# FY 2014 WPI Project Progress Report
# World Premier International Research Center Initiative (WPI)

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| 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 2014 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**
   Upon his retirement from the Howard Hughes Medical Institute at the end of March 2014, the Center Director was employed at the University of Tsukuba from April 1st under a joint appointment with the University of Texas Southwestern Medical Center (UTSW). A foundation has been established whereby the Center Director can concentrate almost entirely on the research and management of IIIS, maintaining a time commitment ratio at the University of Tsukuba and UTSW of 95 to 5, respectively.
2. **Recruitment of Principal Investigators (PIs) and Researchers**
   Yang Dan was appointed as an overseas satellite principal investigator (PI), making her the second female PI alongside Carla Green. In order to develop the achievements of basic biological research to medical applications, we are working to enhance translational research at IIIS. On this front, we have made the preparations to appoint a clinical researcher, Makoto Satoh, as a new PI. As a result, IIIS hosts a total of 116 members, with 20 PI, 26 researchers (not including PI), 13 technicians, 16 administrative staff members and 41 undergrad and graduate students.
3. **Research Objectives and Research System**
   The research objectives that we set out to achieve from the start of the project and are adhering to 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. We have clarified our roadmap and research deliverables to achieve our objectives by showing how three research fields, i.e., basic biology, clinical medicine and pharmaceutical science, are fused to establish integrative sleep medicine.
4. **Research Results**
   In 2014, IIIS published 126 original papers, a roughly 70 percent increase over the previous year. The results were reported in international journals with high impact factors, including papers in *Cell Metab*, *PNAS*, *Cell*, *Neuron*, etc. In addition, 4 PIs received awards for their research achievements.
5. **Securing Competitive Research Funding**
   The total amount of external funds acquired by researchers of the IIIS core group in the 2013 fiscal year was 196,060,000 JPY.
6. **Collaborative Institutions**
   For collaboration with UTSW, a cooperative framework has been well established to promote a research alliance. Qinghua Liu and Robert Greene have built laboratories within the core group of IIIS, while Joseph Takahashi and Carla Green engage in collaborative research with IIIS from the overseas satellites. Furthermore, we have plans for personnel exchanges with Takahashi (UTSW) and Dan (UC Berkeley). Research collaborations with the RIKEN BioResource Center and Niigata University were completed, having achieved the planned results.
7. **Research Support**
   An internal grant system was introduced as start-up support intended for researchers that failed to acquire competitive research funding such as Grants-in-Aid for Scientific Research, etc. Researchers within IIIS were invited to submit research proposals. To ensure the neutrality of the review process, three faculty members in the administration served as reviewers to examine the proposals for prioritization.
8. **Outreach / Research Meetings**
   For the 3rd annual international symposium, we collaborated with Hiroki Ueda, RIKEN Center for Developmental Biology / University of Tokyo and Joseph Bass, Northwestern University, to hold a joint symposium with a theme encompassing sleep, the biological clock, and appetite/obesity. Under the title "Homeodynamics in Clocks, Sleep and Metabolism", the joint symposium was held on September 24, 2014 at Ito Hall, Ito International Research Center, University of Tokyo. The event was a success, with 230 participants in attendance.
   Researchers in the field of sleep and neuroscience from overseas and inside Japan were invited as lecturers for the IIIS Seminar Series, which was held 29 times in FY 2014.
9. **General / Environmental Improvements**
   Construction of the new building (6-stories with 8,000 square meters of floor space), started in February 2014 has been managed as two construction zones according to funds, i.e., MEXT subsidy and university self-financing. The subsidy construction zone was finished as of March 31, 2015, while the self-financing zone will be completed at the end of June.

1. Summary of center project

| <Plan at start of project> | <Results/progress/alternations from 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. | No changes to the plan center project. |

## 2. Research fields

| <Plan at start of project > | <Results/progress/alternations from plan at start of project > |
|---|---|
| Sleep medicine<br>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.<br>As described below we are moving forward steadily with our research activities.<br><br>Principal investigators (PIs) with expertise across different disciplines, 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.<br><br>For fundamental research, we have obtained multiple pedigrees with abnormal phenotypes in sleep/wake behaviors through molecular genetics in mice (forward genetics). We were successful in identifying the "*Dreamless*" mutation showing abnormal REM sleep and the "*Sleepy*" mutation characterized with a remarkable reduction of waking hours. We expect the discovery of additional regulatory genes playing a vital role in the sleep/wake regulation by proceeding with further analysis of the other pedigrees obtained so far.<br><br>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 studies toward the elucidation of the neuronal 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 an artificial regulation.<br><br>Further, we have discovered that the nucleus accumbens of the basal ganglia is a crucial 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 stratum 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.

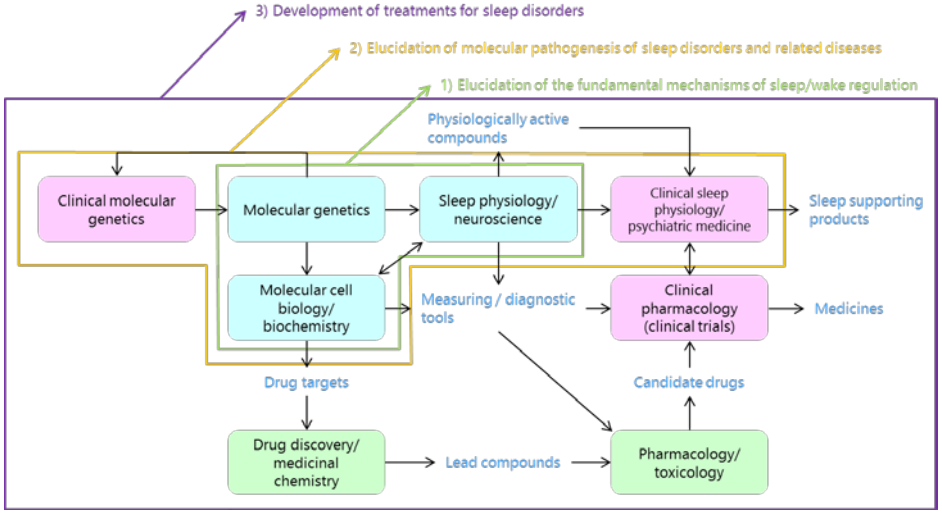
2.) Elucidation of molecular pathogenesis of sleep disorders
[Research objectives to be accomplished by the end of the grant period]

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

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. Roadmap to achieve the research objectives**

The roadmap to achieve the objectives is depicted by illustrating the research framework built with IIIS and collaborators, and showing interactive flows of cross-discipline and cross-research field studies as below.




At IIIS, three research fields (basic biology, clinical medicine and pharmaceutical science) are fused to establish integrative sleep medicine. The field of basic biology aims at objective 1 above (elucidation of the fundamental mechanisms of sleep/wake regulation). For objective 2, close collaboration with disciplines of clinical molecular genetics, clinical sleep physiology and psychiatric medicine, along with basic biology, will be needed. We will have to strengthen the translational research (TR) including pharmaceutical science and clinical pharmacology to achieve objective 3. Towards the development of treatments for sleep disorders, we will have to

- 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.

achieve the deliverables written in blue in the scheme above. Some preliminary results have already been obtained, and we have been establishing the framework of translational research.

## 2. Research discipline of each lab

Each lab of IIIS covers not only a single discipline but multiple ones as listed below.

| | | Liu | Tak | TSa/ MSa | Hay | Laz | RGr/ Vog | CGr | Dan | Ura | Yan/ Fun | Nag | Sat | Shi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Basic Biology | Molecular genetics | ✓ | ✓ | | | | | | | | ✓ | | | |
| | Biochemistry | ✓ | ✓ | | | | | | | ✓ | ✓ | | | |
| | Molecular cell biology | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | | | |
| | Sleep physiology | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | |
| | Neuroscience | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | |
| Pharma-ceutical Science | Pharmacology | | | | | | | | | ✓ | ✓ | ✓ | | |
| | Drug discovery | | | | | | | | | | ✓ | ✓ | | |
| | Medicinal chemistry | | | | | | | | | | | ✓ | | |
| Clinical Medicine | Clinical sleep physiology | | | | | | | | | | | | ✓ | ✓ |
| | Clinical pharmacology | | | | | | | | | | | | ✓ | ✓ |
| | Psychiatric medicine | | | | | | | | | | | | | ✓ |
| | Clinical molecular genetics | | | | | | | | | | | | | ✓ |

Liu: Qinghua Liu
Tak: Joseph S. Takahashi
TSa: Takeshi Sakurai
MSa: Masanori Sakaguchi
Hay: Yu Hayashi
Laz: Michael Lazarus
RGr: Robert W. Greene
Vog: Kaspar Vogt
CGr: Carla Green
Dan: Yang Dan
Ura: Yoshihiro Urade
Yan: Masashi Yanagisawa
Fun: Hiromasa Funato
Nag: Hiroshi Nagase
Sat: Makoto Satoh
Shi: Tetsuo Shimizu

Although some research institutes in the United States and Europe focus on sleep, almost all of those mainly conduct clinical studies. In contrast, IIIS is a unique institute that intensively focuses on fundamental research on sleep medicine. Thus many labs in IIIS conduct basic biological research. We plan to strengthen the research alliances with labs and/or institutes working on clinical medicine, along with the progress in basic research.

For the studies of pharmaceutical science, we also plan to collaborate with; a) pharmaceutical companies, b) governmental institutions such as Japan Agency for Medical Research and Development (AMED) or National Institute of Biomedical Innovation, c) medical institutions promoting sponsor-investigator clinical trials, such as Tsukuba Critical Path Research and Education Integrated Leading Center.

## 3. Goals for project implementation period

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

1) Elucidation of the fundamental mechanisms of sleep/wake regulation
   - Identification of new genes involved in sleep/wake regulation
   - Elucidation of the substances regulating sleep/wake
   - Elucidation of operating principles of the neural networks regulating sleep/wake
   - Elucidation of physiological functions of sleep
2) Elucidation of molecular pathogenesis of sleep disorders and related diseases
   - Elucidation of interactions between the brain and peripheral organs in the sleep/wake regulation
   - Elucidation of intracellular events and molecular association of sleep/wake behavior in the body
3) 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

Some collaborative studies across labs will be needed to achieve some of those goals. We thus started cross-discipline and cross-research field projects such as;

- Evaluation of the effect of exercises to sleep, and elucidation of underlying mechanisms (Yanagisawa/Funato, Sato, Shimizu)
- Development of orexin agonists (Yanagisawa/Funato, Nagase, Urade)
- Evaluation of orexin antagonists (Yanagisawa/Funato, Urade, Sato)
- Development of methods to visualize neural activities (Yanagisawa/Funato, Lazarus, Sakurai/Sakaguchi, Greene/Vogt, Hayashi)
- Development of methods to analyze and identify neural circuits (Yanagisawa/Funato, Lazarus, Sakurai/Sakaguchi, Greene/Vogt, Hayashi)

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| | **4. Research achievements/progress and future plan of each goal up to FY 2014**<br><br>Research achievements/progress and future plans are described in the Supplement (Scientific Reports). |

4. Management

| <Plan at start of project > | <Results/progress/alternations from plan at start of project > |
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| 1) Composition of administrative staff<br><br>*1. Composition of administrative staff*<br><br>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.<br><br>・General affairs section (5 staff members)<br><br>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.<br><br>・Accounting section (4 staff members)<br><br>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.<br><br>・Research fund section (3 staff members)<br><br>The research fund section will be in charge of a wide variety of tasks related | 1) Composition of administrative staff<br><br>*1. Composition of administrative staff*<br><br>The administration is under the leadership of the new Administrative Director, who has research management and research strategy expertise accumulated during his assignment to Senior Director of the research institute of a pharmaceutical company. Supporting the Administrative Director is the new Vice Administrative Director, who has served many years as a section head within the university headquarters. In this capacity, he is intimately familiar with the university administration operational procedures and campus affairs. The four teams within the administration are listed below:<br><br>• General Affairs Team (3 staff members)<br>This team carried out work related to general affairs, human resources, hiring, business trips, office time management, etc. Two full-time university personnel having a long and deep experience with general affairs at the university (including the Vice Administrative Director, holding this concurrent post as Team Leader) were assigned from the headquarters to IIIS. Along with hiring a new staff member with English proficiency to provide 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.<br><br>• Accounting Team (3 staff members)<br>This team carried out work relating to budget management and enforcement, supply purchases, transfer of funds and goods |

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| 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. | domestically and internationally, etc. One full-time university personnel having a long history of familiarity with university budgeting and accounting serves as Team Leader.<br><br>• Research Strategy & Management Team (3 staff members)<br>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 Ph.D. with experiences of drug discovery research as well as liaison office matters at the research division of a pharmaceutical company serves as Team Leader with expertise in contracts and patents.<br><br>• Alliance & Communication Team (3 staff members)<br>This team carried out work relating to public relations (news coverage, press releases and outreach activities), campus seminars, PI meetings, international symposia planning and management, report preparation, etc. A doctoral degree holder with research experience in the private sector as well as experience overseas serves as Team Leader. |
| *2. Use of English as the official language*<br><br>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. | *2. Use of English as the official language*<br><br>English is used as an official language at IIIS. A bilingual environment has been created, with sixty percent of administrative staff members fluent in spoken and written English (except for the people who have specific skills and/or expertise that cannot be replaced by any other people). English is used as the official language for PI, lab and other official meetings. In order to communicate frequently with overseas satellite members, a video conferencing/Skype system has been established in the meeting room. Documentation is 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*<br><br>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 | *3. Recruitment and development of quality staff members*<br><br>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 |

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| 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. | belonging to IIIS. This year a new staff member who is proficient in English was also hired to assign her to General Affairs Team so that they can smoothly communicate with foreign researchers. Many administrative staff members regularly attend the PI meetings and vigorously partake in the discussions using English. Some staff members also join lab meetings, i.e., meetings to report research progress, to receive updates on scientific knowledge and share information. These experiences largely improve the language ability of the staff members on a daily basis. |
| 2) Decision-making system | 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 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.<br><br>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.<br><br>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. | For important matters concerning the administrative management within the Center, all decision-making was done in accordance to the Center Director’s top-down approach. So that the intention of the Center Director takes effect quickly, organizational bylaws and other related regulations continue to be revised or enacted.<br><br>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 video conferencing 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.<br><br>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. |

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| 3) Allocation of authority between center director and host institution<br><br>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 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. | 3) Allocation of authority between center director and host institution<br><br>By positioning IIIS as a research institute that is an independent organization within the University of Tsukuba, we have been able to guarantee a wide range of independent operations, including human resources, environmental improvements, and budget execution. For human resource management at IIIS, we established a human resource committee with decision-making authorization capable of the speedy appointment of researchers.<br><br>Regarding the authority of the host institution to appoint or dismiss the Center Director, the University of Tsukuba President newly established a mixed salary system whereby the Center Director was formally hired by the University of Tsukuba under a joint appointment with the University of Texas Southwestern Medical Center from April 1, 2014. The effort ratio of the Center Director at the University of Tsukuba and the University of Texas, Southwestern Medical Center is 95:5, respectively. The share of intellectual property rights ownership from inventions of the Center Director is inherited to both universities in accordance with the previously specified rate. |

5. Researchers and center staffs

i) “Core” to be established within host institution

Principal investigators

| | At beginning | Final goal | Results at end of FY 2014 | Results at end of April 2015 |
|---|---|---|---|---|
| Researchers from within host institution | 7 | 7 | 7 | 8 |
| Foreign researchers invited from abroad | 0 | 4 | 7 | 7 |
| Researchers invited from other Japanese institutions | 0 | 4 | 6 | 6 |
| Total principal investigators | 7 | 15 | 20 | 21 |

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 staffs in the ( ) brackets.

