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学位の種類	博士（医学）
学位記番号	博甲第　9735　号
学位授与年月	令和　2　年　12　月　31　日
学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Regulation of sleep by traumatic stress （外傷性ストレスによる睡眠制御メカニズムの解明）
主　査	筑波大学教授　博士（医学）　櫻井　武
副　査	筑波大学教授　博士（医学）　高橋　智
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Lou Tingting describes the effect of stressor on sleep architecture and its mechanism. The summary is as follows: Exposure to catastrophic traumatic events could lead to severe mental and behavioural disorders, so-called post-traumatic stress disorders (PTSD), which are characterized by symptoms of re-experiencing, numbing, avoidance and hyper-arousal. Sleep disturbances represent a core symptom of PTSD patients, including insomnia, nightly awakenings, nightmares, sleep paralysis and restless sleep. Although sleep disturbances have long been recognized as a core symptom of PTSD, the PTSD-related sleep phenotypes remain an understudied area. The neural basis of PTSD-related sleep disturbances remains unclear. It has been challenging to establish the causality link between a specific brain region and traumatic stress-induced sleep abnormality.

In this study, the author adopted a standardized single prolonged stress (SPS) paradigm to investigate in depth the effects of traumatic stress on sleep-wake architecture in isogenic wild-type C57BL/6N male mice. SPS is a simple and well-established rodent model of traumatic stress that can reliably induce PTSD-like behavioural and physiological abnormalities.

Firstly, the author examined the effect of the procedure on acute changes in sleep/wake duration and electroencephalogram (EEG). Traumatic stress induced acute changes in sleep/wake duration and EEG

power spectrum. In non-rapid-eye-movement sleep (NREMS), as compared to SD4 treatment, SPS caused a broad suppression in all frequency bands of EEG at ZT4; during rapid-eye-movement sleep (REMS), SPS caused a significant increase in absolute delta, alpha and beta EEG power during the light phase; in wake state, SPS caused a significant reduction in absolute delta and beta power during the light phase. To examine the long-term effect of SPS on sleep-wake architecture, the author compared the EEG/EMG data of the same mice on the seventh day (D7) after SD4 and SPS treatment, and found that SPS, relative to SD4, caused a broad reduction in sleep/wake EEG power density: alpha and beta power of NREMS; theta and alpha power of REMS; alpha and beta power of wakefulness during the light phase. In the dark phase, SPS mice showed a reduction in absolute theta, alpha and beta power of NREMS; theta power of REMS; alpha power of wakefulness. These observations suggest that unlike sleep deprivation, traumatic stress by SPS can lead to long-term sleep/wake EEG abnormalities. Secondly, the author tried to find a specific region which would be persistent active during and after traumatic stress in mice brain and would be related to traumatic-stress sleep regulation. To explore the neurobiological correlates of traumatic stress-induced sleep abnormalities, the author performed a comparative analysis of the expression of c-Fos by immunostaining of mouse brain samples harvested at ZT4.5 and ZT7.5 after SD4/SPS treatment. At ZT4.5, SPS mice, relative to SD4 mice, showed significantly more c-Fos-expressing neurons in multiple subregions of the prefrontal cortex. By two-colour fluoresence in situ hybridization, the author showed that more than 95% of c-fos positive neurons in the mPFC express the excitatory neuron marker vGlut1, but not the inhibitory neuron marker vGat. Moreover, while SD4-induced c-Fos expression dissipated, SPS-induced c-Fos expression could still be observed in the media prefrontal cortex (mPFC), most notably in the PrL, IL and DP at ZT7.5. These results suggest SPS causes persistent hyper-activities of mPFC neurons during and immediately after SPS treatment. The author hypothesized that the persistent hyper-activities of mPFC could contribute to the SPS induced short- and long-term alterations in the sleep-wake architecture and EEG power spectrum. The author also found that chemogenetic inhibition of PrL(a subregion of the prefrontal cortex) activity during SPS could not rescue the SPS-induced acute changes in sleep/wake duration on day 1. However, inhibition of PrL activity could specifically reverse the SPS induced acute suppression of NREMS delta power (1-4Hz EEG). Inhibition of PrL activity could abrogate most of the SPS-induced long-term sleep/wake EEG abnormalities on day 7 as well.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

These results suggest that hyper-activation of mPFC may mediate specific sleep-wake EEG disturbances. This study might represent the first attempt to establish such a direct causal link between dysfunction of a specific brain region and traumatic stress-induced sleep/wake disturbances. These findings might provide attractive targets for future investigations to further elucidate the underlying neural mechanisms of traumatic stress-induced psychiatric disorders. Success in this area suggests that if the neural circuitry underlying functional pathophysiology can be defined, then powerful neuroscience approaches can be effectively translated to the clinic.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on Nov 4, 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 Medical Sciences.