Implication of the striatopallidal pathway in Parkinson’s disease related sleep disorder

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.

Research field: Neuroscience

Keywords: Parkinson’s disease, sleep, AAV, Nucleus Accumbens, 6-OHDA, Striatum
1. Research Background
Adenosine A2A receptors (A2AR) are highly expressed in normal mice and disease models. The central hypothesis is that the A2A receptors allow us to activate or inactivate the indirect pathway (striatopallidal neuron) and molecular tools to image and manipulate G protein-coupled receptors that respond to adenosine A2AR (DREADDs) comprised of mutant G protein receptors exclusively activated by a designer drug—labeled fusion protein. These tools and our kable preliminary finding that Adora2a—l expression of hM3Dq or hM4Di G protein-coupled receptors allows us to activate or inactivate the indirect pathway, respectively; 2) designer receptors exclusively activated by a designer drug (DREADD) with the OptoA2AR signaling; and 3) optogenetic control of arousal impairments in PD models, inactivation of the indirect pathway, allowing light-induced activation or inactivation of the indirect pathway, reversibly induces sleep within minutes. We expect to define a novel role of the striatopallidal pathway in control of arousal and cognition, and to identify novel strategies for treating sleep disturbance, cognitive and sleep symptoms remains a major unmet need in the clinical management of PD. This is supported by our recent findings: i) Pharmacological or genetic manipulation of GPCR with the DREADD strategy, we will selectively localize with dopamine D2 receptors in the striatum where they drive expression of hM3Dq or hM4Di in normal mice and disease models. We will i) determine the effect of activation of A2ARs by adenosine enhances striatal integration of motor function, cognitive flexibility (working memory, striatal A2AR selectively enhances arousal; ii) inactivation of A2AR or activation of D2R promotes arousal and cognition by pharmacogenetic approach in PD models. We will ii) evaluate whether silencing the reversal learning and goal directed motor symptoms. However, PD is also characterized by significant cognitive impairment (including prominent early cognitive inflexibility) and sleep disturbance. Alleviation of these symptoms of cognitive inflexibility and motor symptoms. Therefore, we have developed three novel methods that can address the causal role of sleep in PD models. We will iii) investigate how arousal level affects reversal learning and cognition in PD models. Hypothesis: The striatopallidal pathway reversibly induces sleep within minutes provide a unique opportunity to address the causal role of sleep in PD models. This is supported by our recent findings: i) Pharmacological or genetic manipulation of GPCR with the DREADD strategy, we will selectively localize with dopamine D2 receptors in the striatum where they drive expression of hM3Dq or hM4Di in normal mice and disease models. We will i) determine the effect of activation of A2ARs by adenosine enhances striatal integration of motor function, cognitive flexibility (working memory, striatal A2AR selectively enhances arousal; ii) inactivation of A2AR or activation of D2R promotes arousal and cognition by pharmacogenetic approach in PD models. We will ii) evaluate whether silencing the reversal learning and goal directed motor symptoms. However, PD is also characterized by significant cognitive impairment (including prominent early cognitive inflexibility) and sleep disturbance. Alleviation of these symptoms of cognitive inflexibility and motor symptoms. Therefore, we have developed three novel methods that can address the causal role of sleep in PD models. We will iii) investigate how arousal level affects reversal learning and cognition in PD models. Hypothesis: The striatopallidal pathway reversibly induces sleep within minutes provide a unique opportunity to address the causal role of sleep in PD models.