| | At beginning | Final goal | Results at end of FY 2014 | Results at end of April 2015 |
|---|---|---|---|---|
| Researchers | 41<br><1, 2%> | 115<br><35, 30%> | 46<br><15, 33%><br>[13, 28%] | 48<br><16, 33%><br>[14, 29%] |
| Principal investigators | 7<br><1, 14%> | 15<br><5, 33%> | 20<br><8, 40%><br>[2, 10%] | 21<br><8, 38%><br>[2, 10%] |
| Other researchers | 34<br><0, 0%> | 100<br><30, 30%> | 26<br><7, 27%><br>[11, 42%] | 27<br><8, 30%><br>[12, 44%] |
| Research support staffs | 17 | 40 | 13 | 12 |
| Administrative staffs | 14 | 14 | 16<br>(10, 63%) | 16<br>(10, 63%) |
| Total | 72 | 169 | 75 | 76 |

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| | As a new initiative for enhancement of the research field of clinical medicine, we engaged in negotiations with Makoto Satoh, an experienced clinician of sleep apnea syndrome, to become a Principal Investigator at IIIS. He was invited for the position through the collaboration program with the Ibaraki Prefectural Medical Center of Psychiatry beginning April 1, 2015. While there was a plan to invite Takeshi Sakurai from Kanazawa University to work together in collaboration with Masanori Sakaguchi by establishing Sakurai-Sakaguchi Laboratory, it was difficult for Takeshi Sakurai to move from Kanazawa to Tsukuba for personal reasons, we thus explored an alternative, i.e., joint appointment with Kanazawa University, but the mixed salary system has not yet been introduced in Kanazawa University, delaying the transfer. Negotiations are ongoing for a new transfer time table. |
| ii) Satellites<br><Plan at start of project ><br>Institution (1) University of Texas Southwestern Medical Center (UTSW)<br>- Role<br>Joint research on the relationship between sleep/wake regulation and circadian rhythm, and the ENU Project (molecular genetic research)<br><br>- Personnel composition and structure<br>Carla Green, Joseph Takahashi,<br><br>- Collaborative framework<br>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. | ii) Satellites<br><Results/progress/alternations from plan at start of project><br>Institution (1) The University of Texas Southwestern Medical Center (UTSW)<br>- Role<br>ENU Project (molecular genetic research) and joint research on the relationship between sleep/wake regulation and circadian rhythm<br><br>- Personnel composition and structure<br>Qinghua Liu, Robert Greene, Carla Green, Joseph Takahashi<br><br>- Collaborative framework<br>A satellite 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.<br><br>Qinghua Liu was invited from UTSW as a visiting professor in April 2013 and his laboratory was established on campus at IIIS. We entered into a collaboration and contract research agreement in December 2013. Based on this agreement, the contract research at the laboratory in UTSW for FY2014 was also extended, allowing the continued employment of a postdoctoral researcher. Qinghua Liu was officially appointed under the mixed salary system as a full professor at the University of Tsukuba from April 1, 2014.<br><br>On the other hand, while Robert Greene was also invited as a visiting |

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| | professor at the same time as Qinghua Liu, the installation of his lab was delayed slightly until February 2014. Currently, negotiations are being carried out for Greene's collaborative research and contract research agreement, aiming to go into effect from the beginning of FY 2015.<br><br>Takahashi and Yanagisawa have continued their research collaboration, co-authoring a paper that was published in Neuron in FY 2014. With an aim to further strengthen cooperation, we are applying to the MEXT initiative "The Strategic Young Researcher Overseas Visit Program for Accelerating Brain Circulation" with him and are moving forward with personnel exchanges.<br><br>We expect to improve the visibility of IIIS with the presence of Joseph Takahashi, Carla Green, Robert Greene and Qinghua Liu at the satellite in UTSW. |
| Institution (2) Akita University<br>- Role<br>Joint research in translational research<br><br>- Personnel composition and structure<br>Tetsuo Shimizu, Takashi Kanbayashi<br><br>- Collaborative framework<br>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. | Institution (2) Akita University<br>- Role<br>Collaboration in translational research<br><br>- Personnel composition and structure<br>Tetsuo Shimizu, Takashi Kanbayashi<br><br>- Collaborative framework<br>From the previous fiscal year, a contract research agreement was concluded with Tetsuo Shimizu, clinician/professor at the Department of Neuropsychiatry, Akita University Graduate School of Medicine, and collaborative research is ongoing. Forming bio-resources comprised of the clinical information and samples taken from patients suffering from sleep disorders, particularly focused on narcolepsy, we aim to search for diagnostic markers as well as abnormal sleep pedigrees for the purpose of molecular genetic research. In the future, we plan to elucidate the mechanism of the effect of exercise on sleep and conduct joint clinical research on orexin receptor agonists. |

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| | Institution (3) University of California, Berkeley<br>- Role<br>Collaboration in the study of neural circuits for sleep/wake control<br><br>- Personnel composition and structure<br>Yang Dan<br><br>- Collaborative framework<br>In the wake of her lecture at our FY 2013 international symposium, Yang Dan agreed to research collaboration, and in FY 2014 she became a satellite PI at IIIS. Currently, she is advancing neural circuit network investigations by developing techniques to exert power with optrodes. To further strengthen cooperation in FY 2015, we are applying for the "The Strategic Young Researcher Overseas Visit Program for Accelerating Brain Circulation" with her and moving forward with personnel exchanges. |
| iii) Partner institutions<br>< Plan at start of project ><br>Institution (1) RIKEN BioResource Center, Tsukuba<br>- Role<br>Joint research in ENU screening<br><br>- Personnel composition and structure<br>Shigeharu Wakana<br><br>- Collaborative framework<br>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. | iii) Partner institutions<br><Results/progress/alternations from plan at start of project><br>Institution (1) RIKEN BioResource Center, Tsukuba<br>- Role<br>Collaboration for ENU screening<br><br>- Personnel composition and structure<br>Shigeharu Wakana<br><br>- Collaborative framework<br>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, both sides agreed to end the contract at the end of FY 2013. Further collaborative research focusing on the "Gene Mapping Analysis of Mutant Mice Obtained by ENU Screen" commenced from FY 2014. The relationship will be maintained in the future, with plans for cooperation centered on research progress. |

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| Institution (2) | Institution (2) Niigata University<br>- Role<br>Collaboration for the development of genetically modified mice<br><br>- Personnel composition and structure<br>Kenji Sakimura, Manabu Abe<br><br>- Collaborative framework<br>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. There was success in the production of the 5 lines of genetically modified mice and research goals were being sufficiently achieved. On the other hand, the joint research with our Collaborative PI, Satoru Takahashi, allowed us to use the CRISPR/Cas method to promptly create the necessary mutant mice and therefore the collaboration with Niigata University was not extended further. |

6. Summary of center's research environment

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| < Plan at start of project ><br>1) Environment in which researchers can devote themselves to their research<br><br>1. Support by administrative division<br><br>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, | <Results/progress/alternations from plan at start of project><br>1) Environment in which researchers can devote themselves to their research<br><br>1. Support by administrative division<br><br>Continuing from the previous fiscal year, the administration is under the leadership of the new Administrative Director, who has significant experience of research management and expertise in research strategies from his assignment to Senior Director of the research institute of a pharmaceutical company. Supporting the Administrative Director is the Vice Administrative Director and four teams (General Affairs, Accounting, Research Strategy & Management, Alliance & Communication). The General Affairs Team is led concurrently by the Vice Administrative Director, who has a long experience serving as a section head at the university headquarters. He is involved in negotiations with the Division of Human Resources Development and the Division of General Affairs to coordinate a wide variety of necessary problem-solving at the central administration. The Research Strategy & Management Team is led by a |

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| 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. | faculty member (associate professor) who is familiar with research content, as well as contracts and patents. He is responsible for collaboration research agreements and patent applications, and facilitated their quick processing in cooperation with law firms and patent offices. The Alliance & Communication Team is led by a faculty member (assistant professor) who is familiar with outreach. He actively takes charge of planning for international symposia and IIIS seminars, preparing press releases, responding to media requests and various other activities done smoothly without disrupting the researchers within the institute.<br><br>In addition, the administrative office also contributed to the management of research projects across labs, and led the initiation and negotiation of collaboration projects with profit organizations such as pharmaceutical companies, applications for large grants by forming teams comprised of a few labs, and internal grant reviews. |
| 2. Exempting on-campus researchers from non-research institutional duties, while providing support for their affiliated departments<br><br>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. | 2. Exempting on-campus researchers from non-research institutional duties, while providing support for their affiliated departments<br><br>Continuing from the previous fiscal year, an administrative staff member (secretary) is placed in each laboratory with many members, 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 discussion / decisions made at the PI meetings. During this fiscal year, the University of Tsukuba Research Administration Office arranged for one University Research Administrator (URA) to be placed in IIIS to support the external funding acquisition and subsequent project management assistance. The URA dispatched to IIIS left University of Tsukuba last February and we are currently seeking a replacement. |
| 3. Living support<br><br>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 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 | 3. Living support<br><br>For foreign researchers and their families, living support is provided on campus (Kasuga Plaza Support Center for International Researchers and Families). The support center engages in the following support services: provision of information and consultation to assist with daily living, |

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| 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. | 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 with document preparation. IIIS foreign researchers also receive support through these services.<br><br>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 support from the Japan International Science and Technology Exchange Center (JISTEC), having renewed the contract for foreign researcher support services. This fiscal year we hired a new staff member proficient in English to join the General Affairs Team, serving to strengthen the support for foreign researchers. |
| 2) Startup research funding<br><br>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<br><br>An internal grant system was introduced as start-up research funding. This system is mainly intended for researchers that were not able to acquire competitive research funding such as Grants-in-Aid for Scientific Research. Such researchers were invited to submit research plans for provision of research funding. In order to ensure the neutrality of the review process, three faculty members holding degrees in the fields of medicine and biology in the administration served as reviewers to examine the proposals and prioritize them. Also, we have provided active support for newly invited researchers and were able to successfully deliver results in securing Grants-in-Aid for Scientific Research (research activity start-up support). |
| 3) Postdoctoral positions through open international solicitations<br><br>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. University of Tsukuba's overseas offices; 7. Our overseas Satellite (public | 3) Postdoctoral positions through open international solicitations<br><br>Including the website of WPI-IIIS, along with job boards on sites such as Naturejobs, Federation of European Neuroscience, Sleep Research Society, JREC-IN, Japan Neuroscience Society, Molecular Biology Society of Japan, etc., we have been actively posting job advertisements internationally. In addition, with the support of the University of Tsukuba URA Office, an article showcasing IIIS was placed in the journal Science, |

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| release by the University of Texas Southwestern Medical Center), 8. Personal international networks of the Center Director and Principal Investigators.<br><br>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.<br><br>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.<br><br>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. | including advertisements for postdoctoral fellow and Junior PI positions.<br><br>The total number of applicants for the 2014 fiscal year was 116, with 97% of the applicants from foreign researchers. However, unable to meet the standards set by our institute, the majority of applicants were declined. Out of the group of applicants, one (from Canada) was successfully chosen and began work at IIIS. We employed one postdoctoral research fellow from our overseas satellite at the University of Texas Southwestern Medical Center, having arrived in June 2014. Outside of these channels, we will also engage proactively in recruiting on the occasions of international conferences, etc. We regularly invite speakers from outside for the IIIS Seminar series and make use of the opportunity to look for Junior PI candidates in particular. |
| 4) Administrative personnel who can facilitate the use of English in the work process<br><br>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.<br><br>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<br><br>The administrative services have been continually maintained with the ability to function in English.<br><br>Beginning with documents of experimental plans in various regulatory applications, each type of application form and documents for employment, personnel affairs, and general affairs having been translated into English and are all available in English. Other documents are also converted into English as 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 | 5) Rigorous system for evaluating research performance and system of merit-based compensation<br><br>Continuing for this fiscal year, we are proceeding with the appointment |

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| The President of the University will decide on the renewal and the salary of the Center Director.<br><br>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.<br><br>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.<br><br>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. | of the candidates for the external advisory board. However, due to budget constraints, we were not able to make selections of members and hold the board members meeting. As we regard the major role for the advisory board as scientific evaluation of research programs rather than performance of each researcher, we will continue to carefully investigate how the advisory board's evaluations should be reflected to a system of merit-based compensation.<br><br>The salaries for the administrative staff were determined by the Center Director, based on the opinion of the Administrative Director. Raises are being passed upon due to budget constraints.<br><br>Determinations on salary for researchers invited from outside of IIIS were made by the Center Director, based on their past achievements and salary situations. |
| 6) Equipment and facilities, including laboratory space, appropriate to a top world-level research center<br><br>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.<br><br>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 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. | 6) Equipment and facilities, including laboratory space, appropriate to a top world-level research center<br><br>Starting the construction of the Sleep Medicine Research Building (6-stories with 8,000 square meters of floor space) in February 2014, the steering committee, composed of all stakeholders, including the constructors, designers, construction supervisors, University of Tsukuba Department of Facilities, and IIIS management and secretariat, was organized and its monthly meetings were held regularly for project management, decision-making on important specifications and opinion exchange to facilitate problem-solving. Further meetings are in place with selected members for the purpose of decision-making on detailed specifications, coordination of construction works, and problem-solving. To date, 15 monthly meetings have been held, along with 50 weekly meetings. With the aim of uniting to create a better research environment, these meetings combine staff as well as the active participation of researchers.<br><br>The 5th and 6th floors of the new research building will be equipped with large autoclaves, a cage washer, automatic water supply system with RO water production apparatus, and animal breeding facilities, etc. Development of an appropriate research environment suitable for a |

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.

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

World Premier International Research Center is advancing the tangible aspect of our efforts. In addition, we are collaborating with the Faculty of Art and Design on campus to display works of art integrated into the interior of the new research building. This collaboration is aimed at creating an atmosphere that breathes inspiration and stimulates the intellectual curiosity of researchers. We will work closely with artists, designers, constructors, and manufacturers to advance the art project and plan for its installation to be completed by the opening ceremony on September 2015.

The construction has been managed as two construction zones according to funds, i.e., MEXT subsidy and university self-financing. The subsidy construction zone was finished as of March 31, 2015, while the self-financing zone will be completed at the end of June. After the completion inspection, the building is scheduled for occupancy on July 10th. Until the move into the new building is completed, we will continue to utilize the research laboratories in the Health and Medical Science Innovation Laboratory, Project Research Building, TARA Center and the University of Tsukuba Hospital E Building.

7) International research conferences or symposiums held regularly to bring world's leading researchers together

1. International Symposium
   For the 3rd annual international symposium, we collaborated with Hiroki Ueda, RIKEN Center for Developmental Biology / University of Tokyo and Joseph Bass, Northwestern University, to hold a joint

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.

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

symposium with a theme encompassing sleep, the biological clock, and appetite/obesity. Under the title "Homeodynamics in Clocks, Sleep and Metabolism", the joint symposium was held on September 24, 2014 at Ito Hall, Ito International Research Center, University of Tokyo. The event was a success, with 230 participants in attendance. It was proof that IIIS is recognized as a top runner in sleep studies.

In addition, following the symposium we held a retreat for invited speakers together with young researchers from Hiroki Ueda's lab at RIKEN / University of Tokyo as well as PIs and young researchers from IIIS. Although the retreat was a closed workshop with unpublished research results, it was considered an invaluable opportunity, especially for young researchers, where appeals could be made for deeper insight, with heated debate beginning from early morning until late at night.

2. Public Seminars

The IIIS Seminar series was held this fiscal year 29 times. Since its inception, IIIS has held a total of 56 seminars. A select number have been held as joint seminars with the Ph.D. Program in Human Biology and Ph.D. Program in Life Science Innovation of the School of Integrative and Global Majors (SIGMA), as well as the Ph.D. Program in Human High Performance, Graduate School of Comprehensive Human Sciences (Physical Education, Health and Sport Sciences). This series has seen the attendance of many students and played a major role in the expansion of our human network and recruiting drive for high quality talent.

