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研究課題名（和文）Elucidation of the molecular mechanisms of zinc-induced sleep

研究課題名（英文）Elucidation of the molecular mechanisms of zinc-induced sleep

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研究成果の概要（和文）：亜鉛含有率が高くアスタキサンチンを含む食品の、ヒトの睡眠における効能を調べた。120名の健康な被験者にランダム化・二重盲検・プラセボ対照にて並行群間比較試験を行い、アクティグラフで夜間活動を12週間記録した。
試験は、亜鉛含有率の高い食品が睡眠導入を改善し、睡眠の質を高めると示した。この成果をもとに睡眠の質を向上させるサプリメントを開発し、株式会社富士フィルム　ヘルスケアは「オキシバリアすっとねリッチ」として商品化した。
さらに、研究結果の分子構造を理解するためのマウス実験を行い、亜鉛投与により活発化するニューロン群を特定した。これらのニューロンを刺激すると亜鉛投与に類似して睡眠量が増加した。

研究成果の学術的意義や社会的意義
The annual economic loss to Japanese economy due to sleep disorders is equivalent to 2.92% of the GDP, costing approx. 15兆円. Optimizing the effect of zinc-induced sleep will allow us to develop food supplements specifically designed to counteract sleep restriction and improve life quality.

研究成果の概要（英文）：First, we examined the effect of zinc-rich and astaxanthin-containing food on sleep in humans. We conducted a randomized, double-blinded, placebo-controlled parallel group trial of 120 healthy subjects and recorded their night activity by actigraphy for 12 weeks.
The examination demonstrated that eating zinc-rich food improved sleep onset latency as well as improved the sleep efficiency. Based on the results, we developed a food supplement to promote sleep, and Fujifilm Healthcare Corporation released it on the market as “Oxybarrier Suttone Rich”.
In addition, we performed experiments on mice to understand the molecular mechanisms of the examination, and identified a population of neurons in the brain which are activated by zinc administration. Stimulating these neurons permitted to increase the amount of sleep similarly to zinc administration.

研究分野：neuroscience

キーワード：sleep　zinc　actigraphy　chemogenetics

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

With a daily absorption of 8.3mg/day of zinc (National Survey of MEXT, 2005), the Japanese population barely meets with the zinc intake recommendation from The American National Academy of Sciences and the European Food Safety Authority (8mg/day for women, 11mg/day for men). Furthermore, Japanese consume a lot of beans rich in phytates such as nattō. Phytates are known to drastically reduce the bioavailability of zinc and others micronutrients, with the consequence of causing zinc impairments even in healthy Japanese (Sarukura et al., The Journal of Medical Investigation, 2011). In addition, several global surveys recently concluded that Japanese are the most sleep deprived people in Asia.

２．研究の目的

We expect to define a novel role for zinc in the control of sleep/wake regulation. Not only we want to elucidate the molecular mechanisms by which zinc increases NREM sleep, but we also want determine the deleterious effects of a zinc deprivation as observed in the Japanese population. Finally, optimizing the effect of zinc-induced sleep allowed us to develop food supplements specifically designed to counteract zinc deficiency and improve life quality.

- Elucidate the molecular mechanisms by which zinc is inducing sleep

Sleep is controlled by an incredibly complex amount of systems working in a precise space and time-dependent manners. By using in-situ hybridization (ISH) and immuno-histochemistry methods we decided to determine which brain regions are specifically activated or inhibited after an administration of zinc. In a preliminary experiment, ISH experiment of c-fos mRNA in mice 1 hour after zinc administration demonstrated a specific activation of neurons located in 4 brain regions (unpublished data). We wanted next to inject adeno-associated virus (AAV) that drive the expression of mutant G protein coupled receptors (AAV-DREADD) that respond to otherwise inert compounds CNO to activate (hM3Dq) or inactivate (hM4Di) neurons into these 4 specific regions. These tools provide a unique opportunity to decipher the molecular mechanisms behind zinc-induced sleep.

- Nutritional application of zinc-induced sleep: from “food function” to “functional food”

We already demonstrated that 80mg of zinc-containing yeast can increase NREM in mice. However, the dose necessary for such effect is beyond the Tolerable Upper Intake Levels for Zinc and therefore not compatible with human physiology. Therefore it is necessary to optimize zinc efficiency. We recently discovered that Zinc Gluconate ($C_{12}H_{22}O_{14}Zn$) for example is twice more efficient than our published zinc-yeast to induce sleep (unpublished data). We decided to compare the efficiency of different source of zinc, from organic sources (such as oysters extracts, others zinc-yeast extracts) and from inorganic sources.

During preliminary experiments, while testing the combination of zinc-yeast with potential cofactors, we also found that adding some specific sugar alcohol improves zinc efficiency to increase NREM sleep (Saito Hitomi, Ueda Fumitaka, Urade Yoshihiro and Cherasse Yoan, 亜鉛含有睡眠改善剤、ノンレム睡眠時間増加剤及び鎮静剤, patent WO2015099102-PAMPH-404, July 2015). However the mechanisms involved in such effects remain unclear and must be elucidated.

