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学位論文題目	Slow Wave Sleep and Sleep Need Resolution Through L-type Voltage Gated Calcium Channel （L-type 電位依存性カルシウムチャネルに通じた徐波睡眠と睡眠圧の解消）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, GoEun Han describes the effect of L-type voltage gated calcium channel blocker on slow-wave sleep. The summary is as follows:

（目的 Purpose）

Slow wave or non-rapid eye movement (NREM) sleep is tightly homeostatically regulated and essential for survival. Slow waves are observed in the electroencephalogram (EEG) as oscillations in the delta (0.5-4 Hz) range. Slow wave activity is to date the best indicator for homeostatic sleep regulation; it is increased after prolonged waking and slowly reduced during NREM sleep. The precise mechanisms underlying sleep homeostasis and the generation of slow waves are unknown. Activity-dependent neuronal calcium influx has been hypothesized to play an important

role in generating slow oscillations and might be involved in downstream signaling that mediates sleep function. Dihydropyridine blockers of L-type voltage-gated calcium channels (VGCCs) are in wide clinical use to treat hypertension and other cardiovascular disorders and are readily blood-brain-barrier (BBB) penetrant. The applicant therefore aimed to investigate their potential effect on slow wave generation and homeostatic NREM sleep regulation.

(対象と方法 Materials and Methods)

Experimental procedures were carried out in accordance with local and national regulations and after approval by the animal care and use committee of the University of Tsukuba. Eight- to 25-week-old C57BL/6J male mice and GCamP6f; CamKIICre mice were used for sleep recording and imaging, respectively. Mice were anesthetized with isoflurane and place in a stereotaxic frame. The scalp was removed, and the skull surface cleaned with a scalpel blade. For sleep recording, two EEG screw-electrodes were inserted into the skull: two wire-electrodes were implanted into the neck muscles bilaterally for electromyogram (EMG) recordings. For intracerebroventricular (i.c.v.) injection, a single guide cannula was inserted 1.9mm from the surface of the brain at a 10° angle. Optical windows were inserted in some animals after electrode and i.c.v. cannula insertion. The mice were injected i.c.v. with 100nM of nicardipine.

EEG and EMG electrodes were connected to an amplifier and digitizer board from (Intan technologies) at sampling rate of 1kHz. EEG and EMG were extracted and filtered using MATLAB. As for EEG and EMG recording during imaging, the signals were band-pass filtered and amplified using an analog amplifier. EEG signals underwent fast fourier transformation and further analyses through a custom-made MATLAB-based calculations. The classifications of wakefulness, REM and NREM were based on delta (0.1-4Hz) power, theta (6-10Hz) power to delta power ratio, and the integral of EMG signals. The two-photon laser microscope used was Olympus FluoView FV300/FV1000. After the optical window surgery, mice were given 7 days to recover/habituate to the headplate and a spherical treadmill. The mice could undergo spontaneous wake, NREM and REM sleep states on the spherical threadmill during imaging. Imaging GCaMP6f expressing neurons was conducted in II/III layer.

(結果　Results)

Under baseline conditions, the applicant distinguished how neuronal activity changes during wakefulness, NREM and REM sleep. The imaging data showed that calcium activity increased during NREM sleep than other states. The imaging data under baseline, vehicle-injected and nicardipine-injected conditions showed that after i.c.v. nicardipine injection, the calcium activity decreased significantly. There were total of 100 neurons, found semiautomatically. Because the number of spikes and mean amplitude could vary according to neuronal type; integral of calcium transients over a threshold of 0.5 dF/F was calculated. After 100 minutes nicardipine injection, the calcium activities were reduced, but started to recover after 120 min after the injection.

Although nicardipine successfully blocked calcium influx, it did not influence overall SWA nor the SWA at episode level. Sleep as quantified by EEG and EMG recordings were not affected by nicardipine over vehicle in terms of SWS duration, bout duration, for injections at ZT0 and injections at ZT12, and after sleep deprivation.

(考察　Discussions)

The applicant showed that during NREM sleep, there were more calcium activities in cortical excitatory neurons, suggesting that NREM sleep is composed of active excitatory neurons, and could reflect the ON and OFF oscillations. The author opted for acute pharmacological intervention. Imaging data showed clear block of calcium activity within 100 minutes after the i.c.v. nicardipine injection; however, sleep analysis showed that there was no change in sleep architecture, sleep bouts and duration. Nicardipine also did not affect the slow wave activity over 24 h following application. Time spent in slow wave sleep and episode duration were also not affected. From these results, the applicant concluded that despite evidence that neuronal calcium influx may be involved in NREM sleep function, blocking calcium entry through L-type VGCCs does not interfere with slow wave generation or regulation.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The applicant found that blocking calcium entry through L-type VGCCs does not interfere with slow wave generation or regulation. This is contrary to the hypothesis that neuronal calcium influx may be involved in NREM sleep function. This study raises questions about existing hypotheses and contains very important insights into the regulatory mechanisms of NREM sleep.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on November 15, 2022. The applicant provided an overview of dissertation, addressed questions and comments raised during Q & A session. All 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.