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Interaction between Light-Dark Cycles and Circadian Rhythm on Sleep and Wakefulness in Albino Rats

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ABSTRACT

This study investigated the role of the circadian phase in modulating the effect of short light-dark cycles (LDc) on sleep and wakefulness. Six male albino rats of the Sprague-Dawley strain were implanted with electrodes for standard electrophysiological recordings performed during baseline (12 – 12 h LDc), short LDc treatment, and recovery (12 – 12 h LDc) for 4 days each. In the short LDc treatment, 15 – 15 min LDc were applied, respectively, in mid-periods of inactive and active phases to maintain an entrained circadian rhythm. The results showed that the 15 – 15 min LD ratio of both non-rapid eye movement sleep (NREM) and paradoxical sleep (PS) did not vary with the circadian phase. In contrast, changes in both the NREM and PS amounts in the short LDc treatment varied with the circadian phase. It is argued in the Discussion section that the circadian phase-related changes in the sleep amount did not result from the circadian rhythm effect but from the interactions between the habitual 24 h lighting schedule and the habitual LD distribution of the sleep and wakefulness amounts. On the other hand, this study found that both waking (W) and PS response to short LDc varied with time courses. The 15 min dark period strongly enhanced the W time only when it occurred for the first time in the inactive phase while it consistently facilitated PS across the remaining time periods in both the active and inactive phases. Furthermore, a residual effect of short LDc on PS was revealed in this study. Compared to the baseline, the 12 – 12 h LD ratio of PS was significantly decreased during recovery compared to the short LDc treatment.

Key words: light-dark ratio, arousal states, delta, theta, electroencephalogram (EEG) spectrum, time course

I. Introduction

Light, as a salient natural *zeitgeber*, is actively involved in the regulation of circadian rhythms of cyclic behavior, *e.g.*, sleep and wakefulness, and physiological functions, *e.g.*, body temperature. The discovery by Lisk and Sawyer (1966) of paradoxical sleep (PS) induction through lights-off stimulation in albino rats revealed that light/dark modulates sleep and wakefulness states. Later work applying constant illuminance/dark (Borbely and Neuhaus, 1978; Fishman and Roffwarg, 1972; Tobler *et al*., 1994), short light-dark cycles (LDc) (Alfoldi *et al*., 1991; Borbely, 1976; Borbely *et al*., 1975; Johnson *et al*., 1970; Rechtschaffen *et al*., 1968), light/dark pulses (Benca *et al*., 1996; Rechtschaffen *et al*., 1969), or irregular schedules of alternating light and dark periods (Benca *et al*., 1998) to rats showed that light is advantageous for non-rapid eye movement sleep (NREM) but not for waking (W). Darkness has an opposite effect; i.e., it is advantageous for W but not for NREM. In contrast to the W enhancing effect of darkness in both albino and nonalbino rats, the PS inducing effect of darkness has been observed only in albino rats (Benca *et al*., 1998).

The light/dark effect on sleep and wakefulness in albino rats might or might not depend on a functioning circadian clock system. Lesion studies in certain brain regions show that the circadian clock system can be dissociated from the light/dark effect on sleep and wakefulness. Lesions in the suprachiasmatic nuclei (SCN), functioning together as a circadian pacemaker, completely eliminate circadian sleep rhythm but do not affect PS increases in the short dark period (Sisk and Stephan, 1982). In contrast, the PS inducing effect of darkness is attenuated by lesions in the pretectum although photic entrainment of circadian rhythm remains intact (Miller *et al*., 1998, 1999). Thus, light/dark may directly act on the sleep and wakefulness system without the involvement of a circadian clock system. Furthermore, one report provided behavioral evidence. Neither NREM nor PS in response to one short LDc treatment day varied with the circadian phase (Alfoldi *et al*., 1991). On the other hand, another report showed that the magnitude of light/dark induced changes in sleep and wakefulness was related to the circadian phase (Borbely *et al*., 1975). It is not known whether the different results between the two studies (Alfoldi *et al*., 1991; Borbely *et al*., 1975) were due to free running of endogenous rhythms during the short LDc treatment, which lasted for more than one day (Borbely *et al*., 1975). Thus, this study aimed to clarify the role of the circadian phase in modulating the light/dark effect on sleep and wakefulness. To limit the possible con-

founding effect of phase shifts due to free running of rhythms during a prolonged short LDc treatment, a strategy was developed that involved short LDc applications for four days. With parts of the long light and dark periods of 24 h retained before and after the short LDc period, 15 – 15 min LDc were superimposed in mid-periods of inactive and active circadian phases, respectively, in order to limit the phase shift and to maintain an entrained 24 h rhythm.

