. Direct sequencing of candidate genes located within the mapped chromosomal region found a single nucleotide substitution in a gene, which we termed the *Dreamless* gene. The single nucleotide change was confirmed by whole exome sequencing as the only exonal SNP specific to the mutant mice within the chromosomal region. The nucleotide substitution causes a charge-altering amino acid change at a residue highly conserved from invertebrates to mammals, suggesting a crucial role in the protein function. To prove the causal relationship of the nucleotide change to REM sleep abnormalities, *Dreamless* gene-modified mice are being produced through the CRISPR technology.

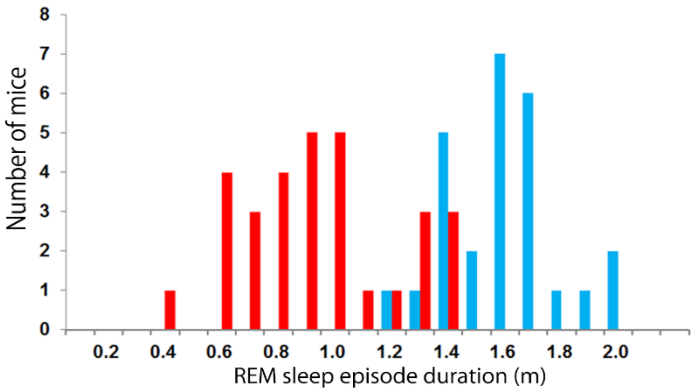

Figure 2. *Dreamless* mutant mice (red) exhibited shorter REM sleep episode duration than wild-type littermates (blue).

[Future directions]

Elucidation of the role of the *Dreamless* gene in the regulation of REM sleep

We have examined only heterozygous *Dreamless* mutant mice. We will examine homozygous mutants to see whether they show a further reduction in REM sleep time. Similar to the *Sleepy* mutants, we will induce the same and related changes in the *Dreamless* gene using the CRISPR technology to prove the causal relationship of the mutation to the phenotype. In *in vivo* and *in vitro* experiments, we will characterize the function of wildtype and mutant forms of Dreamless proteins to understand how the Dreamless protein regulates REM sleep behavior.

d) Identification of the transcription factor responsible for regulating sleep/wakefulness (Urade lab)

We elucidated the transcriptional network involved in sleep-wake regulation in the mouse brain by the GeneChip cDNA microarray analysis with mRNAs obtained from cortexes of sleeping (at 10:00) or waking (at 22:00) animals. After discrimination of

circadian-rhythm-related genes from the gene pool, we identified 55 genes whose mRNAs were up-regulated during sleep by GeneChip and quantitative PCR analyses. The mRNAs of the transcription factor SOX5 increased in a time-dependent manner during the first 4 h of the light period. Among various SOX genes, we found that only SOX5 was up-regulated during sleep and identified a novel splicing isoform of SOX5 with truncation at exon 2, SOX5-t2.

The neurite branching and spine formation was significantly induced in Sox5t2 gene over-expressing human neuroblastoma cells, SH-SY5Y.

Immunofluorescence staining revealed that SOX5-t2 was highly expressed in the nucleus of neurons of the adult mouse cortex. These data indicate that SOX5 is an important regulator of sleep-related functions of neurons.

[Future directions]

Sox5 is involved in the differentiation of oligodendrocytes and the development of neocortical projection neuron. Thus, a variant of Sox5 is possible to induce many neuronal diseases. We will measure the locomotor activities and EEG in Sox5-knock down mice.

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1) Elucidation of the substances and genes of sleep/wake regulation

- **Elucidation of the substances regulating sleep/wake**

a) Elucidation of the fundamental mechanisms in PGD_2 that regulate sleep/wakefulness (Urade lab)

Over the past years, we focused on the molecular mechanisms by which prostaglandin D_2 (PGD_2) is inducing sleep. PGD_2 is the most abundant prostaglandin produced in the brain. Nano-molar injections of PGD_2 in rat brains demonstrated its dose and time dependent somnogenic activity, while PGD_2-induced sleep was indistinguishable from physiological sleep.

PGD_2 can be produced by two distinct types of PGD_2 synthase (PGDS), lipocalin-type PGDS (LPGDS) and hematopoietic PGDS (HPGDS), but only LPGDS is related to sleep. Three potential sites for PGD_2 synthesis by LPGDS have been identified in the brain, i.e. oligodendrocytes (OD), epithelial cells of the choroid plexus (CP) and arachnoid trabecular cells of the leptomeninges (LM).

We have embarked on a program to identify the site of synthesis of somnogenic PGD_2 and generated a transgenic mouse line with the LPGDS gene amenable to conditional deletion using Cre recombinase.

To identify which tissue is responsible for the production of somnogenic PGD_2, we engineered animals lacking the expression of LPGDS specifically in:
- the OD by cross-breeding flox-LPGDS mice with Nestin-Cre mice, inducing a complete KO of LPGDS in the neural but not in the leptomeningeal cells (OD-LPGDS KO mice)
- the CP by injecting adeno-associated virus (AAV), serotype 5, expressing Cre recombinase (AAV5-Cre) in the lateral third ventricle (CP-LPGDS KO mice)
- the LM by injecting AAV, serotype 8, expressing Cre recombinase (AAV8-Cre) into the ventricle of new born mice (LM-LPGDS KO mice)
We recorded electroencephalogram, electromyogram and locomotor activity to measure sleep of 10 weeks old animals with a specific knockdown of LPGDS in one of the 3 target tissues. By using selenium tetrachloride, a specific PGDS inhibitor, we demonstrated that sleep was inhibited in OD-LPGDS and CP-LPGDS KO mice, but not in the mice lacking LPGDS in the LM.
We concluded that leptomeningeal cells, but not OD or CP, are the source of somnogenic PGD_2.
[Future directions]
Leptomeninges are formed by a complex structure of 3 layers of cells: dura mater, arachnoid mater and pia mater. While each of these 3 layers of cells has been proven to express LPGDS, we are not sure yet which one is necessary for the production of PGD_2 regulating sleep. Therefore we are now trying to target the expression of LPGDS into each layer individually in order to better understand the regulation mechanism involved in this process. Furthermore, the release of PGD_2 into the brain has been proven to induce the Adenosine pathway, well characterized as central for sleep regulation. We are now trying to identify which mechanisms and which cells are connecting the PGD_2 and the adenosine pathways.

b) Elucidation of mechanisms regulating sleep by adenosine and its receptors (Lazarus lab)
Key neural substrates for sleep remain to be identified, because finding a neuronal cell group that reliably promotes sleep when stimulated has been the major obstacle to achieving that goal. The Lazarus laboratory, by using optogenetic and pharmacogenetic stimulation of adenosine A2A receptor (A2AR) neurons in the nucleus accumbens (NAc) and the combination of NAc-specific A2AR knockout mice and administration of an A2AR agonist, established in FY2013 that activation of A2AR neurons and adenosine receptors in the NAc strongly promote sleep (Xu Q *et al.*, in preparation). These observations provide the first direct evidence that adenosine and A2AR neurons in the NAc are not

only involved in promoting behavioral inactivity (inhibition of movement), but also play a major role in the regulation of sleep.