8) Other measures, if any

In holding the 3rd annual international symposium, we were able to collect about 2.3 million JPY in sponsorship and donations together with Ueda Lab at RIKEN / University of Tokyo. We were also able to secure over 10 million JPY of donations in support of the new research building through the efforts of the Center Director and Administrative Director. These efforts are expected to continue into the next fiscal year.

In addition, we are preparing to accept a few trainees/interns in the next

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| 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. | fiscal year, with the aim of establishing a short-term stay training system. |

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

| < Plan at start of project > | <Current assessment> |
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| 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.<br><br>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.<br><br>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. | 1) Status of research paper publications<br><br>In the 2014 calendar year, 128 papers were published from IIIS. This is about a 70% increase from the previous year, which reflects the steady progression of our research output. Papers were published regularly in high impact journals such as from Wei et al. in *Cell Metab*. (19(6):927-40), and others in *PNAS*, *Neuron*, etc. With the acceleration of multi-disciplinary projects, the development of orexin agonists and neural activity visualization method, and other results from IIIS are expected to appear in papers soon.<br><br>2) Main awards<br><br>The numerous achievements of IIIS PIs were recognized with the following awards in fiscal year 2014:<br>Masashi Yanagisawa: The Walter B. Cannon Memorial Award, American Physiological Society<br>Hiroshi Nagase: Yamazaki-Teiichi Prize<br>Joseph Takahashi: Election to Institute of Medicine (IOM), National Academy of Sciences<br>Yu Hayashi: Young Teacher Prize for Encouragement, University of Tsukuba |

3) Rise in institutional awareness

In fiscal year 2014, we held the international symposium Tokyo Translational Therapeutics Meeting: "Homeodynamics in Clocks, Sleep and Metabolism". It was held as a joint symposium together in collaboration with Hiroki Ueda, RIKEN Center for Developmental Biology / University of Tokyo and Joseph Bass, Northwestern University, with a theme encompassing sleep, the biological clock, and appetite/obesity. IIIS plays a central role as a research institute leading the world in sleep research, with many researchers, including young members, delivering lectures and presentations.

In addition, orexin, the neuropeptide that controls sleep/wakefulness, discovered by Yanagisawa et al., has contributed greatly to the awareness of IIIS with the launch of the world's first drug targeting it in Japan. Yanagisawa was involved in part with the drug development as he made the discovery of orexin. Besides delivering a large number of talks during the release of the drug, the story was covered significantly in the domestic and overseas media.

4) Status of applicants for Junior PI and postdoctoral fellow positions

In the 2014 fiscal year, we received 27 applications for Junior PI and 120 for postdoctoral fellow positions. More than 90% of the applicants were from foreign researchers, marking a high appeal overseas as an international research center. In particular, we targeted female researchers for Junior PI positions, noting an increase over the number of female applicants from the previous year. Unfortunately, no applicants met the entry standards set by IIIS. Although we have not employed any candidates responding to the public advertisements, we will strive to continue to secure qualified candidates to increase the ratio of foreign researchers without compromising the level of standards that we have set.

5) Efforts to secure research funding

Aside from applications for ImPACT, PRESTO, etc., we are also in the process of applying with JAXA and other organizations for a Grant-in-Aid for Scientific Research in a new scientific area. For Grants-in-Aid for Scientific Research (Kakenhi), Yanagisawa tops the list with a Grant-in-

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| | Aid for Scientific Research (S), and 12 applications accepted. Including the continuing grants, the total comes out to 19, for an amount of 97,843,000 JPY. For Kakenhi applications in the 2015 fiscal year, all qualified individuals were encouraged to apply as with the previous year, for a total of 34 applications filed. |

8. Securing competitive research funding

| <Plan at start of project > | <Results/progress/alternations from plan at start of project> |
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| 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 core researchers in the 2014 fiscal year was 196,060,000 JPY. We aim to further acquire competitive funding from the 2015 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> |
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| Center Director Yanagisawa intends to retire from HHMI promptly after the application is funded. | Under the leadership of Yasuo Miake, Vice President (in charge of research), University of Tsukuba, we formed a task force at the university in November 2013 which includes a patent attorney and lawyer in order to solve the problems surrounding the assignment of intellectual property rights and exome sequencing analysis issues stemming from Center Director Yanagisawa's retirement from HHMI. In December of the same year, we reached a principle agreement for the handling of intellectual property rights after a three-party delegation representing the task force, including Vice President Miake, visited the University of Texas Southwestern Medical Center (UTSW) in person to conclude negotiations. In response to the agreement, Yanagisawa was able to retire from HHMI on March 31, 2014 and was employed at the University of Tsukuba from April 1, 2014. Yanagisawa was placed under a joint appointment from the University of Tsukuba and UTSW having an effort ratio of 95:5, respectively, by using a newly introduced mixed salary system at the University of Tsukuba. This joint appointment was necessary to allow continued use of the Next-Generation Sequencing Core facility at UTSW after retirement from HHMI. Through this agreement, the majority stake of intellectual property rights for Center Director Yanagisawa |

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| | will belong to the University of Tsukuba. With regard to the remaining issue of intellectual property rights arising from the sponsored research performed in UTSW, it was agreed that license revenue based on the rights should be distributed to both UTSW and the University of Tsukuba according to the contribution they made, regardless of the attribution of the rights. This compromise was included in the Research Collaboration Agreement executed as of April 1, 2014, as it would be difficult to assign ownership of the rights from the sponsored research to the sponsor, i.e., University of Tsukuba, even partially according to the Bayh-Dole Act. |

10. Host institution's commitment

| <Plan at start of project ><br>-Provision in host institution's mid-to-long-term plan | <Results/progress/alternations from plan at start of project><br>-Provision in host institution's mid-to-long-term plan |
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| 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."<br><br>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."<br><br>Therefore, the World Premier International Research Center Initiative (WPI) is in complete agreement with the University's Midterm Goals and Midterm Plan.<br><br>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 | Following from fiscal year 2013, we continue university-wide efforts, described on the left, aimed at forming a world premier international research center. |

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| resources, hiring of supporting personnel, and reforming of organizations, and will be aggressively promoted as international centers of research."<br><br>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.<br><br>The first of the Midterm Goals is to make this an "open university" in every 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.<br><br>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.<br><br>-Concrete Measures<br>(1) Competitive grants obtained by researchers participating in the project and in-kind contributions, etc.<br>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.<br><br>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 | - Concrete Measures<br>(1) Competitive grants obtained by researchers participating in the project and in-kind contributions, etc.<br><br>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.<br><br>1) The Organization for the Support and Development of Strategic Initiatives continued the support for IIIS and we received 35 million JPY in management expenses grants.<br>2) Applications for competitive funding were assisted by the Department of Research Promotion.<br>3) Aiming for an independent institute after the program ends; studies have |

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| expenses of center activities.<br><br>2) Competitive research funds obtained by researchers participating in the center<br>Competitive research funds obtained by researchers participating in the center amounted to an average of US$8.86 million in the past 5 years, and it is anticipated that an equivalent amount will be gained. Even after the completion in FY2014 of the FIRST program that founded this center, we anticipate that the center will have about 115 researchers who will gain at least the same level of funds as the previous year. The University will be providing full support for their applications for competitive external and internal research funds.<br><br>3) Support through handling of personnel expenses<br>The personnel expenses of University faculty participating in this center will be treated as University personnel expenses for researchers.<br>University staff (approximately 3 persons) will be assigned to carry out the chief administrative work of the center such as general affairs, accounting, and research funding. The expenses for this staff will be handled by considering them to be administrative staff participating in an administrative support division.<br><br>4) Support through supply of research space<br>The University will use its good offices to facilitate the building of research facilities (more than 5,000$m^2$) according to a plan that will give the center an outstanding research environment and distinctive tangible advantages.<br><br>5) Support for the use of research facilities<br>Various core facilities will be offered for use (see paragraph (5) below). | commenced at the university management level with the president agreeing to allow a number of PI tenured positions in the future.<br>4) Measures were taken to provide personnel costs for 3 administrative positions for university personnel engaging in the key areas of general affairs and accounting.<br>5) As the facilities were limited due to seismic work, etc., support was provided for the research spaces of the Health and Medical Science Innovation Laboratory, University of Tsukuba Hospital E Building, Project Research Building and TARA Center, providing 5,000 square meters divided among the listed venues. The research space currently divided among the 4 locations is less than 6,000 square meters; however, the new research building listed in section (5) below is scheduled for completion in the next fiscal year with a total floor area of 8,000 square meters.<br>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<br><br>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. | (2) System under which the center's director is able to make substantive personnel and budget allocation decisions<br><br>We established the HR Committee in IIIS to develop a system to appoint faculty members. The appointment system is different from the previous personnel system with less steps of the examination accelerated through intensive deliberations, i.e., 2 steps of the examination in total: HR |

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| 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 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. | Committee and Administration Center Appointment Committee. So rapid determination and appointment under the leadership of the Center Director is possible. So far, we have appointed 4 young researchers as Junior PI (1 assistant professor and 3 associate professors).<br><br>For budget execution, we are working to operate an efficient management system by reviewing the system of administrative organization under the leadership of the Administrative Director. |
| (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<br><br>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.<br><br>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.<br><br>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 | (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<br><br>Collaborative PIs invited from within the university campus are engaged in research collaboration varying in scope from PIs in the core group. Namely, each Collaborative PI and core group PI is basically engaged in joint research, however, consideration is given to the department affiliation of each Collaborative PI for his/her research activities. When the scale of collaborative research increases and further support is required for Collaborative PIs, we hire faculty members or researchers at our expense in the applicable laboratories to work on the collaboration projects with IIIS. |

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| created, so as to aid in the nurturing of outstanding new personnel. | |
| (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<br><br>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 and adjustments as necessary.<br><br>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.<br><br>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.<br><br>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. | (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<br><br>All official meetings (such as PI meetings) are held in English, and a system was established to use Skype videoconferencing with satellite institutions abroad regularly. Other meetings, such as lab-wide meetings to 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 setting, are also held in English. Active translation into English of all university system applications and general announcements is also taking place. |
| (5) Accommodation of center's requirements for infrastructural support (facilities, e.g., laboratory space; equipment; land, etc.)<br><br>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 | (5) Accommodation of center's requirements for infrastructural support (facilities, e.g., laboratory space; equipment; land, etc.)<br><br>In order to create an attractive space for our research groups, the university conditioned a 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. In addition, the research environment is continuously being improved. |

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| confidence to be a place for research of the highest level.<br><br>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 renovation of the E Building, the center will be provided with temporary floors on the Laboratory of Advanced Research D.<br><br>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.<br><br>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. | Construction of the new research building started in February 2014, aiming to initiate operations and hold an opening ceremony in September 2015. To ensure the building conforms to standards of a top level research facility, the steering committee (IIIS Liaison Committee for Building Construction) composed of constructors, designers, construction supervisors, University of Tsukuba Department of Facilities, and IIIS management and secretariat, was organized, and its monthly meetings were held regularly. Further, weekly meetings were in place with selected members for the purpose of decision-making on detailed specifications, coordination of construction works and problem-solving. Also, due to soaring costs for materials, personnel and construction influenced by the earthquake reconstruction and the Tokyo Olympics, etc., adjustments had to be made to the original plan with an increase in the target budget of around 6 billion JPY. Owing to the strong leadership of the university president and vice president in charge of financial affairs and facilities, resolution has been achieved in the form of financing secured from the Department of Finance and Accounting and appropriation to the repayment of indirect costs of competitive research funds. |
| (6) Support for other types of assistance<br><br>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, | (6) Support for other types of assistance<br><br>From May to November 2014, the University of Tsukuba URA Office dispatched one member to IIIS to serve as a university research administrator. The URA Office plans to provide additional human resource support from July 2015. |

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| 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. | |

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<br><br>Directive to demonstrate how IIIS research results will be linked to human clinical trials. | 1) Measure 1<br><br>We will accelerate collaborative research from 2015 onwards with a global pharmaceutical company with our drug discovery research that links the seed of orexin receptor agonists to the treatment of patients. In addition, our collaborative research with Akita University is ongoing with a purpose to conduct human molecular genetic analysis of short sleepers. We have already obtained preliminary results from a number of short sleepers, with confirmation taken at the level of the actigraph for short sleep periods, and are continuing to move forward with these studies. From fiscal year 2015 we welcome the clinician Makoto Satoh as a new PI through the collaboration program with the Ibaraki Prefectural Medical Center of Psychiatry, with the aim of conducting research to develop new diagnostics of human sleep. We are also advancing research in cooperation with our Collaborative PI from the University of Tsukuba Faculty of Health and Sports Sciences investigating the effects of bedding environment on sleep. Also, cooperating with the Japan Aerospace Exploration Agency (JAXA), we are applying for the grant to support translational research involving clinical studies. |
| 2) Item 2<br><br>Directive for research at IIIS to focus more on sleep. | 2) Measure 2<br><br>All the joint research being carried out by the 5 Collaborative PIs on campus is focused on sleep relevant studies. With Satoru Takahashi, we are continuing collaborative research producing model mice with sleep abnormalities by using CRISPR/Cas9 technology. Collaborative research investigating the effects of stress on sleep is pressing forward with Ichiyo Matsuzaki. Sleep and metabolism collaborative research is |

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| | being pursued together with Hitoshi Shimano. Akiyoshi Fukamizu studies influence of pregnancy on sleep. With the compulsory retirement of Junichi Hayashi, we have brought on a new Collaborative PI from the University of Tsukuba Faculty of Health and Sports Sciences, Kumpei Tokuyama, to start joint research on exercise and sleep.<br><br>Anticipating commercial viability of the lead compounds created in IIIS, 3 laboratories are cooperating to launch an orexin receptor agonist project, and synthetic chemists and pharmacological researchers have consistently shared information to build a system to perform drug discovery research. We will further accelerate research from fiscal year 2015 on this project, collaborating together with pharmaceutical companies. |
| 3) Item 3<br><br>Directive to employ female PI to core research group, increase the effort rate of Takeshi Sakurai and increase the ratio of foreign researchers. | 3) Measure 3<br><br>Regarding the hiring of female PI into the core research group, we have actively been recruiting with female only PI advertisements on a number of websites from October 2014 and 2 candidates were screened by means of a job seminar. At present, there have been no successful candidates; however, we will continue our recruitment efforts into the future. To improve the effort rate of Takeshi Sakurai, we plan to utilize a mixed salary system and are considering how to gradually increase his effort rate at the University of Tsukuba. As there is currently no mixed salary system at Kanazawa University, implementation will take place hopefully from fiscal year 2015. The ratio of foreign researchers at the end of fiscal year 2014 was 33%, surpassing the benchmark of 30% set by MEXT. |
| 4) Item 4<br><br>Directive for disclosure of gene names and improvement to allow for adequate evaluation. | 4) Measure 4<br><br>We would like to disclose as much information as possible. However, not only gene names, but certain information contains new patent-pending compounds from collaborations with industry and therefore it will be necessary to adopt a prudent measure at the time of the site visit for disclosure. We have consulted with Program Officer Kaibuchi and are considering to have confidentiality agreements signed by all parties during the site visit and as well as explicitly mark presentation |