II. Materials and Methods

1. Subjects and Surgery

Male Sprague-Dawley rats were purchased from the National Laboratory of Animal Breeding and Research Center, Taipei, Taiwan, R.O.C. The surgical procedure for skull electrode implantation was previously described (Bergmann *et al*., 1989). Briefly, six rats (6 – 9 months) were implanted aseptically under pentobarbital (Somnotol) anesthesia (65 mg/kg i.p.) with stainless steel screw electrodes (0 – 80× 3/32, length 2.4 mm; Small Parts, Inc., Miami, FL, U.S.A.) to record cortical EEG and hippocampal theta activity and with multistranded microwires (A-M Systems, Inc., Everett, WA, U.S.A.) to record nuchal electromyograms (EMGS). The EEG recording electrodes were threaded into burr holes in the skull with 2 – 3 turns to come into contact with the dura. The theta activity recording electrodes penetrated the dura and the surface of the neocortex such that the tip finally stayed at the top of the hippocampus. All the electrodes were connected to a miniature connector (Continental Connector Co., Bloomfield, NJ, U.S.A.), and then the connector was mounted using dental cement (Hygenic Co., Akron, OH, U.S.A.) to a skull base made using P-10 resin (3M, St. Paul, MN, U.S.A.). A temperature-sensitive transmitter (VM-type, Mini-Mitter Co., Inc., Sunriver, OR, U.S.A.) was placed in the peritoneal cavity to record the body temperature.

After surgery, each rat was housed in an individual plastic cage ($35 \times 35 \times 55$ cm^3) placed in a sound attenuated recording room. The recording room was illuminated by fluorescent lamps. The switch for the lamps was controlled by a timer. A 12 – 12 h LD schedule (lights on: 09:00 – 21:00, ca. 300 lux vs. <1 lux at lights-off) was employed. Rats were allowed to recover from surgery for a minimum of 7 days, and animal handling was performed on postsurgery days 4 – 7 for at least 15 min a day. Subsequently, each rat was connected via a recording cable to a commutator (Airflyte Electronics, Bayonne, NJ, U.S.A.) suspended from a counterbalanced lever. After 5 more days for adaptation to the recording cable, baseline EEG, hippocampal theta activity, and EMG recordings began. The cage temperature was regulated at 23 – 24°C. Food and water were available *ad libitum*. Food intake, water intake and body weight measurements, and cleaning were performed every other day at around 9:00 to maintain the rats' well-being. All of the animal facilities and care followed the guidelines provided by the *Guide for the Care and Use of Laboratory Animals*, 1996, National Academy Press, Washington, D.C., U.S.A.

2. Recordings

Electrophysiological signals from each rat were passed via a long low-resistance, shielded connection cable to amplifiers (BIOPAC Systems, Inc., Santa Barbara, CA, U.S.A.) in an adjoining room. Amplified hippocampal theta activity was passed to a filter (band pass: 4 – 7 Hz). Amplified EEG and EMG signals and filtered theta activity were then converted into digital signals with 14.65 ms sampling intervals by means of a MP100 data acquisition unit (BIOPAC Systems, Inc.) and DAP2400 e/5 (Microstar Laboratories, Inc., Bellevue, WA, U.S.A.). Digitized signals from the MP100 were displayed on a monitor via AcqKnowledge software (BIOPAC Systems, Inc.) and stored in an optical diskette. The DAP2400 e/5 was used to perform automatic on-line data processing. All the electrophysiological signals were rectified and integrated over 30 s epoch by the DAP. Pulses from an AM radio which continually monitored signals from the implanted temperature-sensitive transmitter were digitized by a radio-blip detector and then sampled and analyzed by the DAP (Bergmann *et al*., 1989). Due to deficiencies in the temperature recording system in four rats, however, temperature data were not reported. Every 24 hours, the transformed data were scored for stages as W, PS, or NREM substages: low EEG amplitude sleep (LS), medium EEG amplitude sleep (MS), or high EEG amplitude sleep (HS), using the Parametric Animal State Scoring system (Bergmann *et al*., 1987). LS is characterized as low-amplitude EEG, EMG and theta. The rest of the NREM is subdivided into MS, the portion with EEG amplitude below the modal EEG amplitude of NREM, and HS, the portion with EEG amplitude above the mode. The scoring criteria of EEG, EMG and theta did not show any significant changes across the experimental period. Samples of computer-generated sleep scores were compared to raw signals stored in the optical diskette to ensure proper performance of the scoring system. EEG spectrum analysis was performed on line by means of Fast Fourier Transform using the FFT command provided by the DAP software with a block size of 2048 sample points. The EEG amplitudes within the delta band (0.8 – 4.25 Hz) were integrated for each 30 s epoch. Although a 30 s window span could violate the prerequisite assumption of stationary signals made by Fast Fourier Transform, the integrated amplitude in the delta band was highly, positively correlated to the mean calculated across eight 256-sample FFTs for each epoch. Due to hardware limitations, the window span was set at 30 s instead of a shorter span.

3. Experimental Design

After at least 4 days of stable baseline data had been

obtained, short LDc were performed for 4 days, followed by at least 4 more days of recovery (back to 12 – 12 h LDc). During the four short LDc treatment days, 15 – 15 min LDc for a total of 12 cycles were performed 3 hours after lights-on and again 3 hours after lights-off. This schedule was intended to limit phase shifts of endogenous rhythms in short LDc (Borbely *et al*., 1975) and to maintain an entrained 24h rhythm as in baseline.