[Future directions]

Whereas the mesolimbic dopamine system from the midbrain to the striatum, including the NAc, has been studied by many laboratories for decades, it remains unknown where or in what cell component adenosine is generated. Adenosine may be released by astrocytes in the NAc or neurons in the medial prefrontal cortex, the amygdala or the ventral tegmental area projecting to the NAc. Studies in the Lazarus and Urade laboratories have previously revealed that caffeine induces wakefulness by blocking the action of adenosine on A2ARs in the NAc (Lazarus M, *et al*., *J Neurosci* 2011, **31**: 10067-10075; Lazarus M, *et al*., *Trends Neurosci* 2012, 35: 723-731). For caffeine to be effective as an antagonist and cause wakefulness, A2ARs must be tonically activated by extracellular adenosine and thus, we hypothesize that the deletion of the neural source of adenosine (astrocytes or neurons) results in attenuation of the arousal effect of caffeine due to the absence of extracellular adenosine. This hypothesis will be tested by the conditional inhibition and lesioning of potential neural sources of adenosine in various cell type-specific cre mice in combination with the stereotaxic microinjection of adeno-associated viral vectors.

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2) Elucidation of regulating neural circuits and functions of sleep

- **Elucidation of operating principle of the neural networks regulating sleep/wake**

a) *In vivo* two-photon calcium imaging of cortical neurons during sleep (Yanagisawa/ Funato Lab)

To reveal the detailed activity patterns of cortical neurons during sleep and wakefulness, we designed and constructed the *in vivo* two-photon microscopy system for unanesthetized mice. In order to avoid drifts in the field of view by the mouse's movement and to simultaneously reduce stress to the mouse, we adopted a trackball system, which allowed the mouse to freely move with the head fixed under the objective (Figure 3). Indeed, our system markedly reduced the restraint stress, so that mice readily slept under microscopy even though they were head-restrained. The system allows us to record calcium dynamics in the cortex at cellular resolutions in naturally awake and sleeping mice (Figure 4).

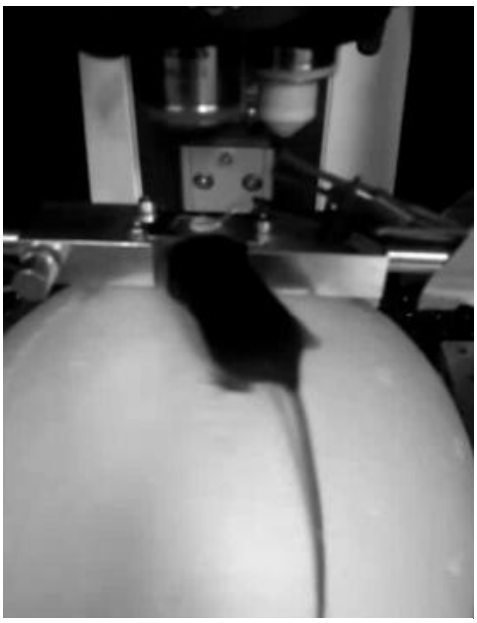

Figure3. Our two-photon imaging system eliminates the drift in field of view and greatly reduces stress to the unanesthetized mouse.

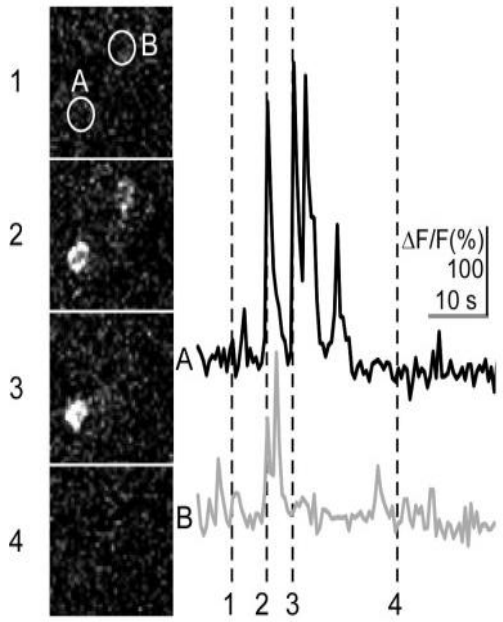

Figure4. Ca^{2}+ imaging of pyramidal neurons from an unanesthetized *Thy1-GCaMP7* transgenic mouse.
Left: Frames from a two-photon time series of pyramidal neurons.
Right: Fluorescence changes in neuronal cell body A and B in left frames.

[Future directions]

Visualization of calcium dynamics of cortical neurons during sleep/wakefulness behavior.

Cortical neurons are divided into excitatory and inhibitory neurons and the inhibitory neurons are further divided into several subpopulations based on neurochemical, physiological and anatomical features. Each subpopulation of neurons may differentially behave during the different sleep/wakefulness stages. By combining *in vivo* two-photon calcium imaging and mouse molecular genetics including the *Cre-loxP* system, we will continue to examine the calcium dynamics of genetically defined subpopulations of cortical neurons in sleep and wakefulness.

b) Analyses on input/output system of orexin-producing neurons (Sakurai/Sakaguchi Lab)

We analyzed input/output systems of orexin-producing neurons. Regarding input systems, we found that orexin neurons receive inhibitory projections by glycinergic neurons (Hondo *et al.*, *PLoS One* 2011). We also showed that orexin neurons express neurotensin, which acts as an autocrine regulatory factor through Ntsr2 (Furutani *et al.*, *PLoS One*, 2013). We confirmed that specific pharmacogenetic stimulation of GABAergic

neurons in the POA leads to an increase in the amount of non-rapid eye movement (NREM) sleep. We also found that optogenetic stimulation of fibers arising from POA GABAergic neurons resulted in rapid inhibition of orexin neurons, suggesting direct connectivity between POA GABAergic neurons and orexin neurons (Saito *et al*., *Front Neurosci*, 2013). Regarding the afferent system, we showed that each orexin receptor subtype plays differential roles in gating NREM and REM sleep through distinct neuronal pathways (Mieda *et al*., *J Neurosci*, 2011). Using the DREADD system, we suggested that excitation of orexin neurons increased the amount of time spent in wakefulness and decreased both NREM and REM sleep times. Likewise, inhibition of orexin neurons decreased wakefulness time and increased NREM sleep time (Sasaki *et al*., *PLoS One*, 2011). We showed that orexin neurons are capable of fast glutamatergic control of their projection targets（Schone *et al*., *J Neurosci*, 2012; Schone *et al*., *Cell Rep*, 2014). We found that NA neurons in the LC showed a higher firing frequency in narcoleptic mice during both wakefulness and NREM sleep as compared with wildtype mice due to functional decrease of GABAergic input to these neurons. These alterations might play roles in the sleep abnormality in narcolepsy (Tsujino *et al*., *PLoS One*, 2013). We found that an OX1R-mediated pathway in the LC is involved in the physiological fear learning process via regulation of noradrenergic neurons (Soya *et al*., *J Neurosci*, 2013). We found that that DR serotonergic and LC noradrenergic neurons play differential roles in orexin neuron-dependent regulation of sleep/wakefulness (Hagegawa *et al*., *J Clin Inv*, 2013). In order to understand the differential contribution of both receptors in regulating sleep/wakefulness states we compared the pharmacological effects of a newly developed OX2R antagonist (2-SORA), Compound 1 m (C1 m), with those of a dual orexin receptor antagonist (DORA), suvorexant in mice and found that an orexin-mediated suppression of REM sleep via potential activation of OX1Rs in the LC may possibly contribute to the differential effects on sleep/wakefulness exerted by a DORA as compared to a 2-SORA. (Etori, *et al*., *Front Neurosci*, 2014).

