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研究課題名（和文）Elucidate the neural mechanisms underlying sleep disturbances in neurodevelopmental disorders-focusing on CDKL5 deficiency disorder-

研究課題名（英文）Elucidate the neural mechanisms underlying sleep disturbances in neurodevelopmental disorders-focusing on CDKL5 deficiency disorder-

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交付決定額（研究期間全体）：（直接経費）　　3,400,000円

研究成果の概要（和文）：CDKL5 欠損症（CDD）患者の大多数（>86%）は重度の睡眠障害を抱えている。 しかし、CDD患者の睡眠障害の根底にあるメカニズムについてはほとんど知られていない。 この研究では、Cdkl5 ノックアウト（KO）マウス モデルにおいて、睡眠表現型検査を実施した。 Cdkl5 KO マウスは、非急速眼球運動睡眠に費やす時間が短く、覚醒している時間が長くなり、睡眠エピソードの持続時間が短かった。 さらに、Cdkl5 KO マウスは、EEG パワースペクトルに異常を示した。 Cdkl5 KO マウスのこれらの特徴は、CDD 患者で観察される睡眠パターンおよび異常な脳波に似ています。

研究成果の学術的意義や社会的意義
We performed for the first time the comprehensive sleep characterization of Cdkl5 KO mice. Cdkl5 KO mice recapitulate sleep disturbances and background EEG abnormality in patients with CDD, suggesting that Cdkl5 KO mice can be a good genetic model to study sleep disturbances in CDD.

研究成果の概要（英文）：CDKL5 deficiency disorder (CDD) is a devastating neurodevelopmental disorder caused by pathogenic mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene. The majority (>86%) of patients have severe sleep problems. However, little is known about the mechanism underlying sleep disturbances in CDD patients. In this study we conducted comprehensive sleep phenotyping in a Cdkl5 knockout (KO) mouse model by electroencephalography (EEG) and electromyography (EMG) recording. Cdkl5 KO mice spent less time in non-rapid eye movement sleep and more time awake over a 24-hour period, and showed shorter sleep episode duration compared to their wild-type littermates. Moreover, Cdkl5 KO mice exhibited abnormality in EEG power spectrum. These features in Cdkl5 KO mice are consistent with the sleep patterns and abnormal EEG observed in patients with CDD.

研究分野：動物生理化学、生理学および行動学関連

キーワード：CDKL5　睡眠　マウス　神経発達障害　脳・神経　遺伝学

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１．研究開始当初の背景

CDKL5 deficiency disorder (CDD) is a devastating X-linked neurodevelopmental disorder caused by pathogenic mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene. The occurrence estimates are about one in 40,000 live births. Common features of CDD include early-onset refractory epileptic seizures which begin within days or months of birth, developmental delay, intellectual and motor disabilities, cortical visual impairment, and autistic-like symptoms. Moreover, the majority (>86%) of patients were reported to have severe sleep problems including sleep difficulties, sometimes dubbed "all night parties", sleep fragmentation, frequent awakening and excessive daytime somnolence, which greatly affect the quality of life for patients and their families. However, little is known about the mechanism underlying sleep disturbances in CDD patients.

To tackle this problem, we analyzed sleep/wake patterns in adult male *Cdk15* knockout (*Cdk15*$^{-/Y}$) mice and their wild-type (WT) littermates by electroencephalography (EEG) and electromyography (EMG). *Cdk15*$^{-/Y}$ mice exhibited significantly increased sleep onset latency, less overall sleep time, shorter sleep episode duration in both non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS), and more frequent transitions between wake and NREMS compared to WT littermates (Fig. 1). These results indicate that *Cdk15*$^{-/Y}$ mice have difficulty in falling asleep, sleep less and wake frequently at night, and their sleep is more fragmented. These phenotypes resemble sleep disturbances observed in human CDD patients, suggesting that *Cdk15* knockout mice may be a useful genetic model for studying severe sleep disturbance in CDD patients.

