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研究課題名（和文）Implication of the striatopallidal pathway in Parkinson's disease related sleep disorder

研究課題名（英文）Implication of the striatopallidal pathway in Parkinson's disease related sleep disorder

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研究成果の概要（和文）：背部線条体に投射するドパミン作動性神経を破壊するために側坐核(NAc) もしくは黒質緻密部に6-OHDAを局所投与したC57BL/6マウスの睡眠解析を行うと、著しい睡眠の減少が認められた。Adora2a発現神経が活動的なパーキンソン病モデルマウスを作るために、AAV-DREADD-hM3DqをAdora2a-CreマウスのNAcに注入した。CNO投与によってAdora2a発現神経を活性化すると、NREM睡眠が増加した。しかし、6-OHDAを前処置すると、CNOを投与しても睡眠量は変化しなかった。さらに、NAcのAdora2a発現神経の活性化によって物体認識能が改善されることが明らかとなった。

研究成果の概要（英文）：To annihilate dopaminergic neurons projecting into the dorsal striatum, we stereotaxically micro-injected 6-OHDA into the Nucleus Accumbens (NAc) or the Substancia Nigra pars compacta of C57BL/6 mice. We recorded sleep and observed a dramatic reduction of sleep compared to control mice. This effect is particularly severe during dark phase.
AAV-DREADD-hM3Dq have been injected into the NAc of Adora2a-Cre mice with 6-OHDA to generate a mouse model of Parkinson’s disease (PD) in which Adora2a positive neurons can be activated. In control animals (no 6-OHDA), activation of the Adora2a positive neurons in the NAc leads to an increase of NREM sleep. However, after the elimination of the dopaminergic activation of the NAc Adora2a positive neurons we could not observe anymore such phenotype and the total amount of sleep remained unchanged. Furthermore, CNO injection improved mice ability to recognize previous object in the New Object Recognition test.

研究分野：　neuroscience

キーワード：　Parkinson'd disease　sleep　AAV　Nucleus Accumbens　6-OHDA　Striatum

１．研究開始当初の背景

We expect to define a novel role of the indirect pathway and striatopallidal adenosine A2A receptors (A2AR) in normal striatal integration of motor function, arousal and cognition, and to identify novel strategies for treating sleep disturbance and cognitive impairment in Parkinson's disease (PD) by using A2AR antagonist.

２．研究の目的

Parkinson's disease (PD) is the second most common neurodegenerative disease in Japan and in the world. At age 55 years, the incidence of PD is approximately 20 per 10,000, increasing significantly to 120 per 10,000 at age 70. It is estimated that the number of PD cases will double by 2030, as the population ages and life expectancy increases. Thus, there is critical need for more effective long-term management of PD to maintain the quality of life for patients.

Most research on Parkinson's disease (PD) has focused on defining disease mechanisms and devising treatments for PD's cardinal motor symptoms. However, PD is also characterized by significant cognitive impairment (including prominent early symptoms of cognitive inflexibility) and sleep disturbance. Alleviation of these cognitive and sleep symptoms remains a major unmet need in the clinical management of PD.

Adenosine A2A receptors (A2ARs) are highly expressed in the striatum where they co-localize with dopamine D2 receptors (D2R) in the indirect pathway. Tonic activation of A2ARs by adenosine enhances the activity of striatopallidal GABAergic neurons, ensuring that target regions in the thalamus, hypothalamus, and ultimately cerebral cortex are maintained under a tight inhibitory control. Our central hypothesis is that the A2A receptors in striatopallidal neurons function as a "brake" mechanism on wakefulness and cognitive flexibility in PD models. This is supported by our recent findings: i) Pharmacological or genetic inactivation of A2AR or activation of D2R promotes arousal; ii) Inactivation of striatal A2AR selectively enhances cognitive flexibility (working memory, reversal learning and goal-directed behavior) in normal mice and disease models.

