Effects of Sodium Lighting on Circadian Rhythms in Rats

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Rodent studies often must be conducted during an animal's active phase (that is, in darkness) yet also during a typical day shift for staff. Low-pressure sodium lighting (LPSL), to which human retinas are more sensitive than rodents' at low intensity, has been used to facilitate study conduct in dark phase. The assumption was that LPSL would be equivalent to total darkness due to low rodent retinal sensitivity but provide enough lighting for safe technical manipulations due to higher human retinal sensitivity. Unlike other light sources, LPSL has been tested for effects on circadian rhythm specific to locomotive activities in albino mice. Whether LPSL affects circadian rhythms in rats is unknown. In this study, circadian endpoints were derived from body temperature and locomotor activity via telemeters in 8 adult male Wistar rats. When moved from a 12:12-h white-light (that is, cold white fluorescent light):dark (LD) cycle to a 12:12-h white-light:sodium-light cycle, rats demonstrated free-running and disrupted circadian rhythms (that is, lengthened circadian period and reduced circadian robustness and amplitude). Body temperature and locomotor activity were significantly lower in the LPSL phase as compared with dark phase under the baseline condition. When exposed to a 12:12 h sodium-light:dark (SD) cycle, rats entrained with a circadian period similar to 12:12-h white-light:dark (LD), but significantly different from the period under constant darkness (DD). Circadian onset and acrophase were delayed under SD compared with LD. When illuminated with a LPSL pulse under DD, rats showed phase shifts similar to white-light pulse effects, consistent with the phase response curve. To determine whether the image-forming photoreceptors are involved in this process, we used electroretinography. Compared with white light, 589-nm light generated during electroretinography elicited rod photoreceptors responses with longer latency and cone photoreceptor responses with lower amplitude. These results indicate that LPSL is a weaker zeitgeber than white light and may alter the circadian system in rats. Furthermore, because LPSL appeared to be visible to rats, it may not be an appropriate substitute for actual darkness.

Abbreviations: DD, constant darkness; ERG, electroretinography; ipRGC, intrinsically photosensitive retinal ganglion cells; LD, white-light:darkness; LPSL, low-pressure sodium lighting; LS, white-light:sodium-light; SD, sodium-light:darkness.

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Circadian rhythm is the outward manifestation of an internal timing system in mammals, and it enables animals to fit physiologic and behavioral mechanisms to external environmental clues, including light. In typical humans, maintaining consistent circadian rhythms may help to improve overall health and prevent chronic diseases. In addition, timing medications to the body's circadian clock may improve drug effectiveness and decrease side effects in patients with cancer, cardiac ailments, allergies, and arthritis.^{21,26} The circadian biology of rats, the most widely used species in biomedical research, is well documented and significantly influences their metabolic, behavioral, immunologic and endocrine processes.^{40,56} In biomedical research, rats' nocturnal behavior and physiology present a significant challenge to the scientific interpretation of results and their translation to humans, given that exposure to low levels of light for human's investigative activities during the animals' dark phase (night time) can bias research results.^{10,11,13} Furthermore, the results of cognitive, nociceptive,⁹ locomotive, and metabolic assays in rat can be light-phase dependent, necessitating study conduct under 'reversed' light-dark cycles, such that the dark phase of the animals' photocycle occurs during typical human

daytime working hours.^{9,10,17,18,30,41,53} For diurnal humans to work with rodents during the animals' dark phase, red lighting (thought to be very weak for regulating circadian responses⁷) and infrared lamps with night-vision goggles have been used in vivaria. But red light is usually too dim for performing fine procedures and might alter animals' physiology and circadian rhythms,^{12,36} especially at sufficiently high intensities.²⁰ In addition, night-vision goggles might impair depth perception in some users²⁸ or be very difficult to use for others.

Based on photobiology and visual physiology, low-pressure sodium lighting (LPSL), which traditionally was used as street lighting (that is, sodium-oxide lamps), emits nearly monochromatic light at 589 nm,⁵¹ which is close to the wavelength (560 nm) at which the human's red cone photoreceptors are most sensitive⁶ but rodents' eyes are relatively insensitive, due to the lack of opsins sensitive to long wavelengths in their cone photoreceptors.⁴² Proven to be less intrusive to mammalian circadian rhythms¹⁹ than other modern light sources, the use of 589-nm monochromatic LPSL has been proposed as alternative illumination in vivaria,³⁸ thus allowing people to work with rodents under sufficient illumination during the animals' dark phase and circumventing the need to use dim red light or night-vision goggles.

Although LPSL has been tested in albino BALB/c mice and lacked significant effects on locomotive activity,³⁸ the

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potential effect of LPSL in rats has not been explored. In addition, the intrinsically photosensitive retinal ganglion cell (ipRGC)–suprachiasmatic nucleus neural pathway is a key regulator of circadian rhythms of mammalian metabolism and physiology,^{5,22,32,46} the classic image-formation photoreceptors remain to be considered play a role in circadian regulations. For example, one mouse study⁴⁵ suggested that the middle wavelength-sensitive cone photoreceptors (M-cones; λ_{max} , 511 nm) may mediate the photic regulation of circadian rhythms. Another study supports the role of rods and cones in the synchronization of circadian rhythms in mice.³⁹ We hypothesized that 589-nm light emitted by LPSL may affect circadian rhythm in rats indirectly through cone or rod photoreceptors that are sensitive to intermediate wavelengths. The main purpose of the current study was to test whether the LPSL alters the circadian system in adult albino rats and to elucidate how image-forming rod and cone photoreceptors respond to 589-nm light.

