様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Direct Optogenetic Investigation of Cortical Excitability and Connectivity in Slow Wave Sleep

研究課題名（英文）Direct Optogenetic Investigation of Cortical Excitability and Connectivity in Slow Wave Sleep

研究代表者

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研究成果の概要（和文）：大脳皮質は意識をつかさどり、深い睡眠中に現れる脳波である徐波の源である。徐波睡眠中は体動も周囲に関する意識もほぼ失われることから、大脳皮質の神経活動は阻害されていると信じられてきた。本研究では果たしてこの説が正しいのかを、神経細胞の活動を光に応答するタンパク質を用いて計測する手法によって検証した。大脳皮質の一部をレーザー光パルスにより活性化すると、神経細胞の興奮は大脳全体に拡がる。この時の神経細胞の反応は覚醒時よりも徐波睡眠時に顕著に大きいことが判明した。この観察から、大脳皮質の神経細胞は睡眠中も活動しており、アセチルコリンのような脳内化学物質の変化によって興奮性が変割ると予想された。

研究成果の学術的意義や社会的意義
Contrary to expectations the outermost brain layer, cortex, is more excitable in deep sleep compared to waking. This may explain the beneficial effect of sleep on memory by making it easier for different areas to communicate. It may also help understand why many seizures happen in deep sleep.

研究成果の概要（英文）：We are not moving and not fully aware of our surroundings during deep slow wave sleep. Many therefore assume that cortex, the outermost layer of the brain, where conscious thoughts are formed, is inhibited during this time. To study this, we investigated cortical circuits in sleep and waking in freely moving mice. We expressed a light-sensitive protein and then excited it with a brief pulse of laser light. Neurons in the cortex were then reacting as the excitation spread. Surprisingly, in deep, non-rapid eye movement (NREM) or slow wave sleep the responses were largest. The change in the response size occurred very rapidly - only about a minute from small responses in waking to large responses in slow wave sleep. This means the brain does not form (or later destroy) connections in this short time, it has to change existing ones. The change in several brain chemicals - most notably acetylcholine - are likely causing this increase in cortex excitability.

研究分野：Neurobiology

キーワード：Sleep　Cortex　Excitation

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

In slow-wave or non-rapid eye movement (NREM) sleep cortical neurons oscillate between silent, hyperpolarized DOWN states and depolarized, active UP states – this activity produces the slow waves that can be observed in the EEG during NREM sleep. This is very different from cortical activity in waking in which the neurons are constantly depolarized and active, without synchronization. Although we know that slow wave sleep is necessary for survival and beneficial for memory consolidation, the link between these benefits and the cortical slow waves is unclear.

The starkly different modes of activity suggest a change in functional network configuration between waking and NREM sleep. However, how exactly this configuration is changed and by what mechanisms is still unknown. Many drugs that induce sleep increase the function of the inhibitory neurotransmitter GABA and it is a common assumption that cortex is inhibited in NREM sleep.

２．研究の目的

We wanted to directly probe cortical activity and the excitability of cortical neurons during natural waking and sleep, without using pharmacological agents. We wanted to know if cortex was more or less excitable in NREM sleep, because past results using indirect methods are contradictory. We also wanted to determine whether prolonged waking would cause an increase in responses, whereas NREM sleep would reduce response sizes. This is a central prediction of the synaptic homeostasis hypothesis (SHY).

３．研究の方法

We used optogenetic tools to excite clearly defined pathways repeatedly during waking and sleep. We transfected thalamocortical projection neurons with ChR2 and then excited their axons at the entry into cortex. Cortical excitability was quantified measuring cortical local field potentials and action potential generation using implanted microwire tetrodes.

Brief, 5 millisecond laser pulses were applied every 4-6 s and the evoked field potential and produced spikes were measured. The vigilance state of the animal was assessed by recording surface electro encephalogram (EEG) and neck muscle electromyogram (EMG) and using standard criteria to distinguish between waking, NREM and REM sleep.

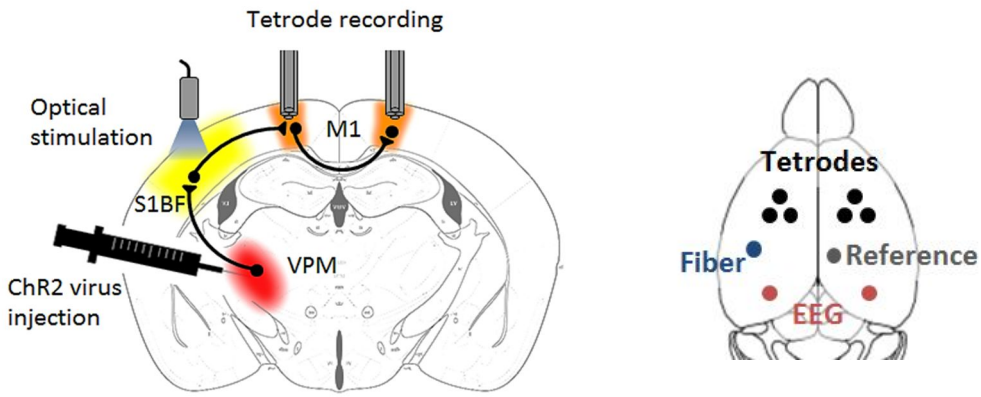

Figure 1: Experimental Setup. Injection of adeno-associated virus (AAV) into VPM, the thalamic relay nucleus for the somatosensory system. Placement of optical fibers over the S1 barrel field, where the thalamocortical fibers terminate. Tetrode recordings from the motor cortex bilaterally.

４．研究成果

Our investigation produced a number of surprising and important findings.

