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学位論文題目	Role of reproductive hormones in sex differences in sleep homeostasis and arousal response in mice （マウスの睡眠恒常性と覚醒反応の性差における生殖ホルモンの役割）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Mr. Jinhwan Choi describes the sexual differences in sleep homeostasis and arousal responses and the involvement of gonadal hormones in these differences. The summary is as follows:

（目的　Purpose）

Sleeping is one of the basic behaviors conserved in most animals. It is considered as an inevitable behavior to sustain life of animals with central nervous system. To date, there have been numerous findings regarding mechanisms of sleep and circadian system using experimental mice which is one of the most widely used experimental subjects in the field. Utilization of commonly shared characteristic of sleep in mammals, a state of continuous transition between non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS), through electroencephalogram (EEG) and electromyogram (EMG) based analysis enhanced our understanding of sleep in mice. Various sex differences in sleep/wake behaviors are observed in mice. However, it still has not been clearly investigated whether there are sexual differences in sleep homeostasis and arousal responses and the involvement of gonadal hormones in these differences. In this study, the author examined sleep/wake behaviors of hormonally intact, gonad removed and

gonadectomized with hormone supplemented male and female C57BL/6 mice under their baseline condition, after sleep deprivation by gentle handling, and arousal responses to repeated cage changes.

（対象と方法　Materials and Methods）

The author used male and female C57BL/6N mice for this analysis. At 7 weeks of age, mice in intact, gonadectomized and gonadal hormone supplemented groups were implanted with EEG/EMG electrode under isoflurane anesthetization for recording sleep/wake behaviors. The implanted EEG/EMG electrodes contains 4 electrode pins and two flexible stainless-steel wires. Under stereotaxic control, EEG/EMG electrodes were lowered to the dura. EEG/EMG electrodes were positioned over the frontal and occipital cortices and thereafter attached to the skull by dental cement. Two flexible stainless EMG wires were inserted into the neck extensor muscles. After the surgery, mice were allowed to recover for at least 7 days. After recovery, mice were tethered to counterbalanced arms in individual cages, which allows free movement and exerts minimal weight to the mice for at least 7 days for habituation to the recording condition. During EEG/EMG electrode implantation surgery, the gonads of male and female mice in gonadectomized and hormone supplemented groups were bilaterally removed. For gonadal hormone supplementation, crystalline testosterone or β-estradiol filled silicon tubes were implanted to EEG/EMG recorded gonadectomized mice. To record sleep/wake behaviors, the author randomly assigned mice into two groups, gentle handling or serial cage changing groups. EEG/EMG were recorded for 3 consecutive days. Baseline sleep/wakefulness was acquired by the mean values of the first and second days without any sleep manipulation. Gentle handling or serial cage changing was performed on day 3 from ZT0. From ZT 0 to ZT6, gentle handling was performed for 6 hours during the light phase to deprive sleep by gently tapping the cage when the mice start to recline and lower their head. For serial cage changing, the mice home cage was replaced with a new cage 6 times, once every hour from ZT0 to ZT5, without any additional stimulation to induce mice wakefulness. After gentle handling or serial cage changing, all mice were left freely to sleep during the recovery period. After 3 days of EEG/EMG recording, gonadectomized mice were subjected to gonadal hormone supplementation. Four weeks later, EEG/EMG were recorded for 3 consecutive days with gentle handling or serial cage change on the third day. The EEG/EMG recordings of intact, gonadectomized, and hormone supplemented mice were carried out at 9, 11, and 15 weeks of age, respectively.

（結果　Results）

In the baseline condition, the author identified that females had higher wake amounts, less non-rapid eye movement sleep (NREMS) amounts, and longer rapid eye movement sleep (REMS) episode durations. Sleep deprivation by gentle handling resulted in an increase of NREMS delta power, NREMS amount and REMS amount in males whereas females exhibited smaller increases. The arousal responses to repeated cage changes were similar between males and females. Gonadectomy strongly enhanced homeostatic sleep regulation in females but only had a modest influence in males. Both males and females showed weakened arousal responses after gonadectomy. Baseline sleep of gonadectomized males and females was similar to that of intact males and females after hormone supplementation. However, supplementation of the gonadal hormones only restored arousal response in males but not in females.

（考察　Discussion）

The author thinks that the results of this study indicate that clear sex differences exist between males and females in their baseline sleep/wake behavior, homeostatic sleep regulation, and arousal responses to external stimuli and that these dissimilarities are differentially influenced by reproductive hormones. Detailed relations of sex with specific sleep parameters after sleep stimulation and sex hormonal manipulations in this study redound to the framework of sexual dimorphism in sleep and provide further understandings of the sex/gonadal hormonal dependent sleep system.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

Sex differences in sleep/wake behaviors are extensively analyzed, but still controversial. The author clearly demonstrated that sex differences exist between males and females in their baseline sleep/wake behavior, homeostatic sleep regulation, and arousal responses to external stimuli and that these dissimilarities are differentially influenced by reproductive hormones. These observations are very useful information to understand sex-dependent disease susceptibility related to sleep.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on Dec 23, 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.