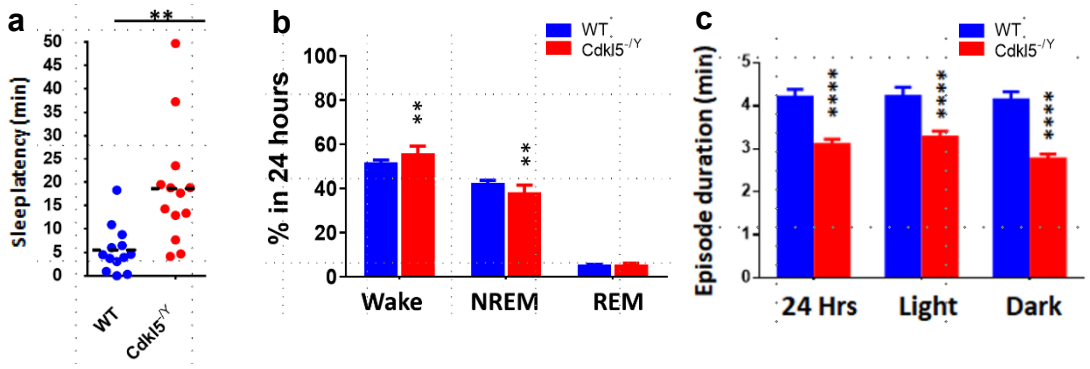

Fig.1. a) Sleep onset latency. b) Time of wakefulness, NREMS and REMS in 24hrs (%). c) Episode durations of NREMS. * P< 0.05, ** P< 0.01, **** P<0.0001.

２．研究の目的

In this study we aimed to: (1) perform comprehensive sleep phenotyping in *Cdk15*$^{-/Y}$ mice including the analysis of sleep architecture and EEG power spectrum, and determine whether *Cdk15* knockout mice can be a good genetic model of sleep disturbances in CDD; (2) investigate the mechanisms underlying sleep abnormalities in CDD by evaluating the sleep regulation function of *Cdk15* knockout mice. The results will provide novel insight into the neuropathology of sleep disturbances in CDD, and will open a new avenue for developing novel and effective therapeutics for sleep problems in CDD patients.

３．研究の方法

(1) Analyze sleep phenotype and EEG power spectrum

Adult *Cdk15*$^{-/Y}$ mice and their littermate controls were stereotaxically implanted with EEG/EMG electrodes under isoflurane. Mice were implanted with an electrode assembly with 4 EEG electrode pins and 2 flexible stainless EMG electrode wires. The EEG electrode pins were placed over the frontal and occipital cortices and fixed to the skull using dental cement. The EMG wires were bilaterally placed into both trapezius muscles. Mice were allowed to recover for at least 7 days before being placed in a recording cage and attached to a recording cable.

(2) Evaluate sleep homeostasis

Sleep is regulated homeostatically, and sleep loss leads to compensatory increases

in sleep duration and intensity. Our preliminary data showed that *Cdkl5*$^{-/Y}$ male mice exhibited overall less sleep time and frequent awakenings (Fig. 1) which could be caused by a dysfunction of sleep homeostasis. We sleep deprived *Cdkl5*$^{-/Y}$ and WT mice for 4 hours to examine the capacity of *Cdkl5* knockout mice to compensate for acute sleep loss by EEG and EMG recording.

(3) Assess circadian function

Sleep is also regulated by the circadian biological clock. *Cdkl5*$^{-/Y}$ mice showed increased sleep latency and fragmented sleep (Fig. 1), which may be attributed to the disruption of circadian rhythms that help to consolidate sleep patterns. To test this possibility, we measured wheel-running activity in Cdkl5 knockout and WT mice. Data were collected for 2 weeks when mice were kept under a 12:12 hr light:dark (LD) cycle, and then the following 2 weeks when mice were kept in constant darkness (DD).

