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

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研究課題名（和文）新規睡眠制御分子SIK３シグナルカスケードの同定

研究課題名（英文）Identification the signaling pathway of a novel sleep regulatory molecule SIK3 protein kinase

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：睡眠覚醒に異常を示す二種類のモデルマウスの脳を用い、大規模なリン酸化プロテオミクス解析を行った。二種類のモデルマウスにおいてリン酸化状態が同じであるタンパク質を80種類同定し，これらをSleep-Need-Index-PhosphoProteins (SNIPPs)とした。SNIPPsのリン酸化は、SIK3遺伝子に変異があり、過度の眠気を示すSleepyマウスでも認められた。SIK3の活性抑制はSNIPPsのリン酸化を減少させた，眠気の指標である周波数の低い脳波の減少が認められた。本研究の結果から，SNIPPsのリン酸化/脱リン酸化のサイクルは睡眠覚醒の恒常性を制御していると考えられた。

研究成果の学術的意義や社会的意義
For the first time, our results suggest that phosphorylation of SNIPPs accumulates and dissipates in relation to sleep need, and the phosphorylation dephosphorylation cycle of SNIPPs represent a major regulatory mechanism underlies sleep wake homeostasis.

研究成果の概要（英文）：We performed quantitative phosphoproteomic studies of whole mouse brains from two models of sleep/wake perturbation. A combined proteome and phosphoproteome data for 9,410 mouse proteins and 62,384 phosphopeptides were examined. Comparison of two models identifies 80 mostly synaptic Sleep-Need-Index-PhosphoProteins (SNIPPs), whose phosphorylation states closely parallel changes of sleep need. Mutant SLEEPY/SIK3 kinase preferentially associates with and phosphorylates SNIPPs. Inhibition of SIK3 activity reduces phosphorylation state of SNIPPs and slow wave activity (SWA) during non-rapid-eye-movement sleep (NREMS), the best known measurable index of sleep need, in both Sleepy and sleep-deprived wild-type mice. Our results suggest that SNIPPs accumulate/dissipate phosphorylation as the molecular substrate of sleep need.

研究分野：sleep biology

キーワード：Sleepy　sleep need

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

(1) Sleep and wake globally impact brain physiology, from molecular changes, neuronal activities to synaptic plasticity. The sleep-wake homeostasis is maintained by generation of a sleep need that accumulates during waking and dissipates through sleep. Homeostatic sleep regulation is a global, intrinsic and cumulative process ultimately involving most of brain cells/regions, which is distinct from executive switching between sleep and wake states controlled by specific neural circuits.

We hypothesize that the molecular substrates of sleep need should satisfy four criteria: 1) globally and similarly regulated in most brain cells/regions; 2) accumulate gradually during waking and dissipate through sleep; 3) change in parallel with sleep need in different contexts; 4) gain/loss of functions of itself causes bidirectional changes of sleep need.

(2) To solve the fundamental mystery of sleep, our collaborator Dr. Masashi Yanagisawa's laboratory is conducting an unprecedented electroencephalogram (EEG) and electromyogram (EMG) based forward genetic screen to isolate sleep mutant mice. After screening ~8,000 ethylnitrosourea (ENU) mutagenized mice, they have established multiple heritable mutant pedigrees with strong sleep abnormalities.

Notably, the first "Sleepy" mutant, which carries a dominant splice site mutation and is predicted to result in exon skipping and in-frame deletion of a regulatory region of Sleepy kinase, exhibits the strongest hypersomnia phenotype reported to date (1), manifested by elevated SWA and duration of NREMS.

２．研究の目的

A "holy grail" of sleep research is to identify the molecular mechanism of homeostatic sleep regulation. Sleep homeostasis describes a basic mechanism for regulating sleep need. For example, most humans need 7-8 hours of sleep/day. A sleep deficit elicits a compensatory increase in the intensity and duration of sleep, whereas excessive sleep reduces sleep propensity or need. This mechanism is conserved from flies to human.

