

<https://doi.org/10.1038/s44323-025-00043-3>

# Modulating light level patterns reduces rest/activity disruption associated with shiftwork



Varun Mandi<sup>1,6</sup>, Haley Miller<sup>2,6</sup>, Jeongkyung Lee<sup>1</sup>, Young-Hwa Goo<sup>3</sup>, Mousumi Moulik<sup>4</sup>, Ke Ma<sup>5</sup>, Antoni Paul<sup>3,7</sup>, Vijay K. Yechoor<sup>1,7</sup> & Mariana G. Figueiro<sup>2,7</sup> ✉

Long-term exposure to nonstandard work schedules can result in circadian misalignment, which has been linked to a series of maladies. To test whether modulating light patterns reduces shiftwork-induced rest/activity disruptions, 30 male C57BL/6 J mice individually housed in cages outfitted with running wheels were exposed to 6 simulated shiftwork light interventions. Mice experiencing high light levels during shiftwork exhibited a significant decrease in activity compared to low light levels during shiftwork and a conventional 12 L:12D condition, indicating circadian misalignment. In contrast, mice experiencing shiftwork in darkness combined with either modulated evening light pulses or circadian blind, vision-permissive light showed similar levels of rest/activity compared to a 12 L:12D condition, with phasor analysis indicating that their 24-h circadian rest/activity patterns were not misaligned. The results show that exposure to light that permits visibility but is below activation of the circadian system during shiftwork can prevent circadian misalignment.

Circadian rhythms are generated by the master biological clock in the suprachiasmatic nuclei (SCN) in the brain's hypothalamus, which regulate the timing of behavioral and physiological rhythms such as the sleep/wake cycle, hormone production, and body temperature. The SCN receive photic input from the retina to entrain the circadian system to the environmental light/dark pattern<sup>1,2</sup>, thus maintaining synchrony between the body's behavioral and physiological rhythms and the external environment<sup>3–5</sup>. Disruption of this synchrony, which can occur among those whose activity schedules and light exposures do not align with their natural biological rhythms (e.g., healthcare professionals, service and industrial sector workers), has been associated with a host of health problems, including various cancers, insulin resistance, type 2 diabetes, and atherosclerotic vascular disease<sup>6–19</sup>. Given that 16% of the United States labor force follows a shifted work schedule<sup>20</sup>, it is important to further understand how circadian misalignment can impact one's health and to find ways to mitigate its harmful effects.

Because the mammalian endogenous circadian period free runs at a period that slightly varies from the 24-h solar day in the absence of exogenous stimuli<sup>21,22</sup>, the master clock needs to be advanced or delayed by

photic stimulus in the beginning of a species' biological day (active period) to maintain circadian entrainment<sup>23</sup>. In humans, light exposures during the biological night (inactive period), conversely, can lead to circadian misalignment<sup>24,25</sup>. Our previous research demonstrated that higher light levels are required for circadian system activation than are required for vision<sup>25,26</sup>. Using a paradigm of changes in lighting conditions for the last four days of the week to mimic what a rotating shift worker would experience (working 3–4 nights per week), we also have previously found that, as in human shift workers, mice develop adipose hypertrophy, insulin resistance, glucose intolerance, and accelerated atherosclerosis with unstable plaque phenotypes<sup>19,25,27–29</sup>. The present study employed a similar lighting pattern in mouse models to test our hypothesis that performing shiftwork under circadian blind, vision-permissive (CBVP) light conditions would help protect against circadian misalignment. To achieve this aim, we employed a lower light level during shiftwork that was vision-permissive but below the threshold for the circadian system response of mice, while also taking into account that the mouse circadian system is more sensitive to optical radiation than the human circadian system<sup>23,26,30</sup>.

<sup>1</sup>Division of Endocrinology and Metabolism, Diabetes and Beta Cell Biology Center, Department of Medicine, University of Pittsburgh, Pittsburgh, PA, 15213, USA.

<sup>2</sup>Light and Health Research Center, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, NY, 10029, USA.

<sup>3</sup>Department of Molecular and Cellular Physiology, Albany Medical College, Albany, NY, 12208, USA. <sup>4</sup>Division of Cardiology, Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, 15213, USA. <sup>5</sup>Department of Diabetes Complications & Metabolism, Beckman Research Institute of City of Hope, Duarte, CA, 91010, USA. <sup>6</sup>These authors contributed equally: Varun Mandi, Haley Miller. <sup>7</sup>These authors jointly supervised this work: Antoni Paul, Vijay K. Yechoor, Mariana G. Figueiro.

✉ e-mail: [mariana.figueiro@mountsinai.org](mailto:mariana.figueiro@mountsinai.org)

To test our hypothesis, we monitored the rest/activity patterns of mice under 6 light interventions that simulated various forms of shiftwork (SW), including the CBVP shiftwork intervention, all compared to a conventional 12 h light:12 h dark (12 L:12D) day shift control condition. These 6 weekly interventions delivered a conventional day shift (DS) 12 L:12D light schedule for 3 days (Monday through Wednesday) followed by 4 days (Thursday through Sunday) of either an inverted 12D:12 L pattern or a 12D:12D pattern. The 3 interventions concluding the week with the inverted 12D:12 L pattern provided light at three photoperiod strengths: high (SW+highL), low (SW+lowL), and circadian blind, vision-permissive dim (SW+CBVP), which were used to test the impact of different light strengths during the shift on rest/activity patterns. The 3 interventions concluding the week with the 12D:12D pattern provided darkness only (shiftwork in darkness [SWD]), darkness with a 30-min morning pulse (SWD+AMpulse), and darkness with a 30-min evening pulse (SWD+PMpulse). These latter interventions were used to test the impact of remaining in darkness during the shift on rest/activity patterns. The addition of a morning or evening pulse was designed to avoid free running from exposure to constant darkness.