[Future directions]

We are going to depict whole connectome of neuronal pathways that regulate sleep/wakefulness states, by means of combination of anterograde/retrograde labelling using AAV and recombinant rabies virus vectors. We also perform functional studies on neurons that constitute these neuronal circuits using electrophysiology and imaging studies. We also plan to search endogenous substances including peptides and small molecules that affect activities of these neurons.

c) Quantitative Mass Spec analysis of wild type and *sleepy* mouse brain proteome (Liu lab)

I recruited a talented postdoc and excellent protein biochemist, Dr. Zhiqiang Wang, to my lab on March 1, 2013. Second, I initiated a close collaboration with an outstanding mass spectrometry expert, Dr. Yonghao Yu, who was a newly recruited faculty at the Department of Biochemistry at UT Southwestern Medical Center at Dallas, USA. Since then, Dr. Wang has been schooled in Professor Yu's lab to learn the art of the cutting-edge quantitative mass spec technologies. If all goes well, Dr. Wang will be able to establish the Mass Spec facility at IIIS in the late spring of 2015 at the newly constructed IIIS building.

Professors Yanagisawa/Funato sent the first batch of wild type and *sleepy* mutant mouse brain samples to UT Southwestern. We prepared these brain samples and performed TMT experiments to compare the brain proteome of wild type and *sleepy* mutant mice. The advantage of TMT is that it allows simultaneous analysis of six brain samples (3 wild type and 3 mutant samples) and thus provides the statistical confidence of the results. In the first round of experiments, we were able to confidently analyze ~4,500 relatively abundant proteins at one time and 99% of these proteins show no statistically significant change between WT and mutant brains. In contrast, the levels of less than ten proteins were up- or down regulated in the mutant proteins. This pilot experiment went surprisingly well because the quality of data from brain tissues (~4,500 proteins) matched that from cultured cell lines (~5,000 proteins).

[Future directions]

We plan to continue this line of investigations to analyze more sleep mutant brains as soon as they are available. We will also begin to analyze post-translational modifications (PTM), such as phospho-proteome. The cross comparison of these mass spec results may reveal the molecular networks that govern sleep/wakefulness regulation.

One caveat is that our proteome analysis will only see the most abundant 4,500 proteins. To solve this problem, we will analyze sub-regions of the brain, such as the hypothalamus, or focus on membrane proteins. Furthermore, Professor Yanagisawa has secured stimulus funds from the Japanese government to purchase the newest Thermo Fusion mass spec instrument, which has arrived at IIIS in March 2014. Thermo Fusion is currently the fastest and most powerful Mass Spec instrument that has three MS. It will allow us to double the depth of proteome analysis to ~9,000 proteins.

d) Investigate downstream signaling pathways of Orexin neuropeptide (Liu lab)

Previously, professors Yanagisawa/Sakurai identified the neuropeptide Orexin (also

known as Hypocretin) as a key regulator for maintaining wakefulness in 1988. The lack of Orexin or its receptors caused Narcolepsy, a disorder characterized by frequent daytime (night time in the case of mice) sleep attacks and cataplexy in mice, dogs, and humans. However, despite intensive studies, the downstream signaling pathways of Orexin/Orexin receptors are not clearly understood. In cultured cell lines expressing Orexin receptors Ox1R or Ox2R, Dr. Wang discovered that Orexin could activate the mTOR pathway, a central regulator of cell growth and metabolism, in HEK293 and hypothalamic cell lines. Orexin-mediated mTOR activation requires extracellular Calcium influx and seems to require a novel pathway independent of known upstream kinases Erk and Akt.

[Future directions]

We are currently investigating the mechanism of Orexin/G protein coupled receptor (GPCR)-dependent mTOR activation. We hypothesize that mTOR plays a central role in mediating the effects of Orexin in promoting wakefulness and energy homeostasis. Hopefully, we will submit a manuscript describing this work in the fall of 2014.

e) Elucidation of neural circuits that generate REM and non-REM sleep (Hayashi Lab)

In addition to sleep/wake regulation, mammalian sleep is composed of two distinct states, REM (rapid eye movement) sleep and non-REM sleep. REM sleep is the major source of dreams, whereas non-REM sleep is characterized by a synchronous brain activity called slow waves. Abnormal balance of the two sleep states is a common symptom in various developmental disorders and psychiatric disorders. Little is known, however, about the identity of the neurons responsible for generation of the two states. While classical lesion studies indicated a central role of brainstem pontine area in both the generation and the stoppage of REM sleep, the heterogeneity and complexity of this brain region has hindered identification of the responsible cells. Currently, two proposed models of mutual inhibition between REM and non-REM

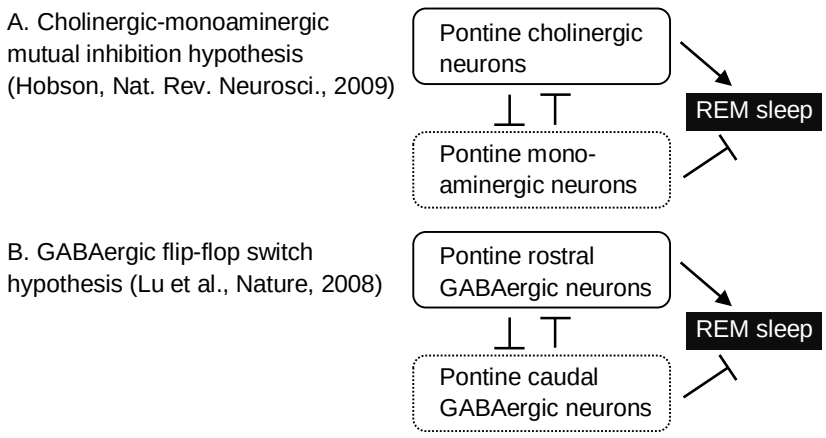

Fig 5 Controversial models of the REM-non-REM sleep switch

promoting neurons (cholinergic-monoaminergic mutual inhibition hypothesis and GABAergic flip-flop switch hypothesis, Fig. 5) are under controversy, but both lack evidence (Hobson, *Nat. Rev. Neurosci*., 2009; Lu *et al*., *Nature*, 2006).