I hypothesize that the "sleep need" of the "*Sleepy*" mutant may be constitutively higher than that of wild-type mice. Therefore, the "*Sleepy*" mutant mouse model presents a unique opportunity to identify the molecular substrate of "sleep need".

３．研究の方法

Advances in multiplexed quantitation, such as tandem mass tag (TMT), have recently revolutionized quantitative proteomics. These cutting-edge mass spectrometry technologies have greatly impact many biology areas, such as cancer and stem cell research, but have not been applied to sleep research.

We investigate the molecular basis of sleep need by quantitative phosphoproteomic analysis of whole mouse brain from several sleep models.

４．研究成果

(1) We found sleep deprivation induces cumulative phosphorylation of brain proteome, which dissipates during recovery sleep. Strikingly, Sleepy mutant brains, with constitutively high sleep need despite increased sleep amount, exhibit a hyper-phosphoproteome mimicking sleep deprived brains, owing to a gain-of-function mutation of protein kinase SIK3.

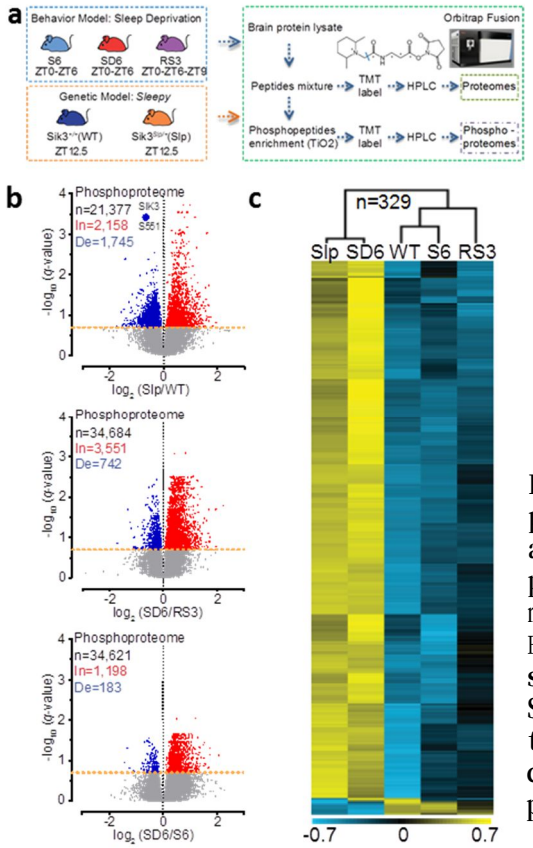

Figure 1 | *Sleepy* brains exhibit hyper-phosphoproteome mimicking sleep deprived brains. a, Experimental design for proteomic/phosphoproteomic analysis of two models (Reprinted with permission of Thermo Fisher Scientific © 2018.) b, Volcano plots showing changes of phosphopeptides in Slp/WT, SD6/RS3, SD6/S6 groups. Multiple unpaired *t*-test (*p*-value) following FDR (*q*-value) analysis. c, Hierarchical cluster analysis of 329 phosphopeptides changed in all three groups.

(2) Comparison of two models identifies 80 mostly synaptic Sleep-Need-Index-PhosphoProteins (SNIPPs), whose phosphorylation states closely parallel changes of sleep need. Mutant SLEEPY/SIK3 kinase preferentially associates with and phosphorylates SNIPPs.