Phasor analysis was used to assess the strength of association between light exposure and locomotor behavior by calculating the degree to which the mice's daily rest/activity rhythm aligned with their daily pattern of

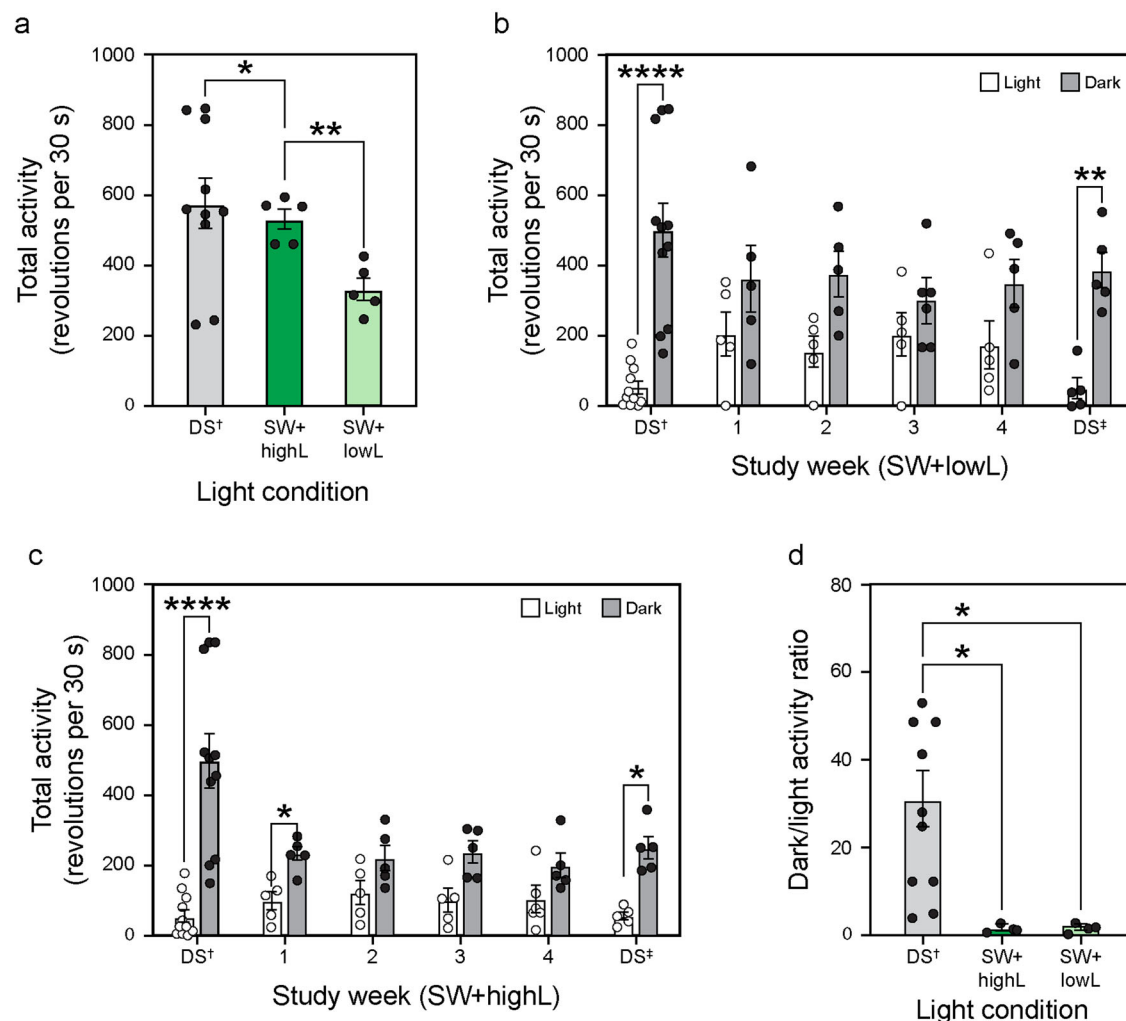
exposures to light (L) and dark (D)<sup>31–33</sup>. Additionally, weekly activity by light/dark cycle during biological rest (periods of lights on) and biological activity (periods of lights off) was calculated for each experimental condition, again compared to the pre-intervention 12 L:12D (DS) condition that served as the experimental control.

## Results

### Shiftwork with high light levels (SW+highL) exacerbates circadian misalignment

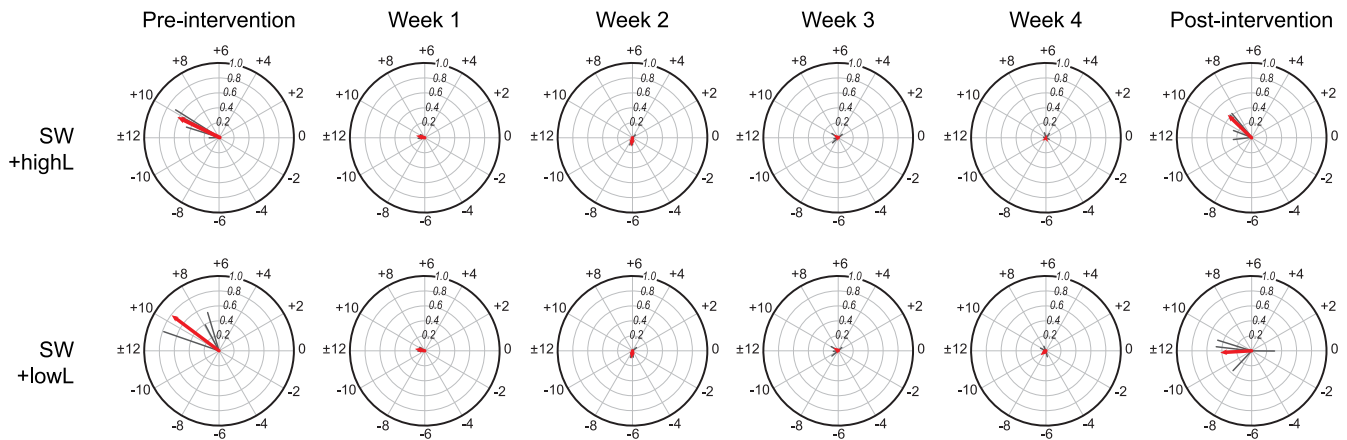
Mice in the SW+highL intervention showed a significant decrease in weekly total activity by the 4th week of the intervention compared to both the pre-intervention DS control condition (a decrease of ~45%;  $p < 0.05$ ) and to the SW+lowL intervention (a decrease of ~42%;  $p < 0.01$ ) (Fig. 1a). There was no significant difference between the SW+lowL group and the pre-intervention, DS control group (Fig. 1a).

In addition, there were significant differences between weekly total activity in the light and weekly total activity in the dark observed during the pre- and post-intervention weeks, when mice were under regular day shift (DS) schedules (Fig. 1b, c). By contrast, there were no significant differences between weekly total activity in the light and weekly total activity in the dark during the intervention weeks (Fig. 1b, c). This difference was lost as early as



**Fig. 1 | Weekly total activity and dark/light activity ratio, as measured through wheel running activity. a** Comparison of weekly total activity between day shift (DS), shiftwork with high light level (SW+highL), and shiftwork with low light level (SW+lowL) conditions. **b** Comparison of weekly total activity between the light and dark phase for the SW+lowL intervention over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). **c** Comparison of weekly

total activity between the light and dark phase for the SW+highL intervention over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). **d** Comparison of dark/light activity ratio between DS, SW+highL, and SW+lowL interventions. Statistical significance: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$ . Notes: † pre-intervention (average of two weeks), ‡ post-intervention (1 week).



