様　式　Ｃ－１９、Ｆ－１９、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２７　年　　６　月　　１　日現在

機関番号：　１２１０２
研究種目：　基盤研究(B)
研究期間：　2012～2014
課題番号：　２４３００１２９

研究課題名（和文）睡眠覚醒における線条体淡蒼球系の役割

研究課題名（英文）Identification of a striatopallidal pathway facilitating sleep and wakefulness

研究代表者
ラザルス　ミハエル（Lazarus, Michael）

筑波大学・国際統合睡眠医科学研究機構・准教授

研究者番号：８０４６９６５０

交付決定額（研究期間全体）：（直接経費）　　14,800,000円

研究成果の概要（和文）：我々はこれまでにカフェインの覚醒効果が側坐核殻部のA2A受容体に依存することを見出した。本研究では側座核A2A受容体の睡眠覚醒調節における役割を調べた。A2A受容体作動薬CGS21680は野生型マウスのノンレム睡眠を増加させたが、側座核特異的A2A受容体欠損マウスの睡眠を変化させなかった。光遺伝学および薬理遺伝学的手法による側座核A2A神経の活性化は、強力にノンレム睡眠を誘発した。これらの結果は側坐核のA2A受容体が睡眠覚醒調節の重要な構成要素であることを強力に示唆する。さらに、腹側線条体が行動プロセスによって睡眠覚醒が調節されるための重要領域である可能性を示唆する。

研究成果の概要（英文）：We have demonstrated that caffeine induces wakefulness by blocking the action of adenosine on A2A receptors (A2AR) in the nucleus accumbens (NAc; Lazarus M, et al., J Neurosci, Vol. 31, No. 27, 2011, pp. 10067-10075). Adenosine promotes sleep, however the extent to which these A2AR-positive NAc neurons contribute to sleep regulation was previously unknown. Pharmacological activation of A2ARs by the agonist CGS21680 increased non rapid eye movement (NREM) sleep in wild type, but not NAc-specific A2AR KO mice. Optogenetic and pharmacogenetic activation of NAc A2AR neurons induces robust NREM sleep. Our observations provide the first direct evidence that A2AR neurons in the NAc are not only involved in promoting behavioral inactivity but also play a major role in regulating sleep. These findings further suggest that the NAc might be a key site through which sleep and wakefulness are regulated by behavioral processes (Lazarus M, et al., Trends Neurosci, Vol. 35, No. 12, 2012, pp. 723).

研究分野：　Systems Neurobiology

キーワード：　Sleep　Optogenetics　Pharmacogenetics　Adenosine　Nucleus Accumbens　Caffeine　Adeno-associated virus

１．研究開始当初の背景

The fundamental governing principles for the regulation of sleep are incompletely understood and neural substrates that, when activated, promote non-rapid eye movement (non-REM, NREM) sleep, also known as slow wave sleep, remain to be identified. 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. We have revealed that caffeine induces wakefulness by blocking adenosine A2A receptors (A2AR) in the nucleus accumbens (NAc; Lazarus M, et al. J Neurosci 31, 10067–10075, 2011). Adenosine promotes sleep and for caffeine to be effective as an antagonist and cause arousal, excitatory A2AR must be tonically activated by endogenous adenosine. A2AR are highly expressed on neurons of the NAc that also express dopamine D2 receptors (D2Rs) and enkephalin (Enk), but the extent to which those A2AR/D2R/ Enk-positive neurons in the NAc contribute to the regulation of sleep is not known.

２．研究の目的

We proposed to identify the organization of the neural circuitry in which locomotion and motivational behavior drives arousal through the reciprocal interaction of somnogenic adenosine and wake-promoting dopamine on striatopallidal neurons of the striatum. By combining a new generation of powerful tools for the remote control of neuronal activity through in-vivo stimulation and inhibition with the sleep bioassay system established in our laboratory, we aim to determine the basal ganglia control of sleep-wake regulation. This area of research is currently considered as a rapidly emerging field in neuroscience. Especially, we proposed to identify a striatopallidal pathway from the nucleus accumbens to basal forebrain and midbrain areas through which adenosinergic excitatory neurotransmission induces sleep.