Materials and Methods

Animals for circadian study. All procedures involving animals were conducted with approval of the Pfizer IACUC. To avoid sex and estrous cycle effect on circadian,^{14,29,59} male Wistar Han (Crl:WI [Han]) rats ($n = 8$; age, 8 to 15 wk) were obtained from Charles River Laboratories (Wilmington, MA) and were free of known rat adventitious pathogens. Rats were housed in static microisolation cages on Alpha-Dri (Shepherd Specialty Papers Watertown, TN) bedding and maintained at $21 \pm 1^\circ\text{C}$, 40% to 70% relative humidity, and 12:12-h white-light:dark cycle (LD; lights on, 0600 to 1800; lights off, 1800 to 0600) in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*.²⁴ The irradiance and illuminance of the room white light (T8 ULTRAMAX Eco XL, 4100 K, 28 W, General Electric, Boston, MA) were $48.5 \mu\text{W}/\text{cm}^2$ and 262 lx, respectively, at the center of the room and 1 m above the ground. An optical power and energy meter (model PM 100D, Thorlabs, Dachau, Germany) and light meter (model LM631A, Amprobe, Everett, WA) were used to obtain these measurements. Rats had free-choice access to both pelleted chow (no. 5053, Purina, Richmond, IN) and chlorinated, reverse osmosis-purified water (Innovive, San Diego, CA) provided in water bottles. Food, water, bedding, and caging were changed weekly.

Surgical implantation of telemetry devices. After a 2-wk acclimation period after receipt from the vendor and under standard lighting conditions, rats were implanted intraperitoneally with a temperature and activity telemeter (model TA-F40, Data Science International, St Paul, MN).

Aseptic technique was strictly applied during all surgical procedures. Rats were anesthetized in a clear plastic box by using 2% to 3% isoflurane in oxygen. During surgery, anesthesia was maintained by using 1% to 3% isoflurane administered through a nosecone. The surgical site was clipped free of hair and prepared for aseptic surgery. Ophthalmic ointment was instilled in each animal's eyes to prevent corneal drying. Anesthetic depth and respiratory rate and pattern were monitored frequently. The body temperature of rats was maintained by using a circulating-water heating pad. After small skin and laparotomy incisions were made along the abdominal midline, a telemetry device was inserted into the peritoneal cavity. The laparotomy incision and subcuticular layers were closed with sterile absorbable suture (catalog no. Z513G, 4-0 PDS II, Ethicon, Somerville, NJ). Rats were returned to clean cages once they had recovered from anesthesia and were fully ambulatory. Opioid analgesics (buprenorphine, 0.04 mg/kg SC; carprofen, 4 mg/kg SC) were provided for a minimal coverage of 72 h postsurgery.

Rats were singly housed and monitored postoperatively for pain, wellbeing, and incisional healing twice daily for 7 to 10 d. Sutures were removed by 14 d postoperatively.

Light cycle design. Circadian studies were initiated 3 wk after surgery, when rats had returned to their presurgical body weight. To prevent potential light contamination and circadian interference,¹⁰ rats were housed in a room that was light-tight to external lights, and light sources on instrument panels within the study room were covered with foil. Standard white light was produced by fluorescent tubes in the ceiling. The spectral power distribution of the cold white fluorescent light (Figure 1) was obtained by using a compact spectrometer (range, 200 to 1000 nm; model CCS200, Thorlabs). Cages were placed on telemetry receivers on a static rack and were positioned to ensure the maximal uniformity of lighting; cages were not rotated during the study, to minimize interruptions to animals' circadian rhythms. The irradiance and illuminance (mean $\pm$ SEM) of the white light were $35 \pm 1.1 \mu\text{W}/\text{cm}^2$ and 170 ± 4.1 lx, respectively, at the outside front surface of cages and $26 \pm 1.5 \mu\text{W}/\text{cm}^2$ and 145 ± 4.4 lx, respectively, within cages at rodent eye level. LPSL bulbs (SOX-E, 18 W, Philips, Amsterdam, Netherlands) were used in series 4000 housings (ABS Lighting, New Haven, CT) mounted on tripods. LPSL produced a virtually monochromatic light at 589.3 nm wavelength (actually, 2 dominant spectral lines very close together at 589.0 and 589.6 nm). The spectral power distribution of LPSL is shown in Figure 1. Two LPSL lamps were placed in the room and adjusted to ensure the uniformity of lighting. The irradiance and illuminance of LPSL was $36 \pm 1.3 \mu\text{W}/\text{cm}^2$ and 174 ± 1.6 lx, respectively, at the outside front surface of cages and $28 \pm 1.2 \mu\text{W}/\text{cm}^2$ and 152 ± 2.6 lx, respectively, within cages at rodent eye level. Light intensity between 130 and 325 lx (19 to $48 \mu\text{W}/\text{cm}^2$) at cage level has been recommended to avoid phototoxic retinopathy.²⁴

Various lighting paradigms (Table 1) were used to assess the effects of LPSL on circadian rhythm in rats. At the start of the experiment, rats were maintained under standard 12:12-h white-light:dark (LD) cycles until baseline (control) circadian data were collected for approximately 2 wk. We then tested the following conditions. First, to determine the effect of LPSL on animal's circadian rhythms under a 'reversed light:dark cycle,' rats were exposed to a 12:12-h white-light:sodium-light cycle (LS; that is, sodium light from 1800 to 0600) for 11 d, after which they returned to standard 12:12-h LD cycles (complete darkness from 1800 to 0600). Second, to determine whether sodium light could entrain rats, they were exposed to a 12:12-h sodium-light:dark cycle (SD; that is, sodium light on from 0600 to 1800) for 3 wk, followed by 2 wk of DD. Finally, to determine whether sodium light can reset circadian time after free running (that is, endogenous non24-h circadian rhythm) has been established in DD, rats received a 2-h pulse of LPSL or white light daily from 0600 to 0800 for 2 wk under DD.

Circadian data acquisition and analysis. Locomotor activity and body temperature data were recorded through telemetry (Dataquest ART System 4.1, Data Science International). Throughout the experiment, telemetry data were collected from individual rats for 30 s during 5-min intervals at a sampling rate of 250 Hz. Mean body temperature (in $^\circ\text{C}$) and locomotor activity (in counts/min) from 30-s recordings were logged into the dataset every 5 min. Actograms and circadian data were generated by using ClockLab 2.61 (Actimetrics, Wilmette, IL) running on MatLab R2012b (MathWorks, Natick, MA). Data are presented as mean $\pm$ SEM. Statistical analysis was performed by using a mixed linear model (PROC MIXED) in SAS version 9.4 (SAS Institute, Cary, NC), and the Fisher

Figure 1. Spectral power distribution of light sources used in this study. (A) Cold white fluorescent light. (B) Low pressure sodium light (LPSL).