**Fig. 2 | Weekly phasor angle and magnitude in mice experiencing the shiftwork with high light level (SW+highL) and shiftwork with low light level (SW+lowL) interventions.** Note: The pre-intervention data represent an average of two weeks.

the first week of the intervention in both the SW+highL and the SW+lowL groups (Fig. 1b, c). This is also demonstrated by a significant reduction ( $p < 0.05$ ) in the dark/light activity ratio during the intervention weeks compared to the DS control condition (Fig. 1d). Weekly total activity levels recovered in the post-intervention period, although they did not fully return to pre-intervention, DS control condition levels.

Mice displayed large weekly phasor magnitudes during the DS control condition (pre-intervention), suggesting a high degree of correlation between light/dark and rest/activity patterns (Fig. 2). As shown by their phasor angles, mice in the DS control condition exhibited a higher proportion of activity near the offset of light exposure rather than the onset of light exposure, which reflects the animal's anticipation of the active, dark phase. In both the SW+highL and SW+lowL interventions, we observed a substantial reduction in phasor magnitude as early as the first week of the intervention. This reduction in phasor magnitude suggests that light-associated circadian locomotor activity, as characterized by the correlation between light/dark and rest/activity patterns, is reduced. Variations in phasor angle on a weekly basis during the SW+highL and SW+lowL interventions suggest the temporal relationship between the start and end of the activity/rest and the start and end of the light/dark period was disrupted. Both phasor magnitude and angle recovered in the post-intervention period, although they did not fully return to pre-intervention, DS control condition levels.

#### **Total darkness with modulated evening pulses of light during shiftwork preserves strength of association between light-dark and rest-activity patterns**

Overall, mice experiencing the SWD intervention did not have a significant decrease in weekly total activity (Fig. 3a). Mice experiencing the SWD+PMpulse intervention exhibited weekly total activity similar to the mice in the DS control and SWD conditions (Fig. 3a). Mice experiencing the SWD+AMPulse intervention, however, had significantly lower ( $p < 0.05$ ) weekly total activity than mice in the SWD and SWD+PM pulse groups. Indeed, when looking at each intervention week separately, the weekly total activity in the light and weekly total activity in the dark during the SWD+AMPulse intervention (Fig. 3c) exhibited greater reductions than during the SWD and SWD+PMpulse interventions (Fig. 3b, d).

Although the dark/light activity ratio in the SWD intervention was higher than in the SWD+AMPulse, SWD+PMpulse, and the DS control conditions, the differences were not statistically significant (Fig. 3e). There were also no significant changes in phasor angle or magnitude between the groups (Fig. 4).

#### **The SW + CBVP intervention prevented shiftwork-induced circadian disruption**

Mice experiencing the SW + CBVP intervention did not show significant differences in weekly total activity, dark/light activity ratio, or weekly total

activity in the light and total activity in the dark over the course of the intervention weeks compared to the DS control week (Fig. 5). Weekly phasor angle and phasor magnitudes in mice experiencing the SW + CBVP intervention were not significantly different from those experiencing the DS control condition (Fig. 6).

