様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ９ 月　 ８ 日現在

機関番号：１２１０２
研究種目：若手研究
研究期間：2019～2021
課題番号：１９Ｋ１９４７９

研究課題名（和文）The elucidation of brain regions and signaling pathways underlying cannabinoid-induced seizures

研究課題名（英文）The elucidation of brain regions and signaling pathways underlying cannabinoid-induced seizures

研究代表者

マリシェフサカヤ　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

交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：本研究開始時の目的は、カンナビノイドによる痙攣発作に関与する脳領域とそのシグナル経路の同定であった。In situハイブリダイゼーションの結果から、視索上核（Supra optic nuleus; SON）が関与していることが示唆された。そこで光遺伝学的手法を用いてSONを活性化したところ、予想に反し痙攣は誘発されなかったが、光刺激時は覚醒時間が延長しNREM、REM睡眠の両方が抑制された。長時間の刺激(4時間以上)においても睡眠は完全に抑制され、刺激後にはリバウンド睡眠が見られた。これらの結果はSONが覚醒状態の誘導や維持に深く関与していることを示している。

研究成果の学術的意義や社会的意義
Investigation of neural circuit underlying arousal effect of SON photostimulation would provide crucial information on sleep-wake circuitry and the role of hypothalamic SON in maintaining wakefulness

研究成果の概要（英文）：Initially, this research project aimed to identify brain regions and signaling pathways underlying cannabinoid-induced seizures. Based on previous in-situ hybridization data we assumed that SO nucleus is involved in cannabinoid convulsive effects and therefore investigated the effect of optogenetic activation of SO nucleus. Unexpectedly, we discovered that bilateral photostimulation of SO nuclei did not induce electrographic seizures, but resulted in increased wakefulness (time in wake) with complete absence of sleep (both NREM and REM) during stimulation. Prolonged photostimulation (4 h and more) resulted in complete absence of sleep and produced massive sleep rebound at the end of stimulation. Prolonged photostimulation (8 h) resulted in the absence of sleep for the first 4 hours and significant decrease of sleep for the rest of photostimualtion time. This suggests that SO nucleus is highly involved in inducing and maintaining arousal state.

研究分野：法医学関連

キーワード：sleep　wakefulness　SON　photostimulation　EEG　sleep rebound

様　式　C-19、F-19-1、Z-19（共通）

1．研究開始当初の背景

In the cannabinoid scientific community there is continuous debate about convulsive potential of cannabinoid agonists. Numerous studies demonstrate increased susceptibility threshold to epileptic seizures (Detyniecki, K. & Hirsch, 2015), while others show some evidences that certain cannabinoids can increase susceptibility to seizure, or trigger first onset seizures in humans (Buonamici, M. et al., 1982; Wade, D. et al., 2006). In our previous work we discovered a seizure-inducing effect for natural cannabinoid Δ^9-THC, and the synthetic cannabinoid JWH-018 (2.5 mg/kg) in mice (Malyshevskaya et. al, Sci. Rep., 2017). These cannabinoid-induced seizures are mediated by CB_1R, because pretreatment with a selective CB_1R antagonist prevents seizure development and CB_1RKO mice are resistant to the cannabinoid convulsive effects. Our preliminary data supported the idea that cannabinoids induce seizures in a very specific manner, because certain brain regions are activated upon cannabinoid administration. Using ISH method we found that *c-fos* (immediate early gene) mRNA gene expression is increased in the hypothalamic paraventricular (PVN) and supraoptic (SO) nuclei, 1 hour after cannabinoid administration. These data implied that SO nucleus is involved in cannabinoid convulsive effects, therefore, during this project we investigated the effect of optogenetic activation of SO nucleus.

2．研究の目的

This research project aimed at identification of brain regions and signaling pathways underlying cannabinoid-induced seizures. Based on c-fos immunostaining data, we aimed at creating “cannabinoid seizure model” mouse by transfecting with Chr2-AAV and further photostimulation of SON (brain region involved in cannabinoid seizures).

3．研究の方法

To create experimental “cannabinoid seizure model” mouse we used photoinduicible AAV-EF1a-hChR2-mCherry-AAV virus and optogenetically activated SON nucleus by means of blue light. Stereotaxic brain microinjections of AAVs in conjunction with optogenetics allows regionally restricted transduction of neuronal cell activation. Finally, for the recording of brain activity, EEG electrodes were placed on the surface of the skull and mounted with the dental cement. EMG electrodes were placed into neck muscles to record muscle activity. After the recovery, we recorded baseline EEG, and then mice were given 10 ms blue light (~460 nm) pulses at 3 Hz for 4 (8) consecutive hours starting from ZT0.

4．研究成果

According to our hypothesis, optogenetic activation of SO nuclei should induce seizures similar to the ones observed after cannabinoid administration. However, unexpectedly, we discovered that bilateral photostimulation of SO nuclei did not induce electrographic or behavior seizures, but resulted in increased wakefulness (time in wake) with massive increase of wakefulness and lost of sleep during stimulation. In detail, prolonged photostimulation (4 hrs~) resulted in decreased NREM sleep time, NREM delta energy, REM sleep and increased wakefulness during stimulation (Fig.1), compared to the baseline. This activation produced massive sleep rebound, that was accompanied by increased NREM sleep amount and delta NREM amount immediately after the end of stimulation. The other parameters, such as behavior or wake state analysis did not show any abnormality. Detailed analysis of EEG spectra did not identify any seizure, suggesting there was no seizure-inducing effect detected. We have also examined the effect of 8 hrs photostimulation (Fig.2), which again resulted in significant

increase of wakefullness with almost absence of sleep for the first 4 hours and significant decrease of sleep for the rest of photostimualtion time (last 4 hrs). The analysis of Mean delta energy showed massive sleep rebound soon after photostimulation finished. Despite the normal behavior of the mice during and after 8-hr stimulation, sleep rebound was still observed during 10 hrs after the end of the photostimulation. This suggest that SON long-term activation disrupts sleep-wake neurocircuit at profound level. The future analysis should include analysis of the next consecutive days following the stimulation.