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| | materials as confidential where applicable. |
| 5) Item 5<br><br>Directive to strengthen system to inspire young researchers to actively join overseas exchanges (business trips, dispatch, etc.). | 5) Measure 5<br><br>Looking at the fiscal year 2014 overseas business trip track record of our institute, of the 30 overall cases, 15, or half the total, were taken by young researchers. We are recommending more young researchers in the next fiscal year to actively take part in overseas exchanges. Concerning dispatch overseas, we have applied to "The Strategic Young Researcher Overseas Visit Program for Accelerating Brain Circulation" for fiscal year 2015, and a few of our young researchers have expressed interest in being posted to our overseas satellites at the University of California, Berkeley, and the University of Texas Southwestern Medical Center. |
| 6) Item 6<br><br>Necessity for early establishment of bioinformatics research core using next-generation sequencing and informatics. | 6) Measure 6<br><br>In order to transfer the functions of the UTSW Next Generation Sequencing Core to Japan, Yanagisawa visited Toyohashi University of Technology, a candidate institute for collaboration, and conducted a feasibility study to confirm the accuracy of the sequence data and the secondary analysis capability of data. Unfortunately, as Toyohashi University of Technology did not meet the requested benchmark level, the prospective collaboration was halted. To respond to the informatics of the sequence data analysis on campus, a bioinformatics researcher who obtained his Ph.D. from UTSW was hired and posted at IIIS from June 2014. In addition, we have proposed the construction of a next-generation sequencing core research organization and submitted a budget request to the University of Tsukuba for fiscal year 2015. |
| 7) Item 7<br><br>Necessity of new path for IIIS in creating a short-term workshop program for young researchers in order to raise the profile of the institute. | 7) Measure 7<br><br>The 3rd Annual IIIS Symposium held in September 2014 presented the first step towards implementation of the short-stay workshop program for young researchers. During the symposium, we conducted a questionnaire to gauge interest and demand for a proposed workshop. 80% of the foreign researchers and students who responded to the questionnaire stated an interest in such a workshop. When asked about desired technical training, the most cited response was for analysis of neural circuits using optogenetics and pharmacogenetic probes, |

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| | followed by EMG and EEG measurements, and multi-electrode measurements used in vivo. It was discovered that many young researchers would like to go on a short-term program and receive technical training. Based on these results, we are considering the implementation of a short-stay workshop program. |

12. FY 2014 funding

i) Overall project funding

(Unit: 1 million yen)

| Cost Items | Details | Costs (1 million yen) |
|---|---|---|
| Personnel | Center director and Administrative director | 39 |
| | Principal investigators (11 persons) | 64 |
| | Other researchers (30 persons) | 191 |
| | Research support staffs (10 persons) | 40 |
| | Administrative staffs (14 persons) | 69 |
| | Total | 403 |
| Project activities | Gratuities and honoraria paid to invited principal investigators: | 0 |
| | Cost of dispatching scientists (2 persons) | 9 |
| | Research startup cost (11 persons) | 36 |
| | Cost of satellite organizations (2 satellite organizations) | 18 |
| | Cost of international symposiums | 3 |
| | Rental fees for facilities | 12 |
| | Cost of consumables | 12 |
| | Cost of utilities | 9 |
| | Other costs | 6 |
| | Total | 105 |
| Travel | Domestic travel costs | 1 |
| | Overseas travel costs | 8 |
| | Travel and accommodations cost for invited scientists (3 overseas scientists) | 2 |
| | Travel cost for scientists on secondment | 0 |
| | Total | 11 |
| Equipment | Depreciation of buildings | 0 |
| | Depreciation of equipment | 229 |
| | Total | 229 |
| Other research projects | Projects supported by other government subsidies, etc. | 0 |
| | Commissioned research projects, etc. | 64 |
| | Grants-in-Aid for Scientific Research, etc. | 89 |
| | Total | 153 |
| Total | | 901 |

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| WPI grant for FY 2014 | | 462 |
| Costs of establishing and maintaining facilities in FY 2014 | | 860 |
| Establishing new facilities (8,000m²) | Costs paid: | 860 |
| Cost of equipment procured in FY 2014 | | 155 |
| Recycling preparativeHPLC system 1 set | | 6 |
| Data projector 1 set | | 1 |
| Automatic rack washer system for laboratory animal use 1 set | | 47 |
| Steam Sterilizer System for Animal Facility 1 set | | 97 |
| Others | | 4 |

ii) Costs of Satellites and Partner institutions

| Cost Items | Details | Costs (1 million yen) |
|---|---|---|
| Personnel | Other researchers (1 person): | |
| | | |
| | | |
| | | |
| | Total | 13 |
| Project activities | | 2 |
| Travel | | 1 |
| Equipment | | 2 |
| Other research projects | | |
| Total | | 18 |

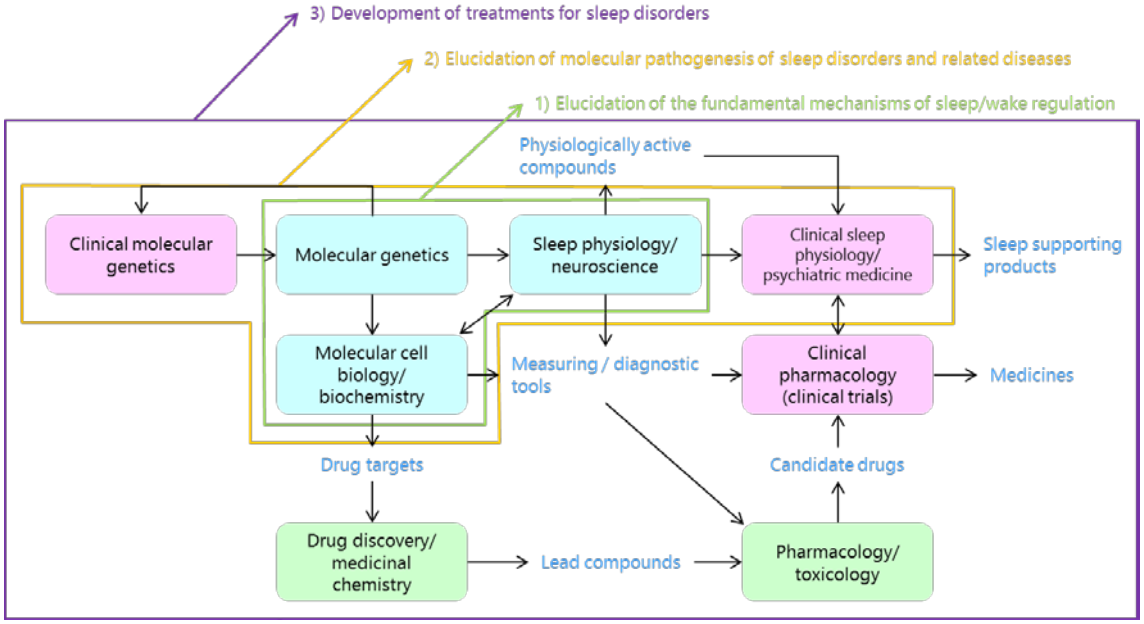
# Research objectives, progress in FY2014 and future perspectives

## 1. Research objectives

IIIS has set three research objectives as follows;

1) Elucidation of the fundamental mechanisms of sleep/wake regulation
2) Elucidation of molecular pathogenesis of sleep disorders and related diseases
3) Development of treatments for sleep disorders

The roadmap to achieve the objectives is depicted by illustrating the research framework built with IIIS and collaborators and showing interactive flows of cross-disciplinary and cross-research field studies as below;




At IIIS, three research fields (basic biology, clinical medicine and pharamceutical science) are fused to establish the integrative sleep medicine. The field of basic biology aims at the objective 1 above (elucidation of the fundamental mechanisms of sleep/wake regulation) primarily. For the objective 2, close collaboration with disciplines of clinical molecular genetics, clinical sleep physiology and psychiatric medicine, along with basic biology, will be needed. We will have to strengthen the translational research (TR) including pharmaceutical science and clinical pharmacology to achieve the objective 3. Towards the development of treatments for sleep disorders, we will have to achieve the deliverables written in blue in the scheme above. Some preliminary results have already been obtained, and we have been establishing the framework of translational research.

Each lab of IIIS covers not only a single discipline but multiple ones as listed below.

| | | Liu | Tak | TSa/ MSa | Hay | Laz | RGr/ Vog | CGr | Dan | Ura | Yan/ Fun | Nag | Sat | Shi |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Basic Biology | Molecular genetics | ✓ | ✓ | | | | | | | | ✓ | | | |
| | Biochemistry | ✓ | ✓ | | | | | | | ✓ | ✓ | | | |
| | Molecular cell biology | ✓ | ✓ | ✓ | ✓ | | | | | ✓ | ✓ | | | |
| | Sleep physiology | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | |
| | Neuroscience | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | | |
| Pharma-ceutical Science | Pharmacology | | | | | | | | | ✓ | ✓ | ✓ | | |
| | Drug discovery | | | | | | | | | | ✓ | ✓ | | |
| | Medicinal chemistry | | | | | | | | | | | ✓ | | |
| Clinical Medicine | Clinical sleep physiology | | | | | | | | | | | | ✓ | ✓ |
| | Clinical pharmacology | | | | | | | | | | | | ✓ | ✓ |
| | Psychiatric medicine | | | | | | | | | | | | | ✓ |
| | Clinical molecular genetics | | | | | | | | | | | | | ✓ |

Liu: Qinghua Liu
Tak: Joseph S. Takahashi
TSa: Takeshi Sakurai
MSa: Masanori Sakaguchi
Hay: Yu Hayashi
Laz: Michael Lazarus
RGr: Robert W. Greene
Vog: Kaspar Vogt
CGr: Carla Green
Dan: Yang Dan
Ura: Yoshihiro Urade
Yan: Masashi Yanagisawa
Fun: Hiromasa Funato
Nag: Hiroshi Nagase
Sat: Makoto Satoh
Shi: Tetsuo Shimizu

Although some research institutes in US and Europe focus on sleep, almost all of those mainly conduct clinical studies. In contrast, IIIS is a unique institute that intensively focuses on fundamental research on sleep medicine. Thus many labs in IIIS conduct basic biological researches. We plan to strengthen the research alliances with labs and/or institutes working on clinical medicine, along with the progress in basic researches.

For the studies of pharmaceutical science, we also plan to collaborate with; a) pharmaceutical companies, b) governmental institutions such as Japan Agency for Medical Research and Development (AMED) or National Institute of Biomedical Innovation, c) medical institutions promoting sponsor-investigator clinical trials, such as Tsukuba Critical Path Research and Education Integrated Leading Center.

## 2. Goals

Goals to be accomplished by the end of the grant period have been set to each research objective as below.

### 1) Elucidation of the fundamental mechanisms of sleep/wake regulation

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

- Elucidation of operating principles of the neural networks regulating sleep/wake
- Elucidation of physiological functions of sleep

**2) Elucidation of molecular pathogenesis of sleep disorders and related diseases**

- Elucidation of interactions between the brain and peripheral organs in the sleep/wake regulation
- Elucidation of intracellular events and molecular association of sleep/wake behavior in the body

**3) 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

Some collaborative studies across labs will be needed to achieve some of those goals. We thus started cross-research field projects such as;

- Evaluation of the effect of exercises to sleep, and elucidation of underlying mechanisms (Yanagisawa/Funato, Sato, Shimizu)
- Development of orexin agonists (Yanagisawa/Funato, Nagase, Urade)
- Evaluation of orexin antagonists (Yanagisawa/Funato, Urade, Sato)
- Development of methods to visualize neural activities (Yanagisawa/Funato, Lazarus, Sakurai/Sakaguchi, Greene/Vogt, Hayashi)
- Development of methods to analyze and identify neural circuits (Yanagisawa/Funato, Lazarus, Sakurai/Sakaguchi, Greene/Vogt, Hayashi)

## 3. Results and progress by the end of FY2014 and future perspectives

For each goal set for three objectives, results and progress by the end of FY2014 and future perspectives are described as follows.

### (1) Yanagisawa/Funato Lab

#### a) Effect of *Sleepy* gene mutation on sleep time

The *Sleepy* mutant pedigree has been established through our screening of randomly mutagenized mice for sleep abnormality. Heterozygous *Sleepy* mutant mice showed approximately a 30% reduction in 24-h wake time and ~35% reduction of dark-phase wake time. Linkage analysis of *Sleepy* N2 generation identified the chromosomal region responsible for reduced total sleep time with a LOD score of more than 20. Whole exome sequencing of mutant and wildtype littermates identified a single nucleotide change specific to *Sleepy* mutant mice within the mapped chromosomal region. The mutation

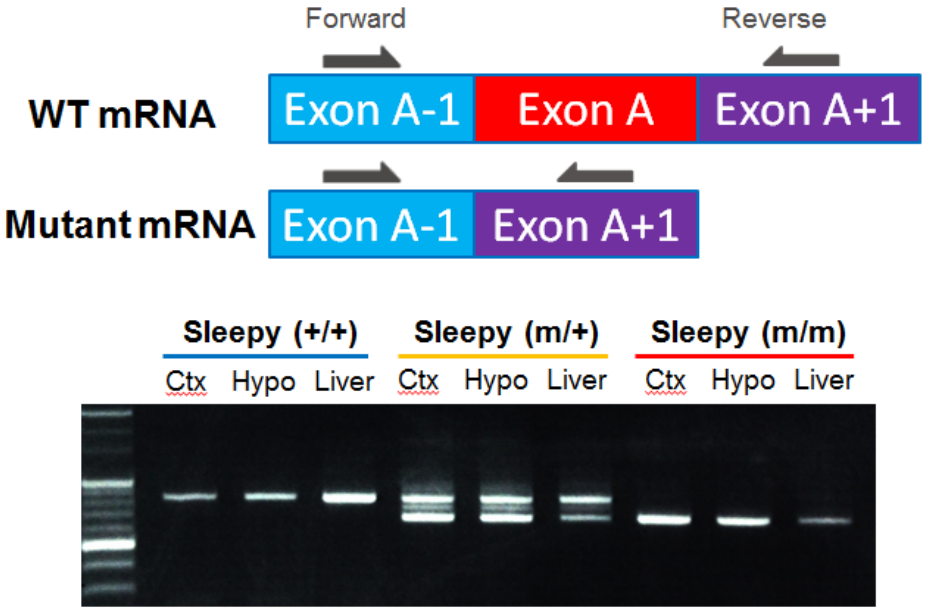



**Figure 1**. RT-PCR of *Sleepy* mRNA: Shorter PCR products are specific to heterozygous *Sleepy* mouse. Homozygous *Sleepy* mutant mouse has only a short form. The size difference between the two bands corresponds to the size of ExonA. Ctx: Cortex, Hypo: Hypothalamus.

destroys a splice donor site of a gene 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. Homozygous mutant mice have the short variant only (Figure 1). Direct sequencing of the short RT-PCR product confirmed exon skipping in the *Sleepy* mutant mRNA due to the splice mutation. To definitively prove the causal role of this mutation in the *Sleepy* phenotype, we made mice in which the splice donor site of the *Sleepy* gene is disrupted by zinc finger nuclease (ZFN) technologies. The ZFN-based *Sleepy* mutant mice showed reduced sleep time similar to ENU-screening based *Sleepy* mutant mice. Although decreased wake time could be due to a decreased arousal response or increased sleep need, *Sleepy* mutant mice have normal arousal response to external stimuli, suggesting the possibility that *Sleepy* mutant mice have increased sleep need.

**[Future directions]**

To identify proteins interacting to *Sleepy* protein in brains, we are making mice in which FLAG and HA peptides are inserted in the amino terminus of *Sleepy* protein using CRISPR technology. This mouse will allow us to reveal differences the interactome profile between *Sleepy* mutant and wildtype mice, and during sleep and wakefulness.

**b) Effect of *Dreamless* gene mutation on REM sleep**

The *Dreamless* mutant pedigree has also been established through our high-throughput screening of ENU-mutagenized mice. *Dreamless* mutants exhibit short REM sleep episode duration and reduced total REM sleep time. 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 and whole exome sequencing found a single nucleotide substitution in a gene we termed the *Dreamless* gene. 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 has been produced through the CRISPR technology. *Dreamless* mutant mice made by CRISPR technology exhibited shorter REM sleep episode similar to ENU screening-based *Dreamless* mutant mice (Figure 2).