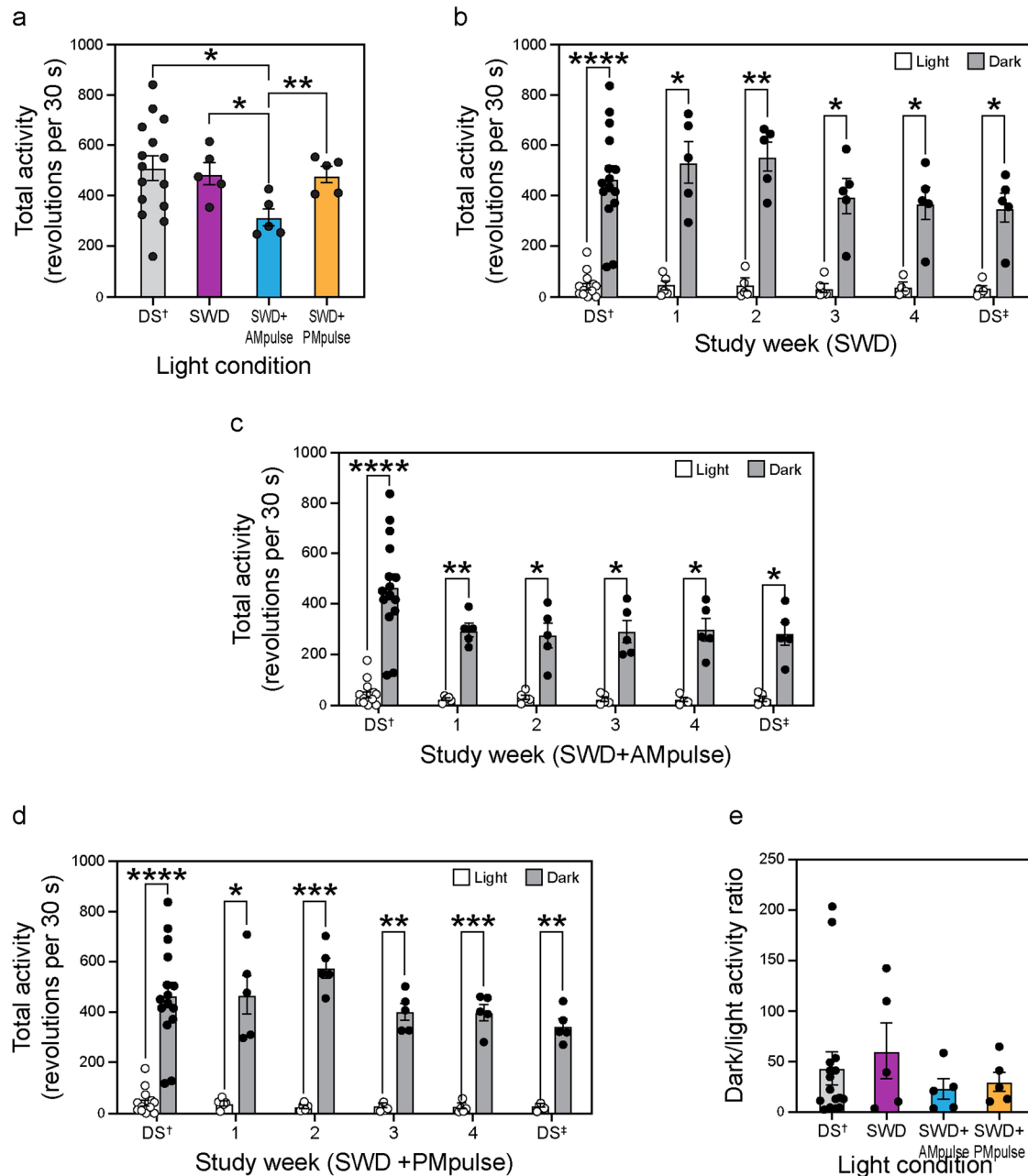
#### **Discussion**

In the present study, we tested a novel lighting intervention designed to mimic a lighting condition that we hypothesized would avoid circadian disruption while still maintaining visibility in the shiftwork population. We used a shiftwork paradigm where animals experienced a 12 L:12D (i.e., day shift [DS]) schedule from Monday to Wednesday, after which we shifted their light/dark pattern by 12 h until Monday and returned the animals to a DS schedule. This schedule was designed to mimic what a rotating shift worker would experience (working 3–4 nights per week). We were able to replicate previous data showing that this shiftwork paradigm leads to light-induced rest/activity rhythms disruption as exemplified by an increase in activity during the light period, a reduction in the dark/light activity ratio, a reduction in weekly total activity in the light and weekly total activity in the dark, and a reduction in phasor magnitude (a measure of the strength of association between light and circadian locomotor activity)<sup>32</sup>.

We also expanded on previous work<sup>19,25</sup> by showing that modulating light levels during a shiftwork schedule reduces rest/activity disruption associated with rotating shifts. Indeed, simply changing the light level during the biological night from 25 lx to 12 lx resulted in a significantly reduced change in light-induced rest/activity rhythms. More importantly, we showed that exposing animals to circadian blind, vision-permissive light (0.05 lx) during their biological night (i.e., the SW + CBVP intervention) maintained rest/activity rhythms similar to a 12 L:12D DS schedule.

One obvious way to mitigate the negative effects of light exposure during shiftwork schedules is to maintain darkness during the shiftwork period. We explored the use of total darkness during the biological night over all the weeks when mice were subjected to the shiftwork paradigm. Each week was comprised of 3 days of a regular 12 L:12D DS cycle followed by 4 days of total darkness (shiftwork biological days). Mice in this SWD group showed rest/activity rhythms similar to the DS control group, although there was an indication that, over the course of the treatment weeks, the mice started to reduce activity during the dark period and increase activity during the light period, suggesting they were beginning to exhibit a deterioration of circadian entrainment. This effect was not induced by masking, given that the animals remained in total darkness during the simulated nightshift days. In fact, mice experiencing SWD also exhibited a greater dark/light activity ratio, given that they were exposed to greater dark than light periods over the course of the week.

Singh et al.<sup>34</sup> previously showed that the impact of constant darkness on brain activity, behavioral outcomes, and hormone outcomes in mice is

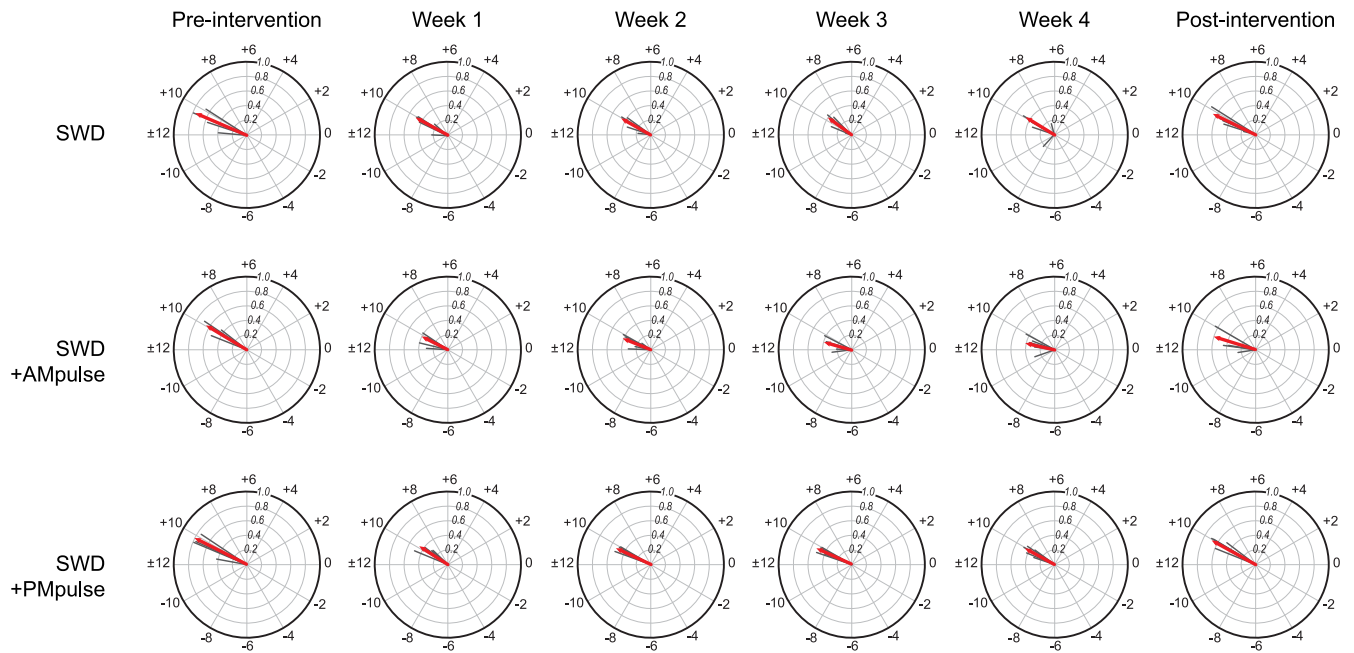


**Fig. 3 | Weekly total activity and dark/light activity ratio for the shiftwork in darkness (SWD) conditions.** **a** Comparison of weekly total activity between day shift (DS) condition and the shiftwork in darkness (SWD), shiftwork in darkness with morning light pulse (SWD+AMpulse), and shiftwork in darkness with evening light pulse (SWD+PMpulse) interventions. **b** Comparison of weekly total activity between the light and dark phase for the SWD intervention over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). **c** Comparison of weekly total activity between the light and dark phase for the SWD

+AMpulse intervention over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). **d** Comparison of weekly total activity between the light and dark phase for the SWD+AMpulse intervention over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). **e** Comparison of dark/light activity ratio between the DS condition and the SWD, SWD+AMpulse, and SWD+PMpulse interventions. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Notes: † pre-intervention (average of 2 weeks), ‡ post-intervention.

dependent on sex and on the length of time spent in darkness. For example, female mice were resilient to 3 weeks in darkness, but by 5 weeks in constant darkness the negative effects on these outcomes were the same for males and females<sup>34</sup>. Our results from male mice did not show significant alterations in rest/activity patterns after 4 weeks of experiencing the shiftwork paradigm, perhaps because the constant darkness for 4 days was alternating with 3 days of the conventional 12 L:12D condition. Further research is needed to determine whether lack of changes in rest/activity patterns is also reflected in loss of alterations in molecular biomarkers.

