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NIGHTTIME DIM LIGHT EXPOSURE ALTERS THE RESPONSES OF THE CIRCADIAN SYSTEM

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Abstract—The daily light dark cycle is the most salient entraining factor for the circadian system. However, in modern society, darkness at night is vanishing as light pollution steadily increases. The impact of brighter nights on wild life ecology and human physiology is just now being recognized. In the present study, we tested the possible detrimental effects of dim light exposure on the regulation of circadian rhythms, using CD1 mice housed in light/dim light (LdimL, 300 lux:20 lux) or light/dark (LD, 300 lux:1 lux) conditions. We first examined the expression of clock genes in the suprachiasmatic nucleus (SCN), the locus of the principal brain clock, in the animals of the LD and LdimL groups. Under the entrained condition, there was no difference in PER1 peak expression between the two groups, but at the trough of the PER 1 rhythm, there was an increase in PER1 in the LdimL group, indicating a decrease in the amplitude of the PER1 rhythm. After a brief light exposure (30 min, 300 lux) at night, the light-induced expression of mPer1 and mPer2 genes was attenuated in the SCN of LdimL group. Next, we examined the behavioral rhythms by monitoring wheel-running activity to determine whether the altered responses in the SCN of LdimL group have behavioral consequence. Compared to the LD controls, the LdimL group showed increased daytime activity. After being released into constant darkness, the LdimL group displayed shorter free-running periods. Furthermore, following the light exposure, the phase shifting responses were smaller in the LdimL group. The results indicate that nighttime dim light exposure can cause functional changes of the circadian system, and suggest that altered circadian function could be one of the mechanisms underlying the adverse effects of light pollution on wild life ecology and human physiology.

Key words: *Per1*, *Per2*, circadian rhythms, suprachiasmatic nucleus, light pollution.

The circadian system, when entrained to the day–night cycle, allows organisms to anticipate and adapt to 24-h daily cycles of the environment, ensuring that behavioral and physiological responses occur during the right temporal niche. In mammals, the suprachiasmatic nucleus (SCN) of the anterior hypothalamus serves as the principal circadian clock orchestrating a vast array of rhythmic responses throughout the body, ranging from the sleep–wake cycle to gene transcription (Klein et al., 1991; Hastings et al., 2003).

The environmental light–dark cycle is the most salient entraining factor for nearly all organisms exposed to the daily fluctuation of sunlight, synchronizing the endogenous clock to the local environment (Aschoff, 1960). The light signal, in mammals, is conveyed through the retinohypothalamic tract (RHT) from the retina to the SCN (Moore and Lenn, 1972). Light exposure at night can reset the clock in the SCN and consequently rhythms in behavior and physiology (Daan and Pittendrigh, 1976; Moore, 1983; Meijer and Schwartz, 2003). This resetting is likely mediated by the light-induced up-regulation of two putative clock genes, Per1 and Per2 (Yan, 2009).

In contrast to brief light exposures that reset the clock, the constant presence of light (LL) alters endogenous clock properties, profoundly affecting rhythms in the SCN and in the behavior of organisms. The effects of LL exposure include changes in the free-running period, arrhythmia and rhythm "splitting" (Aschoff, 1981). It has been shown that the behavioral responses associated with LL exposure are derived from disrupted synchrony and/or altered organization of cellular oscillators within the SCN (de la Iglesia et al., 2000; Ohta et al., 2005; Tavakoli-Nezhad and Schwartz, 2005; Yan et al., 2005). Alterations of circadian rhythms can also be triggered by the naturally occurring variations in the environmental conditions as well. For example, the seasonal photoperiodic variation has been shown to induce changes in electrical activity, gene expression and spatiotemporal cellular organization of the SCN (Inagaki et al., 2007; VanderLeest et al., 2007; Naito et al., 2008; Yan and Silver, 2008).