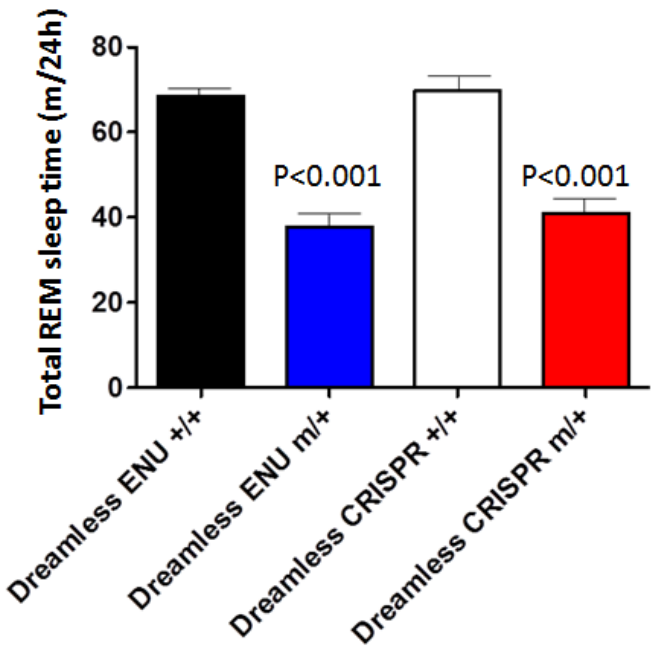



**Figure 2**. CRISPR-based gene-modified mice replicate *Dreamless* mutant phenotype: *Dreamless* mutant mice made by CRISPR technology exhibited shorter REM sleep episode similar to ENU screening-based *Dreamless* mutant mice. One way ANOVA followed by post-hoc Tukey test was performed for statistical analysis.

**[Future directions]**

We will examine homozygous mutants to see whether they show a further reduction in REM sleep time. Through *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.

**c) Identification of novel genes to control sleep/wakefulness**

Identification of *Sleepy* and *Dreamless* genes as novel sleep-regulating genes verifies the power of forward genetic approach in sleep research. We have continued the screening of ENU-mutagenized mice for sleep/wakefulness abnormality. ENU-mutagenized mice are now systematically produced using *in vitro* fertilization by the Laboratory Animal Resource Center.

**[Future directions]**

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

**d) *In vivo* two-photon calcium imaging of cortical neurons during sleep**

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, spontaneously sleeping/waking 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

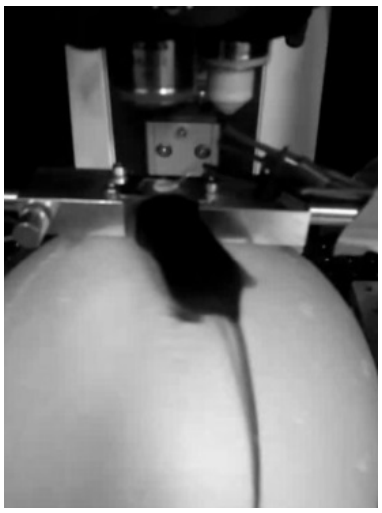


**Figure 3**. Visualization of calcium dynamics of cortical neurons during sleep/wakefulness behavior: Our two-photon imaging system eliminates the drift in field of view and greatly reduces stress to the unanesthetized mouse.

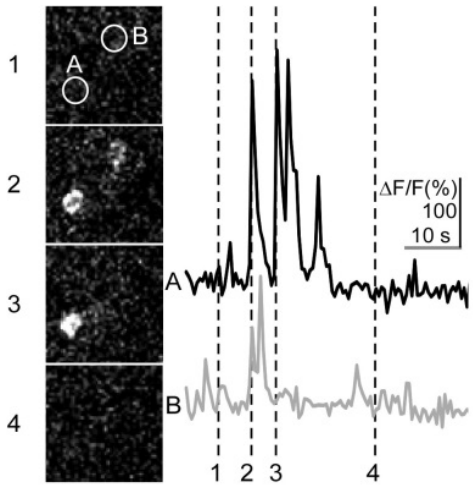



**Figure 4**. $Ca^{2+}$ imaging of pyramidal neurons from an unanesthetized *Thy1-GCaMP7* transgenic mouse; Left: Frames from a two-photon time series of pyramidal neurons.

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). Calcium-imaging of excitatory and inhibitory neurons during wake, NREM sleep and REM sleep is currently underway by microinjecting AAV vectors to the cortex of various Cre driver mice brain to deliver genetically encoded calcium indicators.

**e) Development of chemical compounds which modulate orexin receptor signaling**

Orexin system plays an indispensable role in sleep/wakefulness regulation. Lack of orexin neurons causes the sleep disorder narcolepsy. So, the development of orexin receptor agonist has high therapeutic potential. As collaboration with the Nagase laboratory at IIIS, we have discovered novel candidates for orexin receptor agonist from > 1,500 newly synthesized drug-like compounds, which were originally designed based on the structures of the initial hit compounds we identified through a high-throughput library screening at the University of Texas Southwestern Medical Center. Especially, the compound YNT-185 showed OX2R-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 injection of YNT-185 into orexin-deficient mice caused a significant increase of wakefulness, while OX1R/OX2R double knocked out mice exhibited no difference of wakefulness under the same condition. This demonstrates that YNT-185 increased wakefulness through the activation of orexin receptors.

**[Future directions]**

We will continue to generate and optimize orexin receptor agonists with high potency and blood brain barrier permeability.

**(2) Liu Lab**

**a) Investigate the downstream signaling pathways of Orexin neuropeptide (Wang *et al.*, *JBC* 2014)**

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. Here, we discovered that Orexin could activate the mTOR pathway, a central regulator of cell growth and metabolism, in

HEK293 and hypothalamic N41 cell lines that express either OX1R or OX2R. This orexin/GPCR-stimulated mTOR activation is sensitive to rapamycin, an inhibitor of mTOR complex 1 (mTORC1), but is independent of two well-known mTORC1 activators, Erk and Akt. Rather, our studies indicate that orexin activates mTORC1 *via* extracellular calcium influx and the lysosome pathway involving v-ATPase and Rag GTPases. A transient surge in intracellular calcium is sufficient to mimic orexin signaling in the activation of mTORC1 in a v-ATPase/Rag GTPase-dependent manner.

Thus, our studies suggest that the mTORC1 pathway functions downstream of orexin signaling, which plays a crucial role in many physiological and metabolic processes.

Previous studies showed that the CAG/orexin transgene expressed several fold higher levels of orexin-A and B in the mouse brain, and could rescue the narcolepsy and cataplexy phenotype of mice lacking the endogenous orexin-producing neurons. To study whether orexin activated the mTOR pathway *in vivo*, we examined the effect of orexin overexpression on the mTOR activity in whole brain extracts of wild-type and CAG/orexin mice fed on high fat diet. This *in vivo* experiment suggests that overexpression of orexin can cause hyperactivation of the mTOR pathway in the mouse brain. Narcolepsy patients, who lack the orexin/GPCR signaling in the brain, tend to be overweight. Conversely, prolonged orexin overexpression in mice prevents the high fat diet-induced obesity and insulin resistance by enhancing leptin signaling. Although how orexin signaling interacts leptin signaling remains unknown, the present result suggests that leptin and orexin signaling converge on mTORC1 to negatively regulate metabolism.

**[Future directions]**

We are testing the hypothesis that the orexin-mTOR signaling axis plays a critical role in mediating the effects of Orexin in promoting wakefulness by ICV injection of orexin in the absence or presence of rapamycin.

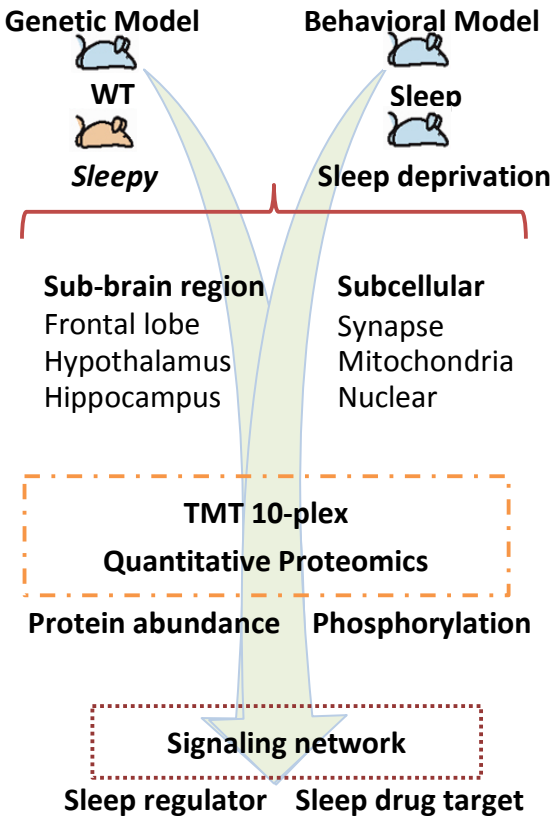



**Figure 5**. Overview strategy of this study

**b) Quantitative proteomics analysis of wild type vs. *sleepy* mutant mouse brains**

A “holy grail” of sleep research is to identify the molecular substrate of the “sleep pressure” that controls total sleep time and drives recovery sleep after sleep deprivation. It is highly possible that the “sleep pressure” of the “*Sleepy*” mutant may be constitutively higher than that of wild-type mice. We propose a novel and powerful strategy of combining forward genetics and quantitative proteomics to identify the molecular substrate of “sleep pressure” and explore the signaling network underlying sleep regulation. The proposed studies represent the first comprehensive quantitative proteomics and phosphoproteomics study of this kind in the field of sleep research (Figure 5).

Tandem mass tag (TMT) and Isobaric tags for relative and absolute quantitation (iTRAQ) are chemical labels used for mass spectrometry based identification and quantification of biological macromolecules. Advances in multiplexed quantitation by TMT or iTRAQ have revolutionized quantitative proteomics (Erickson BK *et al*. *Anal Chem*. 2015). The commercially available TMT 10-plex currently enables simultaneous analysis of up to 10 samples by mass spectrometry (Figure 6).

I recruited a talented postdoc/protein biochemist, Dr. Zhiqiang Wang, to my lab and initiated a close collaboration with Dr. Yonghao Yu, an outstanding mass spectrometry expert at UT Southwestern Medical Center at Dallas, Texas, USA. Since 2013, Dr. Wang has been schooled in Professor Yu’s lab to learn the art of cutting-edge quantitative mass spec technologies. Drs. Yanagisawa/Funato lab sent wild type and *sleepy* mutant mouse brain samples to UT Southwestern. Dr. Wang prepared the brain extracts and performed TMT experiments to compare the brain proteome of wild type and *sleepy* mutant mice. In a single mass spec run, we were able to confidently analyze ~4,500 most abundant proteins, of which 99.9% of these proteins remained unchanged between WT and

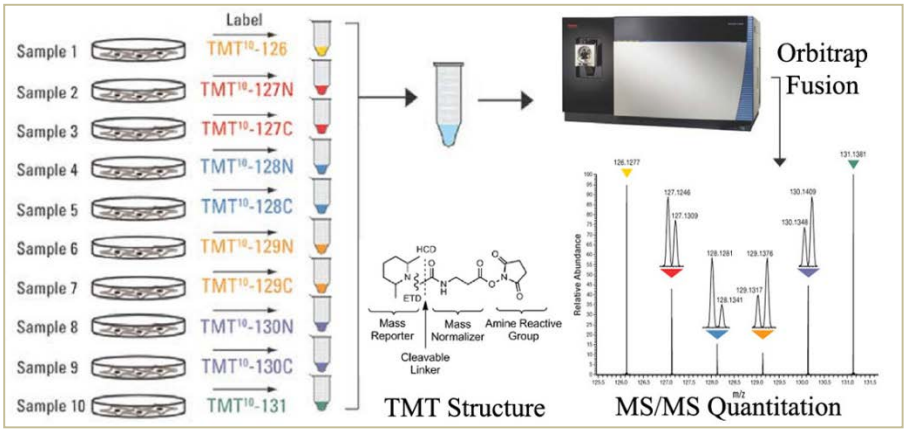



**Figure 6**. A schematic diagram of TMT 10-plex procedure.

mutant brains. In contrast, the levels of less than ten proteins were significantly up-/down-regulated in the Sleepy mutant proteins. These proteins could be candidate effector proteins that cause the hypersomnia or obesity phenotype of *Sleepy* mutant.

WPI-IIIS purchased a state-of-the-art Orbitrap Fusion mass spectrometry instrument from Thermo Inc (delivered and installed). Dr. Wang obtained an invitation fellowship from University of Tsukuba, which allowed him to visit WPI-IIIS for three months from October, 2014 to January, 2015. During this period, he was trained by Thermo specialists and successfully ran a dozen of TMT samples on the "Fusion" machine. The results he obtained were of the highest quality: we could reliably quantify ~9,000 proteins (almost half of the proteome) in a single run of six-sample (3 WT + 3 mutant brains) mixture. Dr. Wang will move to IIIS to take charge of the mass spec facility on May 1, 2015. I want to highlight that this is a remarkable feat since my lab knew nothing about mass spec to begin with and we were able to successfully establish the Mass Spec core facility at IIIS in less than two years.

**[Future directions]**

We will expand the TMT 6-plex method to TMT 10-plex method, which will allows us to simultaneously analyze 10-sample mixture in a single mass spec run.

We will establish methodology to perform quantitative proteomics of sub-regions (e.g. hypothalamus) or subcellular fractions (e.g. synapses or mitochondria) of mouse brains.

We will develop quantitative phosphoproteomics methods to explore specific changes in signaling pathways in the *Sleepy* brain. This is an especially interesting and important direction given that *Sleepy* encodes a protein kinase.

While quantitative proteomics is restricted to most abundant proteins, phosphoproteomics enriches for regulatory proteins that are frequently not so abundant. Moreover, we search in phosphoproteomics for interesting pattern of changes in multiple components of one signaling pathway. By cross examination of phosphoproteomics data, we will identify the common signaling pathways that are affected in different *Sleepy* mutants. It is likely that these signaling pathways play a crucial role in sleep/wake regulation.

**c) Elucidation of the molecular principles of fear and regulation of sleep by emotions such as fear or anxiety**

Sleep is regulated by circadian clock, homeostasis, and emotion. Although it is a well-known phenomenon, how emotion regulates sleep is unclear. Moreover, almost nothing is known about human emotions at the molecular level. Fear is a basic emotion that enhances animal survival by triggering the "fight, freeze, or flight" response to perceived

danger. Uncontrolled fear also underlies many mental disorders, such as anxiety or panic disorders, phobia, depression, and post-traumatic stress disorder (PTSD). Almost nothing is known about the molecular pathways of fear despite of extensive studies. Among various emotions, fear is the most tractable to molecular genetic studies because it can be readily and reliably elicited in a mouse model. For example, lab mice will be instantly afraid of a cat despite being raised away from predators for many generations. This indicates that the basic neurological pathway of fear is innate and must therefore be genetically encoded. To crack the mystery of fear, we have been conducting a forward genetic screen in mice to identify “fearless” mutants using a novel predator odor-based “innate fear” assay that we developed. We believe that this unprecedented and unbiased screen takes a potentially game changing approach to identify the core fear genes, elucidate the molecular mechanism of fear, and explore exactly how emotion regulate sleep/wake cycle. These groundbreaking discoveries may uncover the fundamental molecular principles of human emotions.

Forward genetic screening is one of the most powerful approaches to understanding the molecular basis of human biology and disease (Moresco *et al*, *American Journal of Pathology*, 2013). This is especially true when it comes to dealing with “black box” phenomena that we know little about. Moreover, next generation sequencing (NGS) technologies has recently revolutionized forward genetic screens in mice. The time that it takes to map the causal mutation is dramatically reduced from 8-10 years to 3-6 months. Thus, the “bottle neck” for forward genetic screening is no longer the positional mapping of the mutant gene, but rather the development of a robust assay that is suitable for high-throughput screening in mice.