To manipulate a specific population of neurons out of a heterogeneous population, we focused on the fact that neural function is often closely linked with cell lineage and developmental origin. We established a genetic method that enables activity-manipulation of neurons in a cell lineage-specific manner (Hayashi *et al*., unpublished). With this method, we identified a subpopulation of glutamatergic neurons in two pontine areas that strongly promote or inhibit REM sleep, respectively (Fig. 6, Hayashi *et al*., unpublished). Furthermore, we showed that the same areas contain GABAergic projection neurons that also promote or inhibit REM sleep, respectively (Hayashi *et al*., unpublished). These GABAergic neurons send projections to each other. Thus we conclude that two types of glutamatergic neurons in the pons promote either REM or non-REM sleep, and that mutual inhibition between these two areas mediated by GABAergic neurons regulates the balance between REM and non-REM sleep (Hayashi *et al*., unpublished). Our study dissolves the long debate on the cell types of the REM-non-REM sleep switch, supporting the GABAergic flip-flop switch hypothesis and further indicating the involvement of glutamatergic neurons.

Furthermore, according to the "hourglass model of evolution", developmental origins and cell lineages also provide valuable information about evolution. REM and non-REM sleep are seen only in birds and mammals, yet how these states evolved remain totally unknown. Using our cell lineage specific analyses method, we found that the glutamatergic neurons regulating REM sleep described above share a common developmental origin with the adjacent neurons that promote arousal. Thus, REM sleep might have evolved by the emergence of a specific subpopulation of arousal cells that activate the brain during sleep, not wake (Hayashi *et al*., unpublished).

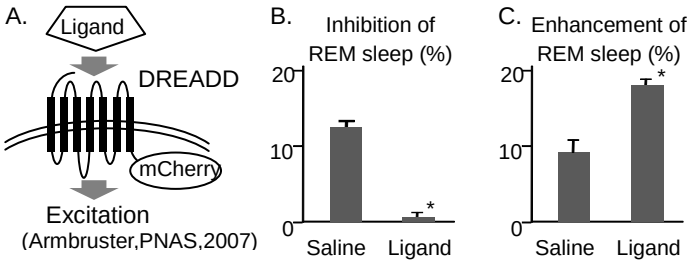

Fig 6. Identification of REM sleep regulating neurons (Hayashi *et al*. unpublished data)

A. Pharmacogenetic induction of neural excitation by expression of the DREADD receptor and administration of its ligand.

B. Inhibition of REM sleep 3hrs following DREADD-mediated stimulation of a specific group of glutamatergic neurons in the rostral pontine tegmental area.

C. Increase of REM sleep 2 hrs following DREADD-mediated stimulation of a specific group of glutamatergic neurons in the caudal pontine tegmental area.

*P<0.01 (Welch's test).

[Future directions]
Here we genetically identified the neurons that are responsible for generating REM or non-REM sleep. We identified their cell types and also found that the REM-non-REM sleep switch shares a close developmental origin with neurons that promote arousal. It remains unknown, however, how a specific population of cells born during development gives rise to subpopulation of cells with totally different functions. To this end, we are conducting microarray analyses to identify genes that are specifically expressed in each subpopulation, and have already successfully identified several candidate genes. Cre-knock-in mice are generated using these genes, and will be utilized to investigate whether the genes or the neurons that they are expressed in actually regulate REM or non-REM sleep.
Furthermore, through identification and manipulation of cells regulating REM and non-REM sleep, we succeeded in inhibiting or increasing REM sleep at a desired time point. We will utilize this method to address the long unsolved mystery of the functional roles of REM sleep.

f) Identification of mechanisms controlling slow wave sleep (Greene/Vogt lab)
In recent years we have studied the role of different interneurons in neural networks and in dendritic integration. In addition we have studied the role of synaptic plasticity in neurodevelopmental disorders.
We have successfully combined optogenetics and voltage sensitive dye imaging in slice preparations as a precursor to applying these techniques *in vivo*. We have successfully performed the first EEG recordings using hardware and software from the open physiology project.
[Future directions]
We are planning to use high resolution imaging techniques and optogenetic/DREADD technology to study cortical networks involved in sleep. In the transition from wakefulness to sleep the cortex undergoes a dramatic reorganization of its network activity from a high level uncoordinated state to low level coordinated activity. This is reflected in the surface EEG as an increased power in the 0.5 to 4.5 Hz range. This slow wave sleep is crucial for sleep function and survival, but the neuronal networks underlying slow wave sleep and their regulation remain poorly understood.
In the first step, we want to study its homeostatic property (increased slow wave power after sleep deprivation) as a local cortical phenomenon. We will control the level of activation in small circumscribed cortical areas by adeno-associated virus-mediated transfection of neurons with designer receptors exclusively activated by designer drugs

(DREADD). Using surface EEG and local field potential recordings we want to investigate how altering activity at this level controls slow wave sleep power. Does hypo-activation during waking lead to less slow wave activity later? How local is this phenomenon? Is dampening through activation of GABAergic neurons equivalent to direct dampening of principal neurons? Does disruption of slow wave activity during sleep result in a rebound in later sleep phases, or does it even substitute for slow wave sleep?

In a second project we want to use *in vivo* calcium and voltage imaging through genetically encoded sensors to study the activity of different neuron species during waking, during slow wave sleep and in the transition between the two. Sensors will be expressed in select subtypes of neurons and activity in these neurons will be correlated with local field potentials in their vicinity.

In a third project we want to use optogenetic tools to 'imprint' defined rhythmic activity on small cortical regions in order to test whether such activity can mimic slow wave sleep and to test which parameters such an imposed rhythm needs.

g) Elucidation of operating principles of neural [-glial] networks regulating sleep/wake (Greene/Vogt lab)

Homeostatic sleep need increases during waking and decreases during sleep. We show

