Effects of a Brief Light Pulse and Intracerebroventricular Injection on Sleep-Wake Activity in Rats

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Objectives. The light/dark (L/D) cycle is an important regulator of the circadian rhythm of sleep-wake activity and neuroendocrine fluctuation. We previously reported that the intracerebroventricular (ICV) injection of corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide during the middle of the dark period altered spontaneous waking; however, it is believed that either the light pulse during the ICV injection or the injection *per se* in the middle of the L/D period may disturb circadian rhythm of rats. The purpose of this study was to further elucidate the effects of ICV injection and the light pulse given during the dark period and during the light period on sleep-wake activity.

Methods. Male Sprague-Dawley rats were implanted with electroencephalogram (EEG) electrodes and an ICV guide cannula. Rats were accustomed to a 12/12h L/D cycle. Naïve rats received a 5-min period of light flash either in the middle of the light period or in the middle of the dark period, whereas the well-habituated rats received a light pulse and ICV injection of pyrogen-free saline (PFS) either in the middle of the light period or in the middle of the dark period.

Results. In naïve rats, the 5-min period of light pulse given during the dark period of the L/D cycle significantly enhanced slow wave sleep (SWS) and decreased rapid eye movement sleep (REMS) during the hour when the light flashed. Sleep was not altered when the light pulse occurred during the light period. There was no change in brain temperature. In well-habituated rats, the light pulse with ICV injection of PFS decreased SWS and REMS during the hour when the manipulation was given; during the subsequent 12-h period, the SWS and REMS significantly rebounded. Brain temperature increased after ICV injection.

Conclusions. Our results indicate that injection and handling, which are considered to be stressors to rats, either during the middle of light period or during the middle of the dark period, mask the effect of light pulse on sleep-wake activities. **(Mid Taiwan J Med 2004;9:211-8)**

Key words

light flash, stress, sleep

INTRODUCTION

The regular light/dark (L/D) cycle contributes to the circadian rhythm of sleep-wake activity and neuroendocrine fluctuation. The direct effects of light and dark on mechanisms of sleep and wakefulness have been studied over the past three decades, especially in albino rats. Several reports have indicated that ultra-short L/D cycles increased rapid eye movement sleep (REMS) immediately following lights-out [1-3]. Many studies have reported that a five-minute dark pulse occurring intermittently during the

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light period of the L/D cycle enhanced REMS [4-7]; on the other hand, REMS decreased when a brief light pulse was given during the dark period of the L/D cycle [8]. Clinically, bright-light treatment is a common therapy for sleep disturbances in aging adults; the treatment has been shown to reduce the frequency and duration of awakening during the night, sleep latency, early morning awakening and daytime napping [9-11]. The effects of bright light may be due to the strong coupling between light, an external time-cue, and the internal sleep-wake rhythm controlled by the hypothalamic nucleus suprachiasmaticus [12]. As for the effects on endocrine fluctuation, Kostoglou-Athanassiou et al have reported that exposure to bright light during the early hours of darkness delayed the nocturnal melatonin peak and altered cortisol, growth hormone, prolactin and nocturnal vasopressin secretion [13].

Our previous observations on sleep alterations were mostly based on the manipulation of intracerebroventricular (ICV) injection of substances either at 20 minutes prior to the beginning of the light period or at 20 minutes prior to the beginning of the dark period. When the ICV injection was given 20 minutes prior to the light period of the L/D cycle, a light pulse coupled with injection was given during the dark period. However, there was no change in sleep-wake activity regardless of the ICV injection time [14,15]. Another of our previous studies has shown that ICV injection of corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide given at the midpoints of 12-hour periods of the L/D cycle reduced spontaneous wakefulness during the dark period, but not during the light period [16]. However, the effect of the light pulse given during the dark period on the circadian rhythm if the ICV injection is given at the midpoint of the dark period is unknown. Therefore, we designed the current study to further elucidate the effects of ICV injection *per se* and the light flash during the dark period and during the light period on sleep-wake activity.