Given the profound effect of lighting condition on the clock properties of the SCN, it is likely that inappropriate light exposures would lead to significant consequences in the circadian system. Since the invention of electric lights, artificial lighting has become essential for our society. However, this has also altered environmental lighting conditions, the most noticeable changes being a brighter night. More than half of the world's population is living under a night sky that is brighter than what would be naturally experienced with a full moon (Cinzano et al., 2001). In the present study, we investigated the effect of brighter nights on the functions of the SCN and the consequent behavioral responses of animals housed in a light: dim light (LdimL) condition. In particular, we determined how chronic expo-

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Abbreviations: LD, light: dark cycle; LdimL, light: dim light cycle; LL, constant light; LP, light pulse; OD, optical density; PRC, phase response curve; RHT, retinohypothalamic tract; SCN, suprachiasmatic nucleus; ZT, Zeitgeber time.

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sure to light at night affects the expression of the clock genes *Per1* and *Per2* in the SCN, as well as the display of overt activity rhythms in mice. The results suggest that brighter nights can alter the function of the brain clock and the overall expression of circadian rhythms.

EXPERIMENTAL PROCEDURES

Animals

Male CD1 mice (28 days old) were purchased from Charles River and were randomly assigned to one of two groups. The control group was housed in a 12:12 h light: dark (LD, 300 lux: 1 lux) cycle. Light phase illumination was produced by cool white fluorescent bulbs. A red safety light, with a maximum light intensity of 1 lux, was on during the dark phase to allow for animal care and maintenance. The experimental group was housed in a 12:12 h light: dim light (LdimL 300 lux: 20 lux) condition, in which the dim light was produced by a fluorescent bulb (F34T12/CW/RS/EW, Philips Electronics, NV, USA) wrapped with a scarlet polycarbonate cover (American Made Plastics Co., NJ, USA). The dim light is mostly red/orange but contains a blue component, with the peak wavelength at 610 nm and the second peak at 440 nm (USB2000+VIS-NIR Miniature Fiber Optic Spectrometers, Ocean Optics Inc. FL, USA). The maximum photon flux density at the cage level is 1 μmol/m^2/s in dim light vs 4 μmol/m^2/s in light phase (MQ-100 Quantum meter, Apogee Instruments Inc.). This dim light was chosen because it was similar to street light (high pressure sodium lamps), which is one of the main sources of light pollution (Chepesiuk, 2009). Food and water were available *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University.

Histological analysis

Animals from each group were group housed in their respective lighting condition for 3 weeks prior to being used in the experiments to investigate the effect of nighttime dim light exposure on the circadian oscillation and to study the light responsiveness of the SCN. To test the effects on circadian oscillation, animals were sacrificed at Zeitgeber times (ZT; ZT0 is lights on) 12 and 23 (n=4/time point/group). Animals at ZT23 were sacrificed in either dark (LD group) or dim light (LdimL group) condition. To evaluate the light responsiveness, animals (n=4/group) were given a 30 min light pulse (LP, 300 lux) starting at ZT16 and were then sacrificed at ZT17.5, 90 min after the beginning of the LP. Control animals (n=4/group) were treated identically, but were not exposed to the LP. In both experiments, mice were overdosed with sodium pentobarbital (200 mg/kg; Vortech Pharmaceutical, Ltd., MI, USA) and perfused intracardially with 20 ml saline followed by 50 ml 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were post-fixed for 12–18 h, and cryoprotected in 20% sucrose overnight. Sections (40 μm) were cut through the entire SCN using a cryostat.

Immunocytochemistry (ICC). For the tissue collected at ZT12 and 23, free-floating sections were incubated with mPER1 antibody (1:5000, gift of Dr. D. R. Weaver, University of Massachusetts, MA, USA, now available as Millipore antibody AB2201) and processed with avidin–biotin-immunoperoxidase technique using 3,3′-Diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St. Louis, MO, USA) as the chromogen. To deal with potential variability in independent ICC runs, animals to be compared directly were processed together with unique cut to mark each brain. After the ICC reaction, sections were mounted on slides, dehydrated with alcohol rinses, cleared with xylene, and coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ, USA).