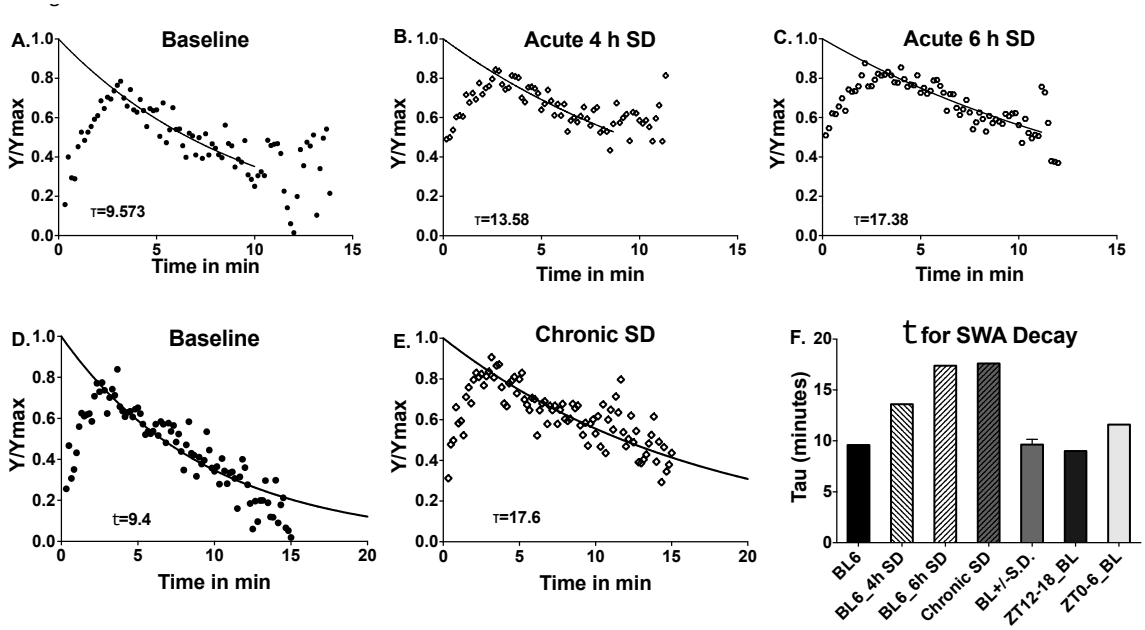

Figure 7. SWA decay rate constant correlates with sleep need in wild-type mice.
The time constant of SWA decay (τ) during an average SWS episode following differing amounts of SD is determined by a single phase exponential, regression fit in wild type mice. The SD conditions for C57BL6 mice (n=4) include "Baseline", 4h and 6h acute SD (**A-C**, respectively). **D-E**. Another group of wild type strains (on a C57BL6 background; n=24) experienced chronic SD (4 h SD with 2 h recovery for 8 consecutive cycles). The plots show time of the SWS episode (plotted for each 10s epoch; X axis) versus normalized SWA power (Y axis). **F.** A histogram of SWS episode, SWA decay, τ, determined for each SD condition shows a graded slowing in proportion to previous enforced W duration. "BL+/-S.D." is a pooled wild type strain (used for chronic SD) that provided a standard deviation (S.D.) of 1.1minute.

that encephalographic slow wave activity (SWA), an index of sleep need, exhibits a single exponential decay during a single slow wave sleep (SWS) episode in mice, and that the decay slows significantly and in direct proportion to the amount of sleep deprivation (SD; labeled figure 7). Conditional knockout of neuronal adenosine A1 receptors demonstrate their necessity for this decay. Furthermore, in mice with reduced expression of the glial adenosine kinase, an adenosine-metabolizing enzyme, the decay rate constant slows to a degree comparable with that induced by 6-hr sleep deprivation. Consolidation of SWS, another index for sleep need, is also decreased with neuronal A1 receptor deletion, and increased with glial adenosine kinase deficiency. These results demonstrate a glial-neuronal circuit mediated by intercellular adenosine, controlling the expression of sleep need. The high affinity, low capacity adenosine kinase is regulated by glial metabolic state, which may then influence the neuronal expression of sleep need as it resolves during sleep (labeled Figure 8).

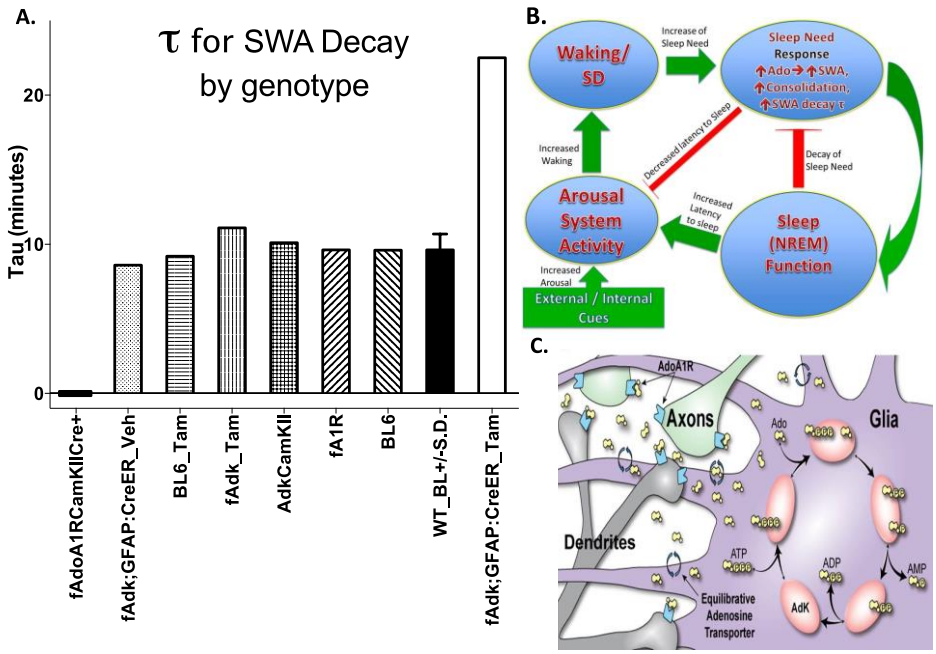

Figure 8. The decay rate constant for SWA during the SWS episode is influenced by the AdorA1 and Adk genes controlling expression of the adenosine A1 receptor and adenosine kinase, respectively.

A. A histogram of the rate constants for decay (τ) of SWA during an average SWS episode under baseline conditions for different genotypes. Loss of neuronal Adora1 (fAdoA1RCamKIICre+) results in loss of decay and reduction of Adk expression (fAdk;GFAP:CreER_Tam) increases τ by more than 10X the standard deviation (S.D.= 1.1min). **B.** Systems level cartoon depicts relationships between waking, which is facilitated by arousal and increases sleep need and sleep function, which decreases sleep need and presumably, enhances arousal. **C.** At the local circuit level, the expression of sleep need is mediated by adenosine acting on neuronal A1 receptors to facilitate rebound SWA in response to prolonged enforced waking. During SWS, adenosine flows down its concentration gradient into glia by glial equilibrative transporters, where it is metabolized by the low capacity but high affinity enzyme, adenosine kinase, thus reducing the activation of neuronal AdoA1Rs that controls the rate of SWA decay.

[Future directions]
In consideration of the close relationship of the buildup and decay of SWA to sleep need, we plan to examine the local circuit mechanisms responsible for firstly, the generation of SWA during SWS and secondly for its modulation by previous waking (W) duration. The local circuit mechanism(s) responsible for its generation are likely to be modulated by both local factors like adenosine concentration, mediated by a neuronal-glial circuit that we have identified (see above) and also by influence of monoaminergic and cholinergic arousal centers, that induce the large increase of SWA on transition from W to SWS. Thus, to maintain physiological local and systemic influences (including long range projections) of SWA, local circuit activity will be monitored with genetically encoded, next generation, calcium and/or voltage sensitive dyes, imaged with 2 photon techniques. This will allow synchronous monitoring of multiple neurons (as well as labeled glia) participating in the generation of emergent local circuit activity responsible for the slow waves associated with sleep need. An understanding at the cellular and intercellular level of sleep need related modulation of the SWA may prove essential to the unraveling of the enigma of the function of sleep.

