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学位の種類	博士（ 人間生物学 ）
学位記番号	博甲第 9662 号
学位授与年月	令和 2 年 8 月 31 日
学位授与の要件	学位規則 第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Progressive changes in sleep and its relations to amyloid-β distribution and learning in App^{NL-G-F} mice （App^{NL-G-F}マウスにおける睡眠異常およびアミロイド β や記憶学習能力との関係についての解析）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Sakura Eri Bautista Maezono describes the sleep/wake and cognitive behaviors of a mouse model of Alzheimer’s disease (AD) at different age stages. The content is summarized as follows:

（目的 Purpose）

Patients with AD often suffer from sleep disturbances. Alterations in sleep parameters, especially those related to rapid eye movement sleep, can precede the onset of dementia. Moreover, findings from recent animal studies provide strong support that insufficient sleep accelerates neurodegeneration. To accurately understand the sleep impairments in patients with AD and their underlying mechanisms using animal models, it is crucial to use models in which the brain is affected in a manner similar to that observed in AD patients.

The study sought the following:

1) To evaluate how the sleep architecture and state-dependent oscillatory brain activities are affected in App^{NL-G-F} heterozygous and homozygous mice
2) To gain insight into the brain areas responsible for the altered sleep patterns by assessing

amyloidosis in several subcortical areas involved in sleep regulation

3) To investigate the relationship between the development of sleep disturbances and cognitive impairment via assessing the learning and memory abilities in these mice and analyzing the correlation between their performance in the behavioral tasks and sleep parameters.

（対象と方法　Materials and Methods）

Sakura Eri Bautista Maezono focused on *App*$^{NL-G-F}$ mice, in which expression levels and expression patterns of mutated amyloid precursor protein follow the endogenous patterns commonly observed in AD patients. She characterized the sleep architecture of the *App*$^{NL-G-F}$ heterozygous and homozygous mice at two ages (6 and 12 months) by recording of the epidural electroencephalogram (EEG) and electromyogram. In addition, cognitive behaviors were investigated with open field and trace fear conditioning tests and mice were immediately killed by an overdose of anaesthesia following the fear conditioning tests for histological analyses.

（結果　Results）

At the age 6 months, the homozygous mice exhibited reduced rapid eye movement sleep (REMS), whereas at an older age (12 months), the homozygous mice exhibited further reduction in REMS together with a slight reduction in non-rapid eye movement sleep. By contrast, the sleep architecture of the heterozygous mice appeared overall normal at both ages. Furthermore, the homozygous mice at the younger age exhibited a decrease in the ratio of EEG gamma power to delta power during REMS, resembling the EEG slowing phenomenon often observed in the preclinical or early stage of AD. Thus, phenotypes related to rapid eye movement sleep exhibited by the homozygous mice resembled the features of preclinical or early stages of AD. Homozygous mice at both the younger and older ages showed learning and memory impairments in the fear conditioning test. Task performance strongly correlated with the amount of rapid eye movement sleep at the older age, but not at the younger age. Finally, measurements of the amyloid-β accumulation in several brain areas revealed that amyloid-β accumulation in the pontine tegmental area and ventral medulla followed a course similar to that of the REMS reduction, i.e., an age-dependent increase in the homozygous mice and low levels in the heterozygous mice.

（考察　Discussion）

This is the first study to describe the sleep abnormalities exhibited by *App*$^{NL-G-F}$ homozygous and heterozygous mice and the association of these sleep abnormalities with learning ability. Sleep is regulated by various brain areas and neuronal subtypes. Thus, addressing the association between sleep and AD using mouse models that overexpress or ectopically express APP or presenilin could complicate interpretations. Unlike previous studies in which the applied mouse models carried either multiple copies of *App* or *presenilin* or use heterologous promoters to express these genes, the present study used a mouse model in which mutated *App* was singly knocked into the original *App* locus. Indeed, homozygous mice faithfully recapitulated several aspects of the sleep abnormalities associated with preclinical or early AD.

The sleep architecture in the homozygous mice at 6 months of age was characterized by a decrease in REMS. At 12 months of age, the reduction of REMS was further pronounced, and NREMS was also reduced. By contrast, the sleep architecture of the heterozygous mice appeared mostly normal, even at 12 months of age. This might be explained by the time course of Aβ accumulation in brain areas crucial for REMS regulation. Accumulating evidence supports an essential role

of the pontine tegmental area and ventral medulla in regulating REMS. In these two areas, in contrast to the hippocampus or cortex, Aβ was almost undetectable in the heterozygous mice. On the other hand, in the homozygous mice, Aβ in these two areas increased with age, consistent with the progressive decrease in REMS. Therefore, damage to the brainstem might be critical for the development of sleep deficits in AD.

According to the results of cortical EEG spectral analyses in AD patients or patients with mild cognitive impairment, alterations in the oscillatory activity during REMS are suggested to be more sensitive biological markers of the disease than alterations during wake. In such patients, EEG slowing, i.e., the simultaneous occurrence of an increase in the power of the slow component and a decrease in the power of the fast component of the EEG power spectrum during REMS was observed. The homozygous mice in this study appeared to well recapitulate the EEG slowing during REMS, which appears to be the first report of this phenomenon in an AD mouse model. The altered oscillatory activities highlight the advantage of using a single *App* knock-in mouse, in which the endogenous expression pattern of *APP* is faithfully recapitulated.

Interestingly, at 7 months, there was no correlation between the REMS amount and learning or memory, whereas at 13 months, there was a strong and positive correlation. Perhaps, the memory deficit and REMS impairment originally develop independently at younger ages, but in the course of disease progression, somehow REMS impairment contributes to worsening of the learning and memory deficit. Post-learning REMS is crucial for memory consolidation. As the 13-month-old heterozygous and homozygous mice displayed learning impairments during training, however, it is unlikely that defects of the post-learning sleep are the major cause. Therefore, if the REMS impairment does contribute to learning and memory deficits, it might be that REMS is somehow involved in the daily maintenance of the brain areas related to learning.

These findings support the notion that changes in rapid eye movement sleep are an early marker of AD and provide a starting point to address the mechanism of sleep deficits in AD and the effects on cognitive function.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

In the final examination, Sakura Eri Bautista Maezono presented clear data on the changes in sleep/wake and cognitive behaviors in an Alzheimer’s disease model mice. Her findings reveal a mouse model of Alzheimer’s disease that can be useful to understand molecular links between REMS disturbances, cognitive impairments and Alzheimer’s disease. Sakura Eri Bautista Maezono has a deep understanding of the subject and fulfills all necessities to graduate from the Human Biology Ph.D. program.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on May 11th, 2020. 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.