We have developed three novel methods that permit us to control the indirect pathway in the striatum at cellular (striatopallidal neuron) and molecular levels (intracellular A2AR signaling): 1) Two optogenetic transgenic lines that allow light- induced activation or inactivation of the indirect pathway, Adora2a-Cre x channelrhodopsin 2 (ChR2) and Adora2a-Cre x halorhodopsin (Arch), respectively; 2) "designer receptors exclusively activated by a designer drug" (DREADDs) comprised of mutant G protein coupled receptors that respond to otherwise inert compounds to activate (hM3Dq) or inactivate (hM4Di) G protein signaling; and 3) optogenetic control of A2AR signaling with the "OptoA2AR" fusion protein. These tools and our remarkable preliminary finding that optogenetic activation of the indirect pathway reversibly induces sleep within minutes provide a unique opportunity to address the causal role of sleep in modulating cognition in intact animals.

Aim 1: Define the role of the striatopallidal pathway in control of arousal and cognitive flexibility by optogenetic manipulation in normal and PD models. Hypothesis: The striatopallidal indirect pathway exerts inhibitory control that is critical for arousal and cognitive flexibility in PD. Using our Adora2a-Cre x Ai35 (Arch) or Adora2a-Cre x Ai32 (ChR2), or in Adora2a-Cre mice injected with AAV5-DIO-ChR2 using the double-floxed inverted open reading frame (DIO) strategy, we will selectively activate or inactivate the indirect pathway in dorsolateral (DLS), dorsomedial (DMS) and ventral striatum (nucleus accumbens, NAc) and assess arousal and cognition (working memory and reversal learning) in normal mice and PD models. We will i) determine the effect of activating the indirect pathway on cognitive and arousal behaviors in normal mice; ii) evaluate whether silencing the indirect pathway can reverse cognitive and arousal impairments in PD models; iii) investigate how arousal level affects learning and cognition in PD models.

Aim 2: Define the role of the striatopallidal pathway in control of arousal and cognition by pharmacogenetic manipulation of GPCR with the DREADD approach in PD models. We will inject AAV that drive expression of hM3Dq or hM4Di into specific striatal regions of Adora2a-Cre mice. The resulting region-specific expression of these receptors allows us to activate or

inactivate G protein signaling in striatopallidal neurons in distinct subregions of the striatum. This approach will address some limitations of optogenetics (such as lack of applicability to longer neurobiological processes, invasiveness and bulky instrumentation) and allow us to evaluate whether indirect pathway inhibition can reverse cognitive and arousal impairments in 6-OHDA PD models and explore the role of motor initiation/drive in arousal.

3．研究の方法

Over the last two years, we optimized the procedure for AAV injection, histological confirmation, optogenetic manipulation, behavioral testing (for sleep-wake cycle and cognitive flexibility) and molecular analysis of postmortem brains (see preliminary results). We expect to accomplish the specific aims without problems. Given the expertise of Dr. Chen (A2AR neurobiology and cognition and PD) and Dr. Huang (sleep-wake physiology and adenosine neurobiology), optogenetic and pharmacogenetic methods will be performed in both labs, with Chen's lab focusing on cognitive flexibility and Huang's lab on sleep-wake cycle in PD models. Both labs will closely collaborate to determine the interdependence and independence of arousal, cognition and motor activity in PD models.