３．研究の方法

Significant research efforts have recently been directed at developing genetic-molecular tools to achieve reversible and cell-type specific *in vivo* silencing of neurons in awake, freely behaving animals. The obvious goal in developing these tools is to help establish a causal relationship between the activity of specific neurons (or neuronal populations) and behavioral and physiological outcomes. One tool that has been developed and come close to achieving the goal of reversible, *in vivo* silencing of neurons is optogenetics technologies. It would not be an exaggeration to say that optogenetics has ushered in a new era of neurobiology by providing a mechanism that directly links *in vivo* neuronal activity with behavioral and physiological outcomes in freely behaving animals. Another recently developed system permits the selective and “remote” manipulation (activation and silencing) of neuronal activity via all three major GPCR signaling pathways (Gi, Gs and Gq). Termed “designer receptors exclusively activated by a designer drug” (DREADD) these systems involve, broadly speaking, mutant GPCRs that do not respond to their endogenous ligands but are responsive to otherwise inert biological compounds. The usefulness of these systems has been demonstrated in several recent studies (Alexander GM, et al. Neuron 2009, 27; Nawaratne V, et al. Mol. Pharmacol. 2008, 1119).

４．研究成果

(1) **Optogenetic (channelrhodopsin, ChR2) activation of A2AR neurons in the NAc induces robust NREM sleep:** We investigated the role of A2AR neurons in the NAc for sleep-wake regulation by using optogenetic stimulation of channelrhodopsin-2 (ChR2) expressed in A2AR neurons of the NAc (Figure 1). To target ChR2 into A2AR neurons located in the NAc, we used a transgenic mouse, in which Cre-recombinase is expressed under the A2AR promoter (A2AR-Cre; Durieux PF, et al. Nat Neurosci 12, 393-395, 2009) and Cre-recombinase-dependent adeno-associated virus (AAV) carrying ChR2 (AAV-EF1α-DIO-ChR2-mCherry) was stereotaxically injected unilaterally into the NAc of A2AR-Cre mice (NAc-ChR2 mice). We generated group of NAc-ChR2 mice as well as control mice by microinjection of A2AR-Cre mice with AAV containing mCherry (NAc-mCherry mice), and implanted electrodes for electroencephalogram (EEG) and electromyogram (EMG) and cannulae for optical fibers above the NAc. Two weeks after the operation, we stimulated both mouse groups with 30 ms pulses of blue light at a frequency of 4 Hz between 22:00 and 23:00 and simultaneously recorded their EEG and EMG (Figure 1). Robust induction of NREM sleep was observed during the dark period after selective activation of A2AR neurons by blue light (Figure 1), resulting in more than 80% NREM sleep during optogenetic stimulation. We confirmed by immunohistochemistry for the red fluorescent protein mCherry (fused to ChR2) and NeuN that the expression of ChR2 was restricted to the NAc and additionally, that

neuronal cell loss at the injection site due to AAV toxicity was absent (data not shown).

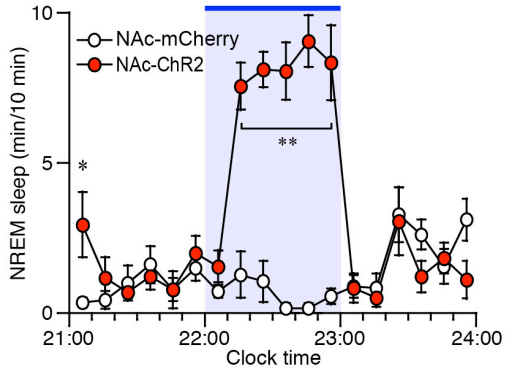

Figure 1. Optogenetic stimulation of A2AR neurons in NAc-ChR2 and NAc-mCherry mice. The time course of NREM sleep. Data are presented as the mean ± SEM (n = 4). *p < 0.05, **p < 0.01 compared between mouse groups, assessed by unpaired Student's t-test. (a and b) Blue bar and area indicates the period of light illumination.

Moreover, after stereotaxic-based brain microinjections of Cre recombinase-dependent AAV carrying DREADD into the NAc of A2AR-Cre mice, robust induction of NREM sleep can be observed during selective activation of A2AR neurons by the small molecule clozapine-N-oxide (data not shown).