All together this data suggest that SON is strongly involved in inducing and possibly maintaining arousal state. We unexpectedly discovered completely new, so far unreported function of SO nucleus demonstrating robust wake-promoting result of SON photostimulation. Importantly, the activation of SON results in complete absence of sleep and normal wakefulness, judged by EEG analysis and animal behavior observation. Investigation of neural circuit and mechanisms underlying arousal effect of SON photostimulation would provide crucial information on sleep-wake circuitry and the role of hypothalamic SON in maintaining wakefulness. In future, we are very interested to investigate how activation of SO nuclei produces arousal, what mechanism underlie this phenomenon and to anatomically dissect inputs and outputs of the structures causing the arousal effect.

Fig.1

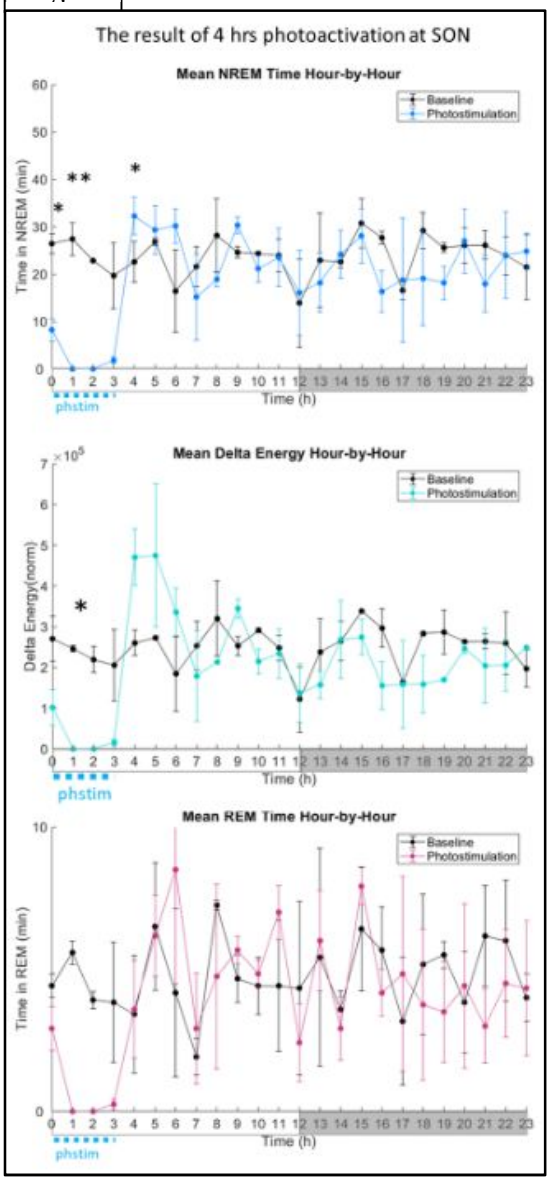
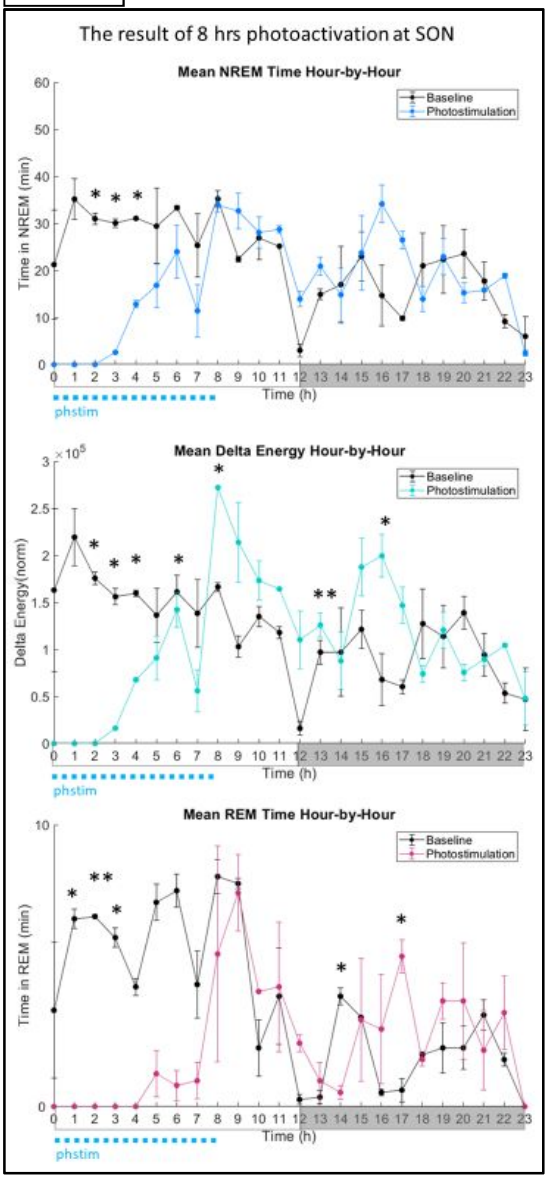


Fig.2



５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関