Aim 1: Optogenetic control of the indirect pathway by Adora2a-Cre x ChR2/Arch mice or AAV-DIO-ChR2. Optogenetic activation and silencing of the indirect pathway by illuminating the subregion of striatum after targeted expression of the Arch/ChR2 expression in the indirect pathway by using the Adora2a-Cre x Ai35/Ai32 mice or by injecting AAV5-DIO-ChR2 into the Adora2a-cre mice. Light-activation (depolarization) and silencing (hyperpolarization) of the striatopallidal neurons during behavioral testing will be achieved by illuminating the striatum with a 100 mW DPSS blue Laser. Preliminary Results: To critically optogenetically activate and silence the indirect pathway in freely behaving animals, the Adora2a-Cre mice (expressing Cre under the control of Adora2a gene regulatory elements) were microinjected with AAV-DIO-ChR2-mcherry and stereotaxically implanted with EEG recording electrodes and the guide cannula. The EEG recording and laser stimulation was performed 2 weeks after the surgery. The results indicated that activation of the indirect pathway in the NAc by blue light stimulation increased NREM sleep in Adora2a-cre mice expressing ChR2 (Fig 2). More recently, we have also developed a novel (Adora2a-Cre x Ai35) transgenic line by crossing Adora2a-creKG139 (expressing Cre under the control of Adora2a gene regulatory elements, (Figure 2D) with the Ai35 (expressing Archaerhodopsin-3, Arch which produces hyperpolarization) or Ai32 line (expressing channelrhodopsin 2, ChR2, which produce depolarization, fused with GFP) to create mice which express Arch or ChR2-GFP selectively in MSNs of the indirect pathway. Light-induced silencing the indirect pathway MSNs in the NAc induced contralateral rotations (Fig. 2E) in Arch-GFP expressing mice. This is in good agreement with a previous study showing that optogenetic activation of the indirect-pathway (using AAV-DIO-ChR2 in D2R-Cre mice) yielded ipsilateral rotations. Predictions and interpretation: The rapid temporal control and reversibility offered by our optogenetic manipulation of the indirect pathway allow us to probe the causal relationship between arousal and reference/working memory as well as reversal learning. The finding of increased NREM sleep amounts and duration correlating with enhanced performance in working memory and reversal learning would provide the direct evidence for the causal role of sleep in promoting cognition. By inducing sleep at different phases of working memory or reversal learning, this would provide novel insight into the specific cognitive domains that are affected by sleep/wake physiology.

Aim 2: Pharmacogenetic control of GPCRs in the indirect pathway by DREADDs: Using Adora2a-Cre line, we will microinject AAV coding for Cre-dependent conditional "DREADD" system into the striatum. This method utilizes modified muscarinic receptors (hM3Dq for excitation and hM4Di for inhibition) that have lost their affinity for endogenous acetylcholine but can still be activated by a synthetic and pharmacologically inert ligand, clozapine-N-oxide (CNO). Preliminary Results: Using Adora2a-Cre mice, mutant muscarinic receptors, (hM3Dq and hM4Di) are expressed solely on the indirect pathway neurons by using stereotaxic microinjection of viral vectors carrying the DREADD systems into the striatum. Figure 3 showed that pharmacogenetic

exciting A2AR-expressing neurons in NAc via hM3Dq by CNO produced a robust increase in NREM sleep in mice. Predictions and Interpretations: We predict that the inhibition of the dorsal striatum by AAV5-DIO-hM4Di will ameliorate sleep and cognitive abnormalities in 6-OHDA mice. The finding of reduced wakefulness in 6-OHDA mice that are reversed by pharmacogenetic inactivation of the indirect pathway would provide the most compelling evidence yet for the indirect pathway in the striatal control of arousal. Pharmacogenetic inactivation of the indirect pathway may also enhance working memory and reversal learning. In fact, similar enhancements of arousal and cognition would suggest their possible link between them. Given demonstrated role of the A2AR in the indirect pathway in NAc in control of caffeine-induced arousal, we expect a major role for the indirect pathway in the NAc and DMS to control arousal and cognitive inflexibility, respectively.

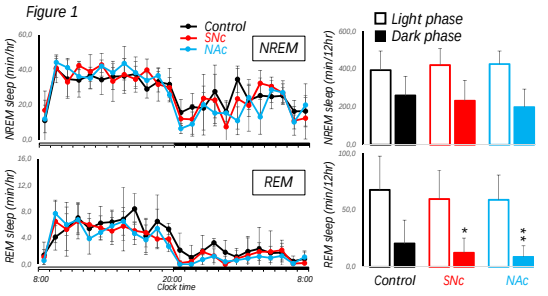
4. 研究成果

Adeno-associated virus production: in order to modulate the activity of neurons located in the dorsal striatum of Adora2a-Cre mice (expressing Cre under the control of Adora2a gene regulatory elements), we successfully generated 800uL of AAV-DIO-eNpHR3.0 (2.5e12 copies/ml) AAV-DIO-ChR2-mcherry (7.2e12 copies/ml), AAV10-DIO-hM3Dq (3.6e11 copies/ml) and AAV10-DIO-hM4Di (2.2e13 copies/ml).