(2) **Pharmacological activation of A2ARs by the agonist CGS21680 increased NREM sleep:** When the highly selective A2AR agonist CGS21680 (2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamido-adenosine) is infused into the lateral ventricle of mice, NREM sleep is induced dose-dependently in wild type (WT), but not in global A2AR KO mice. We hypothesized that the sleep-inducing effect of CGS21680 is mediated by A2ARs on neurons in the NAc. We stereotaxically injected bilaterally a Cre-recombinase-expressing AAV into the NAc of loxP-modified A2AR mice to generate NAc-A2AR KO mice ((NAc; Lazarus M, et al. J Neurosci 31, 10067–10075, 2011) and measured sleep after central administration of vehicle or the A2AR agonist CGS21680 (Figure 2). We infused CGS21680 into the lateral ventricle of WT and NAc-A2AR KO mice at 5 pmol/min for 6 hours (20:00 to 2:00) and recorded their EEG and EMG. Typically, NREM sleep strongly increased in the control group (WT mice), but not in the NAc-A2AR KO mice generated by the AAV-Cre microinjection (Figure 2). Induction of NREM sleep in WT mice by the A2AR agonist during the dark period was followed by a rebound of wakefulness during the subsequent light period (data not shown). These findings indicate that A2ARs in the NAc are specifically required for the sleep-inducing effect of A2AR agonist CGS21680.

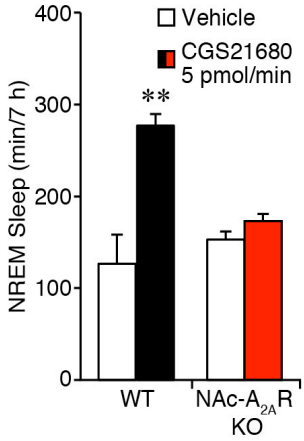

Figure 2. The sleep-inducing effect of A2AR agonist CGS21680 was abolished in NAc-A2AR KO mice. The time course total time of NREM sleep for 7 h after the start of CGS21680 infusion. Data are presented as the mean ±SEM (n = 5). **p<0.01 compared to the vehicle control, as assessed by paired Student's t-test.

(3) **Projections of A_{2A}R NAc neurons to wake-promoting areas:** To better understand the functions of these receptors in sleep, projections of A2AR neurons were mapped utilizing AAV encoding humanized Renilla green fluorescent protein (hrGFP) as a tracer for long axonal pathways (Figure 3).

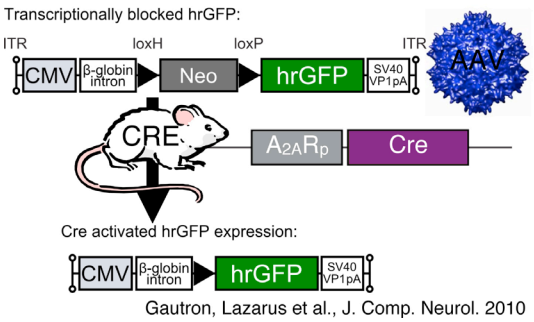

Figure 3. Anterograde tracing of A2AR neurons in the NAc.

The Cre-dependent AAV was injected into the NAc of A2AR-Cre mice. Immuno-histochemistry was then used to visualize hrGFP, highlighting the perikarya of the A2AR neurons in the injection sites, and their axons in projection regions. The data revealed that A2AR neurons exhibit medium-sized and either round or elliptic perikarya with their processes within the NAc. Moreover, the projections from the Acb distributed to wake-promoting nuclei in the forebrain, diencephalon, and brainstem, including the

basal forebrain (substantia innominata and horizontal limb of the diagonal band of Broca), lateral hypothalamus, tuberomammillary nucleus and ventral tegmental area (Figure 4). The results supply a solid base for understanding the roles of the A2AR and A2AR neurons in the NAc in the regulation of sleep (Zhang J, et al. Front Neuroanat 7, 43, 2013).

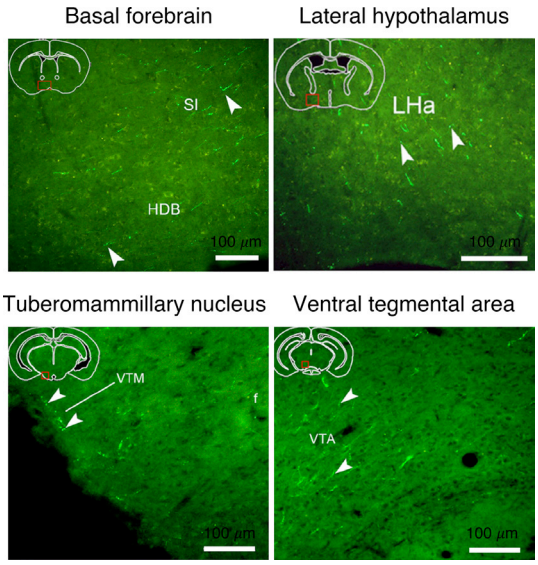

Figure 4. Projections of A_{2A}R NAc neurons to wake-promoting areas. hrGFP-immuno-reactivity in the NAc visualized by fluorescent and ABC techniques. Abbreviations are as follows: SI, substantia innominata; HDB, horizontal limb of the diagonal band of Broca; LHa, lateral hypothalamus;VTM, ventral tuberomammillary nucleus; VTA, ventral tegmental area. Scale bar = 100 μm.