４．研究成果

(1) Analyze sleep phenotype and EEG power spectrum

We analyzed the temporal distribution of sleep and wake within each daily phase in *Cdkl5*$^{-/Y}$ and control mice. *Cdkl5*$^{-/Y}$ mice exhibited significantly more wake time and less sleep time during the transition from dark to light phase (Fig. 2). We also performed EEG power spectral analysis. EEG signal was decomposed into distinct frequency bands, such as low delta (1-2 Hz), delta (1-4 Hz), theta (5-8 Hz), alpha (9-14 Hz) and beta (15-30 Hz), which corresponds to the neuronal activities of different brain regions, and cognitive and mental conditions. *Cdkl5*$^{-/Y}$ mice displayed significant differences in EEG power distribution in both sleep and awake states compared to WT controls. In NREM sleep, *Cdkl5*$^{-/Y}$ mice showed an elevated low delta power across 24 h. In REM sleep *Cdkl5*$^{-/Y}$ mice showed a decreased theta power which correlated with impaired learning and memory. In wakefulness *Cdkl5*$^{-/Y}$ mice showed an increased beta power (Fig. 2). These abnormalities in sleep architecture and EEG power spectrum recapitulate sleep disturbances in patients with CDD.

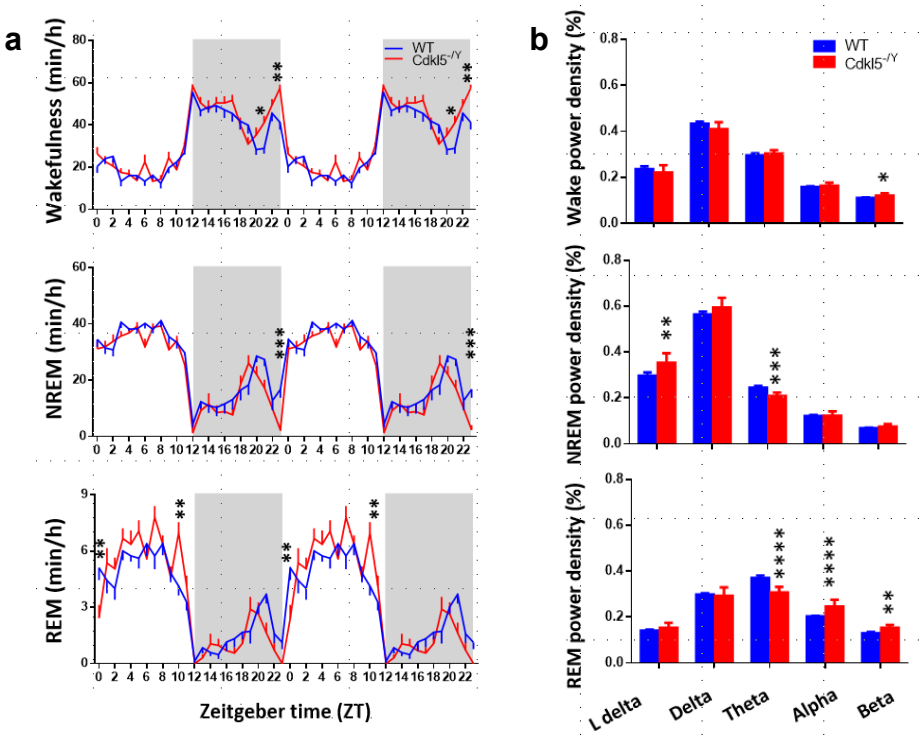

Fig.2. a) Circadian variation in wakefulness, NREMS and REMS. b) Power density during wakefulness, NREMS and REMS.