In terms of mouse behavior screens, Dr. Joseph Takahashi’s “Clock” screen, which uncovered the central clock genes that drive circadian behaviors, remains the quintessential example of this powerful strategy. A key factor for the success of “Clock” screen was a remarkably tight “Clock” assay [relative standard deviation (RSD) = 0.72%]. Previously, scientists have attempted to conduct forward genetic screens using the “learned fear” or “fear conditioning” assays, e.g. mice were trained to be afraid of a harmless sound by pairing this cue with electric foot shock. However, these screens were doomed to failure mainly because the assays were too variable (RSD = 50-100%), such that it was impossible to reliably distinguish mutant vs. wild type mice and map the responsible genes (Takahashi, *Science* 2008 and personal communications).

Mice have poor vision and rely on a sensitive olfactory system to detect predators. Thus, we recently developed a predator odor-based "innate fear" assay that was suitable for high throughput genetic screening. In this assay, a single chemical, which was derived from fox urine, triggers a robust freezing response in the c57/B6J mice-a gold standard strain for behavior screens. Using "Freezeframe" software to quantify freezing, wild-type mice exhibited 5-20% freezing without odor and 60-95% freezing in response to predator odor. This innate fear assay has a tight RSD of ~10%, making it feasible to screen for "fearless" mutants with <47% freezing rate (>3SD below the normal mean 77%). We conducted a pilot "FEAR" screen in collaboration with Dr. Masashi Yanagisawa at WPI-IIIS, University of Tsukuba. After screening ~1,600 ENU-mutagenized F1 (6J/6N) males, we isolated 10 putative "fearless" mutants (Figure 7). Two of ten mutants were shown to be inheritable by backcrossing the F1 males with 6N females to generate the N2 progenies and scoring their phenotype.

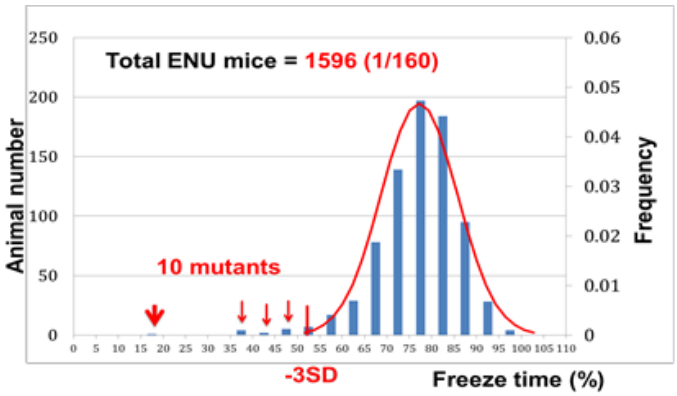



**Figure 7**. Results of a pilot "FEAR" screen.

**[Future Directions]**

1) We will identify the mutant genes by genetic mapping and exome sequencing;
2) We will verify the causality of the "fearless" phenotype by re-creating the ENU-induced mutations by the CRISPR/Cas9 genome-editing technology;
3) We will determine whether these are truly fearless mutants by a host of neuroscience and behavior assays;
4) We will characterize the physiological functions of the gene products in relation to fear behavior by combining biochemical, cell biological, and biophysical approaches.

## (3) Urade Lab

### a) Induction of cataplexy in wild-type mice by orexin receptor antagonists and suppression of cataplexy in orexin KO mice by orexin receptor agonist

Suvorexant was approved for human use in Japan in 2014 for the treatment of insomnia. This is the first drug that induces sleep by targeting orexinergic arousal system. Since narcoleptics are caused by reduced orexin signaling, sustained blockage of orexin

receptors may mimic the condition of orexin KO mice and develop cataplexy. Dr. Kaushik found in 2013 that SOREM and cataplexy were induced by re-challenge with a high dose of suvorexant (100 mg/kg) after 1 week of washout following a chronic treatment for 2 weeks. When mice were treated with a high dose of suvorexant for 1 week, re-challenge after 1 week washout developed catapletic behavior in mice. He repeated the experiment with another dual orexin receptor antagonist (DORA-22) and followed the similar protocol. DORA-22 also induced SOREM and cataplexy by re-challenge after 1 week washout following the chronic treatment for 1 week, similar to suvorexant. Now, he examine the changes in orexin and orexin receptor contents in the mouse brain during chronic treatment and washout of suvorexant and DORA-22.

Dr. Kumagai, the Tokyo Univ., and Ms Nagata used our sleep bioassay system with a continuous intracerebroventral infusion of drug to free-moving mice and confirmed that a small orexin agonist Chemotype 3 efficiently suppressed cataplexy of orexin KO mice.

**b) Identification of somnogenic natural components**

Sleep-promoting natural components become an alternative choice of treatment for insomnia. By using our sleep bioassay system in mice, several natural components have been found to be associated with the sleep-promoting activity. Dr. Kaushik started the collaborating study with scientists of AIST to identify the active component for somnogenic activity of plant extracts of ashwagandha (*Withania somnifera*), which has been used as a traditional medicine for insomnia in India. He found that the water extract containing triethylene glycol (TEG) and the purified TEG induced NREM sleep in a dose-dependent manner after oral administration in mice and also increased total amount of REM sleep during next day. He will elucidate the target neural structure of TEG and neural cell types involved in its somnogenic effect. Dr. Cherasse found by the collaborative study supported by NARO and Fuji film company that Zinc-containing yeast extract dose-dependently increased the total amount of NREM sleep, whereas the extracts containing other divalent cations (manganese, iron, and copper) did not increase NREM sleep. This is the first evidence that zinc induces sleep and opened the way to new types of food supplements designed to improve sleep. Dr. Aritake previously demonstrated that crocin and crecetin, two major carotenoid pigments of saffron, increased the total time of NREM sleep after its intraperitoneal administration. He found that highly glycosylated crocin improved 5-10 fold the absorption after oral administration and the photo-stabilities, and suggested its potential use for the treatment of insomnia. Mr. Nakamura of Lion company found that sake yeast extracts containing S-adenosyl methionine and methyl thio-adenosine induced NREM sleep in

mice in an adenosine $A_{2A}$ receptor-dependent manner.

### c) The effect of cannabinoid ligands on the EEG pattern in mice

Recently many kinds of synthetic cannabinoids appear and spread elsewhere raising the awareness of the society. Based on the request from NIHS, Drs. Aritake and Malyshevskaya measured changes in locomotion, behavior and EEG/EMG characteristics in mice of those psychoactive drugs, such as Δ9-THC (plant-derived cannabinoid), JWH-018 and CP-55,940 (synthetic cannabinoid). After an i.p. injection, those drugs resulted in prolonged hypolocomotion for 4-12 hours and significantly increased the EEG power in a frequency range of 3-4 Hz as compared with control. Moreover, JWH-018 administration induced behavioral and EEG-registered seizure. These data demonstrated important adverse drug effects that should be reconsidered by society, clinical doctors and drug users.

## (4) Lazarus Lab

Our main goals are to identify novel brain circuits that regulate the wake/sleep cycle and elucidate homeodynamics in sleep which ensure that the neuronal processing of motivational, cognitive and emotional behaviors occur under metabolically favorable circumstances.

### a) The role of the nucleus accumbens in sleep-wake regulation

Sleep or sleep-like states seem to exist in all complex organisms that have a central nervous system. The sleeping habits of humans are unique in the sense that we often defy sleep and stay awake for occupational and recreational reasons or other life-style choices, despite experiencing fatigue during that time. The motivation to defy sleep and stay awake for a wide range of life-style choices is often accompanied by the use of psychoactive substances, most prominently caffeine, but also prescription drugs such as Modafinil (Lazarus M, Urade Y; In: The Adenosinergic System - A Non-Dopaminergic Target in Parkinson's Disease, Simola N, Morelli M, Wardas J, eds; 2015, in press).

We have demonstrated that caffeine induces wakefulness by blocking the action of adenosine on $A_{2A}$ receptors ($A_{2A}$Rs) in the nucleus accumbens (NAc; Figure 8a) (Lazarus M, *et al*., *J. Neurosci*. 2011, p. 10067). Adenosine promotes sleep and $A_{2A}$Rs are highly expressed on neurons in the NAc that also express dopamine D2 receptors and enkephalin, however the extent to which these $A_{2A}$R-positive NAc neurons contribute to sleep regulation was previously unknown. Pharmacological activation of $A_{2A}$Rs by the agonist CGS21680 increased non rapid eye movement (NREM) sleep in wild type, but not NAc-specific $A_{2A}$R KO mice (Figure 8b. Optogenetic (channelrhodopsin, ChR2)

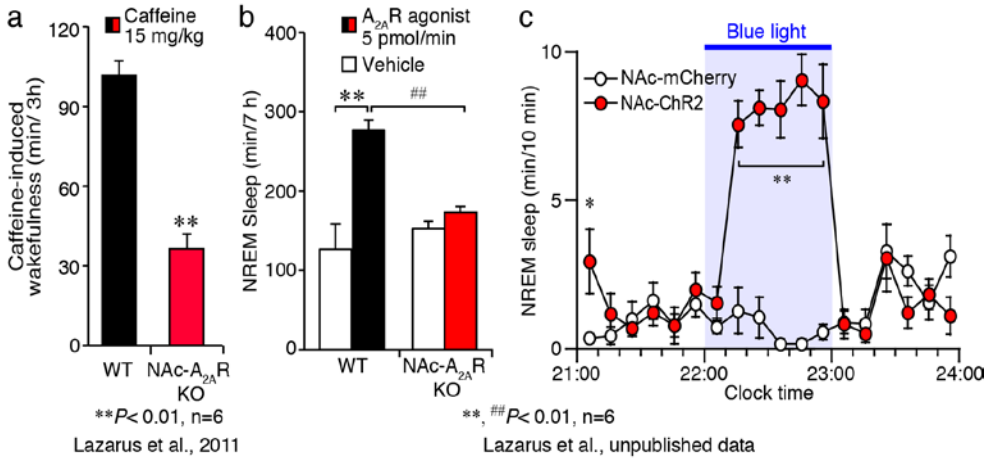



**Figure 8**. Adenosine and $A_{2A}R$ neurons in the NAc play a major role in sleep regulation.

activation of NAc $A_{2A}R$ neurons induces robust NREM sleep (Figure 8c; Lazarus *et al.*, in preparation).

Our observations provide the first direct evidence that adenosine and $A_{2A}R$ neurons in the NAc are not only involved in promoting behavioral inactivity (inhibition of movement) but also play a major role in regulating sleep. These findings further suggest the intriguing possibility that the NAc might be a key site through which sleep and wakefulness are regulated by behavioral processes and, by extension, that motivational state may be an important fundamental regulator of sleep and wakefulness (Lazarus M, *et al.*, *Trends Neurosci*. 2012, p. 723; Lazarus M, *et al.*, *Curr. Opin. Neurobiol.* 2013, p. 780).

**b) The role of prostaglandin (PG) D2 in the regulation of pathological sleep**

The neural and cellular basis of sleep need or, alternatively, "sleep drive" remains unresolved, but has been conceptualized as a homeostatic pressure that builds during the waking period and is dissipated by sleep. One theory is that endogenous somnogenic factors accumulate during wake and that their gradual accumulation is the underpinning of sleep homeostatic pressure. Besides adenosine, several additional putative hypnogenic substances implicated in the sleep homeostatic process have been identified, including prostaglandin (PG) D2 and cytokines. Some clues into the humoral "mechanisms" underpinning sleep regulation have been gleaned from study of individuals exhibiting "sickness behavior", i.e., the fever, malaise, increased pain sensitivity, feeding, and changes in sleep–wake typically observed during a bacterial or viral infection. Sickness behavior links to a cascade of pro-inflammatory mediators,

including a wide range of cytokines and PGs that trigger an array of physiological responses termed the acute phase reaction. It is now an accepted fact that sleep regulation and the production of pro-inflammatory cytokines by the host defense (immune) system activity are strongly interrelated. Rodents treated with proinflammatory cytokines exhibit an enhancement of NREM sleep and a decrease in REM sleep. Although cytokine production is inevitably accompanied by the secretion of PGs, we demonstrated that the increase of NREM sleep during an infection is independent of PGs (Oishi Y, *et al*., *Brain, Behav. Immun*. 2015, in press), a surprising fact considering: 1) the fever response is completely dependent on PGE2 type EP3 receptor signaling (Lazarus M, *et al*., *Nat. Neurosci*. 2007, p. 1131) and 2) PGs have been implicated in the regulation of sleep (Urade Y, Lazarus M; In: The Genetic Basis of Sleep & Sleep Disorders, Shaw PJ, Tafti M, Thorpy MJ, eds; 2013, p. 73-83).

Similar to inflammation, seizures induced in animal models by electroconvulsive shock or administration of pentylenetetrazol increase the content of various PGs in the brain. In collaboration with the Urade Lab, we showed that PGD2 acting on prostaglandin type DP1 receptors is essential for seizure suppression and regulates sleep that follows seizures (Kaushik MK, *et al*., *Exp. Neurol*. 2014, p. 82).

**[Future Directions]**

**a) Elucidation of sleep homeodynamics of the adenosinergic system in the nucleus accumbens**

We revealed an unexpected function of adenosine and $A_{2A}$ receptor ($A_{2A}$R)-possessing neurons in the nucleus accumbens (NAc) in the control of the sleep-wake cycle, however (1) the homeodynamics of $A_{2A}$R neurons across the sleep-wake cycle are unknown and (2) the source of adenosine in the NAc remains a mystery.

Regarding (1), we are developing an endomicroscopic fiber-optic system to visualize changes in adenosine, cyclic adenosine monophosphate (cAMP) and neural spiking patterns in $A_{2A}$R-positive neurons of the NAc in freely behaving mice during the sleep-wake cycle. In collaboration with Nagase Lab, we also aim to establish the spatiotemporal control of the NAc $A_{2A}$R using photo-stimulation of a caged $A_{2A}$R agonist by an endoscopic fiber-optic system. To date, *in vivo* applications of photocaging techniques in non-transparent model organisms have not been reported and thus, 'photopharmacology' in freely moving mice is a frontier technology with a wide range of applicability in the field of neuroscience to dissect the effects of receptors with complex brain distributions. Deep-brain imaging and photopharmacology are part of the IIIS-cross-research-field projects 'Development of methods to visualize neural activities' and

'Development of methods to analyze and identify neural circuits'.

Regarding (2), we hypothesize that the source of adenosine can be identified by the genetic depletion of extracellular adenosine with the intention of dysregulating the sleep-wake cycle. We attempt to selectively deplete extracellular adenosine in the NAc by genetically directing adenosine deaminase (ADA) expression to NAc astrocytes or neurons for removal of intracellular adenosine followed by equilibrating depletion of extracellular adenosine (Figure 9). ADA-driven adenosine depletion has never been attempted before and thus, constitutes a fresh approach to solve one of the most enduring questions in the sleep field, that is 'Where and what is the source of adenosine in the brain?'

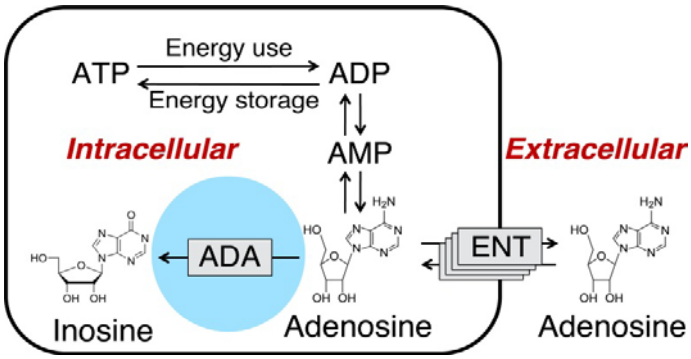



**Figure 9**. Genetic depletion of extracellular adenosine.

**b) Development of a new class of sleeping pills by targeting $A_{2A}R$ (in collaboration with Nagase Lab)**

We expect the $A_{2A}R$ in the NAc to become a new drug target for the treatment of sleep disorders. All currently existing $A_{2A}R$ agonists lack brain-permeability and have side effects, such as hypotension. As brain adenosine levels can be greatly elevated during wakefulness, a freely penetrating allosteric enhancer may effectively increase the sleep-inducing effect of adenosine, especially in the NAc. We are screening chemical libraries in a high-throughput bioassay for human adenosine $A_{2A}R$ based on a luciferase reporter system in combination with a sleep bioassay in freely moving mice to generate lead compounds for a new class of sleeping pills.