(4) **Conclusion and Significance:** The key neural substrates for sleep remain to be identified. Our findings that activation of A2AR neurons and adenosine receptors in the NAc strongly promote sleep provide the first direct evidence for a major role of the adenosinergic system of the NAc in control of the sleep-wake cycle. A2AR activation may be a novel strategy for the treatment of insomnia and brain-permeable A2AR agonists or allosteric enhancers may constitute a new class of sleeping pills. Moreover, adenosine represents a state of advanced energy consumption due to absence of all energy-bearing phosphate groups of adenosine triphosphate (ATP), the ultimate 'energy currency' of all living organisms, and ATP depletion, elevation of extracellular adenosine and increase of sleep are positively correlated. Adenosine inhibits the activity of cholinergic arousal centers in the brainstem via adenosine A1 receptors under conditions of increased metabolic demand. Adenosine also activates A2ARs on inhibitory medium spiny projection neurons in the NAc to restrain the arousal system and induce sleep. Such sleep prevents cognitive underperformance and excessive emotionality, which may lead to wrong decisions or bad choices. Caffeine that blocks the action of adenosine in the NAc counteracts fatigue and increases alertness; for this reason, caffeine is the most widely used psychoactive compound.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 15 件）

(1) Lopez-Huerta VG, Nakano Y, Bausenwein J, Jaidar O, Lazarus M, Cherassse Y, Garcia-Munoz M, Arbuthnott G. The neostriatum: Two entities, one structure? Brain Structure and Function, 査読有, in press, 2015
DOI: 10.1007/s00429-015-1000-4
(2) Lu A, Zuo C, He Y, Chen G, Piao L, Zhang J, Xiao B, Shen Y, Tang J, Kong D, Alberti S, Chen D, Zuo S, Zhang Q, Yan S, Yuan F, Zhou B, Duan SZ, Yu Y, Lazarus M, Su Y, Breyer R, Funk C, Yu Y. EP3 receptor deficiency attenuates pulmonary hypertension through suppression of Rho/TGF-β1 signaling. Journal of Clinical Investigation, 査読有, Vol. 125, No. 3, 2015, pp. 1228-1242
DOI: 10.1172/JCI77656.
(3) Oishi Y, Yoshida K, Scammell TE, Urade Y, Lazarus M, Saper CB. The roles of prostaglandin E2 and D2 in lipopolysaccharide-mediated changes in sleep. Brain, Behavior and Immunity, 査読有, in press 2015
DOI: 10.1016/j.bbi.2014.11.019.
(4) Yazaki-Sugiyama Y, Yanagihara S, Fuller PM, Lazarus M. Acute inhibition of a cortical motor area impairs vocal control in singing zebra finches. European Journal of Neuroscience, 査読有, Vol. 41, No. 1, 2014, pp. 97-108.
DOI: 10.1111/ejn.12757.
(5) Inutsuka A, Inui A, Tabuchi S, Tsunematsu T, Lazarus M, Yamanaka A. Concurrent and robust regulation of feeding behaviors and metabolism by orexin neurons. Neuropharmacology, 査読有, Vol. 85, 2014, pp. 451-460
DOI: 10.1016/j.neuropharm.2014.06.015.
(6) Mogk S, Meiwes A, Shtopel S, Schraermeyer U, Lazarus M, Kubata B, Wolburg H, Duszenko M. Cyclical Appearance of African Trypanosomes in the Cerebrospinal Fluid: New Insights in How Trypanosomes Enter the CNS. PLoS ONE, 査読有, Vol. 9, 2014, e91372