Least Significant Difference posthoc test was used to analyze differences between light cycles. The model included light cycle as fixed categorical factors. Animal ID was included in the RANDOM statement of the MIXED model. Comparisons between light cycles were generated based on model means. A *P* value of less than 0.05 indicated a statistically significant difference between light cycles.

Full-field electroretinogram (ERG) testing. Male Wistar Han (CrI:WI [Han]) rats (*n* = 6; age, 11 to 12 wk; Charles River) were used in the in vivo ERG study. All animals were exposed to a standard LD cycle, with free-choice access food and water. ERG was tested during the daytime (1000 to 1500 in the reversed dark phase). ERG procedures used in the current study were similar to our previously reported method.³¹ Briefly, the rats were dark-adapted for approximately 2 h, a duration reportedly long enough to produce scotopic luminance response curves similar to the results obtained from rats that are dark-adapted overnight,³ and then anesthetized with 1.7% to 2.0% isoflurane and oxygen. Dim red light (generated by red LED 315 headlamps; intensity, approximately 5 μ W/cm²; wavelength, 620 to 645 nm; Energizer Holdings, St Louis, MO) was used briefly to facilitate animal manipulation. Body temperature was maintained using a circulating water heating pad (T/Pump, Gaymar, Orchard Park, NY). Tropicamide (1%; Mydracyl, Bausch and Lomb, Tampa, FL) was applied to the eyes to induce pupil dilation. ERG lens electrodes (Medical Workshop, Groningen, Holland) were placed onto the surfaces of both eyes by using 0.25% hypromellose (Hub Pharmaceutical, Rancho Cucamonga, CA) and sterile saline as a coupling agent. After disinfection the skin by using a 70% alcohol pad, a platinum needle reference electrode (Natus Neurology, West Warwick, RI) was inserted subcutaneously between 2 eyes on the forehead scalp; and the ground was placed on the rat's tail. After scotopic testing, the animals

were exposed to light for 10 min for light adaptation prior to photopic ERG testing. Each ERG test session took about 45 min.

A UTAS BigShot Visual Electrodiagnostics System (LKC Technologies, Gaithersburg, MD) was used to generate spectra similar to sodium light (composed of 589-nm wavelength light), to evoke and acquire the ERG signals (high-pass filtered at 0.3 Hz and low-pass filtered at 500 Hz). ERG protocols were adapted from a previous study⁵⁰ to test scotopic and photopic luminance responses of the retina. The photopic responses were obtained at the background Ganzfeld illumination of 30 cd/m² (white light, generated by the BigShot system and calibrated by LKC Technologies). Among the 6 rats, 2 were tested first with 589-nm light stimuli and followed by white-light flashes in both the scotopic and photopic testing. In the remaining 4 animals, white-light stimuli were tested prior to 589-nm light.

ERG waveforms were analyzed by using manufacturer-provided software under the guidelines of the International Society for Clinical Electrophysiology of Vision.³⁴ a-Wave amplitude was measured from baseline to trough, and latency was measured between stimulus and a-wave trough. b-Wave amplitude was measured from a-wave trough to b-wave peak, and b-wave latency was measured from stimulus to b-wave peak.

Statistical analysis of ERG data. Two-way ANOVA with repeated measures (Prism version 6, GraphPad Software, San Diego, CA) was used to assess ERG responses to white light compared with 569-nm light. Statistical significance of differences was defined at a *P* value less than 0.05.

Results

Effects of 12-h LPSL on circadian rhythms in rats. Under the standard LD lighting condition, our rats displayed similar locomotive patterns (data not shown), as reported previously.²³ Given that the main objective of the current study was to explore whether LPSL could be used in the dark phase in the vivarium to facilitate technical or veterinary procedures, we first tested the rats under a 12:12-h LS cycle. Rats showed a free-running circadian rhythm under the LS condition, with a circadian period (mean $\pm$ SEM, 24.82 $\pm$ 0.04 h) assessed according to body temperature, that was significantly (*P* < 0.05) longer than that under DD condition (24.40 $\pm$ 0.03 h; Figure 2 and 3 D). Therefore the effect of LS on circadian rhythmicity in rats was similar to that of constant light. This observation is consistent with the Aschoff's first rule:² the endogenous free-running circadian period observed in complete darkness will lengthen for nocturnal animals when they are exposed to constant light. In addition, the activity time decreased under LS condition (Figure 2), consistent with the Aschoff's second rule:² under constant bright light, activity time decreases compared with rest time for nocturnal animals.

We also noted significant changes in other circadian parameters under the LS condition. Compared with standard LD conditions, the acrophase of temperature was delayed for approximately 0.8 h, and the circadian robustness and amplitude for body temperature were reduced (*P* < 0.05) by 34% and 29%, respectively, under LS (Table 1). Likewise, rats' circadian robustness and amplitude for locomotor activity under LS were significantly (*P* < 0.05) reduced by 47% and 59%, respectively, during LS, compared with standard light cycles (Table 1). Furthermore, the activity in the LPSL phase decreased (*P* < 0.05) by 31% compared with dark phase; whereas activity in white light increased (*P* < 0.05) by 25% compared with standard lighting conditions.