**c) Elucidation of neuronal mechanisms of metabolic adaptation to sleep loss**

The NAc has the unique capability to integrate cognition and emotion due to glutamatergic innervation from the prefrontal cortex (PFC) and amygdala (Brog JS, et el., *J Comp Neurol* 1993, p. 338). We hypothesize that the NAc may provide crucial functional connectivity between the PFC and the amygdala thereby enhancing appetite for highly palatable foods during prolonged periods of wakefulness. Indeed, sleep deprivation attenuates connectivity between the amygdala and PFC (Yoo S, *et al.*, *Curr*

*Biol* 2007, p. 17) furthering the hypothesis that the amygdala and PFC work in concert with the NAc to regulate sleep and appetite. Dr. Kristopher McEown joint my laboratory in November 2014 as a JSPS postdoctoral fellow. Dr. McEown studied fear behavior in his PhD thesis (McEown K and Treit D, *Neuroscience*. 2013, p. 169; McEown K and Treit D, *Horm Behav* 2011, p. 581) and now aims in my laboratory to develop and validate a novel animal model of sleep and appetite where pharmacogenetic techniques will be used to restrict sleep. Specifically, *in vivo* genetic inhibition will be used to inactivate the NAc with the intention of restricting sleep. We will then compare our 'insomnia mouse model' with caffeine intake or mechanical sleep restriction to observe the effect that each of these sleep restriction methods may have on appetite for weight promoting foods. PFC or amygdala inactivation will also be combined with NAc inactivation to observe potential effects that these manipulations may have on sleep and appetite.

**(5) Sakurai/Sakaguchi Lab**

Goals of our studies are 1) Elucidation of physiological functions of sleep, 2) Development of methods to visualize neural activities, and 3) Development of methods to analyze and identify neural circuits

The optogenetic manipulation of light-activated ion-channels/pumps (i.e., opsins) can reversibly activate or suppress neuronal activity with precise temporal control. Therefore, optogenetic techniques hold great potential to establish causal relationships between specific neuronal circuits and their function during sleep. Due to the critical role of the hippocampal CA1 region in memory consolidation during sleep, we explored the possibility of targeting an inhibitory opsin, ArchT, to CA1 pyramidal neurons in mice. We established a new transgenic mouse line in which tetracycline trans-activator induces ArchT expression. By crossing this line with a CaMKIIα-tTA transgenic line, the delivery of light via an implanted optrode inhibits the activity of excitatory CA1 neurons, which was revealed by single unit recording. We found that light delivery to the hippocampus inhibited the recall of a contextual fear memory. Our results demonstrate that this optogenetic mouse line can be used to investigate the neuronal circuits underlying sleep dependent memory consolidation (Sakaguchi *et al*., submitted). As a spin-out of the above study, we found that, during consolidation period, a fear memory has a vulnerable time window to generalize familiar context (Fujinaka *et al*., will be submitted in April).

**[Future directions]**

By crossing the mice with several driver mouse lines (e.g., c-fos-tTA mouse line), we will specifically target the neurons which were activated during learning, and intervene the activity of the neuronal ensemble during specific stage of sleep. We have already

obtained preliminary results suggesting that sleep plays crucial role in memory consolidation. It will clarify the mechanism of how the memory engram will be formed during sleep.

### (6) Greene/Vogt Lab

We aim to elucidate the cortical neuronal network activity underlying the characteristic rhythmic activity observed in sleep, in particular slow wave sleep. We want to understand the physiological regulation of these networks and reasons for the restorative effect of slow wave sleep on cognitive function.

#### a) DREADD studies for better understanding of slow wave homeostasis

We are using DREADD-based manipulation of neural activity in the cortex to hyper- or hypo-activate small cortical areas. We then study the reaction of these cortical areas in subsequent sleep phases. We are measuring surface EEG and local field potentials from the DREADD transfected and from un-transfected control regions. The main questions are. A) How global is the expression of slow wave sleep. How synchronized are these oscillations. B) Is there local (e. g. at the level of a barrel or functional cortical column) sleep homeostasis in the cortex. C) Can we determine specific elements of the cortical circuitry that are important for slow wave sleep.

The occurrence of the cortical slow waves of sleep is strongly linked with the general level of excitation of cortical networks. Our main strategy is to locally manipulate the excitability of a small cortical area by expressing DREADDs in specific mouse lines.

The possible combinations of the different DREADDs and mouse lines we are using should lead to either a) increased network excitability (excitatory DREADDs (hMdQ3) expressed in excitatory glutamatergic neurons (vGluT-CRE line); or inhibitory DREADDs (hMdQ4) in inhibitory GABAergic cells (vGAT-CRE line)) or b) decreased network excitability (excitatory DREADDs in GABAergic cells, or inhibitory DREADDs in glutamatergic cells). Since increased activity in ascending activating systems is linked to wake and disappearance of slow-waves in the cortex, we expect an increase in network excitability to tend to diminish the occurrence and/or amplitude of slow waves, whereas a decrease of excitability should potentiate these slow-waves.

We have done pilot experiments with DREADD expression in a small cortical area to interfere with local cortical activity, and in particular to try to modify the occurrence of slow-waves. We have injected a virus containing the depolarizing hMdQ3 DREADD in 2 vGAT-CRE mice, as well as the hyperpolarizing hMdQ4 DREADD in 2 vGluT-CRE mice (0.8 ul of a AAV2- DIO-hMdQ3- mCHERRY or AAV2-DIO -hMdQ4-mCHERRY solution in

barrel cortex, Bregma -1,0; Lateral 3,0). These combinations should favor the occurrence of slow waves. Expression of the virus was confirmed by imaging from fixed slices. We recorded the EEG over the injected area and the contralaleral site with screws, as well as the EMG for sleep scoring. In control conditions, the 2 EEGs, though not identical, were extremely similar, and sleep scoring, performed with a sleep scoring application developed in the Greene lab at UTSW, gave extremely similar results independently of the EEG considered. We quantified the impact of DREADD activation by CNO injections by comparing the 2 contralateral EEGs. In preliminary analysis, we have determined the ratio of the delta power of the 2 EEGs for 10 second periods of SWS throughout the course of whole days of recording. So far, with our field recordings with EEG screws, we did not identify an impact of CNO injections on this ratio. The normalized ratio of the delta power of the two sites was not statistically different from the rest of the day following the injections, nor was it different from similar periods in non-injection days. However, we observed in one animal a reproducible spontaneous fluctuation of this ratio in the first hour of the dark phase. This occurred on 6 different days. Even though there is no clear reasons for these fluctuations, this shows that fluctuations in this ratio, for similar periods in control and CNO treatment should be observable, since even spontaneous fluctuations could be identified with this technique.

The absence of effect of CNO in these preliminary experiments is not surprising. This is possibly due to the fact that surface screws sample a large area of the cortex, while the injections were made locally (in only one location, and relatively small volume) and imaging confirmed that the infected areas were small. Thus, the spatially restricted effect of DREADD activation might very well have been masked inside the sampled activity of a larger area. It is also possible that, even if DREADD activation has a local impact, the activity in the non-affected surrounding areas can still be picked up by the electrode located in the affected area, or it might simply overwhelm the local impact of DREADD activation. To address these potential pitfalls, we are now combining more local field recordings with larger areas of DREADD expression. Furthermore, we have so far only tested the decreased excitability combinations of DREADD and mouse line, and we are now also using the increased excitability combinations as a means of counteracting the occurrence of slow waves.

We have now increased the recording power of our sleep chambers to 16 or 32 channels, which will allow us to record from more locations, as well as more local activities.

For the recordings of more local field potential, we have now constructed bipolar electrodes by gluing together along the same axis two electrodes with a separation of

500 μm between their tips in a tetrode configuration. The electrodes were made from 12 μm nichrome wire, and their resistance was reduced to 100-200 kOhms. The electrodes will be placed so that the shorter is on the surface of cortical layer 1 and the longer in layer 5. This should allow us to sample a much more local field potential and detect the local impact of DREADD activation, while also recording surface and depth activity in the same area. Screws for more global EEG recordings and EMGs will also be recorded for sleep scoring.

Furthermore, recording with tetrodes offers the supplementary advantage of being able to extract firing information form neurons and correlate it with field activities. Tetrodes also provide some redundancy for field recordings, in case one connection should go bad.

We will have the possibility of further developing the electrode building technique to eventually glue 4 electrodes together at fixed distances in order to perform Current-Source-Density analysis on the recordings and thus dissect the layer structure of the recorded potentials. Alternatively, we may also construct electrode arrays of diverse configurations to assess the spatial extent of the effect of DREADD activation, as well as the impact of local disturbances on the coherence and synchrony of slow wave activities. This could yield more detailed information on the neuronal mechanisms of the cortical slow-waves of sleep.

**b) *In vivo* imaging**

The main goal of the *in vivo* imaging experiments is the elucidation of cortical neuronal activity patterns in sleep - particularly slow wave sleep. We ultimately want to produce a network blueprint for slow wave sleep oscillations in the cortex. Understanding the cortical network that underlies slow wave sleep will hopefully get us closer to understanding its homeostatic and its restorative function.

We have successfully transformed an existing confocal microscope and scanner to an *in vivo* functional imaging system. Animals are head fixed over an air-suspended styrofoam ball. So far, data was acquired with sedated animals due to

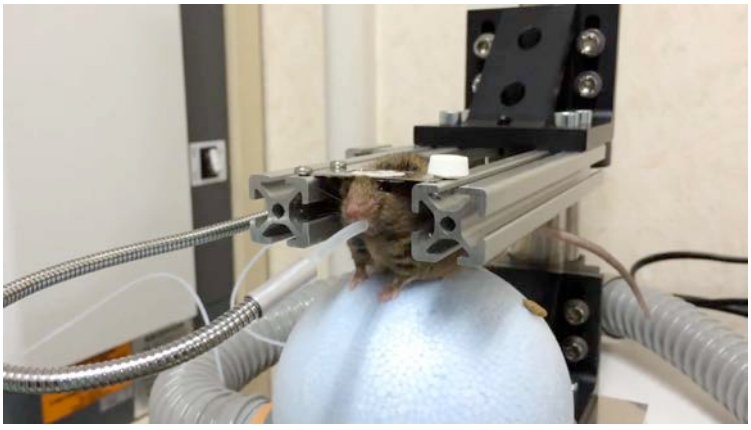


**Figure 10**. Animal with head fixation plate on head fixation training system.

concerns over motion artifacts, however the aim to record from untreated animals in the longer term. We are currently evaluating training schedules to acclimatize mice to the head fixation and to get them to sleep in this configuration (Figure 10).

Animals receive AAV injections into small cortical areas and three weeks later undergo surgery for repeated *in vivo* imaging; consisting of a craniotomy over the injected region, which is covered by a glass window and surface EEG electrodes (contralateral side and occipital reference) and a head fixation plate.

We have obtained (GCamP5) or produced (ArcLightQ329, ArcLightA242) adeno-associated viral vectors for Cre-recombinase dependent expression of genetically encoded calcium and voltage sensors. Recent developments in the field of genetically encoded voltage sensors make them an attractive potential tool for the study of subthreshold network oscillations, which occur during slow wave sleep.

We have successfully transfected VGluT-IRES-Cre and VGAT-IRES-Cre mice with calcium and voltage sensors. The data acquired so far is mainly from voltage sensors (ArcLight). We were able to acquire two-photon signals from ArcLight transfected animals at frame rates around 10 Hz, just above the Nyquist limit for slow wave oscillations (Figure 11A&B). EEG recordings from the contralateral side were time-locked with the imaging data (Figure 11C). Matlab Routines for the analysis of the imaging data and for the correlation with the EEG signals were developed.

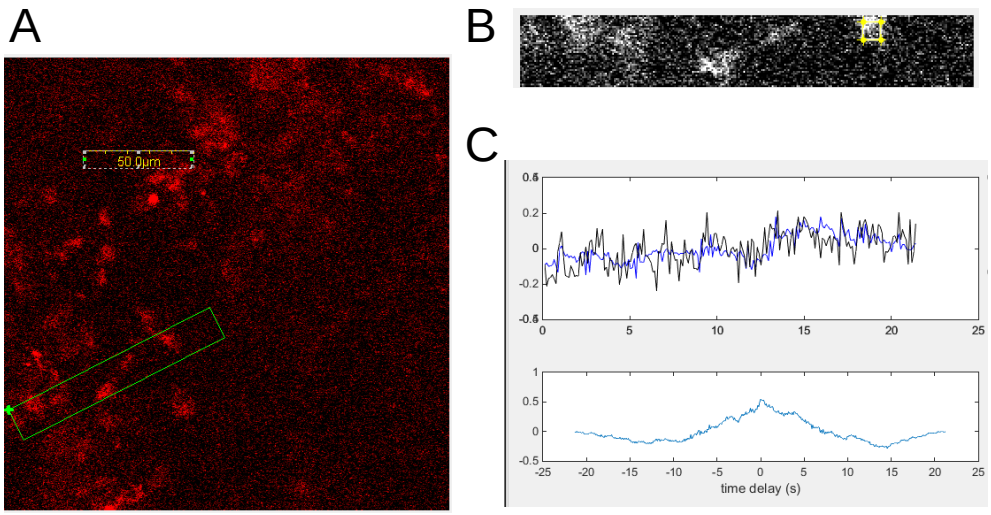



**Figure 11**. A) Overview of ArcLight expressing neurons in cortex of VGAT-Cre mouse. B) 40 by 256 pixel frame, which was scanned at 10 Hz (yellow- region of interest). C top) Imaging data (black) and EEG (blue) C bottom) Cross-correlogram of the two signals.

**[Future directions]**

For 2015 we are planning to refine our protocols and to significantly increase the amount of data collected. In addition *in vitro* control experiments will have to be conducted with all of the newer genetically encoded sensors to be able to properly interpret the acquired data. Animals are adapted to the head fixed situation using replicas of the same system used under the two-photon microscope. The liquid delivery system shown here is used to provide an appetitive stimulus during head fixation. The protocols are currently under development.

## (7) Hayashi Lab

### a) Elucidation of the REM/NREM sleep switch

Mammalian sleep is composed of two distinct states, REM sleep and NREM sleep. Abnormal balance of the two sleep states is a common symptom in various developmental disorders and psychiatric disorders. In attempts to unveil the REM/NREM sleep switch, many lesion or pharmacological studies were executed and revealed a key role for neurons in the mesopontine tegmentum (MPT) (Jouvet, *Arch. Ital. Biol.*, 1962, Hobson, *Nat. Rev. Neurosci.*, 2009). Within this area, the peri-locus coeruleus (LC) α in cats, or the equivalent sublaterodorsal nucleus (SLD) in rodents is critical for REM sleep induction (Vanni-Mercier *et al*, *Arch. Ital. Biol.*, 1989, Lu *et al*, *Nature*, 2006). By contrast, the entity of neurons that negatively regulate REM sleep or those that promote exit from REM sleep is far less understood.