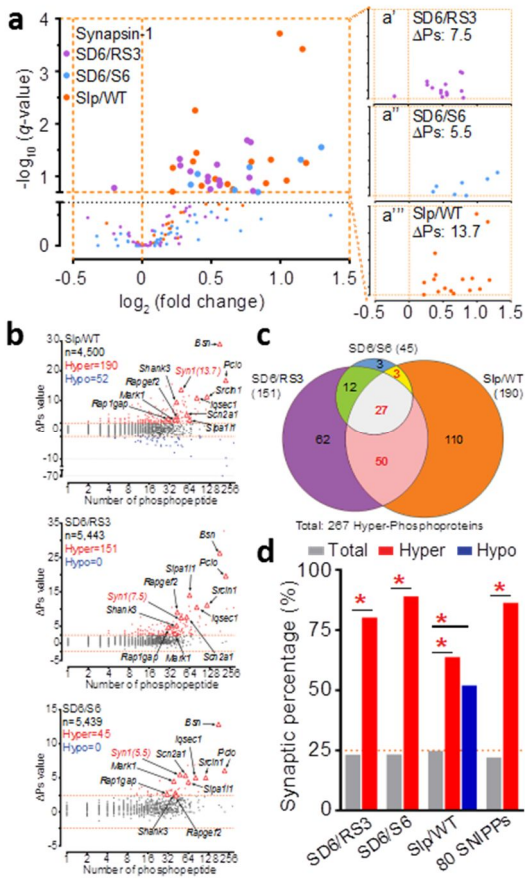

Figure 2 | Phospho-state changes of SNIPPs parallel changes of sleep need. a, Volcano plots of quantified phosphopeptides of Synapsin-1 in SD6/RS3 (violet), SD6/S6 (blue) and Slp/WT (orange) comparisons. Multiple unpaired *t*-test (*p*-value) following FDR (*q*-value) analysis. b, Global ΔPs analysis of phosphoproteins in three comparisons. Dotted lines (ΔPs = +/-2.4). c, A Venn diagram showing overlaps of the Hyper-phosphoproteins (ΔPs > 2.4) between sleep-deprived and *Sleepy* models. d, Percentage of synaptic proteins in total, Hypo-, Hyper-phosphoproteins and 80 SNIPPs. Chi-square test, two-sided. *(red) $P < 0.001$.

(3) Inhibition of SIK3/SLEEPY activity reduces phosphorylation state of SNIPPs and slow wave activity (SWA) during non-rapid-eye-movement sleep (NREMS), the best known measurable index of sleep need, in both Sleepy and sleep-deprived wild-type mice.

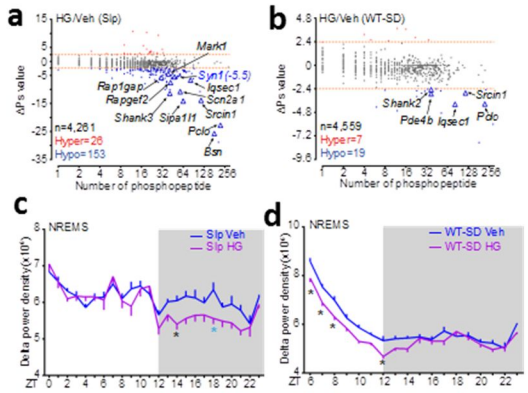

Figure 3 | Inhibition of SIK3 Reduce Phospho-State of SNIPPS and sleep need. a-b, Global ΔPs analysis of HG/Veh (Slp) and HG/Veh (WT-SD) groups. c-d Circadian absolute NREMS delta power analysis of HG/Veh (Slp) (n = 14) and HG/Veh (WT-SD) (n = 16) groups. Mean ± s.e.m., two-way ANOVA, Sidak's (h, j); *(black) $P < 0.05$.

(4) Our results suggest that SNIPPs accumulate/dissipate phosphorylation as the molecular substrate of sleep need. While waking encodes memories by potentiating synapses, sleep consolidates memories and restores synaptic homeostasis by globally downscaling excitatory synapses.

Thus, phosphorylation/dephosphorylation cycle of SNIPPs may represent a major regulatory mechanism that underlies both synaptic and sleep-wake homeostasis.