METEIALS AND METHODS

Animals

Male Sprague-Dawley rats (250 to 300 g; National Laboratory Animal Breeding and Research Center, Taiwan) were used in the present study. Rats were anesthetized by intraperitoneal injection with ketamine/xylazine (87/13 mg/kg), and intraperitoneally administered butorphanol tartrate and penicillin G benzathine to reduce pain and avoid infection. The rats were surgically implanted with electroencephalogram (EEG) screw electrodes, a guide cannula directed into the lateral ventricle, and a calibrated 30-kΩ thermistor (model # 44008, Omega Engineering, Stamford, CT) to monitor brain temperature (Tbr) at the surface of the cortex as previously described [17]. Insulated leads from the EEG electrodes and the thermistor were routed to a Teflon pedestal (Plastics One, Roanoke, VA) cemented to the skull with dental acrylic (Cranioplastic cement and Cyanoacrylate gel, Plastics One, Roanoke, VA). The incision was treated topically with polysporin (polymixin B sulfate – bacitracin zinc) and the rats were allowed to recover for at least one week before the initiation of experiments. The rats were housed in individual recording cages in an environmentally controlled chamber (COCONO model # LE-539; Ron-Fong Technology Corporation, Hsin-Chu, Taiwan). Each chamber possessed two cages. The chambers were maintained at 23 ± 1°C with a 12/12 h light/dark cycle (25 Watt incandescent bulb; approximately 200 lux at cage height). Food and water were available ad libitum.

Two days after EEG electrode implantation, the pedestal was connected to the amplifier system via a connector cable (363-363 cable, Plastics One). On the third postsurgical day, 400 ng angiotensin II, which causes a drinking response mediated by structures in the preoptic area, was administered (Tocris), to assess the free drainage of the intracerebroventricular cannulae. All rats were again injected with angiotensin II at the end of each experimental protocol; only data from those rats that elicited a positive drinking response were included in the subsequent analyses.

Apparatus and Recording

Signals from the EEG electrodes were connected to amplifiers (Colbourn Instruments, Lehigh Valley, PA; models V75-01). EEGs were amplified at a gain of 5000 and analog bandpass was filtered between 0.1 and 40 Hz (frequency response: ± 3 dB; filter frequency roll off: 12 dB/octave). These conditioned signals (EEGs) were subjected to analog-to-digital conversion with 16-bit precision at a sampling rate of 100 kS/s (NI PCI-6033E; National Instruments, Austin, TX). The digitized EEG waveform was stored as binary computer files until subsequent analyses.

Determination of vigilant state was analyzed by visual scoring of 12-s epochs using custom software (ICELUS, Opp MR) written in LabView for Windows (National Instruments). The animal's state was categorized as slow wave sleep (SWS), REMS, or awake based on previously defined criteria [14]. Briefly, SWS is characterized by large-amplitude EEG slow waves and high power density values in the delta frequency band (0.5 to 4.0 Hz). During REMS, the amplitude of the EEG is reduced and the predominant EEG power density occurs within the theta frequency (6.0 to 9.0 Hz). While awake, the rats are generally active and the amplitude of the EEG is similar to that observed during REMS, but the power density values in the delta frequency band are generally greater than those in the theta frequency band.

Experimental Protocol

The rats were separated into two groups; one group of rats (n = 8) was not handled but accustomed to the 12/12-hour L/D cycle; the other group of rats (n = 8) was habituated by daily handling and ICV injections of pyrogen-free saline (PFS) timed to coincide with scheduled experimental manipulation and was also accustomed to the L/D cycle. Figure 1 presents a schematic of the manipulation and recording protocol used in this study, which is consistent with our previous study [16]. We obtained 24-h baseline recordings from undisturbed animals before all manipulations (hours 0-24; Fig. 1).

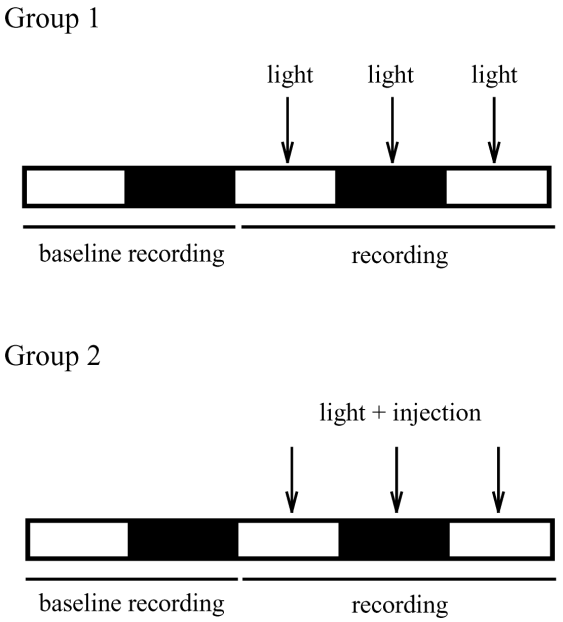

Fig. 1. Scheme of the experimental protocol. The light/dark cycle is depicted by alternating open and filled bars, representing the light and dark periods, respectively. The solid line represents the time of recording. The arrows demonstrate the manipulations; light pulse in group 1 and light pulse plus ICV injection of PFS in group 2.