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2) Elucidation of regulating neural circuits and functions of sleep

- **Elucidation of physiological functions of sleep**

a) Clarifying the effect of sleep on memory (Sakurai/Sakaguchi lab)
Brain-wide neuronal activity can be retrospectively visualized using immediate early gene (IEG) Arc/Arg3.1 compartment analysis of temporal activity by fluorescent in situ hybridization (catFISH) (Guzowski *et al.*, *Nat Neurosci*, 1999). The activity-regulated IEG Arc rapidly accumulates at sites of synaptic activity. We are contrasting expression profiles of Arc transcription during each sleep stage by examining neurons that exhibit one or two dense intra-nuclear foci (indicating neuronal activity within 5 minutes of tissue sampling), only cytoplasmic expression (indicating neuronal activity around 30 minutes before tissue sampling), or both intra-nuclear and cytoplasmic expression (indicating neuronal activity at both time points) (Figure 9).

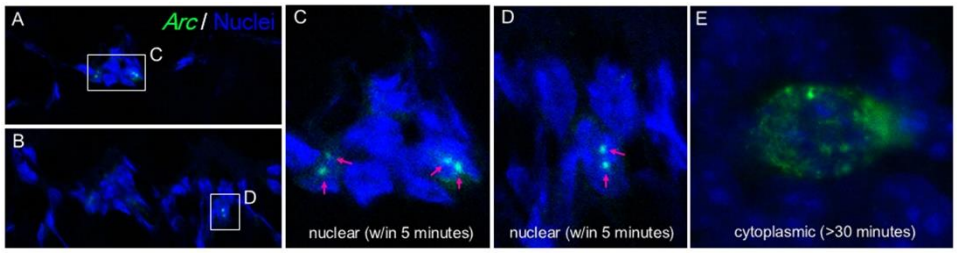

Figure 9. Brain-wide mapping of neuronal activity using catFISH. A,B. Example of Arc RNA signal in adult mouse neurons. C,D. Higher magnification of boxes in A,B showing nuclear Arc signal (allelic), indicating that neurons were activated within 5 minutes of tissue sampling. E. Arc RNA signal in the cytoplasm, indicating that the neuron was activated around 30 minutes before tissue sampling.

We established an experimental schedule that combines fear memory testing and electrophysiological recording during sleep and obtained preliminary behavioral data (Figure 10).

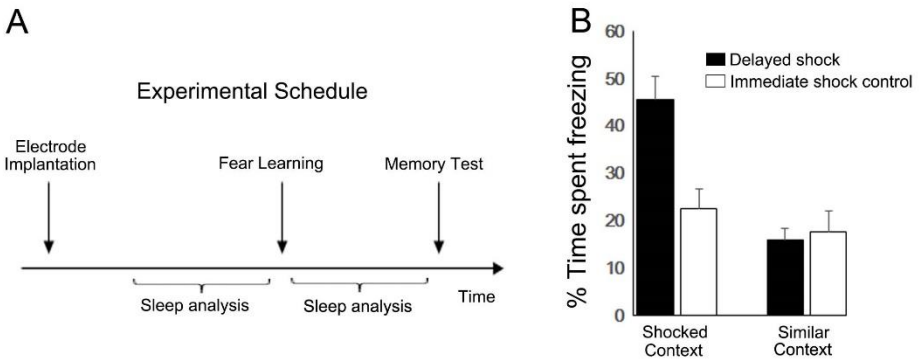

Figure 10. Experimental schedule to examine relationship between sleep and memory. A. EEG/ EMG electrodes were implanted into mouse brains to obtain basal sleep recordings. Sleep recordings were obtained again after fear learning. Interventions (e.g., optogenetic manipulation of target memory circuit) took place during this time period. The next day, a memory test was administered. Because adult-born neurons function in context discrimination, mice were tested not only in the context where learning occurred but also a similar context to test the specificity of context memory. B. Mice that received delayed shock showed context-specific fear memory. By contrast, control mice that received immediate shock did not express fear memory. These mice underwent the same procedure except that, during the learning trial, they were shocked immediately after entering the context, thus preventing an association between the context and the shock. *n* = 8 mice per group.

To examine adult-born neuron activity during sleep, we visualized neurons in the brain using the nestin-promoter-CreERT2 mouse, in which Cre recombinase expression is driven by the nestin promoter and Cre recombinase activity is controlled by tamoxifen (Carvalho & Sakaguchi *et al.*, *J Neurosci*, 2011). These mice are crossed with CAG-flox-stop-flox-eNpH3.0YFP mice so that halorhodopsin and yellow fluorescent

protein (YFP) are selectively expressed (i.e., tagged) in adult-born neurons (Figure 11). This enables us to both examine and manipulate adult-born neuron activity by combining catFISH and optogenetic techniques.

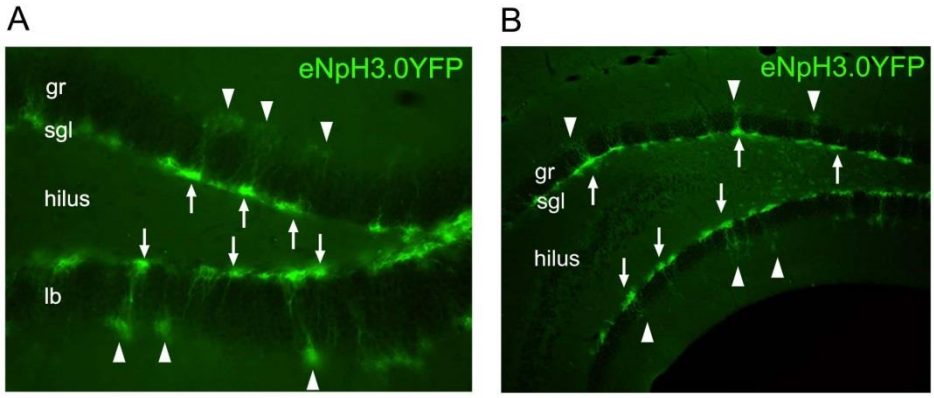

Figure 11. Selective expression of halorhodopsin in adult-born neurons.
Halorhodopsin/YFP fusion protein was selectively expressed in adult-born neurons in the anterior (A) and posterior (B) part of the dentate gyrus. As adult neurogenesis in the hippocampus is confined to the subgranular layer (sgl), the soma of adult-born neurons (arrows) are mainly located in this cell layer, with dendrites extending into the molecular layer (arrowheads) and penetrating the granular layer (gr). lb, lower blade.

To manipulate target neural circuits during specific stages of sleep, it is essential to integrate real-time monitoring of sleep stage with the generation of light pulses during the desired time period. This is achieved by developing a system, in collaboration with Biotex Inc., which is versatile in accepting any light source with pulse modulation control. An experimenter can monitor EEG/EMG activity and video images and push a button to trigger the pulse generator and light source to send a light pulse through optic fiber cables and cannulas to the target brain region.

Patients with post-traumatic stress disorder often generalize between traumatic memory and non-traumatic memory, contributing to a deterioration of quality of life. We found that there may be a time-dependent vulnerability for generalizing between memory for the context where trauma occurred and a similar non-traumatic context. This discovery is being pursued in collaboration with Dr. Szu-Han Wang (Univ. Edinburgh, UK), and its dependence on sleep will be examined in the future.