4. Data Analysis

All the variables obtained from baseline, short LDc, and recovery were averaged across 4 days for each rat, and statistical comparisons then were performed across the six rats. Circadian rhythmicity was evaluated for each rat using a 24 h cosine fitting model (Agren *et al*., 1986) on the averaged hourly data of sleep and wakefulness, EEG delta activity and theta activity, respectively. Three parameters, i.e., intercept, acrophase and amplitude were derived from cosine fits. When data epochs were found to be missing, the hourly data of sleep and wakefulness states were proportionally adjusted for a total of 120 epochs per hour. The LD ratio of each sleep and wakefulness state was quantitatively defined by the formula (epochs of a specific state in light)/(epochs of that state in light + epochs of that state in darkness). The normality of LD ratios was examined using the Kolmogorov-Smirnov test, and the result was not contrary to the assumption of normality.

The interactions between the circadian phase (inactive and active phase) and lighting condition (baseline, short LDc, recovery) in the 15 – 15 min LD ratios was evaluated using a two-factor with repeated measures analysis of variance (RM-ANOVA), followed by post-hoc comparisons using the Newman-Keuls test or Dunnett's test. Whenever neither the interaction nor the circadian phase effect was significant, the lighting condition effect was evaluated using one-way RM-ANOVA, followed by post-hoc comparisons using Dunnett's test. If not specifically noted, the statistical significance level was set at 0.05.

III. Results

1. Circadian Rhythms

The W and NREM time, EEG delta activity and hippocampal theta activity all showed significant 24 h rhythms in baseline in all the rats. Not all the sleep and EEG data for all rats during the short LDc treatment and recovery days reached the significant level; however, some had p values very close to 0.05. Thus, the significant level for cosine curve fitting was loosely set at $p < 0.1$ in order to include more data in the statistical analysis. Under the new criterion, only W showed significant 24 h rhythm across all the lighting conditions in all the rats. Thus, changes in circadian rhythm across the lighting conditions were evaluated using the parameters of W rhythm. The amplitude of W rhythm was significantly lower in the short LDc treatment than in baseline ($F_{(2,10)} = 6.26$, $p = 0.017$), but the intercept and acrophase of W rhythm did not show any changes. Thus, the circadian rhythm was entrained to 24 h, and its phase was maintained throughout the experimental period.

2. 15 – 15 min LD Ratios of Sleep and Wakefulness States in the 6 h Short LDc Period

Significant interactions between the circadian phase and lighting condition were not found in the 15 – 15min LD ratio of any sleep and wakefulness state (Table 1), nor were there significant circadian phase variations. Power analyses were not further applied to Type II error evaluations because the Cohen's *f* measures showed a very small or undetectable effect size. In contrast, significant lighting condition effects on the LD ratio of both PS and NREM were found. The effect was large in both PS and NREM. It was speculated that the circadian phase effect might have been masked by different response patterns of W and NREM during the first hour of short LDc in the inactive phase (see below). Furthermore, the first 15 min dark period in the inactive phase and the first 15 min light period in the active phase followed a longer light period (3 h) and dark period (3 h) than did the remaining 15 min dark and light periods, respectively. Thus, the first hour data of the 6 h short LDc periods were excluded from analysis to determine if the circadian phase effect would then be revealed. Based on the last 5 h data, the circadian phase effect was not significant in any sleep or wakefulness state (Table 2). The effect both the circadian phase and its interaction with the lighting condition remained very small.

Since neither the interaction effect between the circadian phase and lighting condition nor the circadian phase effect was significant, the lighting condition effect was then evaluated using one-way RM-ANOVA (Fig. 1). W was distributed evenly in the 15 min light and dark periods (in the inactive phase: $F_{(2,10)} = 2.797$, $p = 0.108$; in the active phase: $F_{(2,10)} = 1.27$, $p = 0.322$). PS was redistributed more in the dark periods (in the inactive phase: $F_{(2,10)} = 26.494$, $p < 0.0002$; in the active phase: $F_{(2,10)} = 24.413$, $p < 0.0002$) while NREM was distributed more in the light periods (in the inactive phase: $F_{(2,10)} = 17.529$, $p < 0.0006$; in the active phase: $F_{(2,10)} = 12.759$, $p < 0.002$). MS was the main substage of NREM responding to the short LDc treatment (in inactive phase: $F_{(2,10)} = 12.948$, $p < 0.002$; in the active phase: $F_{(2,10)} = 23.585$, $p < 0.0002$).

3. Sleep and WakefulnessAmounts druing the 6 h Short LDc Period

Changes in the sleep and wakefulness amounts during the 6 h short LDc period varied with the circadian phase (Fig. 2). Compared to the baseline, the short LDc treatment did not

Table 1. *F* Ratios and Effect Sizes from the Two-Factor (Lighting Condition × Circadian Phase) RM-ANOVAs Carried out for LD Ratios of Sleep and Wakefulness in the 6 h Short LDc Period

