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Obesity drives temporally distinct physical activity disruptions in mice under a fixed 24-h light-dark cycle

Abigail Usiyevich, Yufan Li, Reema Sharma, Kenny Arbuckle, Jay Shah & Bridget A. Matikainen-Ankney✉

24-h cycles regulate activity and feeding behavior, and obesity and metabolic dysfunction may disrupt such rhythms. Here, using a fixed 24-h light-dark cycle, we investigate sex-specific diurnal physical activity patterns in obese and normal weight mice. We observe that feeding behavior aligns with a mid-dark cycle activity peak. Using passive home cage monitoring and operant feeding tasks, we demonstrate that male and female mice exhibit distinct temporal activity profiles, particularly during the late dark cycle. Diet-induced obesity selectively suppressed mid-dark cycle activity, a temporal window linked to peak food-seeking behavior. These findings highlight temporal disruptions to physical activity in a rodent model of diet-induced obesity and offer insights into potential interactions between feeding behavior and 24 physical activity patterns.

Keywords Feeding behavior, Obesity, Sex differences, Physical activity

Obesity is a growing public health crisis associated with widespread metabolic dysfunction, yet its impact on behavioral rhythms such as physical activity remains incompletely understood. In both humans and rodent models, obesity is associated with decreased overall physical activity[1–4] and with disrupted 24-h cycles[5–7]. However, a direct link between obesity and altered physical activity over the 24-h cycle remains unclear, including whether these activity deficits occur uniformly across the 24-h cycle or are temporally specific. One reason may be due to methods surrounding tracking 24-h activity in mouse models. Traditionally, home cage activity metrics have been collected from rodents using running wheels, where a running wheel is placed in the cage, and wheel rotations are recorded over time. However this methodology can introduce significant confounds, as the presence of a running wheel can itself alter activity levels or influence circadian rhythms[8]. Here we aimed to explore home cage physical activity patterns in mice using a passive infrared monitoring system, and to determine how 24-h physical activity patterns differ in the lean vs. obese state under a fixed 24-h light-dark cycle. We chose a passive infrared monitoring system as our previous work showed that IR detection of home cage activity correlates with locomotion[9], and thus generates data that can be compared to wheel running locomotion in the literature.

Across species, regulation of locomotion and feeding plays a central role in maintaining energy balance. Rodents, like humans, exhibit structured daily rhythms that align activity and food-seeking with the dark (active) phase of a 24-h light/dark cycle[10] and are sexually dimorphic[11,12]. However, the extent to which obesity disrupts these temporal dynamics – especially in the context of spontaneous home cage behavior – is not fully understood. In this study, we show that mice display three temporally distinct peaks of physical activity during the dark cycle, and that these activity peaks are aligned with feeding behavior. Notably, we observe sex differences in the timing and magnitude of these rhythms, suggesting that energy expenditure and intake are finely tuned across the dark cycle and modulated by biological sex.

Within this framework, we find that diet-induced obesity suppresses physical activity selectively during the mid-dark cycle, a window that corresponds to peak food-seeking behavior in lean mice. These findings suggest that obesity does not cause a global reduction in mouse physical activity, but rather disrupts the coupling between energy intake and expenditure in a temporally specific manner. This study reveals a novel aspect of how obesity alters behavioral rhythms and underscores the importance of considering timing when studying the behavioral consequences of obesity. By identifying discrete time windows in which obesity-linked activity suppression occurs in mice, our findings point to potential temporal targets for interventions aimed at restoring metabolic health.

Behavioral and Systems Neuroscience, Psychology Department, Rutgers University, Busch Campus Psychology Building Room 317, 152 Frelinghuysen Road, Piscataway, NJ 08854, USA. ✉email: bridget.m@rutgers.edu

Results

Mice display distinct activity peaks across the a fixed 24h light/dark cycle

We first investigated 24-h activity patterns in singly housed C57BL/6J male and female mice. For 3 weeks we continuously recorded physical activity from singly housed mice with passive infrared monitors in each mouse's home cage (Fig. 1 A). The data reveal enhanced activity exhibited at early and late phases of the dark cycle (Fig. 1B), supporting observations that mice display an early and late burst of activity during the dark cycle, separated by a mid-cycle dip[13–15]. An unbiased peak finding analysis revealed three distinct activity peaks during the dark cycle, at Zeitgeber hours (ZT) 13, 17, and 23 (p1, p2, and p3, respectively) (Fig. 1C, D). This suggests that continuous, non-invasive home cage monitoring reveals a distinct home cage activity profile for mice on a fixed 24-h light-dark cycle.