In situ hybridization. For the tissue collected after the LP and the control (ZT17.5), *in situ* hybridization was performed as described previously (Yan et al., 1999; Yan and Silver, 2002). Briefly, sections were processed with proteinase K at 37 °C and 0.25% acetic anhydride at room temperature for 10 min. The two alternate sets of sections were then incubated in hybridization buffer containing the Dig-labeled *Per1* or *Per2* cRNA probes (0.1 μg/1 ml) overnight at 60 °C in a water bath shaker. After a high-stringency post hybridization wash, sections were treated with RNase A, and then were further processed for immunodetection with a nucleic acid detection kit (Boehringer Mannheim, Indianapolis, IN, USA). Sections were incubated in a blocking reagent diluted 1:100 in buffer 1 for 1 h at room temperature, then incubated at 4 °C in an alkaline phosphatase-conjugated digoxigenin antibody diluted 1:2000 in buffer 1 for 3 days. On the following day, sections were then incubated in a solution containing nitroblue tetrazolium salt (0.34 mg/mL; Roche, Indianapolis, IN, USA) and 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt (0.18 mg/mL; Roche, Indianapolis, IN, USA) for 14 h. The colorimetric reaction was halted by immersing the sections in TE buffer (10 mm Tris–HCl containing 1 mm EDTA, pH 8.0). Sections were mounted and coverslipped as described above.

Data analysis. For quantification, images of serial sections through the SCN were captured using a CCD video camera (CX9000, MBF bioscience, Williston, VT, USA) attached to a light microscope (Zeiss, Gottingen, Germany). Number of mPER1-immunoreactivity (ir) cells was counted bilaterally in three mid-SCN sections using NIH Image J program. At ZT23, numbers of the PER1-ir in the SCN were counted in the ventral and dorsal regions by dividing the nucleus with a straight horizontal line crossing the center of the nucleus, and the average was used to represent the value for each animal. A two-way ANOVA (time×lighting condition) followed by post-hoc *t*-test was used to assess the effect of lighting condition on the expression of PER1. The expression of *mPer1* and *mPer2* mRNA was also quantified using the NIH Image J program. Relative optical density (ROD), assessing the mean gray value per pixel, was used to quantify the intensity of the signal in the SCN compared with the adjacent hypothalamic area. Three mid-SCN sections were measured, and the average difference between the density measurement of the SCN and the background was the ROD value for each animal. Two-way ANOVA followed by post-hoc *t*-test was performed to assess the effect of nighttime dim light housing on the light-induced expression of *mPer1* or *mPer2* (LP×lighting condition).

Behavioral anaylsis

Animals (n=10 per condition) were singly housed in plexiglass cages (34×28×17 cm^3) equipped with running wheels (26 cm diameter, 8 cm width). Wheel revolutions were recorded in 5-min bins using VitalView (Minimitter, Inc.). After 3 weeks of entraining to the lighting cycle and habituating to the apparatus, animals from both LD and LdimL group were transferred into constant darkness (DD) for 2 weeks. Several circadian parameters such as the total daytime and nighttime activities, time for activity onset and offset were examined to investigate the effect of the different nighttime light intensities on behavioral activity rhythms and entrainment. The onset and offsets were determined by creating actograms using the ClockLab (Actimetrics, Inc.). All parameters were averaged across the 7 days in the third week of either LD or LdimL condition for each animal. The free-running period was calculated based on data from first week in DD using the Clocklab program, which estimates the period based on activity onset. The animals were then re-entrained to the LD or LdimL cycle for 3 weeks, and then were examined for their response to light. Specifically, they were given a light pulse (LP, 300 lux, 30 min) at ZT16 and then submersed into DD for the subsequent week (Aschoff, 1965; Albrecht et al., 2001). Phase shifts were calculated based on the

activity onset from 7 days before and after the LP. To assess the effect of nighttime dim light and/or LP on the behavioral parameters *t*-test or two-way ANOVA were performed.