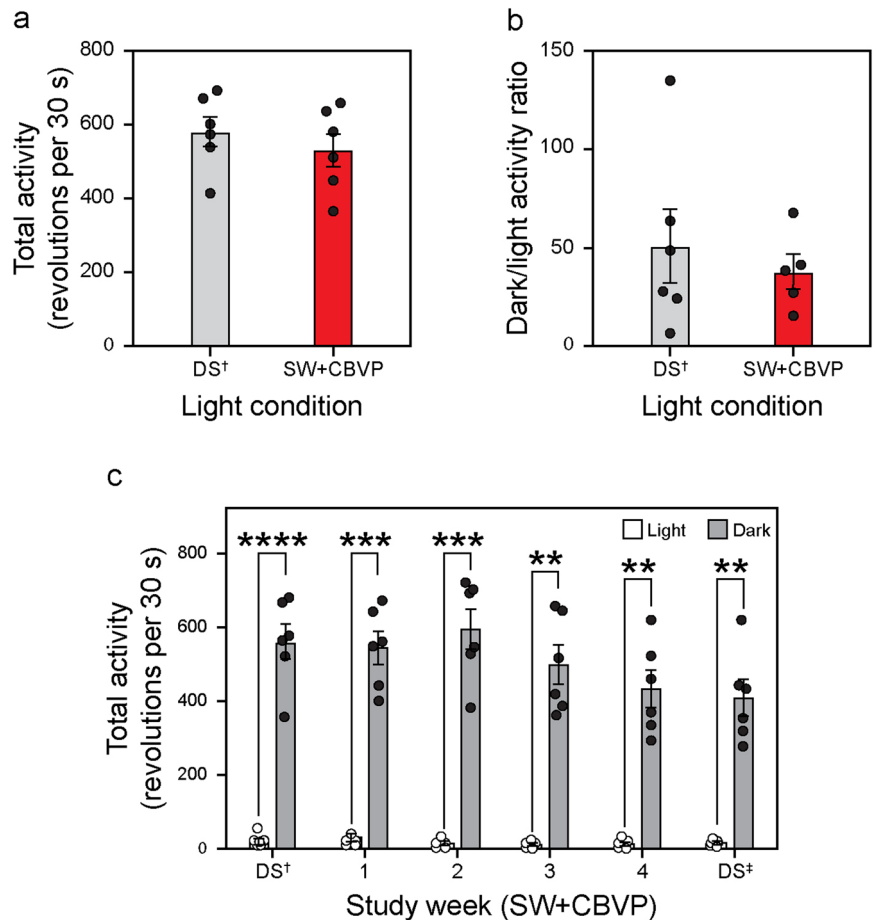
To address the possibility of experiencing free running activity over the course of the treatment weeks during the dark days, we tested whether a brief, daily 30-min pulse of light during shiftwork in darkness at ZT0 (SWD+AMpulse) or ZT12 (SWD+PMpulse) would keep the mice entrained over the course of the interventions. As hypothesized, a pulse at the start of the biological day (evening pulse) promoted entrainment of rest/activity rhythms, while the light pulse at the end of the biological day resulted in decreased rest/activity rhythms. Given that mice have an average free-running period of <24 h, it was hypothesized that a greater degree of



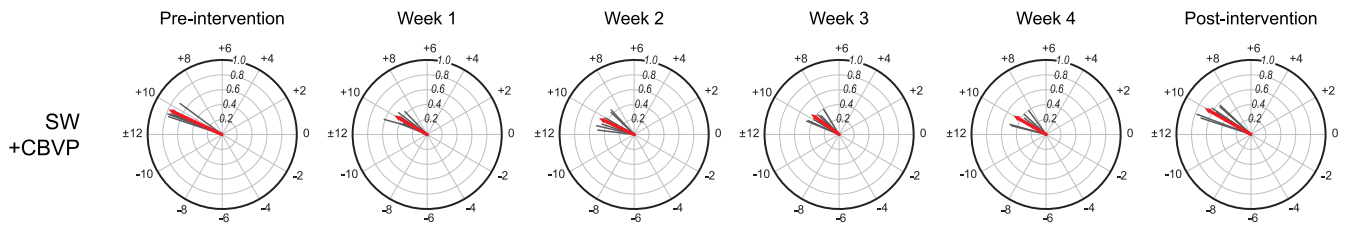
**Fig. 4 | Weekly phasor angle and magnitude in mice experiencing the shiftwork in darkness (SWD), shiftwork in darkness with morning light pulse (SWD+AMpulse), and shiftwork in darkness with evening light pulse (SWD+PMpulse) interventions. Note: The pre-intervention data represent an average of two weeks.**

**Fig. 5 | Weekly total activity and dark/light activity ratio for the shiftwork plus circadian blind, vision-permissive (SW + CBVP) intervention.**

**a** Comparison of weekly total activity between day shift (DS) condition and the SW + CBVP intervention. **b** Comparison of dark/light activity ratio between the DS condition and SW + CBVP intervention. **c** Comparison of weekly total activity between the light and dark phase for the SW + CBVP intervention over the over the pre-intervention week (DS), the 4 intervention weeks, and the post-intervention week (DS). Statistical significance: \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Notes: † pre-intervention (average of two weeks), ‡ post-intervention.







**Fig. 6 | Weekly phasor angle and magnitude in mice experiencing the shiftwork plus circadian blind, vision-permissive (SW + CBVP) intervention.** Note: The pre-intervention data represent an average of 2 weeks.

entrainment would occur with a light pulse at the start of the biological day (evening), given that delaying the circadian clock would be required to maintain entrainment. There were no significant changes in phasor angle or magnitude between conditions. Although mice in the SWD+AMPulse intervention had overall lower total weekly activity, their rest/activity patterns over the course of the treatment weeks seemed to decay sooner in the protocol and reach a plateau, while there was a delay in the decay in activity over the course of the treatment weeks in the SWD and SWD+PMpulse interventions.