We asked whether activity patterns were similar across mice, or whether they would segregate into distinct profiles. To do this, we ran a principal component analysis of 24-h activity patterns across all animals, and identified 3 distinct clusters (Supp Fig. 1A-D). Notably, these clusters appeared to be separated primarily by activity levels during p3 and predominantly aligned by sex; clusters 0 and 2 comprised predominantly male mice (3/4 mice and 9/11 mice, respectively), and cluster 1 comprised female mice (Supp Fig. 1D). Direct comparison of activity between male and female mice shows that animals from both sexes demonstrated 3 distinct activity peaks, but during p3 male mice showed an increase in activity relative to female mice (Supp Fig. 1E-G). This supports previous work demonstrating a surge in physical activity in males in the late dark cycle[16–22]. However, prior investigations of female mice showed an increase in physical activity in the late dark cycle, similar to males[16]. Our data shows decreased female physical activity in p3 relative to males, potentially due to differences in passive recordings vs. using running wheels. Together this suggests that mouse home cage physical activity organizes into distinct peaks during the active cycle, and that these patterns may be sex-specific.

Feeding behavior aligns with mid-dark cycle activity

Metabolism, energy balance, and feeding behavior are regulated on a 24-h cycle in addition to activity levels[7,23]. Therefore we next asked how food seeking patterns varied across a fixed 24-h light and dark cycle. In a new cohort of mice, we assessed feeding patterns using an in-home cage feeding device (FED3[24]) to run a 72-h operant task where a single nose poke (on either a left or right nose poke port) yielded a 20 mg sucrose pellet (Fig. 2A). Mice had ad libitum access to standard chow and water throughout the task. We graphed pellets earned over 24 h, and observed an increase in pellets earned at p2 (Fig. 2B), suggesting mice seek the most pellets during the mid dark cycle. We then identified which hour of the 24-h cycle had the greatest number of pellets earned for each mouse, and plotted that in a histogram, confirming most mice seek most of their pellets in the mid dark cycle during p2 (Fig. 2 C).

Both male and female mice demonstrated increased food-seeking during p2 (Supp Fig. 2 A, B). Further analysis of inter-pellet intervals revealed that most feeding events occurred within 60 s of each other for both sexes, suggesting clustered feeding events which we dubbed "meals" (Supp Fig. 2 C, D). We observed that females consumed fewer meals (Supp Fig. 2E) but ate significantly larger meals compared to males (Supp Fig. 2 F). Thus while meal patterns differ between sexes, both males and females preferentially seek food during p2, aligning feeding with a temporally distinct physical activity bout.

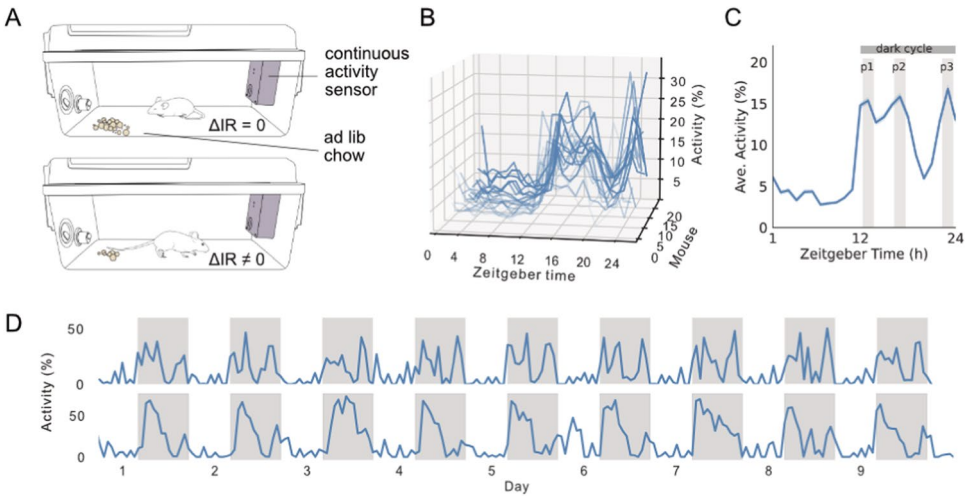

Fig. 1. Monitoring home cage activity in mice reveals distinct dark cycle activity bouts in a fixed 24-h light-dark cycle. (**a**) Schematic showing physical activity monitor in the home cage. Signal corresponds to mouse activity. (**b**) 24-h activity plot for individual mice. (**c**) 24-h activity plot, averaged for all mice. Grey bars indicate dark cycle peak activity. (**d**) Examples of continuous activity trace from a single male (top) and female (bottom) mouse. $n = 8$ female mice, 12 male mice.