RESULTS

Effect of nighttime dim light on the daily oscillation of the SCN

PER1 expression was high at ZT12 and low at ZT23 under both lighting condition (Fig. 1). At ZT12 (Fig. 1A, left column), densely packed PER1-ir nuclei were seen throughout the SCN in both LD and LdimL. At ZT23, scattered PER1-ir nuclei were seen at the center to dorsal but absent in the ventral region of the SCN in LD, showing the distribution characteristic to this time as previously reported (King et al., 2003). However, for the animals housed in LdimL, PER1-ir nuclei were distributed in both dorsal and ventral regions. Quantitative analysis revealed a significant time effect and a dim light effect (Fig. 1B, two-way ANOVA, time effect: $F=509.8$, $P<0.01$; dim light effect: $F=5.3$, $P<0.05$; interaction: $F=1.9$, $P>0.05$). Post-hoc comparison revealed that the difference in the numbers of PER1-ir nuclei between LD and LdimL conditions were not significant at ZT12 (*t*-test, $P>0.05$), but significant at ZT23 (*t*-test, $P<0.01$). To further analyze the difference in the spatial distribution of PER1 at ZT23 between the two groups, PER1-ir nuclei were counted in ventral and dorsal regions of the SCN (Fig. 1C). Two-way ANOVA revealed a significant effect on lighting condition ($F=28.6$, $P<0.05$), a marginally significant effect on region ($F=5.56$, $P=0.056$). The interaction between the two effects was not significant ($F=4.6$, $P>0.05$). Post-hoc comparison found a significant effect of light condition in the ventral region (*t*-test, $P<0.05$).

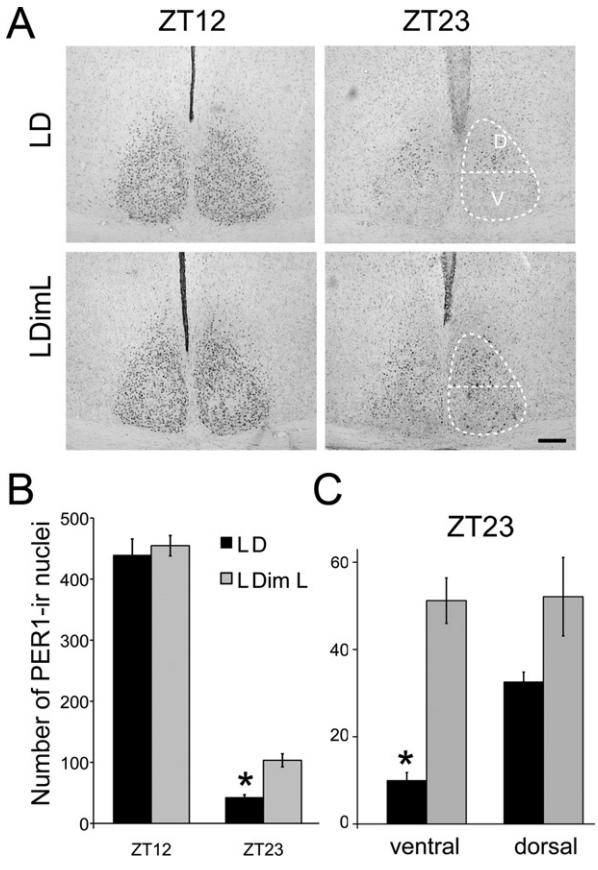

Fig. 1. Representative photomicrographs (A) and quantitative analysis (B, C) of expression of PER1 in the SCN of animals in LD and LdimL at ZT12 and 23. Scale bar=100 μm. White dashed line indicates the outline of the SCN and the ventral and dorsal region defined at ZT23 in the present study. The data are presented as mean±SEM, $n=4$. * $P<0.05$.

Effect of nighttime dim light on the light-responsiveness of the SCN

An LP at night induced both *mPer1* and *mPer2* expression in the SCN (Fig. 2). For *mPer1* (Fig. 2A), at ZT17.5 without an LP, *mPer1* expression was absent in the SCN of animals housed in LD, but was low to moderate in the SCN of animals in LdimL. After an LP, *mPer1* expression was intense in the SCN of LD animals, but only moderate in that of LdimL animals. Quantitative analysis (Fig. 2B) by two-way ANOVA revealed a significant interaction between LP and lighting conditions ($F=12.0$, $P<0.01$). Tests of simple main effect of dim light at night found a significant effect in LP group (*t*-test, $P<0.01$), but not in the no-LP controls (*t*-test, $P>0.05$). Tests of simple main effect of LP revealed significant effect in both LD and LdimL groups (*t*-test, $P<0.01$).