Next, we placed the rats under a 12:12-h sodium-light:dark cycle (SD). Animals entrained to 12:12-h SD cycles had a body

Table 1. Comparison of circadian parameters among LS, SD, and LD lighting protocols

Parameter		Lighting protocol		
		LD	LS	SD
Body temperature	Circadian period (τ , h)	24.01 $\pm$ 0.02	24.82 $\pm$ 0.04 ^a	24.02 $\pm$ 0.04
	Circadian robustness (Qp, %)	51.51 $\pm$ 3.20	33.75 $\pm$ 3.94 ^a	48.16 $\pm$ 3.85
	Circadian onset (h)	12.26 $\pm$ 0.19	12.90 $\pm$ 0.33 ^a	12.89 $\pm$ 0.32 ^a
	Circadian acrophase (h)	16.86 $\pm$ 0.20	17.68 $\pm$ 0.30 ^a	17.67 $\pm$ 0.29 ^a
	Circadian amplitude ($^{\circ}$ C)	0.83 $\pm$ 0.08	0.59 $\pm$ 0.09 ^a	0.82 $\pm$ 0.09
	Mean temperature during light (inactive) phase ($^{\circ}$ C)	36.54 $\pm$ 0.07	36.61 $\pm$ 0.07	36.47 $\pm$ 0.07
	Mean temperature during dark (active) phase ($^{\circ}$ C)	37.06 $\pm$ 0.04	36.95 $\pm$ 0.05 ^a	36.98 $\pm$ 0.05
	Daily mean temperature ($^{\circ}$ C)	36.80 $\pm$ 0.05	36.78 $\pm$ 0.05	36.73 $\pm$ 0.05 ^a
Locomotor activity	Circadian period (τ , h)	24.01 $\pm$ 0.01	24.82 $\pm$ 0.02 ^a	24.03 $\pm$ 0.02
	Circadian robustness (Qp, %)	23.06 $\pm$ 0.87	12.61 $\pm$ 1.38 ^a	23.34 $\pm$ 1.52
	Circadian acrophase (h)	17.80 $\pm$ 0.15	18.42 $\pm$ 0.19 ^a	18.59 $\pm$ 0.26 ^a
	Circadian amplitude (counts/min)	3.66 $\pm$ 0.39	1.49 $\pm$ 0.44 ^a	3.48 $\pm$ 0.46
	Activity in light (inactive) phase (% of total counts)	24.91 $\pm$ 1.52	42.75 $\pm$ 2.13 ^a	24.27 $\pm$ 2.31
	Activity in dark (active) phase (% of total counts)	75.09 $\pm$ 1.52	57.25 $\pm$ 2.13 ^a	75.73 $\pm$ 2.31
	Activity in light (inactive) phase (counts)	256 $\pm$ 13	322 $\pm$ 23 ^a	213 $\pm$ 25
	Activity in dark (active) phase (counts)	779 $\pm$ 56	534 $\pm$ 67 ^a	709 $\pm$ 70
	Daily total activity (counts)	1031 $\pm$ 60	852 $\pm$ 77 ^a	918 $\pm$ 83
Number of animals tested		8	6	7

Data are expressed in mean $\pm$ SEM. A mixed linear model (PROC MIXED) and the Fisher least significant difference posthoc test were used to compare groups (LS compared with LD and SD compared with LD).

^aValue significantly ($P < 0.05$) different from that for LD protocol.

temperature circadian period (24.02 $\pm$ 0.04 h) that was not different from that seen for standard LD lighting (24.01 $\pm$ 0.02 h) but that was significantly ($P < 0.05$) different from the free-running circadian period under constant darkness (24.40 $\pm$ 0.03 h, Figure 3). Compared with LD conditions, both body temperature and activity acrophases were phase-delayed slightly (approximately 0.8 h, $P < 0.05$), and there was no statistically significant differences regarding circadian robustness or amplitude (Table 1, Figure 3, $P > 0.05$).

Under a 12:12-h LS cycle, rats showed a free-running circadian rhythm, as occurs under constant light. During 12:12-h SD cycles, rats entrained to sodium light with a delayed acrophase.

Effect of a 2-h light pulse on circadian rhythms. To further explore the effects of LPSL, a free-running circadian rhythm was established in rats under DD, after which they were exposed to a 2-h pulse (from 0600 to 0800) of sodium light or white light daily for 2 wk under DD. The onset of phase shifts in body temperature induced by a 2-h LPSL were similar to those seen after a 2-h white-light pulse, and there were no statistically significant differences between the 2 groups. Specifically, pulses of LPSL or white-light given at 12 to 19 h circadian time both caused a phase delay, whereas light pulses at 20 to 24 h caused a phase advance (Figure 4).

Scotopic rod ERG response to flashes of 589-nm or white light. a-Wave latency was significantly ($P < 0.05$) prolonged in the rod responses to 589-nm light flashes at all intensity levels tested (Figure 5 E). However, there was no statistically significant change in a-wave amplitude (Figure 5 C) or b-wave amplitude (Figure 5 D) or latency (Figure 5 F) between 589-nm and white-light stimuli.

Photopic cone ERG response to flashes of 589-nm or white light. b-Wave amplitude showed small but significant ($P < 0.05$) reductions in the cone responses to 589-nm light flashes at all

intensities tested (Figure 6 A). However, there was no difference between white and 589-nm flash-evoked photopic ERG b-wave latency (Figure 6 B).

Discussion

In the present study, we observed the expected significant difference in circadian parameters of rats under LS compared with LD conditions. In other words, LPSL applied during the dark phase of the photocycle induced a free-running circadian rhythm, like that under constant light, and affected the magnitude of locomotor activity in our rats. These effects were not reported in a previous study in mice when LPSL was applied during the first 4 h of the dark phase;³⁸ we are unable to further compare the previous findings with our current results because phase changes in motion and temperature were not explored in the previous study.³⁶ This delay in acrophase was considered to be related to a free-running circadian period that was longer than 24 h in rats and resulted from weaker entrainment. These data demonstrated that LPSL is a weaker zeitgeber than white light.^{5,7,12,19,22,31} A wide range of measures of biochemical and physiologic circadian functions (for example, glucose utilization, body temperature, motor activity, blood pressure, sleep-wake cycle, heart rate) are available for chronobiologic studies. Daily oscillations in body temperature and activity are known to be well synchronized with each other under LD and DD conditions⁴ and are relatively easy to monitor in rodent studies. In the current study, the rats presented with basic temperature oscillation within 1 to 2 $^{\circ}$ C, which is consistent with the literature.^{27,49} Interestingly both the onset and acrophase of temperature were delayed for approximately 3 h under the LS schedule compared with LD condition. This finding is somewhat in agreement with a previous rat study,³⁷ in which the temperature cycle was found to be most sensitive to green light and least to blue and red light.