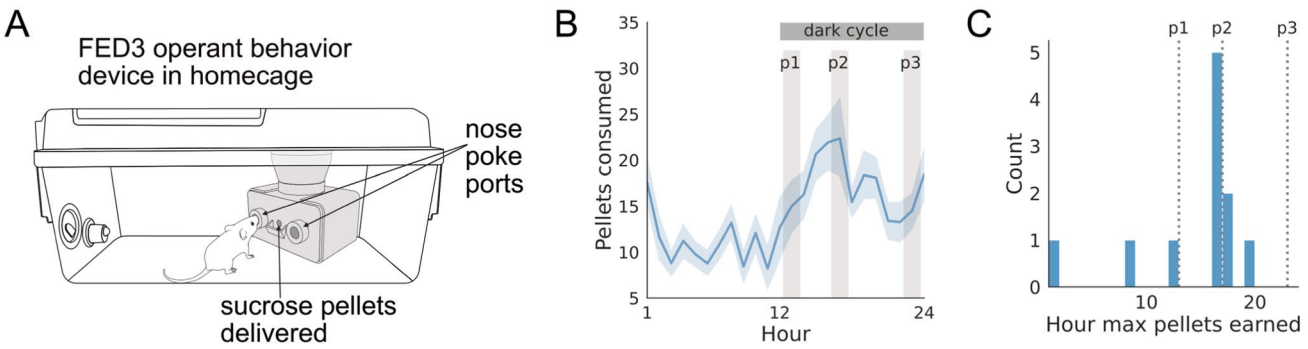

Fig. 2. Food seeking peaks mid dark-cycle in mice. (**a**) Schematic of FED3 in the homecage. (**b**) Average pellets earned over 24 h cycle. (**c**) Histogram of hour bin during which max pellets are consumed. $n = 14$ males, 14 females.

Diet-induced obesity reduces mid-dark cycle activity

Chronic disruptions, such as shift work, irregular eating patterns, or sleep deprivation, are all strongly associated with obesity, and obesity itself can disrupt such rhythms, creating a self-reinforcing cycle that exacerbates weight gain and associated health complications[5–7]. In humans, cycle disruptions such as insufficient or irregular sleep are linked to increased obesity risk due to altered energy balance and metabolic dysregulation[5,6]. Similarly, diet-induced obesity in rodents has been shown to reduce dark cycle activity, as measured by laser beam breaks in metabolic chambers or actimetry[25,26]. Given our earlier observation that food-seeking behavior peaks during p2 (Fig. 2B, C, Supp Fig. 2A, B), we asked whether diet-induced obesity would alter physical activity specifically during this phase. To test this, we exposed male and female mice to either ad libitum high-fat diet (HFD; obese group) or standard chow (control) for eight weeks. As expected, both male and female mice exposed to HFD gained significantly more weight compared to controls (Fig. 3A-C). We then recorded home cage activity during the final week of weight gain (Fig. 3A, D). We observed a significant reduction in activity during p2 in obese male and female mice compared to lean controls (Fig. 3E-J), and obese female mice also exhibited reduced activity during p1 (Fig. 3H). These results confirm that diet-induced obesity leads to significant reductions in home cage physical activity in mice, and demonstrate that the mid-dark cycle (p2) is particularly susceptible to activity suppression.

Discussion

Here we demonstrate that mice exhibit three distinct activity peaks across the dark cycle in a fixed, 24-h light-dark cycle experiment, that such activity patterns are sex-specific, and that activity during the second peak occurs during a bout of increased food seeking. We observed a reduction in activity primarily during the second activity peak in both male and female mice with diet-induced obesity relative to lean controls. Our data highlights key distinctions between home cage activity monitoring methods, and suggests that recorded outcomes from running wheels vs. passive activity monitors may differ significantly.

Running wheels vs. passive IR activity detectors

In studies using running wheels, activity typically surges in two bouts, initially after the onset of the dark cycle, followed by a surge prior to the onset of the light cycle[13–15]. This suggests that rodent activity is bimodal during the wake cycle, though one running wheel study showed that male and female rodents run continuously throughout the dark cycle[17]. Passive IR activity monitors used in the home cage collect movement data that correlates with home cage locomotion[9], which is important to keep in mind when comparing running wheel readouts and IR recordings. Both collect data indicative of locomotion, though running wheels likely reflect vigorous bouts of activity, while IR detectors reflect ambulation around the home cage. Thus, while our observation of three distinct activity peaks across the dark cycle contrasts published work in revealing distinct activity bouts during the dark cycle, it also underscores differences in physical activity measured in the presence or absence of a running wheel. Further, studies using running wheels demonstrate that female rodents exhibit higher levels of running relative to males[16,17,20]. Our data suggest that during the early- and mid-dark cycles there are no obvious sex differences in home cage activity, and in the late dark cycle male mice display higher activity levels. This supports a study in mice using infrared sensors in the home cage to track 24-h activity that revealed a distinct late-dark cycle surge in activity in male mice[21]. Together, this further emphasizes the need for careful interpretation of round-the-clock activity studies that rely on running wheels, as activity measured by voluntary wheel-running behavior may obscure or exaggerate underlying activity patterns.

