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学位論文題目	Enhanced Responsiveness of Cortical Neurons During Sleep (睡眠時における大脳皮質ニューロン応答性の増強)

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author measures local field potentials (LFP) and multi-unit activity (MUA) in the cortex in response to repeated brief optogenetic stimulation of thalamocortical afferents to examine the differences of responsiveness in the cortical neurons between each vigilance states.　The summary is as follows:

Purpose: Cortex exhibits large shifts in its activity depending on the vigilance state. Waking and rapid eye movement (REM) sleep are characterized by the ongoing irregular activity of cortical neurons, while during slow wave sleep (SWS), these neurons show synchronous alterations between silent (OFF) and active (ON) periods, named slow wave activity (SWA). The network dynamics underlying these phenomena are not fully understood. To examine the difference of cortical network activity across vigilance state, cortical response of repeated brief optogenetical stimulation of thalamocortical afferents were investigated using Local field potential (LFP) and multi-unit activity (MUA) recording.

Spike sorting, a data processing to identify single unit activity (SUA) from MUA, for long term recording data remains challenging. To establish long-term SUA analysis, automation of the long-term spike sorting method has also been investigated in this study.

Materials and methods: Channelrhodopsin 2 was expressed in the ventral posteromedial nucleus in the thalamus, which has a specific projection to the sensory cortex through adeno-associated virus infection, and thalamocortical axons were optogenetically stimulated by delivering light to the primary somatosensory cortex. LFP and MUA in the primary motor cortex in response to repeated brief optogenetic stimulation of thalamocortical afferents were recorded by tetrode implantation in freely behaving mice.

Model-based clustering algorithm was repeatedly applied on the recorded MUA data. The clusters that indicate

SUA were visually assessed at every trial until getting a satisfactory clustering label.

Results: Both LFP and MUA responses were considerably increased in sleep compared to wake, with larger responses during SWS than during REM sleep. SUA isolated from a subset of experiments also exhibited increased evoked activity in SWS compared with wake. MUA responses were biphasic in all vigilance states: a brief window with increased unit activity after stimulus followed by a longer period of reduced unit activity. The balance between rapid increase and following reduced amount of unit activity did not differ significantly across states. Responses to stimuli were slightly but significantly larger during SWS-OFF periods than during SWS-ON periods. To precisely interpret the behavior of cortical neurons during the response, the kinetics of MUA responses was evaluated. In SWS, the larger response occurred in a longer time window with a longer time to peak, suggesting the reduced feedforward inhibition as a factor that determines the initial spiking time window. SWS responses showed clear daily fluctuation correlated to light-dark cycle, but no reaction to increased sleep need following sleep deprivation. Model-based clustering methods were applied to 18 recording of 24 hours from 4 mice, 53 tetrodes in total. After repeated applications of clustering, 391 clusters were successfully isolated with intra-spike interval (ISI) < 2 ms less than 1% of all ISI.

Discussion: Sensory evoked cortical responses in humans and animals during natural sleep or under anesthesia are typically smaller. The enhanced cortical response to thalamocortical input that has been shown in this study supports the hypothesis that thalamic filtering significantly contributed to reduced cortical responses to sensory stimulus during sleep. The well-described changes in the neuromodulatory environment between vigilance states might contribute to changes in the cortical response amplitude. Due to the lack of reaction to increased sleep need, potential homeostatic synaptic plasticity was either absent or masked by large vigilance-state effects. Increased cortical network responses may be necessary for SWA during SWS or maintaining cortical activity in the absence of sufficient peripheral input.

The analysis of SUA through long recording may help to further investigate the cortical network activity. SUA obtained from the recording data needs further assessment method for cluster verification to apply further physiological analysis.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The applicant examined the reaction of the cortical network to optogenetic thalamocortical activations to know the precise neuronal activity transmissions during each vigilance state. She clearly showed that cortical responses were increased during sleep as compared with wakefulness. This is an important finding to understand the activity of cortical neurons during sleep.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on January 19th, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.