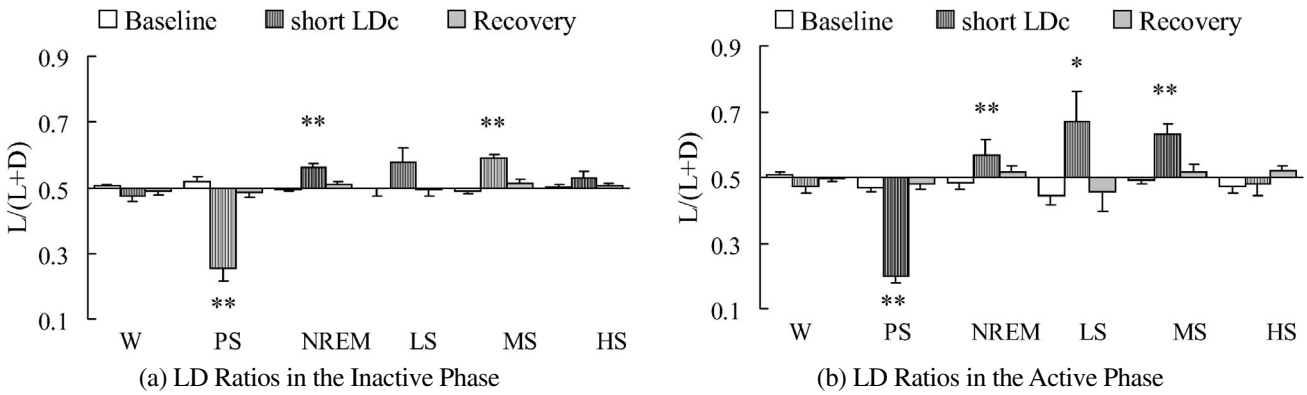
Sleep and Wakefulness State	Factors	*F* ratio	*p*	Cohen's *f* measure of effect size
Waking (W)	lighting condition × circadian phase	0.075	0.928	–
	lighting condition	2.277	0.153	0.066
	circadian phase	0.100	0.755	–
Paradoxical sleep (PS)	lighting condition × circadian phase	0.414	0.666	0.034
	lighting condition	55.383	0.000***	1.138
	circadian phase	2.371	0.136	0.037
Non-rapid-eye-movement sleep (NREM)	lighting condition × circadian phase	0.320	0.729	–
	lighting condition	21.247	0.000***	0.624
	circadian phase	0.017	0.896	–
Low EEG amplitude NREM (LS)	lighting condition × circadian phase	3.276	0.080	0.113
	lighting condition	4.223	0.047*	0.154
	circadian phase	0.000	0.986	–
Medium EEG amplitude NREM (MS)	lighting condition × circadian phase	0.757	0.479	–
	lighting condition	27.813	0.000***	0.747
	circadian phase	1.235	0.277	0.006
High EEG amplitude NREM (HS)	lighting condition × circadian phase	1.307	0.289	0.017
	lighting condition	0.740	0.487	–
	circadian phase	1.784	0.194	0.021

Notes: df of *F* (lighting condition × circadian phase) = (2,25); df of *F* (lighting condition) = (2,25), df of *F* (circadian phase) = (1,25); – : effect size not calculable because the *F* ratio was less than 1.
* $p < 0.05$; *** $p < 0.0005$.

Table 2. *F* Ratios and Effect Sizes from the Two-Factor (Lighting Condition × Circadian Phase) RM-ANOVAs Carried out for LD Ratios of Sleep and Wakefulness during the Late 5 h of the 6 h Short LDc period Sleep and Wakefulness State

Sleep and Wakefulness State	Factors	*F* ratio	*p*	Cohen's *f*measure of effect size
W	lighting condition × circadian phase	1.384	0.269	0.021
	lighting condition	3.431	0.048*	0.120
	circadian phase	0.008	0.930	–
PS	lighting condition × circadian phase	0.027	0.973	–
	lighting condition	63.949	0.000***	1.237
	circadian phase	0.543	0.468	–
NREM	lighting condition × circadian phase	1.283	0.295	0.015
	lighting condition	19.088	0.000***	0.579
	circadian phase	0.011	0.917	–

Notes: df of *F* (lighting condition × circadian phase) = (2,25); df of *F* (lighting condition) = (2,25), df of *F* (circadian phase) = (1,25); – : effect size not calculable because the *F* ratio was less than 1.
* $p < 0.05$; *** $p < 0.0005$.



(a) LD Ratios in the Inactive Phase (b) LD Ratios in the Active Phase

Fig. 1. The 15 – 15 min LD ratio (mean ± SEM) of sleep and wakefulness states in the 6 h short LDc period in the inactive (L, upper panel) and active phases (D, lower panel). The LD ratio in either circadian phase was derived by dividing the total amount of one stage across the twelve 15 min light periods by that of the 6 h short LDc period. The LD ratios during baseline and recovery were calculated based on the corresponding 6 h data, respectively.
* $p < 0.05$, ** $p < 0.01$, one-way RM-ANOVA followed by Dunnett's test for differences between the short LDc treatment and baseline means.