Implications for obesity

Our data shows that obesity-induced decreases in activity are temporally specific, occurring primarily during the mid-dark cycle during a fixed 24-h light-dark cycle. This temporally selective decrease in home cage activity was surprising – while physical activity deficits are well-established in people with obesity as well as rodent models of obesity[1–4], they are typically interpreted as decreases across the 24 h cycle. Such a temporally specific decrease

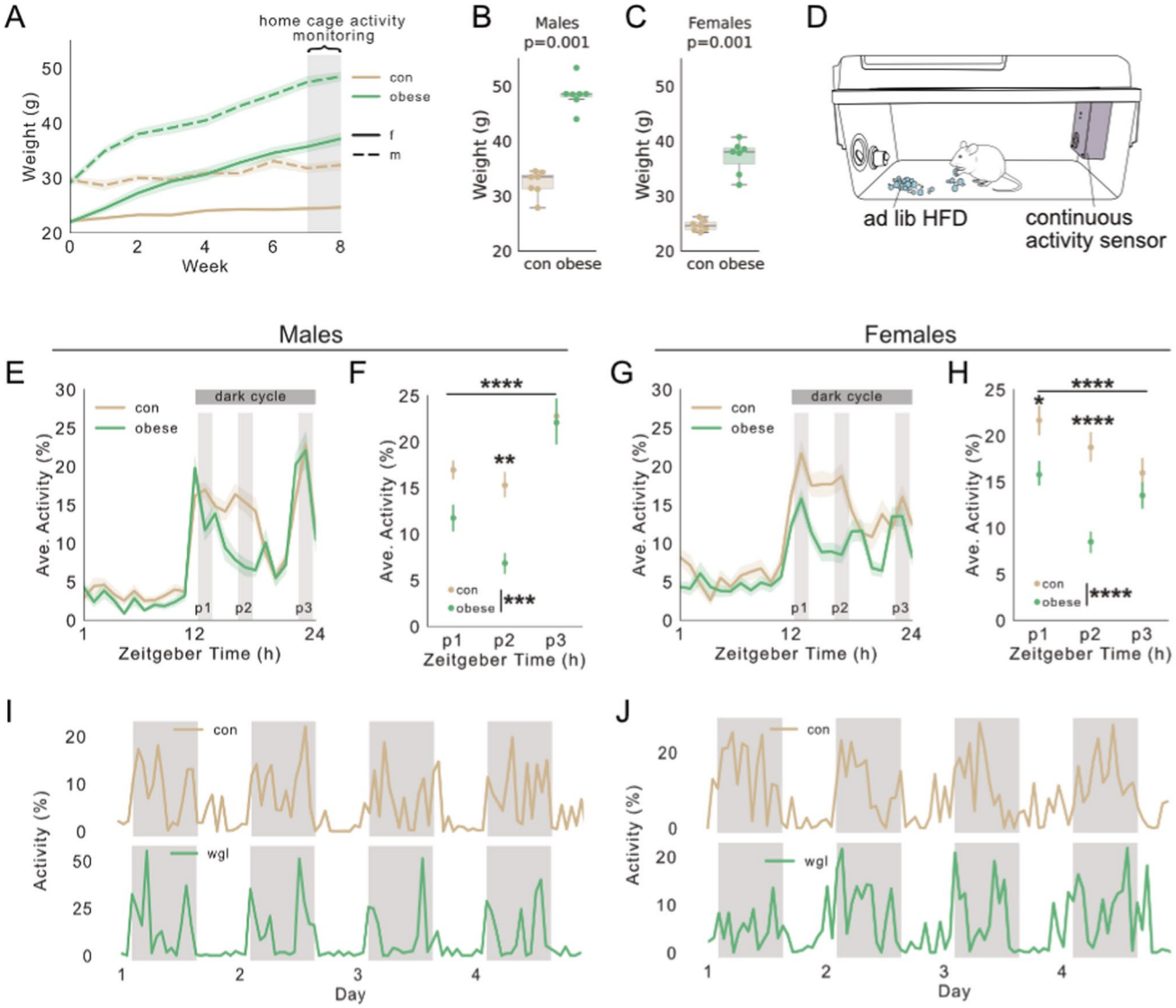

Fig. 3. Diet-induced obesity decreases physical activity in male and female mice mid-dark cycle under fixed 24-h light-dark cycle conditions. **a**. Weight curves over eight weeks for control (tan) and obese (green) male (dotted line) and female (solid line) mice. **b**, **c**. Dotted box plots showing obese male (**b**) and female (**c**) mice weigh significantly more than controls. $p = 0.001$, $p = 0.001$, respectively. **d**. Schematic showing location of activity sensor in home cage. **e**. 24-h activity plot for activty patterns from lean and obese male mice. Grey bars indicate dark cycle peak activity. **f**. Average of activity from lean and obese males across p1, p2, and p3. Significant effect of group ($F(1,270) = 13.4783$, $p = 0.0003$) and significant effect of peak time ($F(2,270) = 25.8207$, $p = 0.0001$). Post hoc tests reveal significant decrease in activity of obese mice relative to control at p2 ($p = 0.0030$). **g**. 24-h activity plot for activty patterns from lean and obese female mice. Grey bars indicate dark cycle peak activity. **h**. Average of activity from lean and obese females across p1, p2, and p3. Significant effect of group ($F(1,225) = 27.5859$, $p = 0.0001$), peak time ($F(2,225) = 3.6424$, $p = 0.0009$), and interaction between group and time ($F(2,225) = 3.6424$, $p = p = 0.0277$). Post hoc tests reveal significant decrease in activity of obese mice relative to control at p1 ($p = 0.0489$) and p2 ($p = 0.0001$). **i**, **j**. Examples of continuous activity traces from control (tan) or obese (green) male (left) or female (right) mice over four days. Linear mixed model with multiple comparisons in **f**, **h**. Tukey post hoc tests in **f**, **h**. $n = 7$ males, 7 females.

in home cage activity in obese mice, occurring during a time of increased food seeking, strongly suggests a link between 24-h activity cycles and over-feeding leading to obesity. However - future studies are needed to determine the nature of such targeted activity suppression, as our experiments do not reveal the nature of the temporally specific decline in activity. For instance, this temporally selective suppression of activity might reflect an energy-conserving adaptation or perhaps coincide with increased time spent consuming food vs. home cage locomotion. Regardless, these findings are consistent with previous reports of reduced dark cycle activity in obese rodents[15,25,26], and underscore the relationship between 24-h activity rhythms and feeding.

Conclusions and limitations of this study