Procedures to determine the effects of ICV PFS injection and the ICV injection with light pulse were initiated at the beginning of the light period. The manipulations (light pulse or light pulse plus ICV PFS injection) were given three times at 12-h intervals; these manipulations were timed to the mid-point of the three consecutive light/dark periods beginning with the light period (Time = 30-, 42-, 54-h relative to the computer recording time). Illuminance (200 lux) was given either during the dark period or during the light period of the L/D cycle. The injection volume was 3 μL over approximately a 2-min period. The light pulse was given for five minutes. Recordings for determination of sleep-wake behavior for each manipulation were initiated at light onset and continued for 36-h.

Statistical Analyses

All values are presented as mean ± SEM. One-way analyses of variance (ANOVA) for the duration of each vigilant state (SWS, REMS, awake) were performed across the two 12-h time blocks comprising the 23-h recording period.

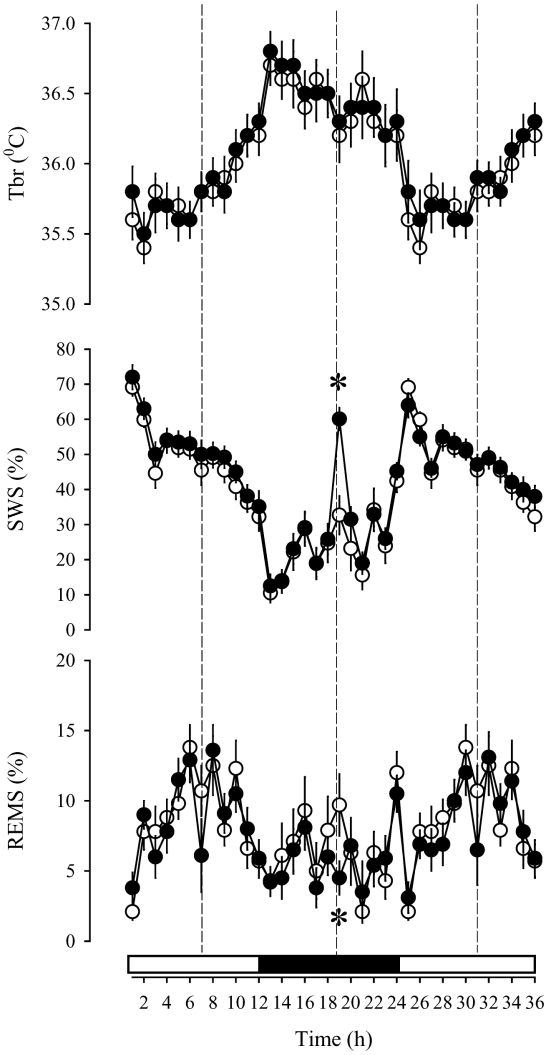

Fig. 2. Effects of light pulse in the middle of 12-h period of L/D cycle on sleep-wake activity. The open circle represents the values obtained from undisturbed rats and the closed circle depicts the values obtained after the light pulse. The dash line represents the time when the light pulse was given. Open and closed bars depict the alternating period of L/D cycle. Asterisks denote hourly time points during which values obtained after light pulse differed statistically from those obtained after baseline recording. SWS = slow wave sleep; REMS = rapid eye movement sleep; Tbr = cortical temperature.

The effects investigated included various manipulations (undisturbed baseline, light pulse, and light pulse + ICV PFS injection). If statistically significant differences were detected, post hoc multiple comparisons were made to determine which condition(s) contributed to the effect. An α level of $p \leq 0.05$ was taken as indicating a statistically significant difference between the two different manipulations.

RESULTS

Effects of Light Pulse on Sleep-Wake Activity in Naïve Rats

Light pulses were timed to the middle of consecutive 12-hour periods of the light/dark cycle. Relative to the baseline values obtained from freely behaving undisturbed animals (hours 1-24), light pulse transiently increased SWS and decreased REMS during the hour when rats received 5-minute light pulse during the dark period, but did not produce any changes when rats received light pulse during the light period. The time spent in SWS increased from 32.7 ± 5.5% obtained from baseline to 60.1 ± 3.2% after 5-minute light pulse; the total amount of REMS decreased from 9.7 ± 2.2% obtained from baseline to 4.5 ± 1.2% after 5-minute light pulse. This brief light pulse did not alter brain temperature (Fig. 2).