DOI: 10.1371/journal.pone.0091372
(7) Kaneko K, Mizushige T, Miyazaki Y, Lazarus M, Urade Y, Yoshikawa M, Kanamoto R, Ohinata K. δ Opioid receptor activation stimulates normal diet intake but conversely suppresses high-fat diet intake in mice. American journal of physiology. Regulatory, integrative and comparative physiology, 査読有, Vol. 306, No. 4, 2014, pp. R265-272
DOI: 10.1152/ajpregu.00405.2013
(8) Kaushik MK, Aritake K, Kamauchi S, Hayaishi O, Huang ZL, Lazarus M, Urade Y. Prostaglandin D2 is crucial for seizure suppression and postictal sleep. Experimental Neurology,査読有, Vol. 253, 2014, pp. 82-90
DOI: 10.1016/j.expneurol.2013.12.002
(9) Xu Q, Xu XH, Qu WM, Lazarus M, Urade Y, Huang ZL. A mouse model mimicking human first night effect for the evaluation of hypnotics. Pharmacology, Biochemistry and Behavior, 査読有, Vol. 116, 2014, pp. 129-136
DOI: 10.1016/j.pbb.2013.11.029
(10) Zhang J, Xu Q, Yuan X, Cherasse Y, Schiffmann SN, De Kerchove D'Exaerde A, Qu W, Urade Y, Lazarus M, Huang ZL, Li R. Projections of nucleus accumbens adenosine A2A receptor neurons in the mouse brain and their implications in mediating sleep-wake regulation. Frontiers in Neuroanatomy, 査読有, Vol. 7, 2013, 43
DOI: 10.3389/fnana.2013.00043
(11) Zhang J, Zou F, Tang J, Zhang Q, Gong Y, Wang Q, Shen Y, Xiong L, Breyer RM, Lazarus M, Funk CD, Yu Y. COX-2-Derived PGE2 Promotes Injury-Induced Vascular Neointimal HyperplasiaThrough the EP3 Receptor. Circulation Research, 査読有, Vol. 113, 2013, pp. 104-114
DOI: 10.1161/ CIRCRESAHA.113.301033
(12) Kaur S, Pedersen NP, Yokota S, Hur EE, Fuller PM, Lazarus M, Chamberlin NL, Saper CB. Glutamatergic Signaling from the Parabrachial Nucleus Plays a Critical Role in Hypercapnic Arousal. Journal of Neuroscience, 査読有, Vol. 33, 2013, No. 18, pp. 7627-7640
DOI:10.1523/JNEUROSCI.0173-13.2013.
(13) Lazarus M, Chen J-F, Urade Y, Huang Z-L. Role of the basal ganglia in the control of sleep and wakefulness. Current Opinion in Neurobiology, 査読有, Vol. 23, 2013, No. 5, pp. 780-785
DOI: 10.1016/j.conb.2013.02.001
(14) Lazarus M, Huang Z-L, Lu J, Urade Y, Chen J-F. How Do the Basal Ganglia Regulate Sleep-wake Behavior? Trends in Neuroscience 査読有, Vol. 35, 2012, No. 12, pp. 723-732
DOI: 10.1016/j.tins.2012.07.001
(15) Kaneko K, Lazarus M, Miyamoto C, Oishi Y, Nagata N, Yang S, Yoshikawa M, Aritake K, Furuyashiki T, Narumiya S, Urade Y, Ohinata K. Orally Administered Rubiscolin-6, a δ Opioid Peptide Derived from Rubisco, Stimulates Food Intake via Leptomeningeal Lipocallin-type Prostaglandin D Synthase in Mice. Molecular Nutrition and Food Research, 査読有, Vol. 56, 2012, No. 8, pp. 1315-1323
DOI: 10.1002/mnfr.201200155

〔学会発表〕（計 14 件）

(1) Lazarus M. Why coffee wakes us up? – Role of adenosine A2A receptors in the nucleus accumbens for sleep-wake regulation. 6th World Congress on Sleep Medicine, Symposium, March 21-25, 2015, Seoul, Korea

(2) Lazarus M. The role of the nucleus accumbens in the control of sleep and wakefulness. The 2014 Fujihara Seminar on Homeodynamics in Clock, Sleep and Metabolism, September 24-27, 2014, Shizuoka, Izu, 招待講演

(3) Lazarus M. 光遺伝学と DREADD を用いたカエフィンの覚醒作用メカニズムの解明. Neuroscience 2014 - 37th Annual Meeting of the Japan Neuroscience Society, Symposium, September 11-13, 2014, Kanagawa, Yokohama

(4) Lazarus M. The role of adenosine A2A receptors in the nucleus accumbens for the control of sleep and wakefulness. The 39th Annual Meeting of Japanese Society of Sleep Research, Symposium, July 3-4, 2014, Tokushima, Tokushima

