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研究課題名（和文）睡眠の「深さ」 の制御機構－徐波睡眠調節を担う皮質ネットワークの解明

研究課題名（英文）How the brain regulates the depth of sleep - Cortical networks for slow wave sleep

研究代表者
Ｖｏｇｔ　Ｋａｓｐａｒ（VOGT, Kaspar）

筑波大学・国際統合睡眠医科学研究機構・准教授

研究者番号：８０７４００３４

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研究成果の概要（和文）：深い徐波睡眠は生命維持に不可欠な現象であるが、徐波と睡眠の恒常性制御機構に関与する神経回路は明らかになっていない。そこで本研究では、DREADDで皮質ニューロンを精密に制御した状態で徐波睡眠を測定し、この神経回路の解明を試みた。
CNO投与はDREADD発現及びコントロール領域の脳波に影響を与えなかった。脳波の相関解析で見られた覚醒・NREM・REM睡眠時の異常に高い相関性は、主に海馬のシータ波由来であると考えられた。そこで新たに双極性局所ワイヤー電極を導入し、記録システムを改善した。その結果、双極性電極のみが真の局所脳波を反映することが明らかとなった。現在、CNO投与実験を再度行っている。

研究成果の概要（英文）：Deep, slow wave sleep is necessary for survival, yet the neural networks underlying slow wave sleep and their homeostatic regulation are still unknown. To find and study these networks, cortical neurons were precisely controlled by DREADDs and subsequent slow wave sleep was measured in affected and control areas.
Initial injections of CNO did not significantly change EEG characteristics in AAV injected and control regions. The pattern of sleep stages also did not change. We presumed the unusually high correlation during waking, NREM and REM sleep to result from volume-conducted signal contamination, mostly from hippocampal theta activity. We changed our recording strategy to ensure that we were recording locally generated signals through bipolar fine wire electrodes. Only bipolar electrodes showed truly local signals. We have now solved these problems and are repeating the experiments with CNO injections.

研究分野：Sleep Research

キーワード：NREM　Slow wave sleep　DREADD　EEG

１．研究開始当初の背景

The longer we are awake, the higher our need for sleep becomes. This homeostatic sleep regulation can be observed by measuring specific frequencies in the EEG. Deep sleep is characterized by a dominance of slow waves (0.4 to 4 Hz) in the EEG. The higher the sleep need (longer prior waking), the more intense these oscillations are once the subject falls asleep. There is evidence that this regulation can work not only at the level of the whole brain, but also in a region-specific manner. Accordingly, sleep need would not be a global parameter, but a local, activity-dependent one. For example, subjects that performed tasks using specific areas of the brain more intensely indeed showed intense slow waves in this area.

The actual biological function of sleep is still unknown. We hypothesize that a better understanding of sleep regulation, in particular activity-dependent sleep homeostasis will get us closer to an understanding of its function.

With the advent of novel pharmaco- and opto-genetic tools, we now have the means to precisely control the excitability and firing patterns of neurons with high spatial and temporal precision.

２．研究の目的

Understanding the regulatory mechanisms underlying sleep is an important step in understanding the role of sleep.

Aim 1. NREM sleep and slow wave activity (SWA) are necessary for the restoration of proper cognitive function and ultimately for survival. It is not known, however, whether SWA 'per-se' is necessary for this process. First, SWA could be simply the lowest default state of cortical activity; second, SWA could be the expression of an underlying mechanism, which could proceed in the absence of SWA; third, SWA could indeed be directly responsible for sleep need reduction. For this purpose, we wanted to silence small cortical areas during SWA and study the amount of SWA during later phases.

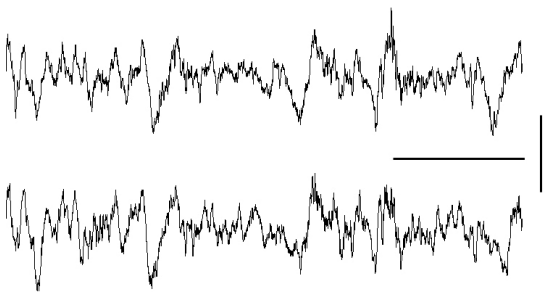
Aim 2. There is substantial evidence that prior activity (and not just waking duration) is an important factor determining sleep need buildup. However, it is unclear at what level of resolution this mechanism works, or which patterns of neural activity efficiently drive this mechanism.

We therefore wanted to interfere with neural activity directly and in a controlled manner during waking and then observe SWA in later sleep phases to determine whether and how increased or decreased activity affected local sleep homeostasis.