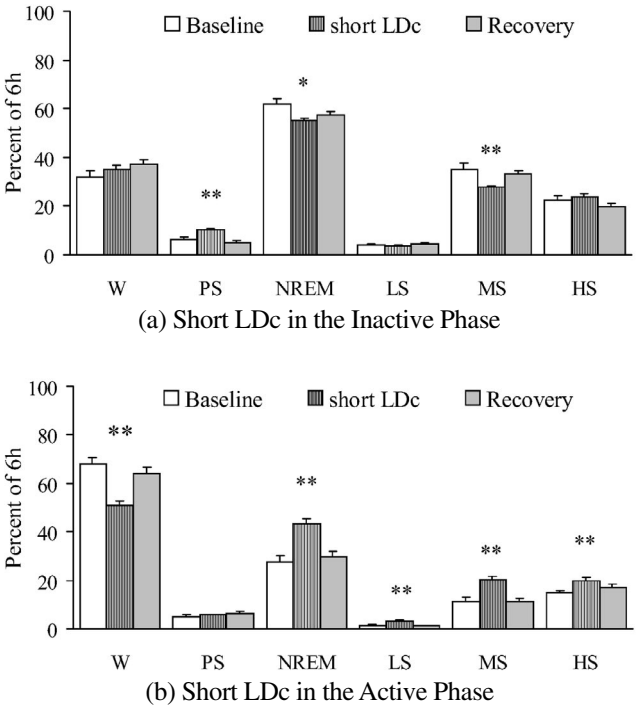


Fig. 2. The period (mean ± SEM) of each sleep and wakefulness state in the 6 h short LDc period in the inactive (a) and active phases (b). Arousal state parameters during baseline and recovery were calculated based on the corresponding 6 h data, respectively.
* $p < 0.05$, ** $p < 0.01$, one-way RM-ANOVA, followed by Dunnett's test for differences between the short LDc treatment and baseline data.

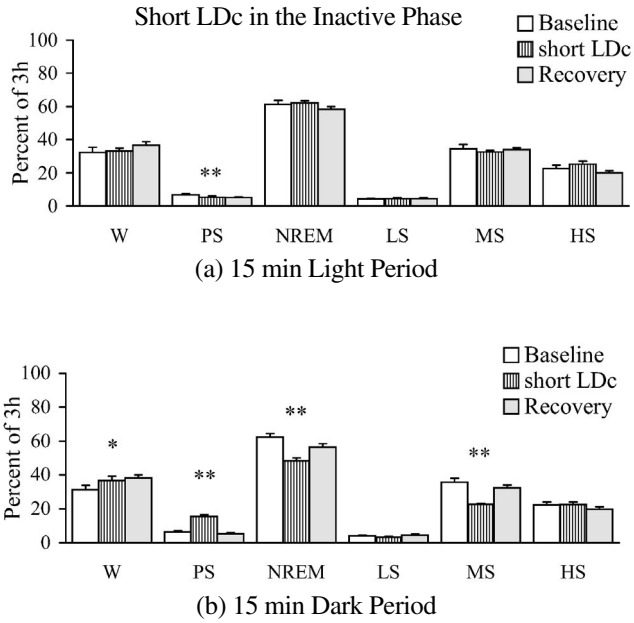


Fig. 3. The period (mean ± SEM) of each sleep and wakefulness state across the twelve 15 min light periods (a) and across the twelve 15 min dark periods (b) in the inactive phase.
* $p < 0.05$, ** $p < 0.01$, one-way RM-ANOVA, followed by Dunnett's test for differences between the short LDc treatment and baseline data

alter the W time in the inactive phase (Fig. 2(a)) but decreased the W time in the active phase ($F_{(2,10)} = 23.126$, $p < 0.0002$; Fig. 2(b)). The PS time increased in the inactive phase ($F_{(2,10)} = 37.576$, $p < 0.0001$; Fig. 2(a)) but did not change in the active phase ($F_{(2,10)} = 2.102$, $p = 0.173$; Fig. 2(b)). NREM ($F_{(2,10)} = 6.025$, $p = 0.019$; Fig. 2(a)), mostly MS ($F_{(2,10)} = 9.202$, $p < 0.006$), decreased in the inactive phase but, including all substages, increased in the active phase ($F_{(2,10)} = 33.439$, $p < 0.0001$; Fig. 2(b)).

The sleep and wakefulness amounts in the 15 min light and dark periods were separately evaluated for the inactive and active phases. In the inactive phase, the 15 min light period only decreased the PS time (Fig. 3(a)) while the 15 min dark period increased both W and PS but decreased NREM, mostly MS (Fig. 3(b)). In the active phase, the 15 min light period increased NREM but decreased W (Fig. 4(a)) while the 15 min dark period increased both NREM and PS but decreased W (Fig. 4(b)).

Taken together, the 15 – 15 min LD ratios of both NREM and PS during the 6 h short LDc period in the inactive phase were comparable to those in the active phase. However, the effect of short LDc on the amounts of sleep and wakefulness varied with the circadian phase, with changes found mainly in PS and NREM in the inactive phase but mainly in W and NREM in the active phase.

4. Time Course of Changes in Sleep and Wakefulness

The effect of short LDc on different sleep and wakefulness states showed different time courses (Fig. 5). Significant interactions between time (15 min period) and the lighting condition were found in all the sleep and wakefulness states (W: $F_{(190,1435)} = 1.985$, $p < 0.0001$; NREM: $F_{(190,1435)} = 2.264$, $p < 0.0001$; PS: $F_{(190,1435)} = 3.004$, $p < 0.0001$). The simple main effect of the lighting condition for each 15 min period was subsequently analyzed with the alpha value set at 0.005. The first 15 min dark period in the inactive phase significantly enhanced and decreased W and NREM, respectively. Except for the first hour in the inactive phase, short LDc consistently altered the LD distribution of both NREM and PS during the remaining treatment period.

5. Delta and Theta Activity

The lighting condition effects on the delta and theta activity were analyzed using one-way RM-ANOVA. Short LDc did not alter the delta activity in any sleep and wakefulness state. In contrast, the 15 min dark period in the inactive phase increased the theta activity in W ($F_{(2,10)} = 9.91$, $p = 0.004$).