Figure 2. Body temperature and activity actograms and profiles under 12:12-h LD and LS cycles. (A). A representative double-plotted actogram (that is, 2 successive days next to each other) of body temperature of a standard 12:12-h LD cycle followed by a 12:12-h LS cycle. Note the free-running circadian rhythm under the LS condition. The dotted line indicates circadian onset phase shift in temperature when the rats were exposed to the LS condition. The experimental condition is indicated on the left of the actograms. (B and C) Group mean body temperature profiles under 12:12-h (B) LD cycle ($n = 6$) and (C) 12:12-h LS cycle ($n = 6$). (D). A representative double-plotted actogram of animal activity. A standard 12:12-h LD cycle is preceded and followed by 12:12-h LS cycles. The dotted line indicates circadian onset phase shift in the activity when the rats were exposed to the LS condition. (E and F) Group mean activity profiles under the (E) 12:12-h LD cycle ($n = 6$) and (F) 12:12-h LS cycle ($n = 6$). Note the free-running activity circadian rhythm under the LS condition. LD, white-light:dark; LS, white-light:sodium-light; white bar, 12-h lights-on phase; black bar, 12-h lights-off phase.

Given that the wavelength of our tested LPSL source was 589 nm, close to the green light range of 560 to 520 nm, and generates similar ERG responses as white light, we believe the circadian clock responded to 589-nm light through cone photoreceptors, which (with rod photoreceptors) have been proposed to be required for maintaining normal circadian rhythms in mice.³³ This finding also is in agreement with our circadian results that 2-h

pulses of LSPS and white light had similar effects on circadian rhythmicity. However, we cannot exclude the possibility that LPSL directly stimulated ipRGC, because mouse melanopsin expressed in HEK293 cells is sensitive to light stimuli as long as 600 nm in wavelength.⁴⁸ Then ipRGC transmit LPSL signals to the suprachiasmatic nucleus and modulate circadian rhythm.^{5,16,46} In addition, LPSL may have excited rod and cone photoreceptors

Figure 3. Body temperature actograms, profile, and circadian period under the 12:12-h SD cycle. (A) Representative double-plotted temperature actogram under 12:12-h LD cycle followed by 12:12-h SD cycles. (B) Representative body temperature actogram under 12:12-h SD cycle followed by 12:12-h DD condition (constant darkness). (C) Mean body temperature profile of rats under the 12:12-h LD condition compared with a 12:12-h SD cycle ($n = 6$ per group). (D) Comparison of circadian period for body temperature ($n = 6$ per group). The circadian period under DD was significantly (*, $P < 0.05$) longer than those under either LD or SD. Circadian period did not differ significantly between LD and SD conditions. Circadian period under LS was significantly (#, $P < 0.05$) longer than under DD.

that modulate the activity of ipRGC indirectly^{32,60} or by means of other pathways that are independent of photoreceptors and the suprachiasmatic nucleus.⁵² This possibility warrants further detailed mechanistic investigation.

To increase our understanding of the mechanism of LPSL-induced circadian changes, we used ERG technology to measure rod and cone photoreceptor responses to 589-nm light and compared these with those to white light. Clinically, this technology is a powerful diagnostic tool for assessing the physiologic integrity of the retina. In addition, ERG is widely used for research, including circadian rhythm studies, in human subjects and other species.⁸ We analyzed the main components of the ERG—the negative-going a-wave and the positive-going b-wave (Figure 5 A and B)—in the current study. Mechanistically, the ERG reflects the light-evoked excitation and inhibition of the retinal network, inducing changes in the concentrations of K^+ , Na^+ , and Cl^- that give rise to multiple field potentials. These field potentials are passively propagated through the volume conductors of the vitreous, lens, aqueous humor, and cornea. Extensive experimental work has determined the sites of origin of the various ERG components. In response to a bright flash (that is, exceeding $0.06 \text{ cd} \times \text{s}/\text{m}^2$) in the 2-h dark-adapted eye, the a-wave largely reflects photoreceptor function; the dark-adapted a-wave primarily

reflects the rod receptor photocurrent.^{43,44} The International Society for Clinical Electrophysiology of Vision's standard ERG³⁵ incorporates a rod-specific response to a dim light under scotopic conditions and a mixed rod-cone response to a bright white flash under dark adaptation. In the spectrum of rod photoreceptors, the peak frequency is around 500 nm, close to the frequency (589 nm) of the sodium light we used in the current experiment. In addition, because approximately 99% of the photoreceptors in the rat retina are rod type,⁵⁵ such a small difference in the light spectrum of the stimulus may not be sufficient to cause overt ERG changes. However, 589-nm light carries a smaller photon energy than a 500-nm light waveform, thus perhaps explaining why the opsin light transduction in the rod outer segment takes a long time for 589-nm light, resulting in a-wave delay in ERG. Even though the rod photoreceptors had similar responses to a 589-nm light flash as white light, as reflected in the a-wave amplitude and in agreement with the literature,¹ we cannot confirm that rat rod photoreceptors are alone in mediating sodium-light-induced circadian changes, given that previously reported retinal degeneration homozygous mice still maintain a circadian phenotype.^{15,16,47} The potential involvement of the ipRGC-suprachiasmatic nucleus sensory pathway in LPSL circadian regulation warrants future investigations.