Our study was novel in its testing of a light treatment strategy for shiftwork that avoids extended periods of total darkness, which is likely impractical for humans, but at the same time does not disrupt the circadian system. We hypothesized that maintaining a minimal, visually permissive level of light (0.05 lx) would allow visibility while being insufficient to disrupt the circadian system (i.e., circadian blind). The visual threshold sensitivity in mice was shown to be 0.005 lx<sup>35,36</sup> and the circadian threshold sensitivity for a 45-min phase shift was shown to be 5 lx of white light on the cage floor<sup>37</sup>. Therefore, the light level used in our study was above the threshold for visibility and below the threshold for circadian activation. Furthermore, our previous work comparing the effect of rotating shift work in humans and similarly altered light patterns in mice demonstrated that this paradigm in mice was translatable to that observed in nurses on a shiftwork schedule<sup>33</sup>. The study's results show that, despite exposure to a shiftwork paradigm, the rest/activity patterns in the SW + CBVP mice were comparable to those in the DS control condition, where mice were on a regular 12 L:12D cycle.

This study has limitations worth noting. The study only measured wheel running activity and did not measure biomarkers of circadian entrainment. We only used male mice and therefore could not determine whether there were sex differences in the responses. Light levels were only measured on the cage floors and not at the animals' eye level, and entrainment, assessed in free-running dark:dark cycles, was limited only to 3 of the 5 conditions tested and did not include the SW+highL and SW+lowL studies. While sleep-wake cycles are associated with light-associated behavioral entrainment, studies involving sleep disruption were beyond the scope of this study. It is not known whether a longer period of the SW + CBVP intervention would have eventually resulted in circadian disruption. Future studies should include female mice, extend the data collection period, and periodically collect biomarkers of circadian disruption. Most importantly, these studies should be extended to determine whether the potentially protective changes in light-induced disruption of rest/activity patterns are reflected in protection against shiftwork related pathologies.

In conclusion, these findings collectively demonstrate that the adverse effects of shiftwork on light-induced rest/activity rhythms disruption can be mitigated by modulating light level, specifically preserving visual perception while not stimulating the circadian clock.

## Methods

### Mouse husbandry

Thirty male C57BL/6J age-matched mice (The Jackson Laboratory, Bar Harbor, ME, USA) were quarantined for 72 h and then single-housed in clear cages placed within light-tight, temperature- and humidity-controlled cabinets (Circadian Cabinet, Actimetrics, Wilmette, IL, USA), with 6 cages

per cabinet. All cages were equipped with a running wheel connected to a wireless node for monitoring wheel activity (at 30-s intervals) using ClockLab chamber control software (version 6, Actimetrics). The cabinets' internal lighting, humidity levels, and air temperature were also controlled and monitored using the ClockLab software. Animals were kept on a regular diet, and regular mouse chow and water were available *ad libitum* for the duration of the study. Animals were not anesthetized or euthanized for the study. The study was approved by the Icahn School of Medicine Institutional Animal Care and Use Committee (IACUC).

### Light conditions and protocol

At the beginning of the experiment, the mice were exposed to a 2-week day shift (DS) control condition (12 L:12D) in which the cage lights were energized to 25 lx from 07:00 to 19:00 and turned off from 19:00 to 07:00, every day of the week. The mice were then randomized into groups and exposed to one of the following 6 light interventions (Fig. 7) for 5 weeks, followed by a return to the 12 L:12D DS schedule for a final week to establish whether the mice would re-entrain to the DS schedule. (Due to an equipment malfunction, valid data are available for only 4 weeks under all light conditions.) Wheel running activity data were collected over the course of the entire study. The spectral power distribution of the white light source used in all conditions is shown in Fig. 8.

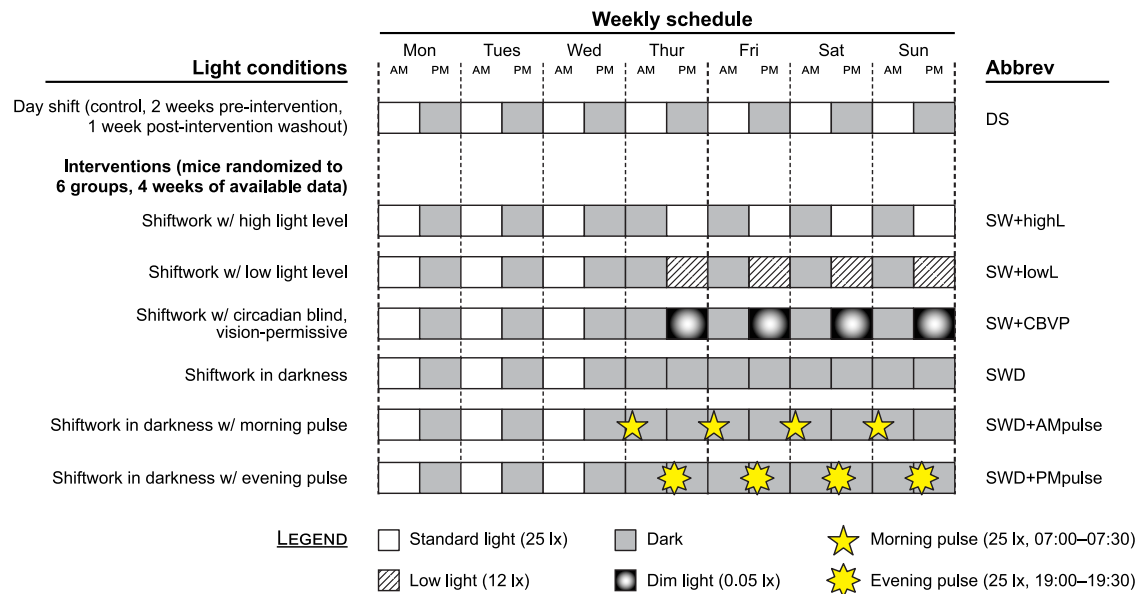
**Shiftwork with high light level (SW+highL).** The mice were exposed to the 12 L:12D schedule from Monday through Wednesday and then to a reversed 12D:12 L schedule in which the lights remained off on Thursday at 07:00 and were reenergized at 19:00. The 12D:12 L schedule was maintained until returning to the 12 L:12D schedule on Monday at 07:00. When energized the lights were set to 25 photopic lux (lx).

**Shiftwork with low light level (SW+lowL).** As with the SW+highL condition, the mice experiencing the SW+lowL condition were exposed to the 12 L:12D schedule from Monday through Wednesday and then to a reversed 12D:12 L schedule, with the lights remaining off on Thursday at 07:00. The lights were reenergized at 19:00 and the 12D:12 L schedule was maintained until returning to the 12 L:12D schedule on Monday at 07:00. In this condition, however, when energized the lights were set to 25 lx Monday through Wednesday and set to 12 lx Thursday through Sunday (see Fig. 7).