３．研究の方法

The main purpose of this research was to elucidate how zinc regulates sleep.

We first wanted to determine which brain regions are activated or inactivated after zinc administration. Furthermore, we wanted to confirm the role of these regions by specifically regulating their neuronal activity by using inducible channel receptors delivered by a stereotaxical injection of AAV-DREADD. And finally we optimized the potency of zinc to induce sleep and collaborated with Fujifilm Corporation to release a food supplement to improve sleep in Japanese population.

We designed our project in order to be completed within 3 years.

- Molecular mechanisms of sleep regulation by zinc: mapping

The most challenging part of this project was to determine the molecular mechanisms involved in the regulation of sleep by zinc. To do so, we first identified which brain regions are specifically activated by zinc. We time-dependently checked the expression of c-fos after zinc administration to determine the sequential activation of the different brain regions identified. These data did provide us a spatial and temporal information

of how zinc is regulating the brain, and demonstrated the key role of these areas in the regulation of zinc-induced sleep.

- Molecular mechanisms of sleep regulation by zinc: functional analysis

We wanted to confirm the involvement of the identified region by stereotaxically injecting AAV-DTA (AAV driving the expression of the neurotoxin DTA, inducing neuronal death). We also used AAVs expressing a mutant G protein coupled receptors that respond to otherwise inert compounds to activate (hM3Dq) or inactivate (hM4Di) G protein signaling of neurons into the zinc controlled brain regions (unpublished data). Finally, after identifying the brain regions involved in zinc-induced sleep regulation we performed double immunostaining to identify and characterize the neurons and neurotransmitters involved in such mechanisms.

- Nutritional application of zinc-induced sleep

Hypothesis: zinc-induced sleep is proportional its bioavailability. We compared the efficiency of different source of zinc, from organic sources (oysters' extracts, others zinc-yeast extracts) and from inorganic sources (zinc carbonate, zinc sulfate, zinc chloride, etc.) as well as potential cofactors amplifying zinc effects. Furthermore, we compared the concentration of zinc in the blood and in the brain of mice submitted to these different compounds.

We recently discovered that Zinc Gluconate (C12H22014Zn) is twice more efficient than our published zinc-yeast to induce sleep (unpublished data). Based on our previous results, Fujifilm released a food supplement to promote good quality sleep (オキシバリア すっとね®), and planned to release optimized products based on our recent and future discoveries.

4．研究成果

We performed experiments in order to understand the molecular mechanisms involved in the regulation of zinc-induced sleep. We observed an increase of neuronal activation into the a specific brain region following zinc administration. We stereotaxically injected AAVs expressing mutant G protein coupled receptors that respond to otherwise inert compounds to activate (hM3Dq) or inactivate (hM4Di) G protein signaling of neurons into the zinc controlled brain region and activated the infected neurons by administering CNO. Neuronal activation of hM3Dq neurons could efficiently increase non-REM sleep, while the activation of hM4Di could efficiently inhibit the sleep inducing effects of zinc (Figure 1). Together, these results demonstrate the essential role of these neurons in the control of zinc-induced sleep. It is especially remarkable that the activation of zinc sensitive neurons with the activatory DREADD (hM3Dq) could permit to increase the total amount of sleep to the same level than the injection of zinc which allowed the expression of DREADD. Conversely, the inhibitory DREADD (hM4Di) did not completely block the effect of zinc administration. Sleep induction was in fact reduced by 50% after activation of hM4Di. However, it is not entirely unexpected and could be the consequence of the fact that (a) another brain region is involved into the regulation of zinc-induced sleep or (b) we could not express hM4Di in all the neurons responding to sleep. Further experiments will be necessary to clarify these results.

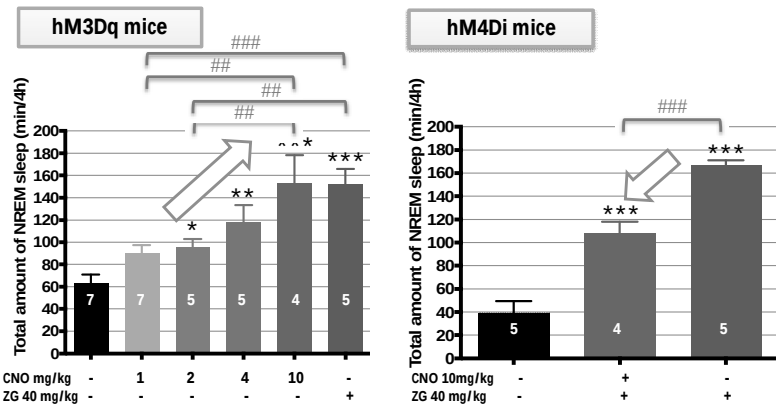

Figure 1: **Chemogenetic regulation of zinc-sensitive neurons modulates sleep and wakefulness**
Expression and activation of stimulating DREADD (hM3Dq, left panel) in the zinc responsive neurons could drastically increase the amount of NREM sleep. Conversely, activation of the inhibitory DREADD (hM4Di, right panel) partially inhibited the sleep-promoting effect of zinc. **p<0.05, **p<0.01, ***p<0.001 compared to vehicle, ##p<0.01, ###p<0.001 between groups.*