Figure 4. Body temperature circadian phase shift induced by 2-h pulses of white light (WL) or low-pressure sodium light (LPSL). A standard 12:12-h LD cycle was followed by constant darkness (DD) for approximately 2 wk and then a daily 2-h (A) WL or (B) LPSL pulse (0600 to 0800) under DD condition. Double-plotted 24-h temperature actograms from rat 1 show that the 2-h pulses of (A) WL or (B) LPSL during DD caused phase advances when light pulses occurred at circadian time 20–24 h. (C and D) Double-plotted temperature actograms from rat 2 show that the 2-h pulses of (C) WL or (D) LPSL pulses during DD caused phase delays when light pulses occurred at circadian time 12–18 h. The circadian time for a light pulse was calculated by taking into account the onset of the temperature rhythm. (E) A temperature onset phase shift caused by a 2-h WL or 2-h LPSL pulse under DD condition (mean $\pm$ SEM, $n = 8$ per group); there are no significant differences between the 2 groups. Open rectangle, light pulse period.

To test the cone-driven (photopic) ERG response to 589-nm and white-light flashes, rod photoreceptors must be suppressed. We took the approach of suppressing rods by imposing a steady background of white light sufficient to saturate their responses (at 25 cd/m²). Under this condition, the amplitude of b-wave represents primarily activation of the depolarization of cone bipolar

cells.^{54,57} In addition, the b-wave is attributed to the interaction of cone bipolar cells and Müller glial cells.⁵⁸ In the rat retina, cones represent only about 0.85% of all photoreceptors; among cone cells, about 93% respond to green light, 7% to blue light, and 0% to red light, and the spectral sensitivity of their medium-wavelength M-cones (green cones) peaks near 510 nm,²⁵ that is, nearly 80

Figure 5. Rats' 2-h dark-adapted scotopic ERG responses to white and 589-nm flash stimuli. (A) and (B) are representative ERG traces recorded after a series of (A) white and (B) 589-nm light stimuli (black arrows), with intensities from weakest ($0.001 \text{ cd} \times \text{s}/\text{m}^2$) to strongest ($7.80 \text{ cd} \times \text{s}/\text{m}^2$). a-Waves were elicited only when the flash intensity exceeded $0.062 \text{ cd} \times \text{s}/\text{m}^2$ ($\sim 20 \text{ dB}$). (C) The a-wave amplitudes did not differ ($P > 0.05$, 2-way ANOVA, $n = 6$) between white compared with 589-nm light stimuli. However, (E) the a-wave latency to the 589-nm light was prolonged significantly (*, $P < 0.05$) compared with the white stimuli-induced responses. No significant difference occurred in either (D) b-wave amplitude or (F) latency between white compared with 589 nm light stimuli (all $P > 0.05$, $n = 6$).

nm different from our test light source of 589 nm in their peak spectrum. This might be the reason why that—even though the photopic b-wave amplitude to 589-nm light is slightly smaller than to white light during the daylight phase—LPSL failed to

affect circadian rhythm. A weakness of the current study is that the light source used for ERG testing was not exactly the same, in regard to in the monochromatic waveform spectrum profile, as the LPSL emitted from an LPSL bulb.

Figure 6. Rats' 10-min light-adapted photopic ERG responses to 589-nm and white-light stimuli. (A) The b-wave amplitude due to 589-nm light was significantly (*, $P < 0.05$) smaller than white stimuli-induced responses. (B) There is no significant difference in the b-wave latency responses between white light and 589-nm light flashes ($P > 0.05$).

In conclusion, the results that we obtained in this study suggest that—to rats—sodium lighting is not the equivalent of complete darkness and that this type of lighting can influence the circadian timing system through imaging-forming photoreceptors.

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References

- Altman CM, Güler AD, Alam NM, Arman AC, Prusky GT, Sampath AP, Hattar S. 2010. Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. *Nat Neurosci* 13:1107–1112. <https://doi.org/10.1038/nn.2617>.
- Beaulé C. 2009. Aschoff's rules. In: Binder DM, Hirokawa N, Winthorst U, editors. *Encyclopedia of neuroscience*. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-540-29678-2_383

