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DIFFERENTIAL EFFECTS OF RETINAL DEGENERATION ON SLEEP AND WAKEFULNESS RESPONSES TO SHORT LIGHT–DARK CYCLES IN ALBINO MICE

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Abstract—This study characterizes the different response patterns of sleep and wakefulness (W) to short light–dark (LD) cycles in albino mice and examines whether retinal degeneration resulting from prolonged bright light treatment and/or *rd/rd* mutation alters such response patterns. Eight young male Institute for Cancer Research (ICR) mice with normal eyes, seven young male *rd/rd* Friend Virus B type (FVB) mice, six young ICR and five young *rd/rd* FVB mice receiving 48-h bright light treatment, and five older *rd/rd* FVB mice were implanted with skull and muscle electrodes to record sleep and W. All the mice were maintained in 12-h–12-h LD cycles at baseline and received 2 days of short LD cycle treatment, which included 5-min–5-min LD cycles for a total of 24 cycles presented 4 h after lights-on and again 4 h after lights-off. All the five mouse groups maintained photo-entrainment of sleep and W rhythms at the baseline and showed a preference for paradoxical sleep (PS) occurrence in the 5-min dark period and non-rapid eye movement sleep (NREMS) in the 5-min light period and a brief alerting effect of light onset on experimental days. Retinal degeneration rising from bright light treatment and/or genetic mutation failed to eliminate or reduce the response of PS and NREMS to short LD cycles, although it enhanced the LD contrast of W, i.e., bright light treatment prolonged the alerting effect of light and the *rd* mutation increased the suppressing effect of the dark on W. These results suggest that sleep responses to short LD cycles and the brief alerting effect of light were independent of the photoreceptors in the outer retina. Furthermore, the residual photoreceptors in the outer retina and/or the photosensitive cells in the inner retina may actively modulate the effect of light and dark signals on W.

Key words: dark pulse, rapid-eye-movement sleep, waking, positive masking, melanopsin, circadian rhythm.

INTRODUCTION

Environmental lighting signals are detected and processed by the eyes to affect sleep and wakefulness (W) via circadian clock-dependent and clock-independent pathways (Borbély, 1978). In mammals, the eye contains three photoreceptive cell types, classical rod and cone photoreceptors, and melanopsin-containing intrinsically photosensitive retinal ganglion cells (Provencio et al., 2000). Three recent studies have consistently shown that melanopsin is required to maintain the direct enhancing effect of light on sleep, but they obtained inconsistent findings with regard to the role played by rod/cone-based photoreception in the sleep effect of light (Altimus et al., 2008; Lupi et al., 2008; Tsai et al., 2009).

Both rats and mice show more sleep, including non-rapid eye movement sleep (NREMS) and paradoxical sleep (PS), and less W in the light period than in the dark period under a 12-h–12-h light–dark (LD) cycle (Johnson et al., 1970; Altimus et al., 2008; Lupi et al., 2008; Tsai et al., 2009), but exhibit a redistribution of PS to the dark period under ultradian LD cycles (Lisk and Sawyer, 1966; Tobler and Borbély, 1978; Deboer et al., 2007). On the other hand, the response of W to light and dark signals is always opposite to NREMS in rats and mice as long as the duration of the dark period is no less than an hour (Borbély et al., 1975; Benca et al., 1998; Obermeyer and Benca, 1999; Altimus et al., 2008; Tsai et al., 2009). However, with dark periods less than an hour, W loses its preference of appearing in dark periods in rats (Borbély, 1976; Tsai, 2001, 2002, 2005). In contrast to the preference of NREMS to occur in the light period, the onset of the light period actually often induces a brief episode of W in rats (Borbély, 1976; Tsai, 2002). It appears that the brief alerting effect of light is also present in mice, since a brief increase in moving distance has been found in them (Morin and Studholme, 2011).

The eyes are necessary for a PS response to dark signals to appear since bilateral enucleation prevents it (Johnson et al., 1970). However, the contribution of the rod/cone and melanopsin-based photoreceptive systems to PS and W in response to light and dark signals is still unknown. In this study, we investigated two albino mouse models of retinal degeneration, a retinal light damage model (Wenzel et al., 2005; Organisciak and Vaughan, 2010; Okano et al., 2012) and *rd/rd* mouse (Carter-Dawson et al., 1978; Lin et al., 2009), to cross-

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Abbreviations: EEG, electroencephalogram; EMG, electromyogram; FVB, Friend Virus B type; ICR, Institute for Cancer Research; LD, light–dark; NREMS, non-rapid eye movement sleep; PS, paradoxical sleep; W, wakefulness.