(2) Evaluate sleep homeostasis

To investigate sleep homeostasis in Cdkl5 KO mice we sleep deprived *Cdkl5*$^{-/Y}$ mice and their WT littermates for 4 h starting at light onset (ZT0-ZT4), and then allowed them to sleep *ad libitum*. After sleep deprivation (SD), the compensatory increase in NREM sleep and REM sleep relative to the baseline period was similar between *Cdkl5*$^{-/Y}$ and WT mice. The same is true for increased NREM delta power in recovery sleep (Fig.

3). These results suggest that *Cdkl5*$^{-/Y}$ mice have normal homeostatic responses to SD.

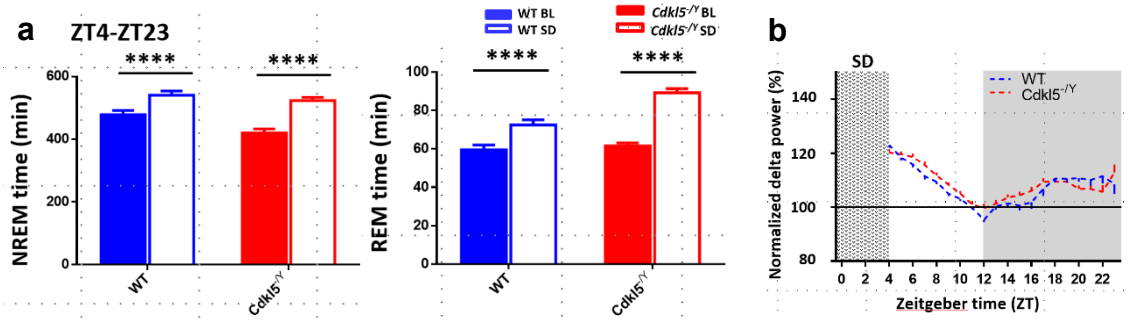

Fig.3. a) The levels of compensatory increase in NREMS and REMS after sleep deprivation (SD) are similar between Cdkl5 KO and WT mice. b) Changes in NREM EEG delta power normalized to their baseline delta power are similar between Cdkl5 KO and WT mice.

(3) Assess circadian function

To assess the circadian phenotype, we placed *Cdkl5*$^{-/Y}$ and their WT littermates in individual cages equipped with running wheels. Under constant darkness, the male *Cdkl5*$^{-/Y}$ showed a circadian period similar to that of their WT littermates (Fig. 4), suggesting that *Cdkl5*$^{-/Y}$ mice appear to have normal circadian rhythmicity.

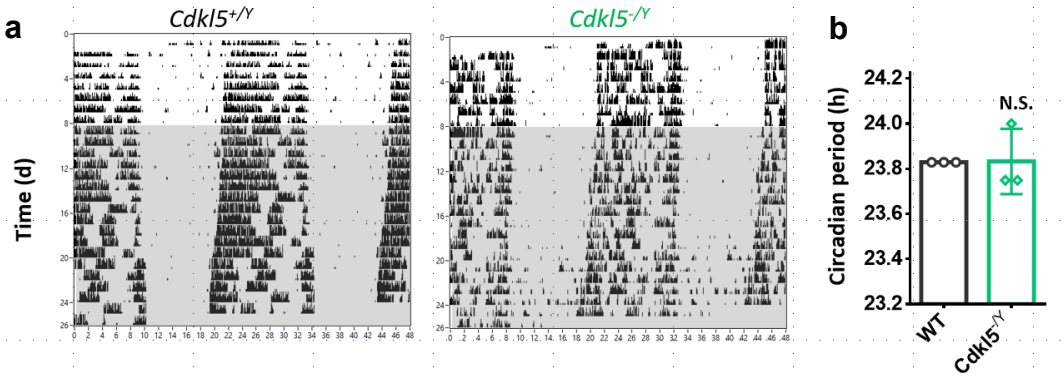

Fig.4. a) Representative double-plotted wheel-running records for male WT and *Cdkl5* KO mice. b) Average circadian free-running periods are similar between WT and *Cdkl5* KO mice.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　0件／うちオープンアクセス　3件）