- Behn D, Doke A, Racine J, Casanova C, Chemtob S, Lachapelle P. 2003. Dark adaptation is faster in pigmented than albino rats. *Doc Ophthalmol* 106:153–159. <https://doi.org/10.1023/A:1022511918823>.
- Benstaali C, Mailloux A, Bogdan A, Auzéby A, Touitou Y. 2001. Circadian rhythms of body temperature and motor activity in rodents: Their relationships with the light-dark cycle. *Life Sci* 68:2645–2656. [https://doi.org/10.1016/S0024-3205\(01\)01081-5](https://doi.org/10.1016/S0024-3205(01)01081-5).
- Berson DM, Dunn FA, Takao M. 2002. Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295:1070–1073. <https://doi.org/10.1126/science.1067262>.
- Bowmaker JK, Dartnall HJ. 1980. Visual pigments of rods and cones in a human retina. *J Physiol* 298:501–511. <https://doi.org/10.1113/jphysiol.1980.sp013097>.
- Brainard GC, Hanifin JP. 2005. Photons, clocks, and consciousness. *J Biol Rhythms* 20:314–325. <https://doi.org/10.1177/0748730405278951>.
- Cameron MA, Barnard AR, Lucas RJ. 2008. The electroretinogram as a method for studying circadian rhythms in the mammalian retina. *J Genet* 87:459–466. <https://doi.org/10.1007/s12041-008-0068-5>.
- Christina A, Merlin N, Vijaya C, Jayaprakash S, Muruges N. 2004. Daily rhythm of nociception in rats. *J Circadian Rhythms* 2:1–3. <https://doi.org/10.1186/1740-3391-2-2>.
- Dauchy RT, Dauchy EM, Tirrell RP, Hill CR, Davidson LK, Greene MW, Tirrell PC, Wu J, Sauer LA, Blask DE. 2010. Dark-phase light contamination disrupts circadian rhythms in plasma measures of endocrine physiology and metabolism in rats. *Comp Med* 60:348–356.
- Dauchy RT, Sauer LA, Blask DE, Vaughan GM. 1997. Light contamination during the dark phase in 'photoperiodically controlled' animal rooms: effect on tumor growth and metabolism in rats. *Lab Anim Sci* 47:511–518.
- Dauchy RT, Wren MA, Dauchy EM, Hoffman AE, Hanifin JP, Warfield B, Jablonski MR, Brainard GC, Hill SM, Mao L, Dobek GL, Dupepe LM, Blask DE. 2015. The influence of red light exposure at night on circadian metabolism and physiology in Sprague-Dawley rats. *J Am Assoc Lab Anim Sci* 54:40–50.
- Dauchy RT, Xiang S, Mao L, Brimer S, Wren MA, Yuan L, Anbalagan M, Hauch A, Frasch T, Rowan BG, Blask DE, Hill SM. 2014. Circadian and melatonin disruption by exposure to light at night drives intrinsic resistance to tamoxifen therapy in breast cancer. *Cancer Res* 74:4099–4110. <https://doi.org/10.1158/0008-5472.CAN-13-3156>.
- Davis FC, Darrow JM, Menaker M. 1983. Sex differences in the circadian control of hamster wheel-running activity. *Am J Physiol* 244:R93–R105.
- Foster RG, Provencio I, Hudson D, Fiske S, De Grip W, Menaker M. 1991. Circadian photoreception in the retinally degenerate mouse (rd/rd). *J Comp Physiol A* 169:39–50. <https://doi.org/10.1007/BF00198171>.
- Freedman MS, Lucas RJ, Soni B, von Schantz M, Muñoz M, David-Gray Z, Foster R. 1999. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284:502–504. <https://doi.org/10.1126/science.284.5413.502>.
- Gritton HJ, Kantorowski A, Sarter M, Lee TM. 2012. Bidirectional interactions between circadian entrainment and cognitive performance. *Learn Mem* 19:126–141. <https://doi.org/10.1101/lm.023499.111>.
- Gritton HJ, Sutton BC, Martinez V, Sarter M, Lee TM. 2009. Interactions between cognition and circadian rhythms: attentional demands modify circadian entrainment. *Behav Neurosci* 123:937–948. <https://doi.org/10.1037/a0017128>.
- Hanifin JP, Brainard GC. 2007. Photoreception for circadian, neuroendocrine, and neurobehavioral regulation. *J Physiol Anthropol* 26:87–94. <https://doi.org/10.2114/jpa2.26.87>.
- Hanifin JP, Stewart KT, Smith P, Tanner R, Rollag M, Brainard GC. 2006. High-intensity red light suppresses melatonin. *Chronobiol Int* 23:251–268. <https://doi.org/10.1080/07420520500521988>.
- Hastings MH, Reddy AB, Maywood ES. 2003. A clockwork web: circadian timing in brain and periphery, in health and disease. *Nat Rev Neurosci* 4:649–661. <https://doi.org/10.1038/nrn1177>.

22. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* **295**:1065–1070. <https://doi.org/10.1126/science.1069609>.
23. Honma KI, Hiroshige T. 1978. Endogenous ultradian rhythms in rats exposed to prolonged continuous light. *Am J Physiol* **235**:R250–R256.
24. Institute for Laboratory Animal Research. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press. <https://doi.org/10.17226/12910>
25. Jacobs GH, Fenwick JA, Williams GA. 2001. Cone-based vision of rats for ultraviolet and visible lights. *J Exp Biol* **204**:2439–2446.
26. Kaur G, Phillips C, Wong K, Saini B. 2013. Timing is important in medication administration: a timely review of chronotherapy research. *Int J Clin Pharm* **35**:344–358. <https://doi.org/10.1007/s11096-013-9749-0>.
27. Kleitman N, Ramsaroop A. 1948. Periodicity in body temperature and heart rate. *Endocrinology* **43**:1–20. <https://doi.org/10.1210/endo-43-1-1>.
28. Knight KK, Apsey DA, Jackson WG, Dennis RJ. 1998. A comparison of stereopsis with ANVIS and F4949 night-vision goggles. *Aviat Space Environ Med* **69**:99–103.
29. Krizo JA, Mintz EM. 2014. Sex differences in behavioral circadian rhythms in laboratory rodents. *Front Endocrinol (Lausanne)* **5**:1–4.
30. Liu CN, Berryman E, Zakur D, Shoieb AM, Pardo ID, Boucher M, Soms CJ, Bagi CM, Cook JC. 2017. A novel endpoint for the assessment of chemotherapy-induced peripheral neuropathy in rodents: biomechanical properties of peripheral nerve. *J Appl Toxicol* **38**:193–200. <https://doi.org/10.1002/jat.3513>.
31. Liu CN, Mathialagan N, Lappin P, Fortner J, Soms C, Seitis G, Johnson TR, Hu W, Matsumoto D. 2014. Crizotinib reduces the rate of dark adaptation in the rat retina independent of ALK inhibition. *Toxicol Sci* **143**:116–125. <https://doi.org/10.1093/toxsci/kfu213>.
32. Lucas RJ, Peirson SN, Berson DM, Brown TM, Cooper HM, Czeisler CA, Figueiro MG, Gamlin PD, Lockley SW, O'Hagan JB, Price LL, Provencio I, Skene DJ, Brainard GC. 2014. Measuring and using light in the melanopsin age. *Trends Neurosci* **37**:1–9. <https://doi.org/10.1016/j.tins.2013.10.004>.
33. Lupi D, Cooper HM, Froehlich A, Stanford L, McCall MA, Foster RG. 1999. Transgenic ablation of rod photoreceptors alters the circadian phenotype of mice. *Neuroscience* **89**:363–374. [https://doi.org/10.1016/S0306-4522\(98\)00353-4](https://doi.org/10.1016/S0306-4522(98)00353-4).
34. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M; International Society for Clinical Electrophysiology of Vision. 2009. ISCEV Standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* **118**:69–77. <https://doi.org/10.1007/s10633-008-9155-4>.
35. Marmor MF, Holder GE, Seeliger MW, Yamamoto S, International Society for Clinical Electrophysiology of Vision. 2004. Standard for clinical electroretinography (2004 update). *Doc Ophthalmol* **108**:107–114. <https://doi.org/10.1023/B:DOOP.0000036793.44912.45>.
36. McCormack CE, Sontag CR. 1980. Entrainment by red light of running activity and ovulation rhythms of rats. *Am J Physiol* **239**:R450–R453.
37. McGuire RA, Rand WM, Wurtman RJ. 1973. Entrainment of the body temperature rhythm in rats: effect of color and intensity of environmental light. *Science* **181**:956–957. <https://doi.org/10.1126/science.181.4103.956>.
38. McLennan IS, Taylor-Jeffs J. 2004. The use of sodium lamps to brightly illuminate mouse houses during their dark phases. *Lab Anim* **38**:384–392. <https://doi.org/10.1258/0023677041958927>.
39. Mrosovsky N. 2003. Contribution of classic photoreceptors to entrainment. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **189**:69–73.
40. Navara KJ, Nelson RJ. 2007. The dark side of light at night: physiological, epidemiological, and ecological consequences. *J Pineal Res* **43**:215–224. <https://doi.org/10.1111/j.1600-079X.2007.00473.x>.
41. Pechlivanova DM, Stoynev AG. 2007. Diurnal variation in the effect of angiotensin II on nociception. *Methods Find Exp Clin Pharmacol* **29**:423–426. <https://doi.org/10.1358/mf.2007.29.6.1119171>.
42. Peirson SN, Brown LA, Pothecary CA, Benson LA, Fisk AS. 2018. Light and the laboratory mouse. *J Neurosci Methods* **300**:26–36. <https://doi.org/10.1016/j.jneumeth.2017.04.007>.
43. Penn RD, Hagins WA. 1969. Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature* **223**:201–204. <https://doi.org/10.1038/223201a0>.
44. Penn RD, Hagins WA. 1972. Kinetics of the photocurrent of retinal rods. *Biophys J* **12**:1073–1094. [https://doi.org/10.1016/S0006-3495\(72\)86145-9](https://doi.org/10.1016/S0006-3495(72)86145-9).
45. Provencio I, Foster RG. 1995. Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Res* **694**:183–190. [https://doi.org/10.1016/0006-8993\(95\)00694-L](https://doi.org/10.1016/0006-8993(95)00694-L).
46. Provencio I, Rollag MD, Castrucci AM. 2002. Photoreceptive net in the mammalian retina. *Nature* **415**:493. <https://doi.org/10.1038/415493a>.
47. Provencio I, Wong S, Lederman AB, Argamaso SM, Foster RG. 1994. Visual and circadian responses to light in aged retinally degenerate mice. *Vision Res* **34**:1799–1806. [https://doi.org/10.1016/0042-6989\(94\)90304-2](https://doi.org/10.1016/0042-6989(94)90304-2).
48. Qiu X, Kumbalasiri T, Carlson SM, Wong KY, Krishna V, Provencio I, Berson DM. 2005. Induction of photosensitivity by heterologous expression of melanopsin. *Nature* **433**:745–749. <https://doi.org/10.1038/nature03345>.
49. Refinetti R. 1996. Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *J Exp Zool* **275**:67–70. [https://doi.org/10.1002/\(SICI\)1097-010X\(19960501\)275:1<67::AID-JEZ10>3.0.CO;2-U](https://doi.org/10.1002/(SICI)1097-010X(19960501)275:1<67::AID-JEZ10>3.0.CO;2-U).
50. Rosolen SG, Rigaudière F, Le Gargasson JF, Brigell MG. 2005. Recommendations for a toxicological screening ERG procedure in laboratory animals. *Doc Ophthalmol* **110**:57–66. <https://doi.org/10.1007/s10633-005-7344-y>.
51. Rowse EG, Harris S, Jones G. 2016. The switch from low-pressure sodium to light emitting diodes does not affect bat activity at street lights. *PLoS One* **11**:1–14. <https://doi.org/10.1371/journal.pone.0150884>.
52. Sakamoto K, Liu C, Tosini G. 2004. Circadian rhythms in the retina of rats with photoreceptor degeneration. *J Neurochem* **90**:1019–1024. <https://doi.org/10.1111/j.1471-4159.2004.02571.x>.
53. Shih KC, Liu LY, Kwok CF, Hwu CM, Juan CC, Hsu YP, Ho LT. 2007. Effect of reversing dark-light cycles on normal diurnal variation and related metabolic disturbance in rats. *Chin J Physiol* **50**:69–76.
54. Stockton RA, Slaughter MM. 1989. B-wave of the electroretinogram. A reflection of ON bipolar cell activity. *J Gen Physiol* **93**:101–122. <https://doi.org/10.1085/jgp.93.1.101>.
55. Szél A, Röhlich P. 1992. Two cone types of rat retina detected by antiviral pigment antibodies. *Exp Eye Res* **55**:47–52.
56. Takahashi JS, Hong HK, Ko CH, McDearmon EL. 2008. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet* **9**:764–775. <https://doi.org/10.1038/nrg2430>.
57. Tian N, Slaughter MM. 1995. Correlation of dynamic responses in the ON bipolar neuron and the b-wave of the electroretinogram. *Vision Res* **35**:1359–1364. [https://doi.org/10.1016/0042-6989\(95\)98715-L](https://doi.org/10.1016/0042-6989(95)98715-L).
58. Wen R, Oakley B 2nd. 1990. K⁺-evoked Muller cell depolarization generates b-wave of electroretinogram in toad retina. *Proc Natl Acad Sci USA* **87**:2117–2121. <https://doi.org/10.1073/pnas.87.6.2117>.
59. Wollnik F, Turek FW. 1988. Estrous correlated modulations of circadian and ultradian wheel-running activity rhythms in LEW/Ztm rats. *Physiol Behav* **43**:389–396. [https://doi.org/10.1016/0031-9384\(88\)90204-1](https://doi.org/10.1016/0031-9384(88)90204-1).
60. Wong KY, Dunn FA, Graham DM, Berson DM. 2007. Synaptic influences on rat ganglion-cell photoreceptors. *J Physiol* **582**:279–296. <https://doi.org/10.1113/jphysiol.2007.133751>.