For *mPer2* (Fig. 2C), the expression in the animals without an LP (ZT17.5) was absent under LD but moderate under LdimL condition. After an LP, intense *mPer2* expression was observed in the SCN of animals housed in both conditions. Quantitative analysis (Fig. 2D) revealed a significant interaction between LP and lighting conditions ($F=25.4$, $P<0.01$). Tests of simple main effect of LP found significant effect in both LD and LdimL conditions (*t*-test, $P<0.05$). Test of simple main effect of lighting condition found significant effect in no LP control group (*t*-test, $P<0.05$), but not in the LP group (*t*-test, $P>0.05$).

Effect of nighttime dim light on behavioral responses

We next examined the behavioral consequence of the altered SCN responses from LdimL condition (Fig. 3). Animals were entrained under both LD and LdimL conditions and showed free-running rhythms after being released into constant darkness (Fig. 3A). Quantitative analysis revealed that during the daytime animals in the LdimL condition were more active, but at night there was no significant difference in the activity level between the two groups (*t*-test, day time activity: *t*-test, $P<0.01$; night time activity: *t*-test, $P>0.05$; Fig. 3B). There was no difference in the temporal distribution of the daytime activities between the two groups (Two-way ANOVA, effect of time: $F=36.36$, $P<0.01$; effect of lighting condition: $F=4.2$, $P>0.05$; interaction: $F=3.05$, $P>0.05$). In both groups, the majority of the daytime activities occurred in the last hour particularly the last 15 min prior to the dark or dim light phase (LD group: 54.4±13.6%, LdimL group: 69.8±4.6%), suggesting an entrainment effect. Animals in the LdimL condition