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clarify how rod/cone-based photoreception influences different responses of PS, NREMS, and W to short LD cycles. In both forms of retinal degeneration, the primary retinal pathological event is the loss of the rod photoreceptors and this eventually leads to the degeneration of the cone photoreceptors (Carter-Dawson et al., 1978; Lin et al., 2009; Organisciak and Vaughan, 2010; Okano et al., 2012). Light-induced retinal degeneration proceeds quickly and takes 10 days or less from light exposure to the loss of photoreceptors (Wenzel et al., 2005). In non-albino C57BL mice carrying the *rd* mutation, no rod cells are present by post-natal day 36 and less than 40% of the cone cells remain at day 65 (Carter-Dawson et al., 1978). Albino rodents are more susceptible than non-albino rodents to light-induced retinal damage (Noell et al., 1966; LaVail et al., 1987) and to inherited retinal degeneration (LaVail and Battelle, 1975). Thus, more severe retinal degeneration is expected in albino mice than in non-albinos; this makes albino mice a suitable model for studying the role of the photoreceptors located on the outer retina in sleep and W responses to light and dark signals.

EXPERIMENTAL PROCEDURES

Animals

Male Institute for Cancer Research (ICR) (Bltw:CD1) and Friend Virus B type (FVB)/NJNarl mice were purchased from BioLasco Taiwan and the National Laboratory Animal Center (Taiwan, ROC), respectively. FVB mice are homozygous for the *Pde6b*^{rd1} allele, which leads to early onset retinal degeneration (Yu et al., 2001; Kim et al., 2005). Since wild-type or heterozygous mutant FVB mice were not available, we thought that ICR mice may be better suited than other albino strains to serve as a ''wild-type'' control for the FVB mice since both strains descend from Swiss mice (Brayton et al., 2012). The age of the animals on arrival in the laboratory was 6–8 weeks. The mice were group housed before the experimental manipulations in standard plastic cages with Lignocel® soft wood bedding (JRS, Holzmühle, Germany) on the bottom in a sound-attenuated room (lights on: 09:00–21:00, 23–25 °C). Food and water were available *ad libitum*. The animal facility was routinely sanitized every week at around 9:00. All the animal facilities and care followed the guidelines provided by the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996, and all the procedures and experimental protocols were approved by the National Chung Cheng University Institutional Animal Care and Use Committee.

Experimental design

In Experiment 1, we studied eight young adult ICR mice (10–12 weeks old at the time of receiving electrode implantation, 34–42 g; ICR_Y) to examine their response patterns of PS, NREMS, and W to short LD cycles. In Experiment 2, six ICR mice (ICR_LL) received bright light (~1700 lux) for 48 h at 10–12 weeks old and underwent electrode implantation surgery 2–4 weeks later (35–42 g). In Experiment 3, we used three groups of FVB mice to examine whether the sleep and W responses to light and dark varied with the degree of retinal degeneration. The first group included seven young FVB mice (11 weeks old at the time of receiving electrode implantation, 24–29 g; FVB_Y). Previous studies showed that FVB mice abruptly lose the photoreceptor cells in the retinal outer nuclear layer at post-natal day 14 and almost completely lose the outer nuclear layer by day 28 (Yu et al., 2001; Kim et al., 2005). The second group consisted of five young FVB mice (FVB_LL) that received 48 h of bright light (~1700 lux) at 11–12 weeks old and underwent electrode implantation surgery 1–7 days later (23–30 g). Considering that cone degeneration proceeds more slowly than rods and continues in adulthood when the rods in the *rd/rd* mice almost disappear (Carter-Dawson et al., 1978; Jiménez et al., 1996; Gouras and Tanabe, 2003), we examined a group of five older FVB mice (20 weeks old at the time of receiving electrode implantation, 30–33 g; FVB_M).

After the daily amount of W was stable for at least 4 days (see Section 'Animals' Surgery), each mouse underwent 2 days of a short LD cycle treatment, which included 5 min–5 min LD cycles (~300 lux in L vs. 0 lux in D) for a total of 24 cycles (lasting for 4 h) presented 4 h after lights-on (~300 lux) and again 4 h after lights-off (Fig. 1). This schedule was intended to reduce the phase shifts of circadian rhythms that occur with continuous short LD cycles (Tsai, 2001) and to evaluate circadian phase-related changes in sleep and W.

Surgery

The mice were implanted aseptically under ketamine (100 mg/kg) and xylazine (15 mg/kg) anesthesia (i.p.) with stainless steel screw electrodes (0–96 × 2/32, Plastics One, Roanoke, VA, USA) to record unilateral bipolar frontal–parietal electroencephalograms (EEGs) and with multi-stranded microwires (A-M Systems, Sequim, WA, USA) to record nuchal electromyograms (EMGs). All the electrodes were connected to a miniature connector (MS363, Plastics One), and then the connector was mounted using dental cement to a skull base made of AB resin. After surgery, each mouse was housed in an individual plastic cage (30 × 30 × 50 cm^3) placed in a sound-attenuated,

Fig. 1. Light schedules at baseline and during the short light–dark (LD) cycle treatment days. The white and black sections of the horizontal bar indicate lights-on (~300 lux) and lights-off periods, respectively. Zeitgeber time 0 (ZT0) is defined as being the onset of the 12-h lights-on period at the baseline. A total of 24 5 min–5 min LD cycles were performed 4 h after lights-on and again 4 h after lights-off during the short LD cycle treatment days.