(5) Lazarus M. Why coffee wakes us up? – The role adenosine A2A receptors in the nucleus accumbens for sleep/wake regulation. 8th Congress of the Chinese Sleep Research Society, Beijing, China, 招待講演

(6) Lazarus M. Why coffee wakes us up? – The role adenosine A2A receptors in the nucleus accumbens for sleep/wake regulation. SLEEP 2014 - 28th Annual Meeting of the Associated Professional Sleep Societies, LLC (APSS), Symposium, May 31-June 4, 2014, Minneapolis, USA

(7) Lazarus M. Why coffee wakes us up? - The role of adenosine A2A receptors in sleep-wake regulation. Tsukuba Global Science Week 2013 ~EXPLORING HUMAN SCIENCES~, International Life Science Workshop, October 2-4, 2013, Ibaraki, Tsukuba

(8) Lazarus M. Why coffee wakes us up? - The role of adenosine A2A receptors in the nucleus accumbens for sleep-wake regulation. The 38th Annual Meeting of Japanese Society of Sleep Research, Symposium, June 27-28, 2013, Akita, Akita

(9) Lazarus M. Why coffee wakes us up? - The role of adenosine A2A receptors in the nucleus accumbens for sleep-wake regulation. 36th Annual Meeting of the Japan Neuroscience Society, Symposium, June 20-23, 2013, Kyoto, Kyoto
(10) Lazarus M. Role of adenosine A2A receptors in the nucleus accumbens for sleep-wake regulation. The 7th Congress of Asian Sleep Research Society, Symposium, November 30-December 2, 2012, Taipei, Taiwan
(11) Lazarus M. Is there an intrinsic role of adenosine A2A receptors in the nucleus accumbens for sleep wake regulation? NEUROSCIENCE 2012 - 42nd Annual Meeting of the Society for Neuroscience (SfN), Minisymposium, October 13-17, 2012, New Orleans, USA
(12) Lazarus M. Role of the A2A receptors in the nucleus accumbens in sleep-wake regulation. The 37th Annual Meeting of Japanese Society of Sleep Research, Symposium, June 28-30, 2012 Kanagawa, Yokohama

〔図書〕（計 2 件）
(1) Lazarus M, Urade Y. Adenosinergic regulation of sleep-wake behavior in the basal ganglia. In: Morelli M, Simola N, Wardas J, editors. The adenosinergic system – A non-dopaminergic target in Parkinon's disease. Springer, 2015, in press
ISBN: 978-3319202723
(2) Urade Y, Lazarus M. Prostaglandin D2 in the regulation of sleep. In: Shaw PJ, Tafti M, Thorpy MJ, editors. The Genetic Basis of Sleep & Sleep Disorders. Cambridge University Press, 2013, Ch. 8, pp. 73-84
ISBN: 9781107041257

〔産業財産権〕
○出願状況（計 0 件）
名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計 0 件）
名称：
発明者：
権利者：
種類：
番号：
出願年月日：
取得年月日：
国内外の別：

〔その他〕
ホームページ等:
http://www.wpiiiislazaruslab.org

６．研究組織
(1)研究代表者
ラザルス　ミハエル（Lazarus Michael）
筑波大学・国際統合睡眠医科学研究機構・准教授
研究者番号：80469650

(2)研究分担者
シェラス　ヨアン（Cherasse Yoan）
筑波大学・国際統合睡眠医科学研究機構・研究員
研究者番号：60544319
（Ｈ２５：連携研究者）

大石　陽　（Oishi Yo）
筑波大学・国際統合睡眠医科学研究機構・研究員
研究者番号：10321704
（Ｈ２５-Ｈ２６）

(3)連携研究者
裏出良博（Urade Yoshihiro）
筑波大学・国際統合睡眠医科学研究機構・教授
研究者番号：10201360

高田陽子（Takata Yohko）
筑波大学・国際統合睡眠医科学研究機構・研究員
研究者番号：60435740

カウシク　マヘシュ（Kaushik Mahesh）
筑波大学・国際統合睡眠医科学研究機構・研究員
研究者番号：20648274

ミタムラ　コ　エリザベス
（Mitamura Ko Elizabeth）
大阪バイオサイエンス研究所・研究員
研究者番号：20450249

マリシェフスカヤ　オリガ
（Malyshevskaya Olga）
筑波大学・国際統合睡眠医科学研究機構・研究員
研究者番号：20739429