Previously, to functionally dissect the complex mixture of neurons populating the pons and isolate neurons involved in REM/NREM sleep switching, we established a method to manipulate neurons of a specific embryonic cell lineage. As a result, we identified a glutamatergic cell lineage that inhibits REM sleep and promotes NREM sleep. However, it was yet unknown how these neurons constitute a REM/NREM sleep switch by interacting with REM sleep promoting-neurons in the SLD. Therefore, we expressed GFP in these neurons and tracked their axons. Axonal projections were detected within the midbrain, in the dorsal area of the deep mesencephalic nucleus (dDpMe). Furthermore, GABAergic neurons in this area sent axons to the SLD. Therefore, we examined the roles of these dDpMe GABAergic neurons by combining the Vgat-ires-Cre mice with Cre-dependent DREADD receptor-expressing viral vectors. As a result, pharmacogenetic activation or inhibition of these neurons strongly suppressed or enhanced REM sleep, respectively (Figure 12A, B). Thus we conclude that the REM/NREM sleep switch comprises two types of glutamatergic neurons that either promote REM or NREM sleep, and GABAergic neurons in the dDpMe mediate the interaction (Figure 12C). Our study

dissolves the long debate on the cellular entity of the REM/NREM sleep switch.

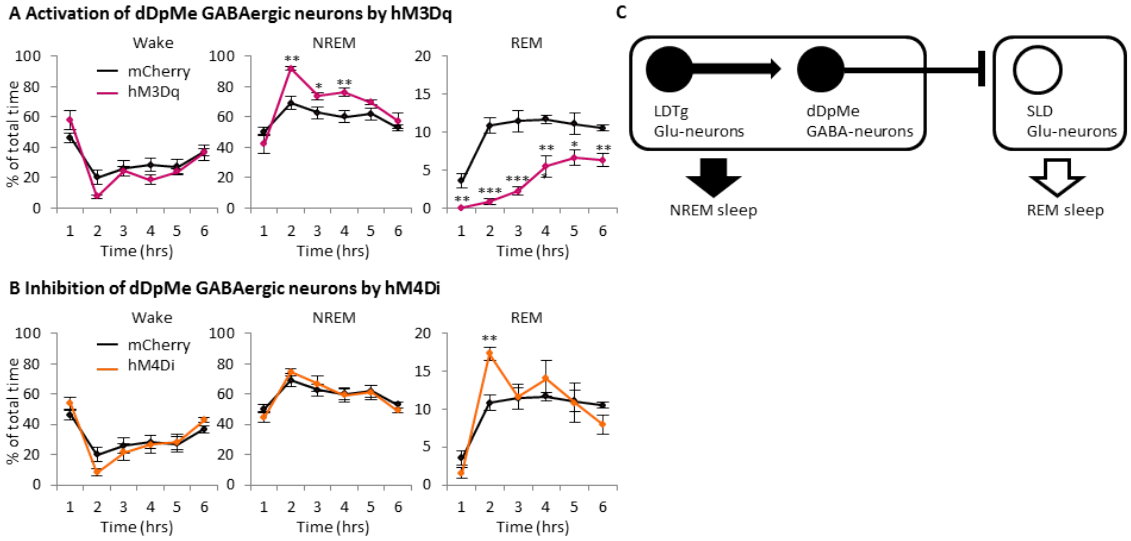



**Figure 12**. Model of the REM/NREM sleep switch based on identification of GABAergic neurons that inhibit REM sleep

[Future directions]

While we successfully identified neurons that constitute the REM/NREM sleep switch, the activity pattern of these neurons during natural sleep remain elusive. To this end, we will combine optogenetics and *in vivo* electrophysiology to elucidate when and how these neurons are activated.

**b) Investigation of the effect of REM sleep on sleep quality control**

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). Sleep deprivation causes a rebound in slow wave activity (SWA) during subsequent NREM sleep and the total time of REM sleep, suggesting that these two components of sleep are important. Indeed, SWA is correlated with growth hormone release during sleep. Moreover, SWA itself contributes to synaptic plasticity and memory consolidation (Chauvette *et al*, *Neuron*, 2012; Marshall *et al*, *Nature*, 2006). By contrast, the function of REM sleep was less understood. Some features of REM sleep, such as its dispersed emergence within NREM sleep, led to proposals that REM sleep somehow contributes to the regulation of NREM sleep quality (Feinberg, *J Psychiat Res*, 1974). Here, we assessed the possibility that

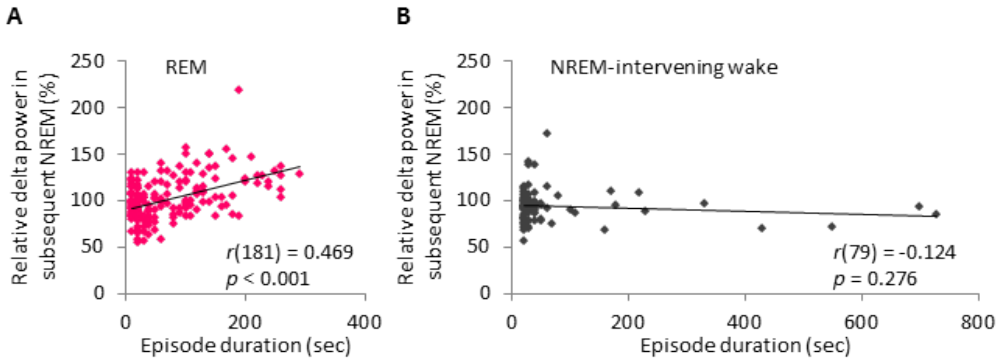



**Figure 13**. Correlation between REM sleep duration and slow wave activity during subsequent NREM sleep

REM sleep enhances SWA during subsequent NREM sleep. First, we observed a significant positive correlation between the episode duration of REM sleep and delta power in the subsequent NREM sleep (Figure 13A).

During the late light phase, where all SWA data were collected, NREM sleep episodes were occasionally intervened by wake episodes, which were relatively short not exceeding ~13 minutes. In case of these NREM-intervening wake episodes, no obvious correlation was observed between episode duration and subsequent delta power (Figure 13B). Thus, in contrast to the well-known SWA enhancing effect of long wake periods lasting several hours (Suzuki *et al*, *PNAS*, 2013), the NREM-intervening episodes observed here, which were relatively short, were unable to increase SWA.

To further test a causal relation between REM sleep occurrence and NREM sleep SWA enhancement, we examined the effect of non-disturbingly manipulating REM sleep by pharmacogenetic activation or inhibition of our identified sleep regulatory circuit. As a result, prolonged REM sleep was followed by enhanced SWA in subsequent NREM sleep, whereas shortened REM sleep was followed by reduced SWA in subsequent NREM sleep. Our results are in part consistent with a study showing that selective REM sleep deprivation in humans reduces SWA during NREM sleep (Beersma *et al*, *Electroencephalogr Clin Neurophysiol*, 1990). A unique cortical activity or neuromodulator release mode during REM sleep might underlie the strong enhancement of SWA during ensuing NREM sleep.

**[Future directions]**

Based on our findings that REM sleep enhances SWA, we will next address the underlying mechanism. We will first focus on adenosine, as it is well established to affect SWA. Currently, there are no tools that allow measurement of adenosine levels *in vivo* at a high temporal resolution. To this end, we will establish culture cell lines that express

fluorescent indicators of adenosine, and transplant these cells to mouse brains for *in vivo* monitoring of adenosine release. This might help understand not only to the function of REM sleep but also a long-lasting mystery of the source of adenosine in the neocortex.

## (8) Shimizu Lab

### a) Study of symptomatic narcolepsy

We have been studying symptomatic narcolepsy from 2001. Fifty cases of narcolepsy due to medical conditions with low orexin levels are selected from 345 cases reported in the literature and our database. The percentage of each medical condition was displayed (Figure 14). (a) Tumors (n = 7, 14%), demyelinating disorders (NMO (n = 6, 12%), MS (n = 3, 6%), ADEM, (n = 3, 6%), genetic/congenital disorders (n = 8, 16%) and neuro-degeneration (n = 8, 16%) are the four most frequent causes. Several categories showed distinct hypothalamic lesions (A, n = 22, 44%), including tumors and demyelinating disorders, while genetic/congenital disorders (PWS, NPC, MYD), neuro-degeneration, paraneoplastic autoimmune syndromes (anti-Ma associated encephalitis) and head trauma did not show distinct lesions (B, n = 28, 56%) (Kanbayashi, 2015 in press).

Among inherited disorders, Niemann-Pick type C (NPC), Myotonic dystrophy type 1 (MYD) and Prader-Willi syndrome (PWS) were mainly reported. PWS is an acquired neurodevelopmental disorder associated with chromosome 15q11-q13. Patients with

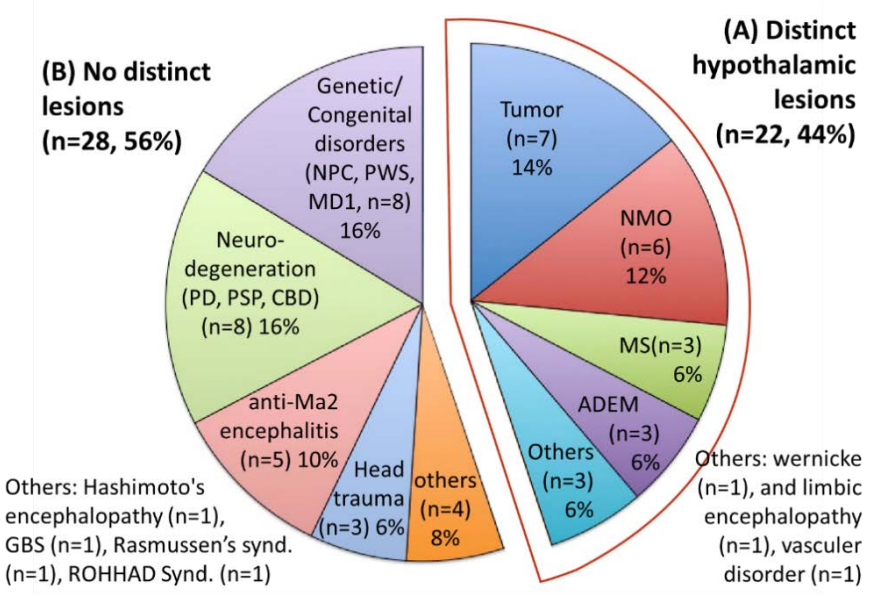



**Figure 14**. Symptomatic narcolepsy due to medical condition with low orexin (n = 50)

PWS often exhibit excessive daytime sleepiness (EDS), excessive appetite, and obesity. As well as in narcolepsy, orexin may be responsible for these symptoms. However, reports regarding CSF orexin levels in PWS patients have been limited. The aim of this study was to examine the relationship between these characteristic symptoms of PWS and the CSF orexin levels. We clinically identified 14 PWS patients, and examined their CSF orexin levels. A total of 37 narcoleptic patients and 14 idiopathic hypersomnia patients were recruited for comparison. CSF orexin levels in the 14 PWS patients were intermediate (192 pg/ml), higher than those in the narcoleptic patients (40 pg/ml), and lower than those in the idiopathic hypersomnia patients (280 pg/ml). Body mass index of the PWS patients (31.5) was higher than in the narcoleptic (22.5) and idiopathic hypersomnia patients (22.3). There was a negative correlation between the Epworth Sleepiness Scale scores and orexin levels in the PWS patients, but not in the narcoleptic and idiopathic hypersomnia patients. These results suggested that the severity in obesity and EDS in PWS patients may be partially, but not fully, due to disrupted orexin levels (Omokawa 2015 SLEEP abstract).

NPC is an autosomal recessive and congenital neurological disorder characterized by the accumulation of cholesterol and glycosphingolipids in the peripheral tissues and of the glycosphingolipids in the brain. Some cases frequently display narcolepsy-like symptoms, including cataplexy. Treatment by Miglustat was started from 2012 in Japan. This is only one curative treatment for NPC.

The subjects were 8 patients with NPC (4 Male and 4 Female). Two cases were low orexin levels (<110pg/ml), 3 cases were intermediate (110-200pg/ml), 3 cases were normal. Four subjects having cataplexy had low or intermediate orexin levels. Three patients were treated by Miglustat. Before medication, one case with cataplexy had intermediate orexin level, and two other cases without cataplexy had normal levels. After 1 year treatment, all three cases got better their symptoms. Two cases with normal range have no change about the orexin levels, but in one case with intermediate orexin level increased the concentration to the normal and her cataplexy was disappeared. Cataplexy and orexin measurement would make a chance to early diagnose and treatment for NPC. It is also suggested that Miglustat is effective in NPC, especially in young and onset early stage of patients (Imanishi 2014, 2015 SLEEP abstract).

**[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.

In addition, we are studying the clinical effects of orexin antagonist (Belsomra) for insomnia patients.

### b) Basic research

We have shown that a simple piezoelectric transducer PZT system is useful as a non-invasive sleep and behavior monitoring system to analyze the developmental aspects of sleep and movement disorders in mice models (Sato 2014, *Exp Neurol*).

## (9) Nagase Lab

The dysfunction of orexin neuronal system leads to serious sleep disorder, narcolepsy. The small molecules showing agonist activity for orexin receptors (OX1R, OX2R) have been expected as therapeutic agent for narcolepsy. We have been trying to discover the novel candidates for orexin receptor agonist and synthesized over 2,000 synthetic samples, which were originally designed based on the structure of the hit compound found in Yanagisawa Lab. Recently, we discovered a water-soluble OX2R selective agonist YNT-185 ($EC_{50}$ = 28 nM; selectivity ratio to OX1R over 100 times, Figure 15) and the icv injection of YNT-185 to wild-type mice significantly induced arousal from sleep in a dose-dependent manner. Moreover, we successively applied YNT-185 to narcoleptic model mice. The icv injection of YNT-185 (260 nmol in 6 µL) to orexin knockout (OXKO) mice improved the frequency of chocolate induced sleep-onset REM periods (SOLEMPs) (Figure 16), but not in orexin receptor double knockout (DKO) mice. Furthermore, the ip injection of the compound (40 mg/Kg) to OXKO mice showed significant decrease of narcoleptic attack in dark phase but not in DKO mice. These data indicated that YNT-185 would be a lead compound for further development of orexin receptor agonist.

In the next stage, we have tried further improvement of agonistic activity for reduction of applied dose (40 mg/Kg). We reinvestigated the detailed structure-activity relationship

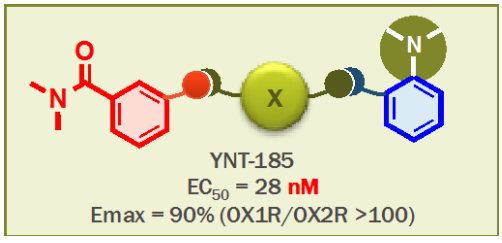



**Figure 15**. Structure of YNT-185.

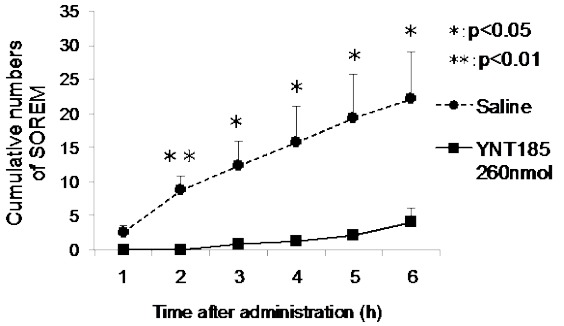



**Figure 16**. The effect of icv injection of YNT-185 on cumulative numbers of SOREMP.

including the evaluation of antagonistic activities for orexin receptors and found that YNT-185 and its structurally related compounds showed weak antagonistic activity counteracting to agonistic activity for orexin receptor (partial agonistic activity). These results suggested that the flexible partial structure of YNT-185 contributes to antagonistic activity and it might consequently disturb the conformational change of receptor protein. Finally, by substitution of the flexible structure for rigid scaffold, we have achieved to obtain a series of more potent full agonists such as YNT-529 ($EC_{50}$ = 7.5 nM) and YNT-530 ($EC_{50}$ = 8.9 nM).

**[Future direction]**

We are going to improve not only pharmacodynamics but also pharmacokinetics such as the penetration of blood-brain barrier and ADME for the development of orexin receptor agonist. The forecasting calculation of physicochemical properties would be introduced for effective drug design.