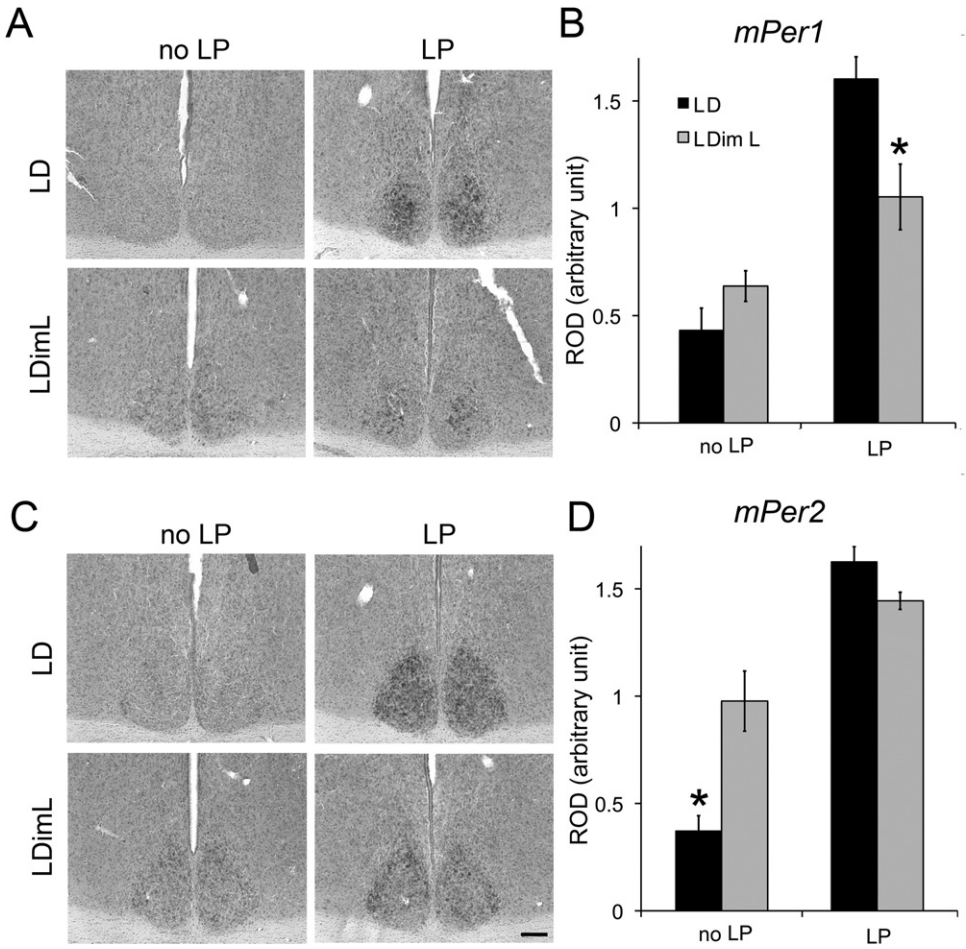

Fig. 2. Representative photomicrographs (A, C) and quantitative analysis (B, D) of expression of *Per1* and *Per2* in the SCN of animals in LD or LdimL group at ZT 17.5 without a light pulse (no LP) and after a light pulse at ZT16 (LP). Scale bar=100 μm. The data are presented as mean±SEM, n=4. * $P<0.05$.

also showed earlier onset and offset for their nighttime activities (*t*-test, $P<0.05$, Fig. 3B). However, there was no difference in the duration of activity (*t*-test, $P>0.59$, data not shown). When the animals were placed into constant

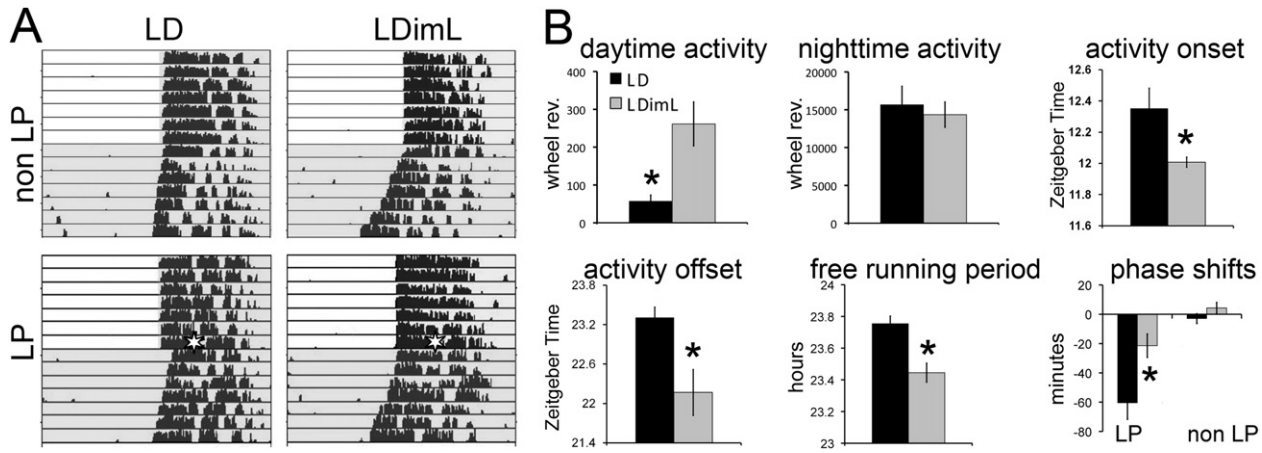

Fig. 3. Representative actograms (A) and quantitative analysis (B) of the behavioral parameters. (A) The actograms show the wheel-running activities from two animals, one in each condition. The gray shadow indicates the dark phase, the stars in the lower panel represent the light pulse. (B) Quantitative analysis of daytime activity, nighttime activity, time for activity onset and offset, free-running period and phase shift after an LP. The data are presented as mean±SEM, n=7 for LP in LdimL, n=10 for the rest of the data points. * $P<0.05$.

darkness, the LdimL groups had a significantly shorter free-running period than LD group (*t*-test, $P<0.05$, Fig. 3B). The transition from either LD or LdimL to DD produced little phase shifting, while an LP at ZT16 produced phase shift in both groups (Fig. 3A). The magnitude of shifts in the LdimL condition was smaller than those in the LD condition (Two-way ANOVA, LP effect: $P<0.01$; effect of condition: $P<0.05$; interaction: $P<0.05$, Fig. 3B). It should be noted that in the LdimL group three animals showed advances instead of delays and were removed from this analysis.