１．著者名 Tingting Lou, Jing Ma, Zhiqiang Wang, Yuka Terakoshi, Chia-Ying Lee, Greg Asher, Liqin Cao, Zhiyu Chen, Katsuyasu Sakurai, Qinghua Liu	４．巻 14
２．論文標題 Hyper-Activation of mPFC Underlies Specific Traumatic Stress-Induced Sleep-Wake EEG Disturbances	５．発行年 2020年
３．雑誌名 Frontiers in Neuroscience	６．最初と最後の頁 883
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fnins.2020.00883	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Can Liu, Chia-Ying Lee, Greg Asher, Liqin Cao, Yuka Terakoshi, Peng Cao, Reiko Kobayakawa, Ko Kobayakawa, Katsuyasu Sakurai, Qinghua Liu	４．巻 12
２．論文標題 Posterior subthalamic nucleus (PSTh) mediates innate fear-associated hypothermia in mice	５．発行年 2021年
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オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Tomohiko Matsuo, Tomoko Isosaka, Yuichiro Hayashi, Lijun Tang, Akihiro Doi, Aiko Yasuda, Mikio Hayashi, Chia-Ying Lee, Liqin Cao, Natsumaro Kutsuna, Sachihiro Matsunaga, Takeshi Matsuda, Ikuko Yao, Mitsuyoshi Setou, Dai Kanagawa, Koichiro Higasa, Masahito Ikawa, Qinghua Liu, Reiko Kobayakawa, Ko Kobayakawa	４．巻 12
２．論文標題 Thiazoline-related innate fear stimuli orchestrate hypothermia and anti-hypoxia via sensory TRPA1 activation	５．発行年 2021年
３．雑誌名 Nature Communications	６．最初と最後の頁 2074
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-021-22205-0	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計7件（うち招待講演　3件／うち国際学会　7件）

１．発表者名 Liqin Cao
２．発表標題 Altered sleep architecture in CDKL5 KO mouse models & possible clinical applications
３．学会等名 2021 CDKL5 Forum（招待講演）（国際学会）
４．発表年 2021年

１．発表者名
Liqin Cao, Tingting Lou, Xin Zhang, Jing Ma, Zhiqiang Wang, Teruyuki Tanaka, Hiromasa Funato, Qinghua Liu, Masashi Yanagisawa

２．発表標題
Natural history study of sleep disturbances in CDKL5 deficiency disorder mice

３．学会等名
JST-CREST "Optobio" / WPI-IIIS Joint Symposium（国際学会）

４．発表年
2022年

１．発表者名
Liqin Cao, Tingting Lou, Jing Ma, Zhiqang Wang, Teruyuki Tanaka, Qinghua Liu

２．発表標題
Sleep disturbances in young and older CDKL5 knockout mice

３．学会等名
2020 CDKL5 Forum（国際学会）

４．発表年
2020年

１．発表者名
Liqin Cao

２．発表標題
The natural history of epilepsy in CDKL5 KO mouse models.

３．学会等名
2022 CDKL5 Forum（招待講演）（国際学会）

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Liqin Cao, Xin Zhang, Tingting Lou, Teruyuki Tanaka, Qinghua Liu, Hiromasa Funato, Masashi Yanagisawa

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Sleep disturbances in mice with a CDKL5 kinase-dead missense mutation, a novel mouse model of neurodevelopmental disorder

３．学会等名
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2023年

１．発表者名 Liqin Cao, Tingting Lou, Xin Zhang, Jing Ma, Zhiqiang Wang, Teruyuki Tanaka, Hiromasa Funato, Qinghua Liu, Masashi Yanagisawa
２．発表標題 Sleep disturbances in a mouse model of CDKL5 deficiency disorder
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２．発表標題 Sleep disturbances in young and older CDKL5 knockout mice
３．学会等名 2020 CDKL5 Forum（招待講演）（国際学会）
４．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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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