1. Cortical excitability as determined by optogenetically evoked fields and neuronal spiking (multi-unit activity) was severalfold larger in NREM compared to waking and REM. Cortex

in REM was slightly more excitable than waking.
2. Transitions between low and high excitability were very rapid and followed within a minute of the vigilance state transition.
3. Cortical UP and DOWN states did not affect excitability significantly. This shows that the cortical DOWN state is not a state of ongoing depression or inhibition.
4. There was a weak influence of circadian time on response size, compatible with SHY, but sleep deprivation did not enhance responses in a way predicted by SHY. In the face of the very large and unexpected modulation of cortical excitability, our approach may not be suitable to test the SHY hypothesis.
Our findings are most compatible with a neuromodulatory effect on cortical excitability, driven by vigilance state changes. The largest responses were found in NREM, which is characterized by low cholinergic and monoaminergic tone. REM sleep is characterized by low monoaminergic tone, but high cholinergic tone. Our first hypothesis is therefore that low cholinergic tone is responsible for the increased responsiveness of the cortex to optogenetic stimuli.
Increased cortical excitability may be crucial for memory consolidation in NREM sleep. By allowing better communication between different brain areas, memories might be better distributed, an important step in consolidation. On the other hand, it may help explain the strong tendency of many seizures to occur during NREM sleep.

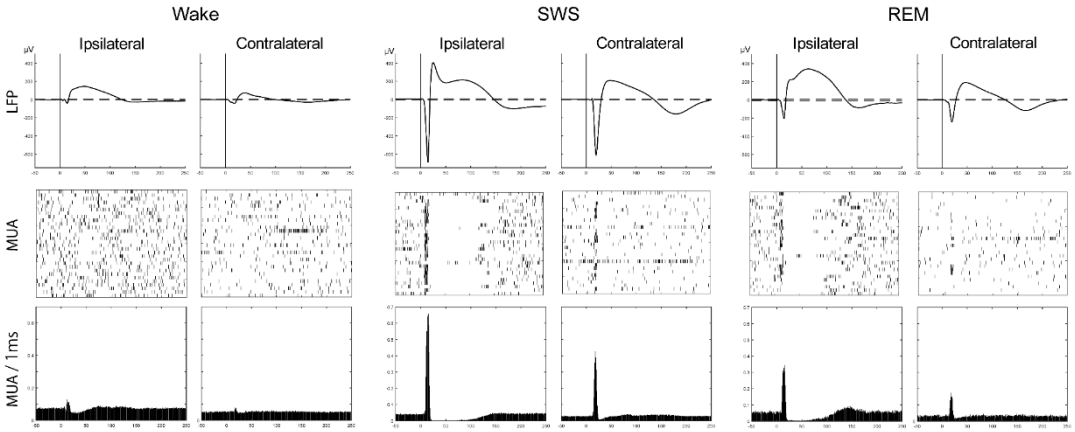

Figure 2: Example of local field potential (LFP, top) and spike response (middle and bottom MUA multi-unit activity) to the same stimulus in waking NREM or slow wave sleep (SWS) and REM sleep. Traces are the average of 50 responses.

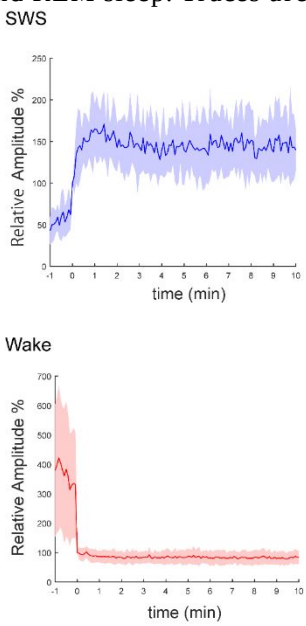

Figure 3: Rapid transition of the response size (LFP amplitude) in the transition from waking to NREM or slow wave sleep (SWS) (top trace in blue) and from NREM sleep to waking (bottom trace in red). In both cases the change in response occurs in less than a minute after the vigilance state transition. This limits the biological processes that could be responsible and indicates a change in neuromodulatory tone as the most likely mechanism.

５．主な発表論文等

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

１．著者名 Matsumoto Sumire、Ohyama Kaoru、D?az Javier、Yanagisawa Masashi、Greene Robert W.、Vogt Kaspar E.	４．巻 10
２．論文標題 Enhanced cortical responsiveness during natural sleep in freely behaving mice	５．発行年 2020年
３．雑誌名 Scientific Reports	６．最初と最後の頁 1-12
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-020-59151-8	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 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 SUMIRE MATSUMOTO, KAORU OYAMA, JAVIER DIAZ, ROBERT GREENE, KASPAR VOGT
２．発表標題 Cortical responsiveness across natural wake and sleep in mice
３．学会等名 The 10th IBRO World Conference of Neuroscience（国際学会）
４．発表年 2019年

１．発表者名 Sumire Matsumoto, Keiichi Morikuni, Momo Matsuda, Kotaro Sakamoto, Kaoru Ohyama, Tetsuya Sakurai, Kaspar Vogt
２．発表標題 Semi-automatic Spike Sorting of Long-term Tetrode Recording in Mice
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１．発表者名 Matsumoto S, Ohyama K, Diaz J, Vogt K
２．発表標題 Communication of Cortical Neuron during Slow Wave Sleep
３．学会等名 The 41th Annual Meeting of Japan Neuroscience Society. Kobe, Japan.
４．発表年 2018年

１．発表者名 Matsumoto S, Ohyama K, Diaz J, Greene R, Vogt K
２．発表標題 Cortical Neuronal Communication During Natural Slow Wave Sleep in Mice
３．学会等名 NEUROCSIENCE 2018 (Annual meeting of Society for Neuroscience)（国際学会）
４．発表年 2018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