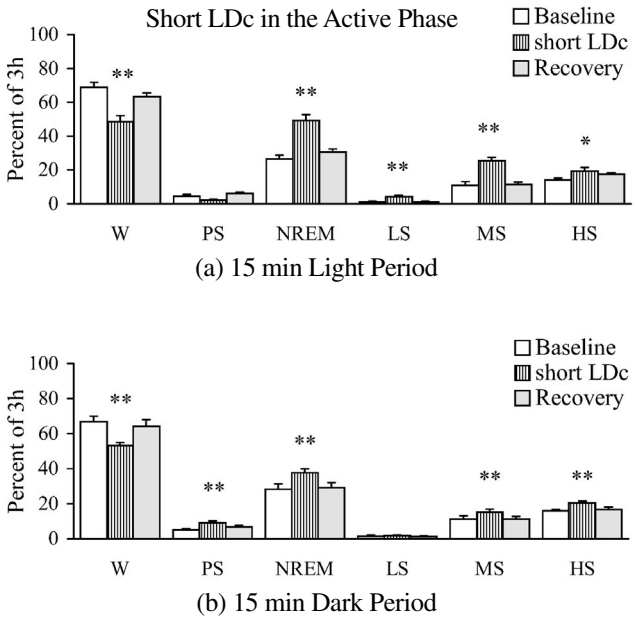


Fig. 4. The period (mean ± SEM) of each sleep and wakefulness state across the twelve 15 min light periods (a) and across the twelve 15 min dark periods (b) in the active phase.
* $p < 0.05$, ** $p < 0.01$, one-way RM-ANOVA, followed by Dunnett's test for differences between the short LDc treatment and baseline data.

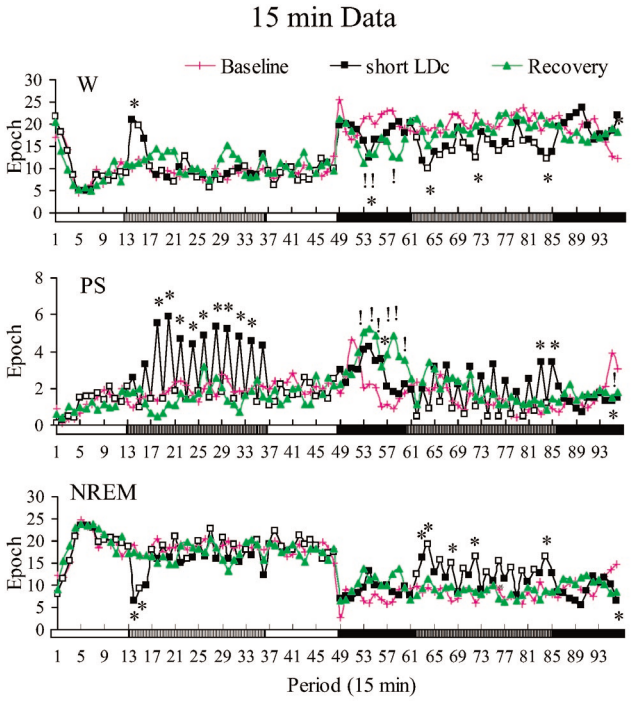


Fig. 5. The periods (one epoch = 30 s) of the sleep and wakefulness states during 15 min periods across the baseline, the short LDc treatment, and recovery. The open square and closed square symbols represent the 15 min light and dark periods, respectively, during the short LDc treatment. The blank section of the horizontal bar below the x-axis indicates the 3 h light period, the black section indicates the 3 h dark period and the vertical line section indicates the 6 h short LDc period during the short LDc treatment days.
* $p < 0.005$, two-factor RM-ANOVA, followed by Dunnett's test for differences between short LDc treatment and baseline data.
! $p < 0.005$, Dunnett's test for differences between recovery and baseline data.

6. Recovery

The amplitude and acrophase of W rhythm and the 24 h total sleep and wakefulness times during recovery (lighting schedule back to 12 – 12 h LDc) were all comparable to those in the baseline. Compared to the baseline, however, some changes in sleep and wakefulness at some time points occurred during recovery (Fig. 5). W time decreased while PS time increased during the second and third hours of the 12 h dark period. Furthermore, the 12 – 12 h LD ratio of PS was lower during recovery than during baseline ($F_{(2,10)} = 13.55$, $p = 0.0014$; Fig. 6). The PS time in the 12 h dark period (active phase) was significantly increased during recovery (lighting condition × circadian phase effect: $F_{(2,25)} = 6.189$, $p = 0.006$. Although it did not reach statistical significance, the PS amount tended to decrease during the 12 h light period ($p < 0.1$). The 12 – 12 h LD ratio of PS was still higher on recovery day four than in baseline. Since sleep and wakefulness data were collected until recovery day five in one rat and day six in three rats, the 12 – 12 h LD ratio of PS on the last recording day averaged across the four rats. The mean LD ratio of PS on the last recording day was recovered to the baseline level ($F_{(2,6)} = 8.547$, $p = 0.018$; Dunnett's test, $p < 0.05$ for the short LDc treatment, $p > 0.05$ for the last recording day). Although the 12 – 12 h LD ratio of W or of NREM during recovery was not significantly altered, both ratios showed a trend of reduced LD differences relative to the baseline levels. Compared to the baseline, W time significantly decreased during the 12 h dark recovery period (lighting condition × circadian phase effect: $F_{(2,25)} = 5.645$, $p = 0.009$). However, the amplitude of the W rhythm derived from the 24 h cosine-fit curve was not different between recovery and baseline. In addition, the NREM time did not show significant differences during the 12 h light or dark periods during recovery. Thus, it seemed that the LD redistribution of PS during recovery could not be fully attributed to a flattened sleep and wakefulness rhythm.