Our results demonstrate that 24-h physical activity patterns are sex-specific, and are temporally coupled to feeding. Further, diet-induced obesity selectively disrupted activity during the mid-dark cycle, a temporal window associated with enhanced food-seeking. These findings further link 24-h activity rhythms and feeding, and suggest that the two may interact in obese states. However, there are limitations to our study. While we observed that food seeking coincided with a mid-dark cycle activity bout in mice, we did not confirm this via infrared video recordings. Without such a direct measure we cannot definitively link mid-dark cycle activity levels and feeding. Relatedly, our observations that obese mice show decreased activity in the mid-dark cycle are correlative, and do not allow us to conclude anything about a specific behavior in which obese mice are participating, simply that diet-induced obesity drives decreased home cage activity during the mid-dark cycle. Studies restricting or granting access to an obesogenic diet during the mid-dark cycle, for instance, would shed light on whether there is a causal link between mid-dark cycle feeding and weight gain, and should be conducted in the future.

Additionally, our studies were conducted under a fixed 24-h light-dark cycle, and therefore our results should not be interpreted differential responses to light cues, and not necessarily linked to internally generated circadian signals or endogenous circadian rhythms. For instance, a hallmark phenotype of obesity is a blunting of motivated behavior[1,27]. Thus the decrease in physical activity we see in obese mice during the mid-dark cycle may simply be a decrease in home-cage activity several hours after dark-cycle onset, and not reflective of endogenous circadian mechanisms linked to food consumption. Alternatively, differences in metabolic or hunger cues associated with the mid-dark cycle surge in food seeking may drive the mid-dark cycle decrease in activity in obese mice. Thus our results may not be reflective of internal circadian rhythm differences between normal weight or obese conditions, but rather differences in cue responses. In order to attribute our results to endogenous mechanisms, these studies would need to be conducted under constant light conditions, which we did not perform here.

Further, in our study female mice exposed to an ad lib obesogenic diet gained weight steadily at a rate on par with male mice. Evidence demonstrating that female mice reliably gain weight when exposed to an obesogenic diet is mixed, with some studies reporting weight gain in females[28–30] yet others demonstrating female mice are resistant to weight gain[31–33]. One explanation for the reliable weight gain in females observed here may be social isolation. Though we did not directly test how group-housing vs. single-housing affects weight gain in this study, evidence suggests singly housing mice may facilitate weight gain. Singly housed male mice gained weight more quickly than group housed males when exposed to HFD[34], and singly housed female mice exposed to HFD showed steady weight gain[35].