[Future directions]

As these projects and achievements are interrelated, they could be accelerated by increasing the number of personnel in the lab. Our results will be widely disseminated through domestic and international conferences and journals.

b) Elucidation of the physiologic roles of sleep, with emphasis on REM sleep (Hayashi lab)

Rats that were sleep-deprived exhibit drastic symptoms such as excess body weight loss and severe skin lesions, and invariably die within a couple of weeks (Rechtschaffen *et al.*, *Sleep*, 1989; Rechtschaffen and Bergmann, *Sleep*, 2002). It is yet unclear, however, why sleep is so essential. While recent studies suggest sleep involvement in memory consolidation, growth hormone secretion, or brain metabolite clearance, all these functions are attributed to non-REM sleep (Takahashi *et al.*, *J Clin Inv*, 1968; Rasch *et al.*, *Science*, 2007; Xie *et al.*, *Science*, 2013). Currently, the function of REM sleep is almost totally unknown.

Based on our identification of REM sleep-regulating neurons, we established transgenic mice in which REM sleep can either be increased or shut down at a desired time point (Hayashi *et al.*, unpublished). Such systems provide an excellent opportunity to address the function of REM sleep.

[Future directions]

In the future, we will conduct *in vivo* imaging of spines and axons under REM sleep inhibition or enhancement to directly observe what is happening in the brain during sleep and address the contribution of REM sleep.

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3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases

- **Elucidation of sleep/wake regulation in the brain and in associated peripheral organs**

a) Sleep/wakefulness behavioral analyses in mitochondrial disease model mice (J. Hayashi/Tanaka Lab)

Examinations of sleep/wakefulness behaviors were conducted in mice (Mito-MiceND6^{M}) possessing point mutations in mitochondrial gene ND6 (NADH dehydrogenase subunit 6). The production of active oxygen is increased in the mice due to the mutations, resulting in reduction of glucose tolerance and tumorigenesis upon aging.

We examined the sleep/wakefulness behaviors, 1) in basic and stationary states, 2) after 4-hour of sleep deprivation and 3) during 24-hour food deprivation and re-nourishment. No significant difference has been detected in the fundamental EEG between young Mito-MiceND6^{M} and wildtype mice.

[Future directions]

It was suggested that Mito-MiceND6^{M} shows the disorder of carbohydrate metabolism

upon aging (Hashizume *et al.*, *PNAS*, 2012). We therefore keep evaluating the changes in sleep/wakefulness behaviors accompanied by aging in every half year, as long as EEG measurement is applicable.

b) Analyses of the effects of lipid metabolism disorder on sleep/wakefulness behavior (Shimano Lab)

We continue to investigate behavioral changes in sleep/wakefulness in the knockout mice lacking Elovl6 (elongation of long chain fatty acid member 6).

The orexin gene is strongly expressed only in a group of nerve cells in the hypothalamic area, but the mechanism of limited transcription regulation is still unknown. The identification of transcription factors regulating orexin gene expression is in progress, using TFEL (transcription factor expression library) established by Assistant Professor Naoya Yahagi.

[Future direction]

We continue to investigate on sleep/wakefulness behavior in the model mice showing abnormal lipid metabolism. Also, the identification of transcription factors using TFEL will be expanded to the genes other than orexin.

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3) Elucidation of the molecular pathogenesis of sleep disorders and associated diseases

- **Elucidation of intracellular events and molecular association of sleep/wake behavior in the body**

a) Elucidation of the molecular mechanisms of excessive sleep (Urade Lab)

Epilepsy is a neurological disorder with the occurrence of seizures, which are often accompanied by sleep. Prostaglandin (PG) D2 is produced by hematopoietic or lipocalin-type PGD synthase (H- or L-PGDS) and involved in the regulation of physiological sleep. Here, we show that H-PGDS, L/H-PGDS or DP1 receptor (DP1R) KO mice exhibited more intense pentylenetetrazole (PTZ)-induced seizures in terms of latency of seizure onset, duration of

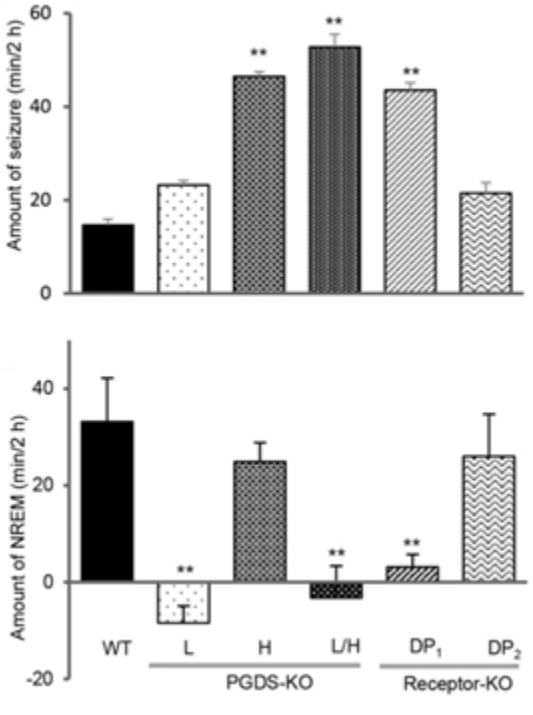

Figure 12

generalized tonic–clonic seizures, and number of seizure spikes. Seizures significantly increased the PGD2 content of the brain in wild-type mice. This PTZ-induced increase in PGD2 was attenuated in the brains of L- or H-PGDS KO and abolished in L/H-PGDS KO mice. Postictal non-rapid eye movement sleep was observed in the wild-type and H-PGDS or DP2R KO, but not in the L-, L/H-PGDS or DP1R KO, mice. These findings demonstrate that PGD2 produced by H-PGDS and acting on DP1R is essential for seizure suppression and that the L-PGDS/PGD2/DP1R system regulates sleep that follows seizures (Figure 12).

[Future directions]

In central nervous systems, H-PGDS is expressed in microglia, L-PGDS is highly expressed in the subarachnoid membrane, choroid plexus and oligodendroglia and DP1 receptor is localized in the subarachnoid membrane, a part of glia limitans and activated astrocyte. We constructed floxed-HPGDS mice, floxed-L-PGDS mice and also floxed-DP1 mice. Using these floxed-mice, we identify the cells involved in anti-epilepsy and induction of excessive sleep after seizures. We examine the excretion and fate pathway of PGD2 produced after PTZ-treatment.

b) Construction of Clinical Biological Resources (Shimizu Lab, Akita University)

The role of Akita University, as a satellite institution, is to function as a hub to bridge results obtained by IIIS in animal model to medical practice in human. Although one cannot expect what kind of findings will be achieved from IIIS, we, members of the Division of Bioregulatory Medicine, Department of Neuropsychiatry, Doctoral Course in Medicine, Graduate School of Medicine and Faculty of Medicine, Akita University, will flexibly and efficiently collaborate with IIIS with our experienced clinical researchers.