To efficiently annihilate dopaminergic neurons projecting into the dorsal striatum, we stereotaxically micro-injected 6-OHDA (4ug diluted into 1uL of artificial CSF) bilaterally into the Nucleus Accumbens (NAc) or directly into the Substancia Nigra pars compacta (SNc) of C57BL/6 male mice. As a control we also injected mice with vehicle into the SNc.

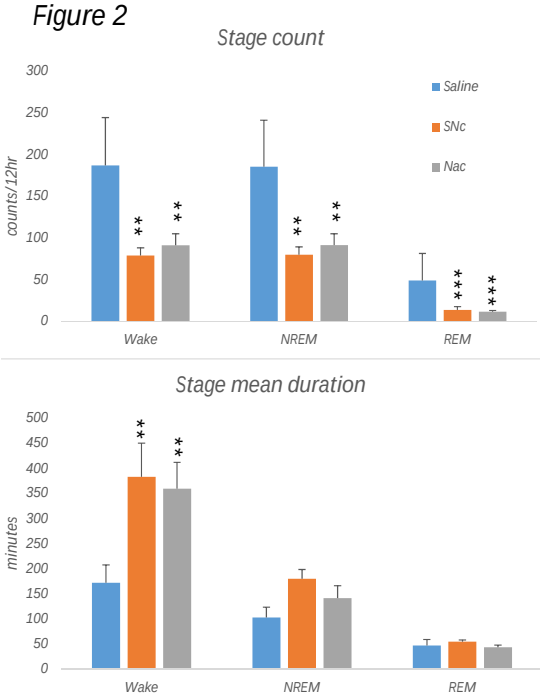
After implantation of sleep recording apparatus, we recorded sleep in these animals and observed a dramatic reduction of the total sleep amount in mice injected with 6-OHDA compared to control mice (figure 1). This effect is particularly severe during dark phase (NREM: -11% in SNc injected mice, -24% in NAc injected mice; REM: -40% in SNc injected mice, -58% in NAc injected mice).

Stages transition analysis reveals that the difference between control animals and



6-OHDA treated animals comes from a reduction of sleep episode numbers (REM: -72% in SNc injected mice, -76% in NAc injected mice) but not duration (figure 2).

Figure 2



All in all, 6-OHDA treated animals show a decrease in sleep and wakefulness episodes fragmentation compared to control animals, resulting in longer episode stages for wakefulness and NREM sleep.

Adeno-associated virus (AAV) expressing the neuron activating "designer receptors exclusively activated by a designer drug" (DREADDs) hM3Dq have been bilaterally injected into the nucleus accumbens (NAc) of Adora2a-Cre mice with 6-OHDA (4ug diluted into 1uL of artificial CSF) to generate a mouse model of Parkinson's disease (PD) in which Adora2a positive neurons can be activated.

After implantation of sleep recording apparatus, we recorded sleep in these animals. In wild type animals, activation of the Adora2a positive neurons in the NAc leads to an increase of NREM sleep. However, after the elimination of the dopaminergic activation of the NAc Adora2a positive neurons we could not observe anymore such phenotype and the total amount of sleep remained unchanged after saline or CNO

injections (0.1, 0.3 or 1mg/kg). We may hypothesize that the dopaminergic activation of NAc neurons is important for the sleep inducing effect observed after the activation of A2aR neurons in NAc. Furthermore, we tested the memory ability by performing the New Object Recognition (NOR) test on the same mouse model after A2aR positive neurons activation by CNO injection. Compared to saline, CNO injection improved mice ability to recognize previous object: number of activation was reduced by 33.3% (versus 23.1% for saline), time spent near the object was reduced by 44.1% (versus 25.3% for saline) and the latency to the 1st activation was increased by 205% (versus 19% for saline).

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　1　件）
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〔図書〕（計　0　件）

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〔その他〕
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http://urade.wpi-iiis.tsukuba.ac.jp/
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