Lastly, our findings showing that mid-dark cycle activity is selectively decreased in mice with diet-induced obesity may differ from genetic models of obesity, which we did not test here and which may impart distinct disruptions. For example, leptin-deficient mice (ob/ob), which are obese with exposure to normal lab chow, show disrupted cyclic hormonal regulation[36]. This suggests increased adiposity regardless of diet consumed can skew 24-h cycles. Future studies should expand upon these limitations.

Methods

Animals and Diets. We investigate the intricate relationship between the development of motivated behavior and obesity under high-fat diet (HFD) exposure. To achieve this, we employ advanced behavioral assays in mice. Male and female C57BL/6J mice (12–14 weeks old, purchased from Jackson Labs) were individually housed under a 24-h light-dark cycle (dark phase: 7 PM–7 AM) with ad libitum access to food and water. Each cage was enriched with two items: wooden blocks and plastic cuddlehuts. Mice were provided either a standard laboratory chow diet (5001 Rodent Diet) or a 60% HFD (Research Diets, D12492). In the weight gain experiment, the HFD group was provided HFD for 8 weeks. Sample size of 14 was calculated to ensure power > 0.8 with an expected outcome of > 80% reduction in motivated behavior assays seen in obese mice[37,38]. Data from all subjects were included in the analyses presented here. Cage temperature and humidity were continuously monitored using sensors to ensure a stable environment across the study. Experimental group assignments were clearly indicated on cage cards to ensure accurate daily diet administration. Throughout the study, no signs of distress or pain were observed in any animal. At the conclusion of the study, mice were put under deep anesthesia with exposure to isoflurane (4% induction in an airtight chamber) for 2–3 min. Upon confirmation of lack of responsiveness to a toe pinch, mice we euthanized with decapitation. All procedures were approved by the Rutgers University Animal Care and Use Committee, and were performed within guidelines put forth by the National Institutes of Health. The present study was performed in accordance with ARRIVE guidelines (https://arriveguidelines.org). We hereby confirm that all experimental procedures were conducted following the above regulations and guidelines. All methods were performed in accordance with the relevant guidelines and regulations set forth by the Rutgers University Animal Care and Use Committee, and by the National Institutes of Health.

Continuous Activity Monitoring. Home cage activity was monitored continuously using Pallidus MR1 passive infrared (PIR) detectors (Pallidus), with activity data collected and binned on an hourly basis. PIR sensors detect movement of warm objects (such as mice) through their sensing zone. Critically, the sensing zone cannot extent through plastic, and as such is limited to inside the home cage. Previously, we used video recordings to calibrate PIR sensors, and observed a correlation between the speed of a mouse moving around an arena with logged PIR activity[9], suggesting activity correlates with locomotion in the cage.

Operant Feeding Assay. Operant behavior was assessed using the Feeding Experimentation Device 3 (FED3) system[24]. Mice were trained to perform nosepokes on an active port to receive a conditioned stimulus (tone and light) followed by a 20 mg sucrose pellet (TestDiet). Nosepokes on the inactive port were logged but had no consequence. Acquisition was conducted under a fixed ratio (FR) schedule, where one nosepoke yielded one

pellet. For progressive ratio (PR) sessions, the required number of nosepokes to earn a reward increased by one after each completed trial. Both FR and PR sessions ran continuously for 24 h.

Data Analysis and Statistics. Data were processed and visualized using custom Python scripts. Bartlett's test was used to assess homogeneity of variance. Depending on the data, appropriate statistical tests, including Student's t-tests, Mann-Whitney tests, and two-way ANOVA, were performed using Python's StatsModels package. All subjects' data were analyzed uniformly. To identify local maxima during the dark phase, we applied the Scipy find_peaks function, defining maxima as an increase followed by a subsequent decrease. Activity data was rescaled to amplitudes of 0–1 for principal component analyses (PCA) and Kmeans clustering. Cluster number was determined using a silhouette analysis, with the silhouette score for 3 clusters = 0.516. PCA and Kmeans clustering was performed on activity data scaled to a min-max of 0–1 absolute scale. The top 3 PCs contributed 66.236% of the total sample variance. Experimenters were not blinded to mouse group, as obesity is an obvious condition in mice. However, experimenters were blinded to group during data analysis. No animals were excluded from this study.

Data availability

All data and analysis files will be made available on OSF: https://osf.io/gnrzb/.