IV. Discussion

As previously shown (Alfoldi *et al*., 1991; Borbely, 1976; Borbely *et al*., 1975; Johnson *et al*., 1970; Rechtschaffen *et al*., 1968), this study confirmed that short LDc induced LD redistribution of both PS and NREM. The 15 – 15 min LD ratios of all the sleep states during the short LDc treatment in inactive phase were comparable to those in the active phase. It is concluded that the effect of short LDc on sleep does not depend on the circadian system. An LD ratio computed usingy the formula (min/hr in dark)/(min/hr in dark + min/hr in light)

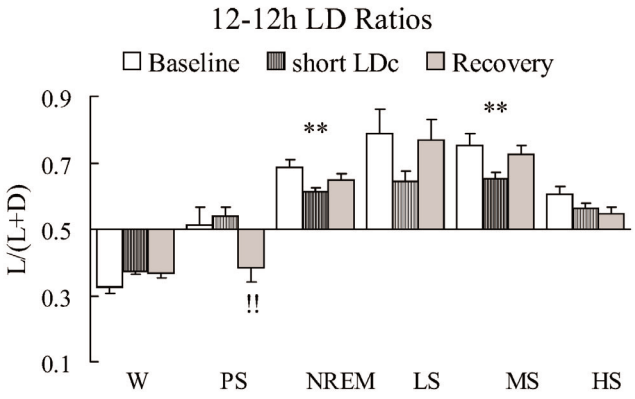


Fig. 6. The 12 – 12 h LD ratios (mean ± SEM) of the sleep and wakefulness states. The LD ratio was derived by dividing the total amount of one stage in the 12 h light period by that of the 24 h LD period during baseline and recovery, respectively. LD ratios during the short LDc treatment days were calculated based on the corresponding 12 - 12 h data.

** $p < 0.01$, one-factor RM-ANOVA, followed by Dunnett's test for differences between short LDc treatment and baseline data.

!! $p < 0.01$, one-factor RM-ANOVA, followed by Dunnett's test for differences between recovery and baseline data.

was previonsly used by Sisk and Stephan (1982) to assay changes in sleep in response to short LDc following lesions in several brain regions. Lesions in the SCN abolish the entrainment of sleep rhythm to 12 – 12 h LDc but do not abolish the response of either NREM or PS to 1 – 1 h LDc. The characteristic LD distributions of both NREM and PS in circadian and ultradian cycles were concluded to be controlled separately by the brain. Therefore, this study, using the LD ratio as an indication of the LD distribution of sleep, provided behavioral evidence corresponding to the neuronal findings.

On the other hand, this study showed that changes in the sleep amounts in the short LDc period was related to the circadian phase. This result corresponds to the finding of Borbely *et al.* (1975). However, the circadian phase-related change in the sleep amount could not be simply attributed to a confounding effect of phase shift of circadian rhythm. This is because the specific short LDc schedule applied in this study, possibly along with help provided by the regular animal care schedule sustained throughout the experimental period, successfully maintained an entrained W rhythm to 24 h. On the other hand, circadian phase-related changes in the sleep amount could not be fully attributed to the circadian rhythm effect. At least two more factors needed to be considered. They were the habitual 24 h lighting schedule and the habitual LD distribution of sleep and wakefulness.

Short LDc applied in different circadian phases had various effects on the lighting background. Short LDc applied in the inactive phase meant adding periodic dark pulses to, periodically withdrawing light from and periodically adding L-D and D-L transitions to the habitual lights-on period. Given that darkness enhances PS, and that light facilitates NREM, the PS time could be increased by periodically adding dark pulses, but NREM time could be decreased by periodic light withdrawal. In contrast, short LDc applied in the active phase meant adding periodic light pulses to, periodically withdrawing darkness from and periodically adding L-D and D-L transitions to the habitual lights-off period. Thus, the NREM time could be increased by periodically adding light pulses, but the PS time could be decreased by periodic dark withdrawal. Secondly, the habitual amount of sleep and wakefulness could modulate the sleep response to changes in the lighting conditions. For example, lights-off induces a shorter PS latency and a longer PS duration when it occurs in NREM than it does when it occurs in W (Sisk and Sawyer, 1966). Since lights-off would be more likely to occur during NREM in the inactive phase than in the active phase, the PS amount would be increased more in the inactive phase, as confirmed in this study.