Zinc is an essential mineral that plays an important role in the body. We previously reported that orally feeding zinc-enriched yeast to mice induces non-rapid-eye-movement sleep. During our previous experiments, while testing the combination of zinc-yeast with potential cofactors, we also found that adding some specific sugar alcohol improves zinc efficiency to increase NREM sleep (patent WO2015099102-PAMPH-404, July 2015). In both case, we demonstrated with our collaborator Dr Saito (Fujifilm Corporation) that zinc blood concentration was higher compared to their respective controls (unpublished data). In addition, astaxanthin, an antioxidant abundant in seafood such as salmon and krill, is able to chelate minerals and may promote zinc absorption, which in return may also improve sleep. The purpose of our study was to examine the effect of zinc-rich and astaxanthin-containing food on sleep in humans.

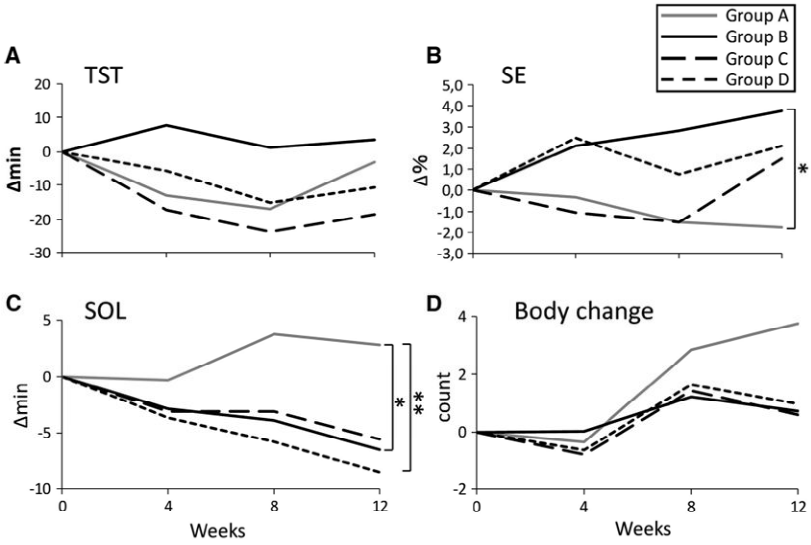

Figure 2: **Zinc-rich and astaxanthin-containing food improved sleep efficiency and sleep onset.** (A) Total sleep time: no significant difference could be observed in the total sleep time of the four groups during the 12 week trial. (B) Sleep efficiency: the group submitted to zinc-rich food (group B) exhibited a statistical improvement of their SE after 12 weeks of treatment compared to the control group A (respectively3.78 ± 7.80 versus −1.74 ± 4.94; p = 0.025). (C) Sleep onset latency: the group submitted to zinc-rich food (group B) or zinc and astaxanthin supplements (group D) exhibited a statistical improvement of their SOL after 12 weeks of treatment compared to the control group A(−6.46 ± 12.29 and −8.57 ± 8.38 versus 2.78 ± 10.55; p = 0.032 and p = 0.004, respectively). (D) The number of body positional changes was lower after 12 weeks of administration of zinc food or supplement (groups B, C, and D) but did not differ significantly from the placebo group A. **p<0.05, **p<0.01 compared to placebo.*

Methods and results: We conducted a randomized, double-blinded, placebo-controlled parallel group trial of 120 healthy subjects and recorded their night activity by actigraphy for 12 weeks. These subjects were divided into 4 groups: placebo (A), zinc-rich food (B), zinc- and astaxanthin-rich food (C), and placebo supplemented with zinc-enriched yeast and astaxanthin oil (D). Compared with the placebo group, the zinc-rich food group efficiently decreased the time necessary to fall asleep and improved sleep efficiency, whereas the group that ingested zinc-enriched yeast and astaxanthin oil significantly improved the sleep onset latency (Figure 2).

Conclusion: Actigraphic sleep monitoring demonstrated that eating zinc-rich food improved sleep onset latency as well as improved the sleep efficiency in healthy individuals.

We then focused on designing a new improved food supplement to promote sleep. It was released on the market as Oxybarrier Suttone Rich by Fujifilm Healthcare Corporation.

５．主な発表論文等

〔雑誌論文〕（計　　4 件）

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# Saito H and Cherasse Y contributed equally to this work

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〔図書〕（計　　0 件）

〔産業財産権〕
○出願状況（計　　0 件）

名称：
発明者：
権利者：
種類：
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出願年：
国内外の別：

○取得状況（計　　0 件）

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〔その他〕
ホームページ等

６．研究組織