京都大学	博　士（　　薬科　学 ）	氏名	前川　洋太
論文題目	Regulation of Siesta by the Central Circadian Clock in the Brain and its Physiological Role in Memory Consolidation (脳内中枢時計による昼寝の制御機構とその記憶形成における役割)		

（論文内容の要旨）

Siesta has been regarded as an evolutionally conserved, brain circadian clock-regulated fundamental physiology. A number of putative physiological benefits of siesta have been reported in humans, which include increased performance, improved alertness, and enhanced memory formation. Epidemiological studies even suggest that taking a proper siesta reduces the incidence of Alzheimer's disease. However, these potential benefits and their underling molecular and/or neuronal mechanisms are currently not experimentally demonstrated.

The master clock in the hypothalamic suprachiasmatic nucleus (SCN) plays a critical role in regulating the sleep/wake cycle. It has been reported that SCN neurons expressing vasoactive intestinal polypeptide are involved in the suppression of locomotor activity during siesta. However, a direct mechanism regulating sleep in siesta is not known. Our laboratory previously identified an SCN-enriched siesta-associated gene (*SSAG*) that mediates regulation of body temperature during siesta. Because the regulation of sleep and circadian changes in body temperature are intimately linked to each other, I assumed that *SSAG* in the SCN might play a role in inducing sleep for siesta.

In **Chapter 1**, using electroencephalogram (EEG), I found that *SSAG* deletion in the SCN causes a deficit in siesta sleep. In **Chapter 2**, using infrared thermography, I found that *SSAG* in the SCN regulates simultaneously sleep and body temperature during siesta. In **Chapter 3**, I employed synaptic inhibitor tetanus toxin and revealed that SSAG-positive SCN neurons mediate regulation of sleep and body temperature during siesta. In **Chapter 4**, using step-down passive avoidance test and novel object recognition test, I showed that siesta sleep promotes episodic memory formation in mice.

Chapter 1: *SSAG* in the SCN is required for proper siesta sleep.

I generated conditional *SSAG* SCN knockout mice (*SSAG* SCN-KO mice) and examined circadian sleep/wake profile using EEG. I found that *SSAG* SCN-KO mice were normal in spectral distribution of EEG power, total time, episode number and duration for wake, non-rapid eye movement (NREM), and rapid eye movement sleep in the entire 24-hour day. I also observed that locomotor activity of *SSAG* SCN-KO mice was comparable to that of control wildtype mice. However, a significant deficit in siesta was observed for *SSAG* SCN-KO mice: *SSAG* SCN-KO mice stayed awake in the middle of night/active phase of mice. These results demonstrate that *SSAG* in the SCN is essential for proper siesta regulation.

Chapter 2: Correlated dysregulation of siesta sleep and body temperature in *SSAG* SCN-KO mice.

To elucidate the temporal relationship between behaviour and body temperature during siesta, I employed infrared thermography. I found that *SSAG* SCN-KO mice often stayed in a "sphinx-like" posture, with their head up, suggesting arousal; and their body temperatures, either interscapular skin surface or core body temperature, were not adequately decreased as compared to control mice.

Chapter 3: *SSAG*-positive SCN neurons mediate regulation of sleep and body temperature in siesta.

Next, instead of using *SSAG* SCN-KO mice, I analysed *SSAG-Cre* knock-in mice injected with adeno-associated virus encoding Cre-dependent tetanus toxin transgene into the SCN. I found that

expression of tetanus toxin in SSAG-positive SCN neurons resulted in a lack or very low degree of siesta sleep and body temperature drop. These results indicate that SSAG-positive neurons in the SCN mediate sleep and body temperature during siesta.

Chapter 4: Siesta sleep is necessary for memory formation.

Epidemiologic studies suggest that siesta may help enhance learning and memory formation in humans. I took advantage of our mouse model to investigate the effects of siesta sleep on memory formation. To this end, I used novel object recognition test and step-down passive avoidance test. I found that *SSAG* SCN-KO mice trained at Zeitgeber time (ZT) 14 (i.e., before siesta time) and tested at ZT22 (i.e., after siesta time) showed reduced memory performance, as compared with control *SSAG* floxed mice (ZT0 denotes lights-on and ZT12 lights-off). In contrast, memory formation of *SSAG* SCN-KO mice trained at ZT22 and tested at ZT6 (i.e., memory formation during extra-siesta time) was not impaired. Sleep profiles during ZT22–6 were comparable between *SSAG* SCN-KO and *SSAG* floxed mice. These results demonstrate that *SSAG*-mediated siesta sleep is required for memory formation during the dark/active phase in mice.

Based on the results from **Chapters 1–4**, I have, for the first time, experimentally demonstrated the physiological contribution of siesta to memory formation by identifying and studying the function of the siesta-regulating gene in the central circadian clock structure in the brain.

（続紙 ２ ）

（論文審査の結果の要旨）

「午睡」というとマウスは夜行性動物であるため語弊があるが、一般に、一日の中における活動期（マウスは暗期）の途中において体温と行動レベルが低下する時間帯がヒトを含め多くの生物種に認められる。この活動期中期の睡眠がもたらす生理的あるいは心理的効果として、ヒトでは認知機能の亢進や、記憶形成能の上昇、免疫応答や心血管機能の改善との関連性が報告されている。しかし、活動期中期の睡眠の正確な生理的役割は、午睡（シエスタ）を特異的に欠如した適切な遺伝子改変モデルマウスが無いため、現在、実験動物を用いた実験的証拠に乏しい状態である。このような中、前川洋太氏は本論文の第一章において、体内時計の中枢器官である脳内の視交叉上核に発現している SSAG を欠損したマウスの脳波測定を実施し、視交叉上核の当該遺伝子がシエスタの睡眠に不可欠であることを明らかにした。さらに第二章では、赤外線サーモカメラを用いたマウスの体温と活動の同時計測系を駆使し、視交叉上核の SSAG がシエスタの睡眠と体温を同時に制御していることを明らかにした。第三章では、SSAG 陽性視交叉上核ニューロンがシエスタの睡眠と体温の調節を行うことを破傷風毒素を用いたシナプス伝達抑制実験により示した。第四章では、ステップダウン型受動回避テストと新規物体認識テストにより、シエスタの睡眠がマウスの日中における正常なエピソード記憶形成に必須であることを示した。これら一連の研究成果は、脳内に存在する午睡制御ニューロンの実体を明らかにするとともに、シエスタの睡眠が日中の正常な記憶形成に必須であることを実験科学的に証明した初めての報告である。ヒトの臨床疫学調査から適切な午睡がアルツハイマー病や心臓病のリスクの軽減につながることが期待されており、本研究は体内時計の基本的神経機構の解明を通じて医学・薬学に貢献する重要な知見をもたらしたといえる。よって、本論文は博士（薬科学）の学位論文として価値あるものと認める。また、２０２２年２月１８日に、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。なお、本論文は、京都大学学位規程第１４条第２項に該当するものと判断し、公表に際しては、（令和７年３月２３日までの間）当該論文の全文に代えてその内容を要約したものとすることを認める。

要旨公表可能日：　　年　　月　　日以降