Since the sleep amount in the short LDc period was determined by multiple factors besides short LDc, it could change differently across studies. Indeed, inconsistent circadian phase-related changes in the PS amount were found in previous studies and in the individual rats in this study (data not shown). For example, one study (Borbely, 1976) found that a short LDc treatment increased the PS amount in the active phase but not in the inactive phase, whereas another study (Alfoldi *et al.*, 1991) found that it increased PS in the inactive phase but not in the active phase. Thus, the circadian phase-related changes in sleep response to the short LDc treatment did not necessarily lead to the conclusion that the effect of light/dark on sleep depends on the circadian phase. Taken together, the results for the 15 – 15 min LD ratio, in this study do not support the assumption that the circadian phase modulates the light/dark effect on sleep.

In contrast to a longer light-dark cycle period (Benca *et al.*, 1998; Borbely *et al.*, 1975), the 15 – 15 min LDc treatment did not induce an LD redistribution in W, as indicated by the mean LD ratio value of W, which was close to 0.5. This result corresponded to one previous study, where both the total W time and the onset frequencies of W did not differ between short (≤ 10 min) light and dark periods (Borbely, 1976). Nonetheless, since the first 15 min dark period in the inactive phase in this study dramatically increased the W time, the difference between the short and long LDc effects on the W time distribution might not depend on the duration of the dark period. Furthermore, the W amount in the first 15 min dark period in the inactive phase was comparable to that at the onset of the habitual 12 h dark period. Thus, it is concluded that whether or not a dark period effectively triggers W depends on the length of the preceding light exposure. However, a longer dark period length could still induce alonger W time.

Although the short LDc treatment induced an LD redistribution in both PS and NREM, it did not alter the intensity of either. EEG results for the intensity of NREM and PS,

i.e., the delta (0.8 – 4.25 Hz) and theta activity (4 – 7 Hz), respectively, did not change during the short LDc period. This result is partially in agreement with and partially contradicts that of a previous study (Alfoldi *et al*., 1991) which applied 1 – 1 h LDc. A 1 h light period did not alter the delta activity (0.75 – 4 Hz) in NREM but increase the theta activity (4.25 – 6.0 Hz) in PS (Alfoldi *et al*., 1991). Furthermore, it has been shown that the light effect on the EEG spectrum depends on the duration of light exposure (Alfoldi *et al*., 1991). Under 12 – 12 h LDc, both the delta (1 – 4 Hz) and theta (5 – 9 Hz) activity in NREM were found to be higher in darkness than in light (Gaztelu *et al*., 1994). Changes in the lighting schedule from 12 – 12 h LDc to continuous darkness increased the delta activity (0.75 – 4 Hz) in NREM in young (Alfoldi *et al*., 1990) and adult (Tobler *et al*., 1994) rats. Thus, prolonged light/dark periods indeed correlate with changes in the theta and delta activity in sleep. This relationship might or might not depend on the circadian clock system. On the other hand, the theta and delta activity in W failed to show LD differences under 12 – 12 h LDc (Gaztelu *et al*., 1994) or to show changes in constant darkness (Alfoldi *et al*., 1990). This study showed that the theta activity in W increased during the 15 min dark period in the inactive phase. This contradicts the findings of Alfoldi *et al*. (1991), where a 1 h light period increased the W theta activity (4.25 – 8 Hz) in both the active and inactive phases. Currently, no convincing explanation is available for the different W theta activity results in response to short LDc.

The final main finding of this study was a residual effect of short LDc on the LD distribution of PS. Relative to the baseline, the mean 12 – 12 h LD ratio of PS was reduced during recovery. The PS time during recovery decreased insignificantly during the 12 h light period by increased significantly during the 12 h dark period. Since the 12 – 12 h LD ratio of PS gradually returned to the baseline level, the LD redistribution of PS during recovery seemed to be a specific residual effect of the short LDc treatment. The question of whether an increase in PS sensitivity to darkness during short LDc is carried over to recovery needs further study. Since the W time during recovery decreased during the 12 h dark period, it could be argued that both the W and PS mechanisms in the active phase were altered by the short LDc treatment. On the other hand, the finding of reduced 12 – 12 h LD differences in both NREM and W during recovery suggests that the circadian sleep rhythm was somewhat flattened by the short LDc treatment, thereby permitting the expression of dark-enhancing/light-suppressing effects on PS. It is also possible that both of the proposed mechanisms, enhanced PS sensitivity to darkness and a flattened circadian rhythm, helped increase PS during the 12 h dark period during recovery.

V. Conclusion

This study obtained several significant results. (1) The application of short LDc during the mid-periods of the active and inactive phases successfully maintained an entrained W rhythm for 24 h. Accordingly, the sleep and wakefulness responses to short LDc in both activity phases could be assayed separately. (2) The 15 – 15 min LD ratio of any sleep state during the short LDc treatment did not vary with the circadian phase. (3) Darkness had different effects on W and PS. The 15 min dark period dramatically triggered W only when it first occurred during the inactive phase; whereas it consistently increased PS during the remaining time periods in both the active and inactive phases. (4) Ouring recovery from the short LDc treatment, the 12 – 12 h LD ratio of PS decreased significantly. PS increased during the 12 h dark period (active phase). These findings confirm that the light/dark effect on sleep can be dissociated from the circadian control system and suggest that the W triggering effect of darkness is related to the preceding light period length. Furthermore, this is the first study to demonstrate a residual effect of short LDc on the LD distribution of PS. Future studies on the mechanism responsible for the different effects of darkness on W and PS in albino rats and for the residual effect of short LDc treatment on PS are needed.

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