**Shiftwork in darkness (SWD).** The mice followed the 12 L:12D schedule from Monday through Wednesday, but rather than experiencing the reversed 12D:12 L schedule used for the 3 SW interventions, the lights remained off on Thursday at 07:00 until Monday at 07:00, after which the energized lights were again set to 25 lx.

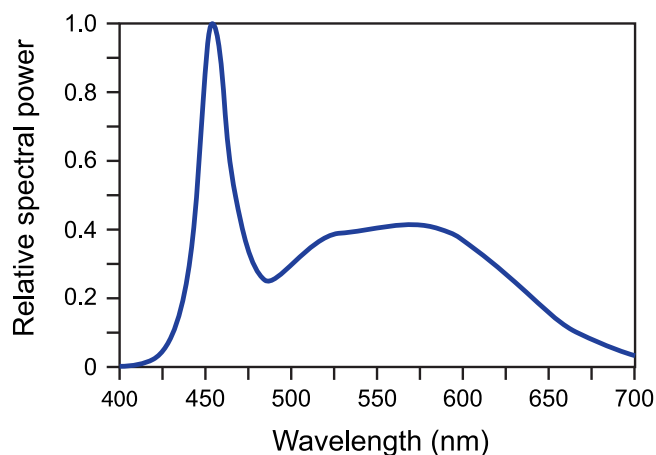
**Shiftwork in darkness with morning (beginning of biological night) light pulse (SWD+AMPulse).** The mice followed the SWD schedule described above. From Thursday through Sunday, a 30-min light pulse (25 lx) was delivered to the darkened cage from 07:00 to 07:30.

**Shiftwork in darkness with evening (beginning of biological day) light pulse (SWD+PMpulse).** The mice followed the SWD schedule



**Fig. 7 | The weekly schedule for the light conditions. In this schedule, AM represents the daytime (07:00 to 19:00) and PM represents the nighttime (19:00 to 07:00) administration of the light conditions. The morning and evening 30-min**

light pulses were administered at the beginning of the biological night (07:00) and day (19:00), respectively.



**Fig. 8 | Relative spectral power distribution of the white light source used in all light conditions.**

described above. From Thursday through Sunday, a 30-min light pulse (25 lx) was delivered to the darkened cage from 19:00 to 19:30.

**Shiftwork with circadian blind/vision-permissive light (SW + CBVP).** The mice followed a schedule that was similar to the SW+highL and SW+lowL. During the reversed schedule (12D:12 L, Thursday at 07:00 through Monday at 07:00), however, the energized lights were set to only 0.05 lx (dim light).

#### Light measurements

Light measurements were collected using a photometer (model X9-1, Gigahertz Optik, Amesbury, MA, USA) with its remote sensor positioned on the floor of each cabinet, between the cages, and with the cabinet doors closed to ensure accurate readings. The light levels were also measured at the bottom of each cage to ensure that all of them received the same amount of light on the cage floors. ClockLab Chamber Control was used to set the light levels and patterns in each of the cabinets. Cabinet light levels were re-measured between experiments to ensure no shifts had occurred. To reduce

the light levels to 0.05 lx on the cage floor for the SW + CBVP intervention, neutral-density light filters (colors 210 and 211, Rosco Laboratories, Stamford, CT, USA) were used to dim the cabinet lights.

#### Data analyses

Wheel running activity data were analyzed on a per animal basis except where otherwise noted. The following analyses were performed:

**Rest/activity patterns.** Rest/activity data from the wheel running device (see Supplementary Material for actograms) were used to calculate: (1) weekly total activity (the sum of all recorded activity data points [wheel revolutions per 30 s] during the entire week); (2) weekly total activity in the light and weekly total activity in the dark (the sum of all recorded activity data points during lights off and the sum of all recorded activity data points during lights on for the entire week); and (3) dark/light activity ratio (the sum of all activity during lights off divided by the sum of all activity during lights on). Weekly spans were defined from zeitgeber time zero (ZT0) on Monday to ZT0 on the following Monday.

**Phasor analysis.** Phasor analysis calculates the vector relationship (phase and amplitude) between the circadian light/dark stimulus pattern and rest/activity response pattern, following methods proposed by Rea et al for quantifying circadian entrainment/disruption<sup>32</sup> in animals and humans. The phasor quantifies how well the light/dark and rest/activity patterns are correlated over a 24-h cycle (phasor magnitude) as well as their relative temporal position (phasor angle). Phasor magnitude is used as an operational, quantitative measure of circadian entrainment for both diurnal humans and nocturnal rodents; the longer the phasor magnitude, the greater the degree of circadian entrainment. Phasor angle depicts the temporal relationship between light/dark onset and rest/activity onset.

#### Statistical analyses

A two-way repeated measures ANOVA was performed with light condition and intervention duration (weeks) as the independent variables, followed by a Tukey correction for multiple comparisons for the outcomes described above.

## Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 27 January 2025; Accepted: 12 June 2025;