DISCUSSION

The results from the present study revealed that both the time-keeping and entrainment functions of the SCN were altered by a brighter night and that these changes had consequences downstream, in the form of changes in the overt behavioral rhythms of the animals. One of the main findings of the present study was an elevated baseline expression of clock genes in the SCN. At ZT23, which is the trough time for PER1 expression, the number of PER1-ir cells in the LdimL group was significantly higher than that in the LD group (Fig. 1). The increase in PER1 expression occurred mainly in the ventral portion of the SCN, where the nucleus receives dense retinal input (Abrahamson and Moore, 2001). Although the number of PER1-ir cells at the peak time (ZT12) remained the same between the two groups, the increased PER1 expression at the baseline level in LdimL group suggests that there was a reduction in the amplitude of circadian oscillation within the SCN. The increased baseline level was also observed for the expression of *Per2* mRNA, at ZT17.5 in the animals that did not receive an LP (no-LP group).

The mechanisms underlying how the brighter nights of the current LdimL condition affected the circadian oscillation within the SCN is unclear and warrants further investigation. It is possible that the elevated light intensity at nighttime increased the basal level of clock gene expression within individual SCN neuron. However, we favor the alternative hypothesis that LdimL housing disrupted the intercellular coupling of the SCN neural network causing desynchrony among the oscillator cells. The SCN sits at the top of the circadian hierarchy and conveys temporal information to other brain regions and to the periphery, ensuring coherent circadian oscillations throughout the body (Davidson et al., 2003; Hastings et al., 2003). The robust circadian rhythms in the expression of clock genes and the downstream events are essential for effective output control. The attenuation in the amplitude of the clock gene expression under LdimL may indicate a weakening in the time-keeping function of the SCN, which can subsequently cause an overall deregulation of the entire circadian system.

By monitoring wheel-running activity, we observed an alteration in behavioral rhythms as revealed by the increase in daytime activity in the LdimL group. Although it is currently unclear what SCN signals control behavioral rhythms, the output signals from the SCN are expected to inhibit the daytime activity in nocturnal animals (Silver et al., 1996; Kramer et al., 2001; Cheng et al., 2002; Lambert et al., 2005; Kraves and Weitz, 2006). Therefore, the increased daytime activity observed in the LdimL group likely reflects an overall decline in the output control of the circadian system, possibly originating from a dampened SCN signal. About 70% of the daytime activity in the LdimL group occurred prior to dark onset, suggesting that there is also an effect on the entrainment mechanism. The claim of an effect of night light exposure on entrainment is also supported by the differences in the time of activity onset and offset between the groups. The free-running periods differed between the two groups as well (Fig. 3). Since both the LD and LdimL groups were released into the same total darkness, the free-running periods are likely reflective of the after-effects of their prior lighting conditions.

The results on activity onset and offset time, as well as those about the free-running period collectively suggest that the endogenous rhythm of the LD and LdimL group were at slightly different phases. This raises the question of whether the phase differences caused the variation in PER1 expression. It should be noted though, that the differences in phase relationship between the two groups is relatively small, only about 20 min. Using male CD1 mice, the same strain as in the present study, it has been shown that the level of PER1 expression at ZT22 and ZT24 are not significantly different (Hastings et al., 1999; Field et al., 2000). Therefore, it is unlikely that the difference in PER1 expression seen at ZT23 was due to a phase difference at the time of sampling, but rather signals an alteration in the intrinsic clock function.