Effects of Light Pulse Coupled with ICV Injection of PFS on Sleep-Wake Activity in Well-habituated Rats

Relative to baseline values obtained from freely behaving undisturbed animals (hours 1-24), light pulse and injection procedures induced transient alterations in sleep-wake behavior and brain temperature in well-habituated rats. SWS decreased transiently and immediately during the hour when light pulse and ICV injection were given in the midpoint of the L/D cycle, but a rebound in SWS was observed in the following recording hours. REMS was also suppressed during the hour when light pulse and ICV injection were given in the midpoint of the L/D cycle, but the rebound in REMS only occurred when the manipulation was given during the dark period (Fig. 3). The light pulse plus injection transiently increased brain temperature at the midpoint of the L/D cycle (Fig. 3).

DISCUSSION

The effects of bright light therapy on human sleep have been extensively discussed

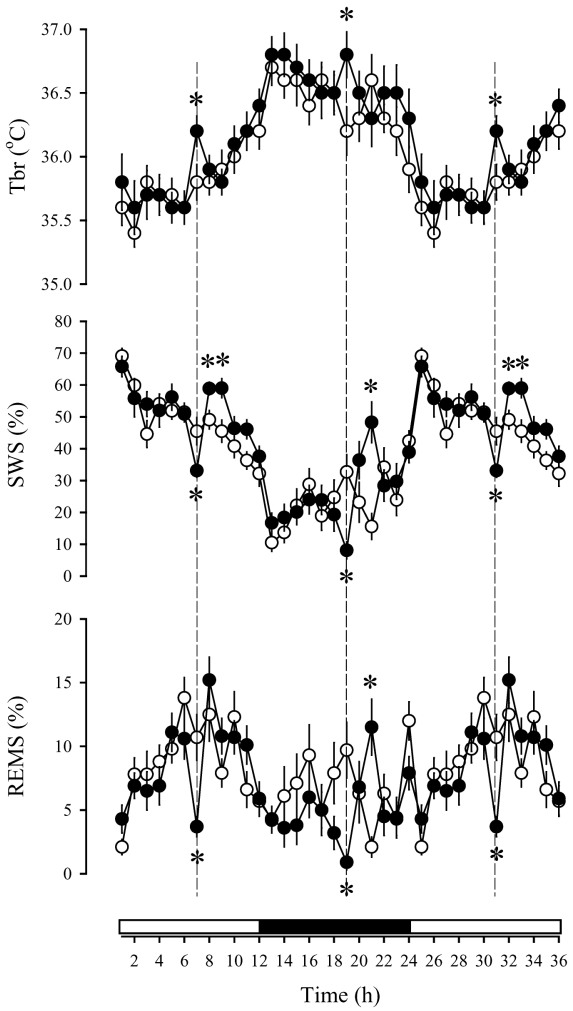

Fig. 3. Effects of light pulse coupled with ICV injection of PFS in the middle of 12-h period of L/D cycle on sleep-wake activity. The open circle represents the values obtained from undisturbed rats and the closed circle depicts the values obtained after giving light pulse with ICV injection. The dash line is the time when the light pulse with ICV injection was given. Asterisks denote hourly time points during which values obtained after manipulations differed statistically from those obtained after baseline recording.

[9,10,18,19]. Murphy and Campbell have found that bright light treatment improved sleep efficiency and delayed the phase of the body temperature rhythm; they also reported that the improvements in performance were related only to sleep and not to the circadian phase [19]. Fetveit et al have shown that bright light therapy substantially improved sleep in nursing home patients; bright light increased sleep efficiency, reduced nocturnal wake time and reduced sleep onset latency [9]. The direct effect of light and dark on sleep and wakefulness has been studied most extensively in albino rats rather than in pigmented rats for the past three decades. It has been shown that five-minute dark pulses presented intermittently during the light period of the L/D cycle produced massive and brief increases in REMS [4-7]. Conversely, if the light pulses were given to albino rats during the dark period of the L/D cycle, REMS was suppressed [8]. Both basic and clinical observations have suggested that a brief light pulse given during the dark phase of the L/D cycle may transiently alter the sleep-wake pattern.