We hereby describe the achievements we have made to accomplish the future collaborations with IIIS in FY2013. We have initiated the construction of a biological resource database to translate the results obtained in IIIS into human studies.

(i) Our expanding biological resources cover human spinal fluid, blood and DNA samples along with clinical data, obtained from sleep disorder patients such as hypersomnia (narcolepsy).

(ii) Those also include samples from other sleep disorder patients, such as idiopathic hypersomnia, recurrent hypersomnia (Kline Levin Syndrome), Perkinson's disease, myotonic dystrophy-accompanied hypersomnia, Niemann-Pick disease type C and others.

We provide those resources, as a part of collaborative investigations, by measuring orexin levels in the samples without compensation.

(iii) We will further expand measurement items (e.g. anti-NMDA receptor antibody levels) to contribute in translating results in IIIS into human clinical studies.

[Future directions]

Based on the knowledge obtained from experimental work to verify the results with samples in practical studies, we have already integrated samples from various patients with sleep diseases. It is also noteworthy that we succeed in preparing collecting samples from short sleepers. To achieve the "bench-to-bed" clinical studies, we will provide resources more rapidly and appropriately.

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4) Development of treatments for sleep disorders

- **Development of sleep disorder therapy drug-candidate**

a) Medicinal chemical studies on the orexin receptor antagonist (Nagase Lab)

Nagase group has discovered the novel candidates for orexin receptor agonist from >1,500 synthetic samples, which were originally designed based on the structure of hit compounds found at the University of Texas. Especially, YNT-185 showed the orexin-2 receptor selective agonistic activity (EC_{50}=28 nM, OX1R/OX2R=96.2) and high solubility in water (>1.3 M in saline). Moreover, the results from intracerebroventricular (ICV) injection of YNT-185 hydrochloride into the orexinergic neuron knocked out mouse indicated the significant extension of alertness, while the orexin receptor double knocked out mouse exhibited no difference of alertness under the same condition. Thus, we could demonstrate that YNT-185 conducted the extension of alertness through the orexin receptor activation pathway (Figure 13).

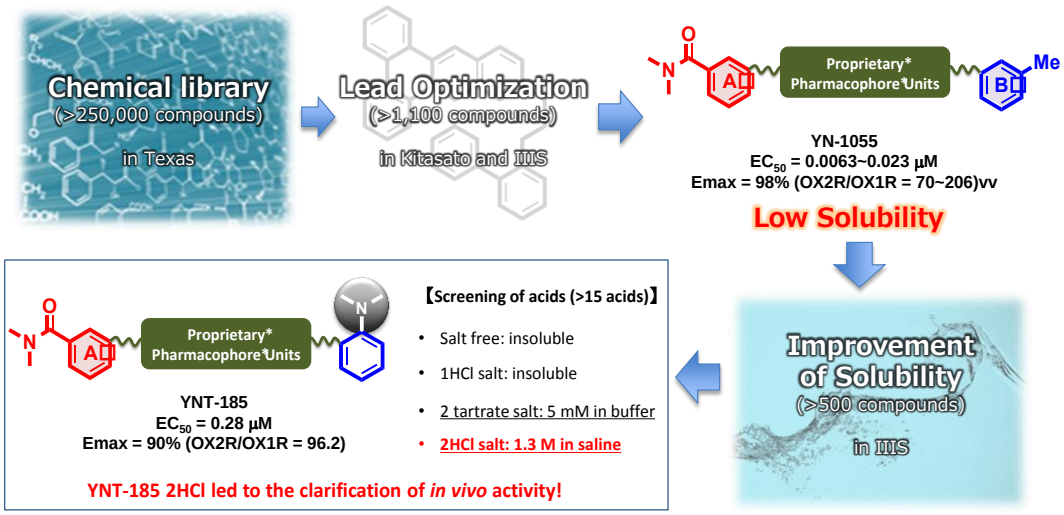

Figure 13

[Future directions]
One of our missions is to establish a basis for chemotherapy of "Narcolepsy" by means of orexin receptor agonist administration. The effect of YNT-185 for SOREMP (sleep onset REM period, which is a specific symptom) under ICV infusion and/or IP injection will be evaluated. In addition, we will also synthesize more potent compounds expected to be more easily penetrated to the blood-brain barrier.

b) Medicinal chemical studies on the adenosine receptor antagonist (Lazarus Lab)
The observation by the Lazarus Laboratory that the activation of A2ARs in the NAc by a specific A2AR agonist strongly induced NREM sleep in mice suggest that pharmacological stimulation of A2ARs may be a novel strategy for the treatment of insomnia and A2AR agonists or agonist-like compounds may constitute a new class of sleeping pills.
[Future directions]
Due to poor brain-permeability and side effects (e.g. hypotension), all currently existing A2AR agonists are however not suitable as safe and effective sleeping pills. For pharmacological A2AR activation in the brain, where adenosine levels can be greatly elevated during wakefulness, a freely penetrating allosteric enhancer is likely to effectively increase the sleep-inducing effect of endogenous adenosine. However, an allosteric enhancer for the A2ARs remains to be developed. The Lazarus Lab will start a new venture to develop a positive allosteric enhancer for A2ARs aiming to create a new class of sleeping pills. The initial goal is to establish a cell-culture bioassay for testing positive allosteric effects of a wide range of compounds on human A2ARs and subsequently, screen chemical libraries (e.g. University of Tokyo or UT Southwestern Chemical library) for suitable lead structures.

c) Studies on candidate compounds derived from natural products for the remedy of sleep disorders (Urade Lab)
Crocetin and crocin (crocetin-di-(β-D-digentiobiosyl)-ester are carotenoid pigment of saffron. Crocin is a major natural form of saffron and a number of pharmacological studies have demonstrated that crocetin and also crocin have a wide range of activities. We examined the sleep-promoting activity of crocetin and crocin by monitoring the locomotor activity and electroencephalogram after administration of these components to mice. Crocin increased the total time of non-rapid eye movement (non-REM) sleep during a 4-hr period from 20:00 to 24:00 after its intraperitoneal administration at a lights-off time of 20:00. Crocin did not change the amount of REM sleep or show any

adverse effects, such as rebound insomnia, after the induction of sleep. We examine the molecular mechanism of crocin-induced sleep by using various gene-manipulated mice, e.g. knock-out (KO) mice of receptors for adenosine, histamine, or dopamine, and found that crocin-induced sleep was markedly reduced in histamine H1 receptor KO mice. These results suggest that crocin may modulate the histaminergic or cholinergic arousal system to induce non-REM sleep.

[Future directions]

We will examine the effects of crocin on the synaptic release of histamine or acetylcholine. Carotenoid pigments are generally an unstable substance. We transglycosylate the crocin to modify the stabilities and ADME and examine the effects on sleep.

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4) Development of treatments for sleep disorders

- **Development of multi-faceted "Good Sleep" program that does not use drugs for the prevention of sleep disorders**

No major progresses were made in FY2013. A grant application to seek supports for the development of a system for sleep diagnosis, which is comprised of a simple EEG device, a smart phone and the cloud computing, was submitted at the end of March 2014.