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)

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Contents

Introduction

Siesta has been regarded as an evolutionally conserved, brain circadian clock-regulated fundamental physiology[1-3]. A number of putative physiological benefits of siesta have been reported in humans, which include increased performance[3-5], improved alertness[6-8], and enhanced memory formation[9-14]. Epidemiological studies even suggest that taking a proper siesta reduces the incidence of Alzheimer's disease[15-17]. However, these potential benefits and their underlying 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[18-20]. It has been reported that SCN neurons expressing vasoactive intestinal polypeptide are involved in the suppression of locomotor activity during siesta[21]. However, a direct mechanism regulating sleep in siesta is not known. Our laboratory previously identified 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[22,23], 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.

Results and Discussion

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.

Normally, sleep is associated with a decrease in body temperature[22,23], suggesting a possibility of coregulation. 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[24-26]. 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 (**Fig. 1**).

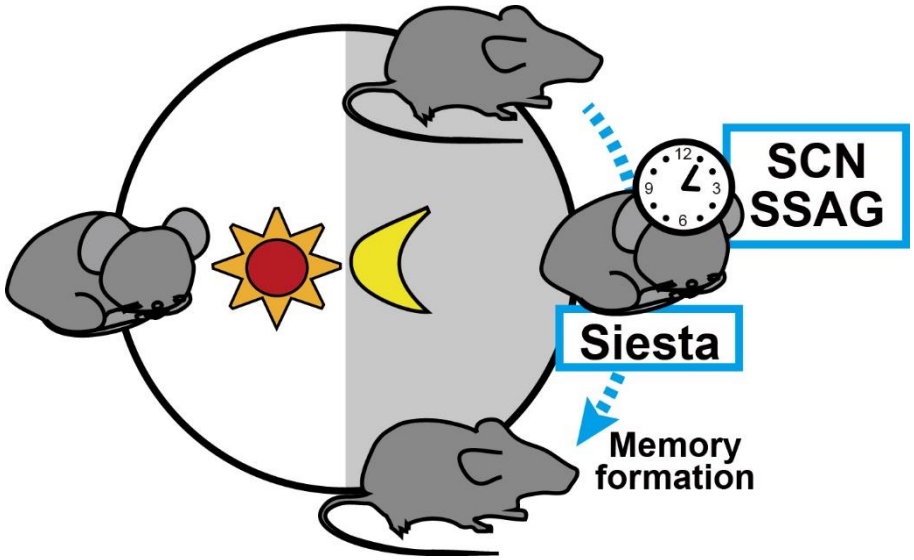

Fig. 1 | Physiological contribution of siesta to memory formation. SSAG in the SCN is essential for proper siesta regulation and SSAG-mediated siesta sleep is required for memory formation during the dark/active phase in mice.

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