Without any external cues, the endogenous rhythms generated in the SCN are not exactly 24 h, therefore, the circadian clock in the SCN has to be reset daily by environmental light in order to maintain synchrony with the local time (Aschoff and Pohl, 1978). The photic entrainment of the circadian clock has been well characterized at multiple levels, including behavioral, physiological and molecular responses (Pittendrigh and Daan, 1976; Moore, 1983; Meijer and Schwartz, 2003; Yan, 2009). A brief light exposure given in early or late night can cause phase delays or advances, respectively, which are likely mediated by the acute induction of *Per1* and *Per2* genes. By assessing the light-induced expression of *mPer1* and *mPer2* gene and behavioral phase shifts, the results of this study revealed attenuated light responsiveness in the animals housed under brighter nights. Within the SCN, after an LP, the levels of light-induced expression of *Per1* and *Per2* genes were both lower in the LdimL group than those in the LD group, indicating an attenuated light-responsiveness of the SCN. The reduced responsiveness was also observed at the behavioral level, animals in the LdimL group shifted less in response to an LP (Fig. 3). The magnitude of phase shifts can be affected by photoperiod to which the animals are exposed (Pittendrigh et al., 1984; Evans et al., 2004). In nocturnal animals, the duration of their daily activity expands in short day and compresses in long day (Refinetti, 2002). However, the duration of their daily activity for the LdimL animals was not different from

the LD animals, suggesting photoperiodic effects were not the possible cause underpinning the smaller phase shifts in LdimL group. The free-running period of animals in LdimL group was shorter than that of animals in the LD group. The shortening in free-running period may alter the phase response curve (PRC) of these animals, such that the pulse of light would then fall at a different portion of the PRC eliciting a different response. The changes in PRC are further suggested by the fact that in the LdimL group, three out of 10 animals showed phase advances instead of delays. In addition, changes in free-running period caused by photoperiod length aftereffects and by the intensity of the light stimuli, have been shown to cause deviations in response to light, altering the magnitude of the shift (Daan and Pittendrigh, 1976; Sharma, 2003). Taken together, our results indicate that dim light at night alters the light responsiveness of the circadian system.

This altered light responsiveness could be derived from changes in input pathways or more likely, the intrinsic properties of the SCN. Melanopsin-containing ganglion cells along with rod and cone photoreceptors constitute the three groups of light-detecting cells in the retina (Hattar et al., 2003; Panda et al., 2003). Photoreceptor transduction can be modulated by changes in the light intensity, and the sensitivity of the photoreceptor can also be reduced by previous light exposure due to photopigment bleaching (Fain et al., 2001). These processes can potentially affect the photic input in the LdimL group. The other factor ultimately underpinning the altered light responsiveness is the clock itself. The increased level of clock gene expression at the through time in the LdimL group indicates a less coherent phase relationship among SCN neurons. It has been shown that the SCN neuronal network organization can determine the phase shifting capacity of the circadian clock (vanderLeest et al., 2009). In mice housed in long or short photoperiods, not only the amplitude of the behavioral phase shifts following an LP differs, the shifts of rhythms in electrical activities of the SCN following N-methyl-D-aspartate (NMDA) treatment also show different responses that correlate to the behavior. The results suggest that the differences in behavioral phase shifts are derived from the SCN rather than the alteration in the input pathways.

In contrast to the bright light exposure, the effect of dimmer light during nighttime is less understood. The dim light in the present study was largely dimmed by spectrum shift, as the photon flux was about only four times lower, but the peak wavelength was at 610 nm opposed to the white light in daytime. In a series of studies, Gorman and colleagues have investigated the effects of dim narrow-band green light ($\sim$0.01 lux, peak λ=560 nm) and have shown that the dim green light at night can facilitate the re-entrainment of rhythms following a shift of the LD cycle (Gorman et al., 2006; Evans et al., 2007, 2009; Frank et al., 2010). These intriguing results suggest that the dimly lit night, even below the threshold for circadian visual system, is not functionally equivalent to the complete darkness.

Increase in ambient light at night can affect ecology of wildlife and lead to health consequences for humans (Chepesiuk, 2009; Hastings et al., 2003; Haus and Smolensky, 2006; Erren and Reiter, 2009). The benefits of artificial lighting to our society are obvious and enormous, however, the impact of nocturnal light on our body needs to be acknowledged and understood. Our results provide evidence of the behavioral and neurobiological effects caused by unnaturally bright nights on the circadian system.

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