３．研究の方法

We implemented a chronic in-vivo EEG and EMG recording system. Because we wanted to be able to compare several recording sites, we were aiming for a multi-channel solution. We decided to purchase and implement a system recently developed by Intan Inc.

The above figure shows a mouse tethered to our continuous recording system. In this example, the mouse has been implanted with multiple bipolar electrodes. The amplifier chip is directly connected to the animal's head. The system offers direct on-chip amplification and digitization. The resulting EEG and EMG recordings were of sufficient quality to use for sleep scoring and analysis.



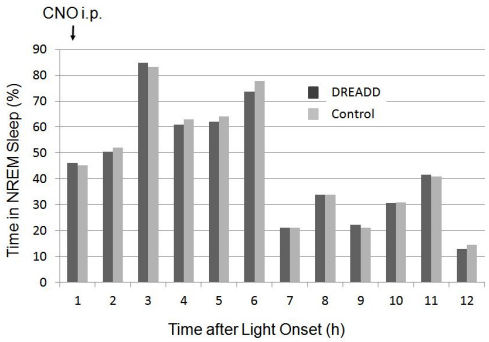
EEGs from two recording sites are shown in the above figure. This mouse is in slow wave sleep (scale bars are 1 s and 200 uV).

Adeno-associated viruses (AAV) carrying plasmids for designer drugs exclusively

activated by designer receptors (DREADD) were injected in one hemisphere (predominantly in the somatosensory cortex). Expression was confirmed with co-expression of a fluorescent marker. DREADDs can be selectively activated by clozapine N-oxide (CNO) and can result in excitation in case of Gq activation or neural inhibition in the case of Gi coupling. We used both types to try to increase or dampen the activity in a small circumscribed cortical area.

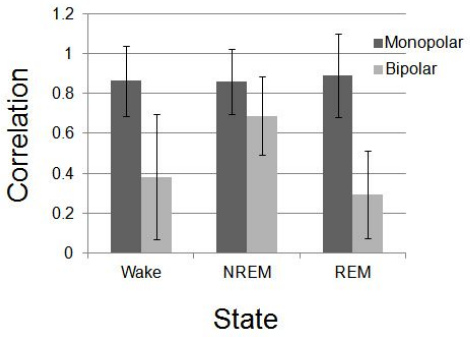
4．研究成果

After initial tailoring of the recording system we were able to record from two surface EEG electrodes, one over the DREADD injected area and one over a contralateral control region. These EEGs were used to sleep score the animals using established parameters. Scoring from both EEGs showed almost identical results, confirming that under control conditions sleep is expressed globally. CNO was injected at the beginning of the light-phase with the highest sleep pressure in the hope of locally perturbing sleep. However, under these circumstances, the sleep scores and other aspects of the EEG were not significantly altered.



As can be seen in this figure DREADD-mediated local activation did not significantly alter sleep scoring.

This result could be the consequence of several factors: DREADD expression might have failed, DREADD activation might have been insufficient, or the recording setup might have been insufficiently selective. Since DREADD expression was confirmed and the same virus was shown to reliably affect neural firing at our institute, we focused on the local sensitivity of the recording setup. Surface electrodes are routinely used in larger mammals to record localized EEG phenomena, however the area covered by a surface electrode in a mouse might be too large. This was indicated by a close analysis of the two signals. Cross correlation between the two EEGs showed persistently elevated levels that did not reflect the classical de-synchronization during waking and REM sleep (dark in the figure below). We now interpret these signals as contaminated by volume conductance (i.e., strong theta activity in the hippocampus) and have switched to a local bipolar recording setup, that should allow us to avoid this problem in the future. This is supported by our finding of significantly reduced correlation in the signals particularly during waking and REM sleep in accordance with findings in larger animals



Cross correlation coefficients between contralateral EEG signals recorded with monopolar surface (dark) and local bipolar (light) electrodes.

Changing to the locally inserted bipolar electrode system required extensive re-engineering of the recording system and the necessary surgery. After successful control experiments we can now repeat our CNO experiments.

5．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　　件）

〔学会発表〕（計 1 件）

Grenier F, Ohyama K, Sezaki M, Oishi Y, Lazarus M, Vogt K, Greene R
Studying the locality of sleep slow wave activity and its homeostasis
4th Annual IIIS Symposium, Tsukuba, 2016

〔図書〕（計　　件）

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権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕

６．研究組織
(1)研究代表者
フォークト・カスパル　（VOGT, Kaspar）
筑波大学・国際統合睡眠医科学研究機構・准教授
研究者番号：80740034

(2)研究分担者
グレニエ・フランソワ　（GRENIER, Francois）
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： 90738692

(3)連携研究者