Our previous study of CRH antisense oligodeoxynucleotide on spontaneous sleep-wake activity was based on the injection of antisense and sense oligodeoxynucleotides at the midpoint of the L/D cycle [16]. Although our results suggested that there was a lack of nonspecific responses to administration of these nucleic acids *per se* [16], there may be transient alterations in rat behavior associated with the brief light pulse given during the dark period, and the handling and injection procedures. Our present results indicate that a brief five-minute light pulse given at the midpoint of the 12-hour dark period of the L/D cycle significantly enhanced SWS and suppressed REMS immediately during the hour the light pulse was given; however, no alteration occurred when the light pulse was given to naïve rats at the midpoint of the light period. This observation suggests that the brief light pulse given during the dark phase changes sleep-wake activity in rats. The circadian temperature rhythm was not altered by the light pulse according to brain temperature measurements. However, the handling and injection procedures produced a transient suppression of SWS and REMS in well-habituated rats during the hour of manipulation, regardless of when the handling and injection procedures were given during the L/D period. There was a corresponding enhancement in brain temperature during the hour when the animals were handled and the injection procedures

were given. A rebound in SWS followed the suppression of SWS during both the dark and light phases; however, REMS only rebounded during the dark phase of the L/D cycle. Our previous results suggested that handling of well-habituated rats and injection prior to either the onset of the dark or light period had no significant effect on sleep-wake activity in the following period [14]. This result indicates that handling and injection either prior to the dark period or prior to the light period may not be stressors to well-habituated rats. However, when the handling and injection coupled with light pulse was given at the midpoint of the light or in the middle of the dark period, the sleep-wake activity was transiently altered and brain temperature increased immediately in response to the manipulation. During the dark period, the effect of handling and injection masked the effect of light pulse on sleep-wake activity. This result may suggest that handling and injection during the midpoint of the L/D cycle are stressors to well-habituated rats. The increase in SWS followed by a decrease in SWS may be a way of compensating for SWS loss after handling and injection, since the total sleep-wake activity in the 12-h period of the L/D cycle was not significantly altered.

In conclusion, our data indicate that a brief light pulse during the dark (active) period significantly enhances SWS, which is somewhat consistent with clinical observations of bright light in treatment of sleep disturbance. The mild stressor, the ICV injection with light pulse, given during the midpoint of the L/D cycle decreases SWS and REMS and increases brain temperature in well-habituated rats.

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光照與側腦室注射對睡眠之影響

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目的 十二小時光照期/十二小時黑暗期的重複性週期是維持一定睡眠週期的重要因素。我們先前的結果顯示在光照期/黑暗期的中點於側腦室給予促腎上腺皮質激素釋放激素(corticotropin-releasing hormone，CRH)的反意核酸(antisense oligodeoxynucleotide)會改變老鼠的睡眠，然而由於在側腦室注射時會有光照與注射本身等因素對睡眠的作用，因此在本實驗要來進一步探討光照與側腦室注射本身對睡眠的作用。

方法 大白鼠經過手術後安裝了腦波電極以及側腦室注射的導管，並讓老鼠適應十二小時光照期/十二小時黑暗期的重複性週期。一組未經操作的老鼠在光照期或黑暗期的中點給予五分鐘的光照並記錄睡眠的變化；另一組經過一星期在光照期或黑暗期的中點側腦室注射生理食鹽水的適應期後，在光照期或黑暗期的中點給予五分鐘的光照以及側腦室注射生理食鹽水並記錄睡眠的變化。

結果 結果顯示在黑暗期的中點給予短暫的光照，在未經操作的老鼠中會在給予光照的那一小時內明顯的增加慢速波睡眠及減少快速動眼期睡眠，但不會改變大腦皮質的溫度。在經過適應期的老鼠中，給予光照及側腦室注射生理食鹽水，會在給予的那一小時內顯著的減少慢速波睡眠及快速動眼期睡眠的量，並在接下來的一、二小時會回復增加慢速波睡眠及快速動眼期睡眠的量；另外，大腦皮質溫度亦會在側腦室注射生理食鹽水時增加。

結論 目前的結果顯示當在光照期或黑暗期的中點給予光照及側腦室注射生理食鹽水對於經過適應期的老鼠依然是某種程度的壓力，而這種壓力會覆蓋過光照本身對於睡眠的影響。（中台灣醫誌 **2004;9:211-8**）

關鍵詞

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