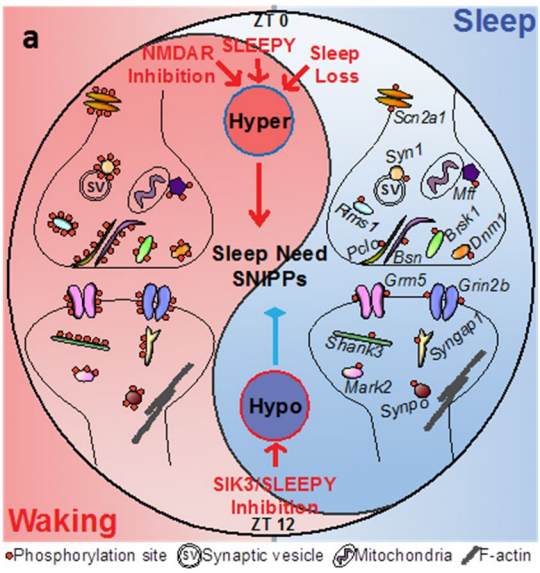

Figure 4 | A molecular model of synaptic homeostasis and sleep-wake homeostasis.

<引用文献>

① Funato, H. et al. Forward-genetics analysis of sleep in randomly mutagenized mice. Nature 539, 378-383 (2016).

(5) In the research period, we also performed a research on post-traumatic stress disorders (PTSD). Sleep disturbances have been recognized as a core symptom of PTSD. However, the neural basis of PTSD-related sleep disturbances remains unclear. It has been challenging to establish the causality link between a specific brain region and traumatic stress-induced sleep abnormality. Here, we found that single prolonged stress (SPS) could induce acute changes in sleep/wake duration as well as short and long-term electroencephalogram (EEG) alterations in the isogenic mouse model. Moreover, the medial prefrontal cortex (mPFC) showed persistent hyper-activity during and immediately after SPS. Chemogenetic inhibition of the prelimbic region of mPFC during SPS could specifically reverse the SPS-induced acute suppression of delta power (1-4Hz EEG) of non-rapid-eye-movement sleep (NREMS) as well as long-term EEG abnormalities. These findings suggest a causality link between hyper-activation of mPFC neurons and

traumatic stress-induced sleep-wake EEG disturbances.

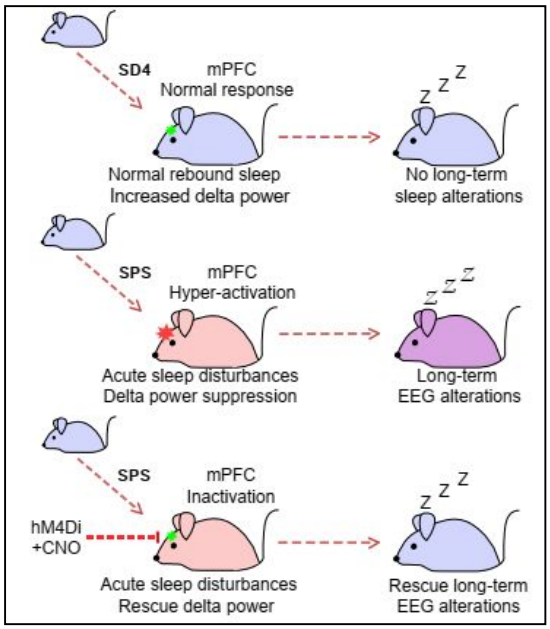

Figure 5 | A model showing that hyper-activation of mPFC contributes critically to the SPS-induced sleep-wake EEG disturbances.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Wang Zhiqiang、Ma Jing、Miyoshi Chika、Li Yuxin、Sato Makito、etc.	４．巻 558
２．論文標題 Quantitative phosphoproteomic analysis of the molecular substrates of sleep need	５．発行年 2018年
３．雑誌名 Nature	６．最初と最後の頁 435～439
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1038/s41586-018-0218-8	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Tingting Lou、Jing Ma、Zhiqiang Wang、Yuka Terakoshi、etc.	４．巻 印刷中
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 -
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〔学会発表〕　計0件

〔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