THE HIDDEN RHYTHM THAT COULD CONFOUND MOUSE STUDIES

Mouse activity and physiology varies through the day, but researchers have learned to control for it. Now a seasonal oscillation could make mouse experiments harder to reproduce.

Life has its rhythms, and that's as true for lab mice as it is for humans. Mice tend to sleep all day and feed, run, mate and explore at night. The time of day can affect frequently studied mouse behaviors such as wheel running and feeding, and can even affect how drug compounds are absorbed, or how the compounds interact with target receptors. For these reasons, a mouse study performed in the morning can generate different results from one performed in the afternoon.

Brun Ulfhake, a neuroscientist at the Karolinska Institute in Stockholm, knew that circadian rhythms, which are tuned to the 24-hour day/night cycle, could cause such variability. In this way they regulate an array of biological functions, including sleep, metabolism, hormone releases and digestion.

But in April Ulfhake's team reported a hidden biological rhythm in lab mice that oscillates over months rather than hours, and seems to make mice far more active than usual. "These variations were totally unexpected," Ulfhake says. The new rhythm could affect the reproducibility of mouse experiments everywhere.[1]

Seeking reproducibility

Circadian rhythms originate from signals in the hypothalamus, and they act on circuits expressed by nearly every cell in the body. As researchers have learned about these rhythms, they've sought to control for circadian variability. Drug researchers now consider how circadian rhythms influence treatment efficacy and side effects when developing dosing regimens, says Marcello Raspa, a pharmacologist at the Italian National Research Council in Rome. Other researchers take measurements at the same time of day, and make sure that collaborators keep their animals on the same light-dark schedules, says Ryan Logan, a neuroscientist at Boston University.

Ulfhake took this quest for reproducibility up a notch by monitoring activity levels in 64 animals day and night for 19 months. His team housed the animals in a home-cage monitoring system developed by Tecniplast, an Italian maker of laboratory animal equipment. Outfitted with 12 floor-mounted electrodes, the company's Digital Ventilated Cage (DVC®) collects movement data multiple times per second. As such, it generates a digital marker of real-time activity and reveals unusual patterns that would otherwise be undetectable, according to Guido Gottardo, Tecniplast's digital product manager.

Over those 19 months, the animals' environment remained the same—light cycles, temperature and feeding routines were all held constant. Yet their activity levels would increase, plateau, and then suddenly drop off. During these cyclical episodes, which lasted from two and four months at a time, the mice were two-thirds more active than their corresponding baseline values.[1] "That's a magnitude that can affect physiological readouts," Ulfhake says.

"THESE VARIATIONS WERE TOTALLY UNEXPECTED."

"Clearly, there's a persistent pattern here that came from somewhere," says Benjamin Smarr, a neurobiologist at the University of California, San Diego, who specializes in biological rhythms and was not involved in the study. Biological rhythms are concerning for lab animal research since they drive significant experimental variability, Smarr says. Gene expression, for instance, varies at different points in a rhythmic cycle. "Then you're asking how much of the effect you observe is due to your intervention or to the effects of a biological rhythm that you may not be aware of."

Seasonal synching

Biological rhythms longer than 24 hours are called infradian rhythms, and they can range from days to years in length. Certain species of birds will molt at the same time every year, even in controlled experimental conditions during which they're exposed to the same daily intervals of light and dark. Bears hibernate on an infradian cycle. The human menstrual cycle is infradian.

Ulfhake suspects his team detected a seasonal infradian rhythm. Yet he acknowledges this conclusion is controversial. Ordinarily, animals with endogenous seasonal clocks are also exposed to environmental cues such as day length that push their clocks forward or backward to synchronize them to outside changes, yet lab mice are housed indoors and do not receive these cues.

What's more, the C57BL/6 mice that Ulfhake studied are deficient in melatonin, a hormone with key roles in seasonal rhythms. German researchers recently compared seasonal variations in C57B1 mice with those in C3H lab mice, which are not melatonin-deficient. The mice were housed in cages outdoors for a year, and monitored with detectors that gathered data every ten minutes. But only the C3H mice showed clear seasonal differences in activity patterns, which suggests that melatonin is what enables mice to respond to environmental cues.[2]

Nevertheless, Ulfhake and his coauthors believe the rhythm they've spotted is endogenous. In their study, they generated heat maps of mouse activity, which revealed that mice in different cages showed elevated activity that started at different times and varied in duration. This suggests the rhythm was generated by some sort of internal clock instead of an external cue, which would have yielded a synchronized response, Ulfhake says.

Circadian rhythms are well understood. Longer-cycle infradian rhythms are more mysterious, but researchers may need to control for them.

Credit: Anthony Bradshaw / Getty Images

The C57BL/6 mice used in the study experienced an infradian rhythm, even though they could not produce melatonin (shown), which an earlier study suggested was necessary. This may point to an unknown biological mechanism.

Credit: Sergey Tarasov / Alamy

Mystery driver

After years of research, biologists understand a lot about circadian rhythms. But seasonal rhythms are comparatively more mysterious, and ways to control for them are not obvious. "We're investigating oscillators and temporal patterns in physiology and behavior that are outside the bounds of circadian regulation," Logan says. "But it's very difficult to isolate and discern what those mechanisms are."

Scientists who study seasonal phenomena usually attribute them to thyroid signaling and melatonin regulation, Logan says. But since the C57BL/6 mice Ulfhake studied make very little circulating melatonin, it has to be something else. "It could originate with the thyroid, or perhaps from some other seasonal signaling mechanisms that have yet to be discovered," Logan argues. Ulfhake, for his part, speculates that an endogenous oscillator located in the nervous system controls the rhythm.

Ulfhake acknowledges that the results, which were published in *Scientific Reports*, were met with some resistance, which he says was to be expected given controversies over seasonal rhythms in lab rodents. But the burden of proof now is on those denying the rhythm's existence, who must come up with an alternate explanation for the unbiased DVC recordings, he says.

Meanwhile, Ulfhake is investigating further. "We still have a lot of work to do to validate our results and extend our observations to other mouse species," he says. "And most importantly, we have to find the oscillator." ■

1. Pernold K., Rullman E. & Ulfhake B. Sci Rep. **11**(1):4961 (2021).

2. Metzger J. et al. J. Biol. Rhythms **35** (1): 58–71 (2020).