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References

1. Friend, D. M. et al. Basal ganglia dysfunction contributes to physical inactivity in obesity. *Cell. Metab.* **25**, 312–321. https://doi.org/10.1016/j.cmet.2016.12.001 (2017).
2. Berglind, D. et al. Women undergoing roux-en-y gastric bypass surgery: Family resemblance in pre- to postsurgery physical activity and sedentary behavior in children and spouses. *Surg. Obes. Relat. Dis.* **11**, 690–696. https://doi.org/10.1016/j.soard.2014.10.018 (2015).
3. Bond, D. S. et al. Pre- to postoperative physical activity changes in bariatric surgery patients: Self report vs. objective measures. *Obes. (Silver Spring)* **18**, 2395–2397. https://doi.org/10.1038/oby.2010.88 (2010).
4. Ramirez-Marrero, F. A., Miles, J., Joyner, M. J. & Curry, T. B. Self-reported and objective physical activity in postgastric bypass surgery, obese and lean adults: Association with body composition and cardiorespiratory fitness. *J. Phys. Act. Health* **11**, 145–151. https://doi.org/10.1123/jpah.2012-0048 (2014).
5. Chaput, J. P. et al. The role of insufficient sleep and circadian misalignment in obesity. *Nat. Rev. Endocrinol.* **19**, 82–97. https://doi.org/10.1038/s41574-022-00747-7 (2023).
6. Depner, C. M., Stothard, E. R. & Wright, K. P. Metabolic consequences of sleep and circadian disorders. *Curr. Diab Rep.* **14**, 507. https://doi.org/10.1007/s11892-014-0507-z (2014).
7. Oosterman, J. E., Kalsbeek, A., la Fleur, S. E. & Belsham, D. D. Impact of nutrients on circadian rhythmicity. *Am. J. Physiology-Regulatory Integr. Comp. Physiol.* **308**, R337–R350. https://doi.org/10.1152/ajpregu.00322.2014 (2015).
8. O'Neal, T. J., Friend, D. M., Guo, J., Hall, K. D. & Kravitz, A. V. Increases in physical activity result in diminishing increments in daily energy expenditure in mice. *Curr. Biol.* **27**, 423–430. https://doi.org/10.1016/j.cub.2016.12.009 (2017).
9. Matikainen-Ankney, B. A. et al. Rodent Activity Detector (RAD), an open source device for measuring activity in rodent home cages. *eNeuro* https://doi.org/10.1523/eneuro.0160-19.2019 (2019).
10. Jiang, Y., Shi, J., Tai, J. & Yan, L. Circadian regulation in diurnal mammals: Neural mechanisms and implications in translational research. *Biology* https://doi.org/10.3390/biology13120958 (2024).
11. Anderson, S. T. & FitzGerald, G. A. Sexual dimorphism in body clocks. *Science* **369**, 1164–1165. https://doi.org/10.1126/science.abd4964 (2020).
12. Fischer, D., Lombardi, D. A., Marucci-Wellman, H. & Roenneberg, T. Chronotypes in the US - Influence of age and sex. *PLoS One.* **12**, e0178782. https://doi.org/10.1371/journal.pone.0178782 (2017).
13. Mitler, M. M., Lund, R., Sokolove, P. G., Pittendrigh, C. S. & Dement, W. C. Sleep and activity rhythms in mice: A description of circadian patterns and unexpected disruptions in sleep. *Brain Res.* **131**, 129–145. https://doi.org/10.1016/0006-8993(77)90033-6 (1977).
14. Eckel-Mahan, K. & Sassone-Corsi, P. Phenotyping circadian rhythms in mice. *Curr. Protoc. Mouse Biol.* **5**, 271–281. https://doi.org/10.1002/9780470942390.mo140229 (2015).
15. Kohsaka, A. et al. High-Fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabol.* **6**, 414–421. https://doi.org/10.1016/j.cmet.2007.09.006 (2007).
16. Blattner, M. S. & Mahoney, M. M. Circadian parameters are altered in two strains of mice with transgenic modifications of estrogen receptor subtype 1. *Genes Brain Behav.* **11**, 828–836. https://doi.org/10.1111/j.1601-183X.2012.00831.x (2012).
17. Brockman, R., Bunick, D. & Mahoney, M. M. Estradiol deficiency during development modulates the expression of circadian and daily rhythms in male and female aromatase knockout mice. *Horm. Behav.* **60**, 439–447. https://doi.org/10.1016/j.yhbeh.2011.07.011 (2011).
18. Duffy, J. F., Viswanathan, N. & Davis, F. C. Free-running circadian period does not shorten with age in female syrian hamsters. *Neurosci. Lett.* **271**, 77–80. https://doi.org/10.1016/s0304-3940(99)00519-4 (1999).
19. Iwahana, E., Karatsoreos, I., Shibata, S. & Silver, R. Gonadectomy reveals sex differences in circadian rhythms and suprachiasmatic nucleus androgen receptors in mice. *Horm. Behav.* **53**, 422–430. https://doi.org/10.1016/j.yhbeh.2007.11.014 (2008).
20. Roper, T. J. Sex differences in circadian wheel running rhythms in the Mongolian gerbil. *Physiol. Behav.* **17**, 549–551. https://doi.org/10.1016/0031-9384(76)90121-9 (1976).
21. Stowie, A. C. & Glass, J. D. Longitudinal study of changes in daily activity rhythms over the lifespan in individual male and female C57BL/6J mice. *J. Biol. Rhythms.* **30**, 563–568. https://doi.org/10.1177/0748730415598023 (2015).
22. Schull, J. et al. Effects of thyroidectomy, parathyroidectomy and lithium on circadian wheelrunning in rats. *Physiol. Behav.* **42**, 33–39. https://doi.org/10.1016/0031-9384(88)90256-9 (1988).
23. Engin, A. Circadian rhythms in *Diet-Induced Obesity. In Obesity and Lipotoxicity*, (eds Engin, A. B. & Engin, A.) (Springer International Publishing), 19–52. https://doi.org/10.1007/978-3-319-48382-5_2. (2017).
24. Matikainen-Ankney, B. A. et al. An open-source device for measuring food intake and operant behavior in rodent home-cages. *Elife* **10**, e66173. https://doi.org/10.7554/eLife.66173 (2021).
25. Kentish, S. J., Vincent, A. D., Kennaway, D. J., Wittert, G. A. & Page, A. J. High-fat Diet-Induced obesity ablates gastric vagal afferent circadian rhythms. *J. Neurosci.* **36**, 3199–3207. https://doi.org/10.1523/JNEUROSCI.2710-15.2016 (2016).
26. Bravo, R. et al. Body weight gain in rats by a high-fat diet produces chronodisruption in activity/inactivity circadian rhythm. *Chronobiol. Int.* **31**, 363–370. https://doi.org/10.3109/07420528.2013.859151 (2014).