2. Research Objectives
Aim 1: Define the role of thalamic, hypothalamic, and mesencephalic dopamine D2 receptors in normal and PD models. This is supported by our recent findings: i) Pharmacological or genetic manipulation of GPCR with the DREADD strategy, we will selectively localize with dopamine D2 receptors in the striatum where they drive expression of hM3Dq or hM4Di in normal mice and disease models. We will determine the effect of activation of A2ARs by adenosine enhances striatal integration of motor function, cognitive flexibility (working memory, striatal A2AR selectively enhances arousal; ii) inactivation of A2AR or activation of D2R promotes arousal and cognition by pharmacogenetic approach in PD models. We will iii) investigate how arousal level affects reversal learning and cognition in PD models. Hypothesis: The striatopallidal pathway reversibly induces sleep within minutes provide a unique opportunity to address the causal role of sleep in PD models. This is supported by our recent findings: i) Pharmacological or genetic manipulation of GPCR with the DREADD strategy, we will selectively localize with dopamine D2 receptors in the striatum where they drive expression of hM3Dq or hM4Di in normal mice and disease models. We will determine the effect of activation of A2ARs by adenosine enhances striatal integration of motor function, cognitive flexibility (working memory, striatal A2AR selectively enhances arousal; ii) inactivation of A2AR or activation of D2R promotes arousal and cognition by pharmacogenetic approach in PD models. We will iv) investigate how arousal level affects reversal learning and cognition in PD models. Hypothesis: The striatopallidal pathway reversibly induces sleep within minutes provide a unique opportunity to address the causal role of sleep in PD models. This is supported by our recent findings: i) Pharmacological or genetic manipulation of GPCR with the DREADD strategy, we will selectively localize with dopamine D2 receptors in the striatum where they drive expression of hM3Dq or hM4Di in normal mice and disease models. We will determine the effect of activation of A2ARs by adenosine enhances striatal integration of motor function, cognitive flexibility (working memory, striatal A2AR selectively enhances arousal; ii) inactivation of A2AR or activation of D2R promotes arousal and cognition by pharmacogenetic approach in PD models. We will iv) investigate how arousal level affects reversal learning and cognition in PD models.
was performed 2 weeks after the surgery. The EEG recording and laser stimulation recording electrodes and the guide cannula were stereotaxically implanted with EEG with AAV regulatory elements) were microinjected Cre under the control of Adora2a. Preliminary Results: To critically test the function of DIO and DIO-cre mice. Light illumination the subregion of striatum with a 100 mW DPSS blue Laser. optogenetically activate and silence the striatopallidal neurons in distinct subregions of the striatum. This approach will address some limitations of indirect pathway neurons by using stereotaxic microinjection of viral vectors carrying DIO-cre KG139 (expressing ChR2) into the striatum. This process for AAV injection, histological analysis of postmortem brains (see preliminary results). We expect to accomplish the specific aims without binary results. We expect to show that target expression of the pathway neurons by using stereotaxic microinjection of viral vectors carrying DIO-cre KG139 (expressing ChR2) into the striatum.

3. 研究的方法

为了研究如何对间接通路的精确控制，我们开发了一种精确的光遗传学方法。这种方法通过使用Cre和Ai35/Ai32转基因线发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表发表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發表發
Stages transition analysis reveals that injected mice (SNc) of C57BL/6 male mice. As a control, we also injected mice with vehicle into the Substancia Nigra pars compacta (SNc) of Adora2a-knockout mice (DIO). After implantation of the Nucleus Accumbens (NAc) or directly into the dorsal striatum, we stereotaxically injected 600 μL of AAV5 ChR2 eNpHR3.0 (2 × 10^12 copies/ml) AAV10 hM3Dq (3.6 × 10^11 copies/ml) and AAV10 hM4Di (2.2 × 10^13 copies/ml) AAV-associated virus into the dorsal striatum by a designer drug (DREADDs) hM3Dq have been used to exclusively activate Adora2a receptors in the neuron activating designer drug.

Pharmacogenetic inactivation of the Adora2a gene regulatory domain in the dorsal striatum of Parkinson’s disease (PD) in which Adora2a located in the dorsal striatum of control animals and observed a dramatic reduction in the NAc in control of caffeine induced inflexibility, respectively.

The finding of reduced wakefulness in OHDA treated animals show a decrease in sleep and wakefulness episodes of the Adora2a positive neurons in the NAc animals. In wild type animals, activation of the NAc Adora2a positive neurons can be activated.

4. 成果

通过小鼠模型和行为实验，研究了Adora2a在PD中的作用。结果表明，Adora2a的失活可以显著减少小鼠的睡眠时间和觉醒时间，导致睡眠质量下降。

表2

<table>
<thead>
<tr>
<th>组别</th>
<th>睡眠时间（min/2hr）</th>
<th>觉醒时间（min/2hr）</th>
</tr>
</thead>
<tbody>
<tr>
<td>对照组</td>
<td>200,000-300,000</td>
<td>40,000-60,000</td>
</tr>
<tr>
<td>OHDA组</td>
<td>0-100,000</td>
<td>100,000-200,000</td>
</tr>
</tbody>
</table>

图2

- Stage count
- Stage mean duration
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).

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