Published online: 03 July 2025

## References

- Duffy, J. F. & Czeisler, C. A. Effect of light on human circadian physiology. *Sleep Med. Clin.* **4**, 165–177 (2009).
- Appleman, K., Figueiro, M. G. & Rea, M. S. Controlling light-dark exposure patterns rather than sleep schedules determines circadian phase. *Sleep Med* **14**, 456–461 (2013).
- Duffy, J. F. & Wright, K. P. Entrainment of the human circadian system by light. *J. Biol. Rhythms* **20**, 326–338 (2005).
- Wright, K. P. et al. Entrainment of the human circadian clock to the natural light-dark cycle. *Curr. Biol.* **23**, 1554–1558 (2013).
- Figueiro, M. G. & Pedler, D. Cardiovascular disease and lifestyle choices: Spotlight on circadian rhythms and sleep. *Prog. Cardiovasc. Dis.* **77**, 70–77 (2023).
- Hansen, J. Increased breast cancer risk among women who work predominantly at night. *Epidemiology* **12**, 74–77 (2001).
- Schernhammer, E. S. et al. Rotating night shifts and risk of breast cancer in women participating in the Nurses' Health Study. *J. Natl. Cancer Inst* **93**, 1563–1568 (2001).
- Schernhammer, E. S. et al. Night-shift work and risk of colorectal cancer in the Nurses' Health Study. *J. Natl. Cancer Inst* **95**, 825–888 (2003).
- Karlsson, B., Knutsson, A. & Lindahl, B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup. Environ. Med* **58**, 747–752 (2001).
- Karlsson, B. H., Knutsson, A. K., Lindahl, B. O. & Alfredsson, L. S. Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. *Int. Arch. Occup. Environ. Health* **76**, 424–430 (2003).
- Muller, J. E. et al. Circadian variation in the frequency of sudden cardiac death. *Circulation* **75**, 131–138 (1987).
- Pan, A., Schernhammer, E. S., Sun, Q. & Hu, F. B. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLoS Med.* **8**, e1001141 (2011).
- McAlpine, C. S. & Swirski, F. K. Circadian influence on metabolism and inflammation in atherosclerosis. *Circul. Res.* **119**, 131–141 (2016).
- Marcheva, B. et al. Circadian clocks and metabolism. *Handb. Exp. Pharmacol.* [https://doi.org/10.1007/978-3-642-25950-0\\_6](https://doi.org/10.1007/978-3-642-25950-0_6) (2013).
- Kang, W. et al. Coronary artery atherosclerosis associated with shift work in chemical plant workers by using coronary CT angiography. *Occup. Environ. Med.* **73**, 501–505 (2016).
- Scheer, F. A. J. L., Hilton, M. F., Mantzoros, C. S. & Shea, S. A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. USA* **106**, 4453–4458 (2009).
- Wang, A., Arah, O. A., Kauhanen, J. & Krause, N. Work schedules and 11-year progression of carotid atherosclerosis in middle-aged Finnish men. *Am. J. Ind. Med.* **58**, 1–13 (2015).
- Silva-Costa, A. et al. Time of exposure to night work and carotid atherosclerosis: a structural equation modeling approach using baseline data from ELSA-Brasil. *Int. Arch. Occup. Environ. Health* **91**, 591–600 (2018).
- Figueiro, M. G. et al. Light-dark patterns mirroring shift work accelerate atherosclerosis and promote vulnerable lesion phenotypes. *J. Am. Heart Assoc.* **10**, e018151 (2021).
- Bureau of Labor Statistics, U. S. *Table 7. Workers by shift usually worked and selected characteristics, averages for the period 2017–2018* <https://www.bls.gov/news.release/flex2.t07.htm> (2019).
- Czeisler, C. A. & Gooley, J. J. Sleep and Circadian Rhythms in Humans. *Cold Spring Harbor Symp. Quant. Biol.* **72**, 579–597 (2007).
- Wideman, C. H. & Murphy, H. M. Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. *Nutr. Neurosci.* **12**, 233–240 (2009).
- Foster, R. G., Hughes, S. & Peirson, S. N. Circadian photoentrainment in mice and humans. *Biology.* **9**, 180 (2020).
- Fonken, L. K. & Nelson, R. J. The effects of light at night on circadian clocks and metabolism. *Endocr. Rev.* **35**, 648–670 (2014).
- Figueiro, M. G., Radetsky, L., Plitnick, B. & Rea, M. S. Glucose tolerance in mice exposed to light-dark stimulus patterns mirroring dayshift and rotating shift schedules. *Sci. Rep.* **7**, 40661 (2017).
- Bullough, J. D., Rea, M. S. & Figueiro, M. G. Of mice and women: Light as a circadian stimulus in breast cancer research. *Cancer Cause Control* **17**, 375–383 (2006).
- Xiong, X. et al. Chronic circadian shift leads to adipose tissue inflammation and fibrosis. *Mol Cell Endocrinol* **521**, 111110 (2021).
- Lee, J. et al. Bmal1 and  $\beta$ -cell clock are required for adaptation to circadian disruption, and their loss of function leads to oxidative stress-induced  $\beta$ -cell failure in mice. *Mol. Cell. Biol.* **33**, 2327–2338 (2013).
- JE, L. L., Trujillo, R., Sroga, G. E., Figueiro, M. G. & Vashishth, D. Circadian rhythm disruption with high-fat diet impairs glycemic control and bone quality. *FASEB J* **35**, e21786 (2021).
- Tir, S. et al. in *Circadian and Visual Neuroscience Vol. 273 Progress in Brain Research* (eds Nayantara Santhi & Manuel Spitschan) Ch. 6, 97–116 (Elsevier, 2022).
- Rea, M. S. & Figueiro, M. G. Quantifying light-dependent circadian disruption in humans and animal models. *Chronobiol. Int.* **31**, 1239–1246 (2014).
- Rea, M. S., Bierman, A., Figueiro, M. G. & Bullough, J. D. A new approach to understanding the impact of circadian disruption on human health. *J. Circadian Rhythms* **6**, 7 (2008).
- Radetsky, L. C., Rea, M. S., Bierman, A. & Figueiro, M. G. Circadian Disruption: Comparing humans with mice. *Chronobiol. Int.* **30**, 1066–1071 (2013).
- Singh, D., Pandey, S., Ghosh, A. & Aich, P. Effects of constant darkness on behaviour and physiology of male and female mice. *Eur. J. Neurosci.* **57**, 1498–1515 (2023).
- Hayes, J. M. & Balkema, G. W. Visual thresholds in mice: Comparison of retinal light damage and hypopigmentation. *Vis. Neurosci.* **10**, 931–938 (1993).
- Daly, G. H., Dileonardo, J. M., Balkema, N. R. & Balkema, G. W. The relationship between ambient lighting conditions, absolute dark-adapted thresholds, and rhodopsin in black and hypopigmented mice. *Vis. Neurosci.* **21**, 925–934 (2004).
- Bullough, J. D., Figueiro, M. G., Possidente, B. P., Parsons, R. H. & Rea, M. S. Additivity in murine circadian phototransduction. *Zool. Sci.* **22**, 223–227 (2005).

## Acknowledgements

This study was funded by the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK128972). It was also funded in part by the Beckman Research Institute/City of Hope (R01DK137515) and an AR-DMRI Innovative Award (to K.M.). The funders played no role in study design, data collection, analysis, and interpretation of data, or the writing of this manuscript.

## Author contributions

V.M. processed and analyzed all experimental data, designed the figures, and assisted with interpreting findings and manuscript writing. H.M. performed the animal experiments and assisted with data analyses, interpretation of findings, and manuscript writing. A.P. participated in study design and provided critical input on data analyses, interpretation of findings, and manuscript writing. Y-H.G. provided critical input for study



design and data presentation and edited the manuscript. K.M. contributed to the study design and manuscript editing. J.L. and M.M. contributed to data interpretation and manuscript editing. V.Y. participated in study design and provided critical input on data analyses, interpretation of findings, and manuscript writing. M.G.F. participated in study design and led the data analyses, data interpretation, and manuscript writing. V.Y., A.P., and M.G.F. secured funding for the research. All authors reviewed and approved the final version of the manuscript.

### Competing interests

The authors declare no competing interests.

### Additional information

**Supplementary information** The online version contains supplementary material available at

<https://doi.org/10.1038/s44323-025-00043-3>.

**Correspondence** and requests for materials should be addressed to Mariana G. Figueiro.

**Reprints and permissions information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025