27. Sharma, S., Fernandes, M. F. & Fulton, S. Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int. J. Obes.* **37**, 1183–1191. https://doi.org/10.1038/ijo.2012.197 (2013).
28. Pettersson, U. S., Waldén, T. B., Carlsson, P. O., Jansson, L. & Phillipson, M. Female mice are protected against High-Fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. *PLOS ONE.* **7**, e46057. https://doi.org/10.1371/journal.pone.0046057 (2012).
29. Guerra-Cantera, S. et al. The metabolic effects of resumption of a high fat diet after weight loss are sex dependent in mice. *Sci. Rep.* **13**, 13227. https://doi.org/10.1038/s41598-023-40514-w (2023).
30. Gelineau, R. R. et al. The behavioral and physiological effects of high-fat diet and alcohol consumption: Sex differences in C57BL6/J mice. *Brain Behav.* **7**, e00708. https://doi.org/10.1002/brb3.708 (2017).
31. Stapleton, S., Welch, G., DiBerardo, L. & Freeman, L. R. Sex differences in a mouse model of diet-induced obesity: The role of the gut Microbiome. *Biology Sex. Differences* **15**, 5. https://doi.org/10.1186/s13293-023-00580-1 (2024).
32. Casimiro, I., Stull, N. D., Tersey, S. A. & Mirmira, R. G. Phenotypic sexual dimorphism in response to dietary fat manipulation in C57BL/6J mice. *J. Diabetes Complicat.* **35**, 107795. https://doi.org/10.1016/j.jdiacomp.2020.107795 (2021).
33. Murtaj, V. et al. Brain sex-dependent alterations after prolonged high fat diet exposure in mice. *Commun. Biol.* **5**, 1–17. https://doi.org/10.1038/s42003-022-04214-x (2022).
34. Queen, N. J. et al. Social isolation exacerbates diet-induced obesity and peripheral inflammation in young male mice under thermoneutrality. *iScience* https://doi.org/10.1016/j.isci.2023.106259 (2023).
35. Bowen, H., Mitchell, T. D. & Harris, R. B. S. Method of leptin dosing, strain, and group housing influence leptin sensitivity in high-fat-fed weanling mice. *Am. J. Physiology-Regulatory Integr. Comp. Physiol.* **284**, R87–R100. https://doi.org/10.1152/ajpregu.00431.2002 (2003).
36. Guo, X. et al. Obesity induced disruption on diurnal rhythm of insulin sensitivity via gut microbiome-bile acid metabolism. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids.* **1869**, 159419. https://doi.org/10.1016/j.bbalip.2023.159419 (2024).
37. Matikainen-Ankney, B. A. et al. Nucleus accumbens D1 receptor–expressing spiny projection neurons control food motivation and obesity. *Biol. Psychiatry* **93**, 512–523. https://doi.org/10.1016/j.biopsych.2022.10.003 (2023).
38. Serdar, C. C., Cihan, M., Yücel, D. & Serdar, M. A. Sample size, power and effect size revisited: Simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem. Med. (Zagreb)* **31**, 010502. https://doi.org/10.11613/BM.2021.010502 (2021).

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Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to B.A.M.-A.