The Harvard Gazette

SCIENCE & TECHNOLOGY

Why we need female mice in neuroscience research

Findings reveal that despite hormonal fluctuations, female mice exhibit more stable exploratory behavior than their male peers

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DATE
March 8, 2023

Mice have long been a central part of neuroscience research, providing a flexible model that scientists can control and study to learn more about the intricate inner workings of the brain. Historically, researchers have favored male mice over female mice in experiments, in part due to concern that the hormone cycle in females causes behavioral variation that could throw off results.

But new research from Harvard Medical School challenges this notion and suggests that for many experiments, the concern may not be justified.

The study results, published March 7 in Current Biology, reveal that female mice, despite ongoing hormonal fluctuations, exhibit exploratory behavior that is more stable than that of their male peers.

Using a strain of mice commonly studied in lab settings, the researchers analyzed how the animals behaved as they freely explored an open space. They found that the hormone cycle had a negligible effect on behavior and that differences in behavior between individual female mice were much greater. Moreover, differences in behavior were even greater for males than for females, both within and between mice.

The results underscore the importance of incorporating both sexes into mouse studies, the research team said.

"I think this is really powerful evidence that if you're studying naturalistic, spontaneous exploratory behavior, you should include both sexes in your experiments — and it leads to the argument that in this setting, if you can only pick one sex to work on, you should actually be working on females," said Sandeep Robert Datta, professor of neurobiology in the Blavatnik Institute at HMS, who co-led the study with Rebecca Shansky of Northeastern University.

From rodents to humans: A history of bias

As neuroscientists strive to better understand the human brain, they routinely turn to the mouse, which Datta considers "the flagship vertebrate model for understanding how the brain works."

This is because mouse and human brains share a considerable amount of structural organization and genetic information, so scientists can easily manipulate the mouse genome to address specific experimental questions and to build models of human diseases.

"Much of what we understand about the relationship between genes and neural circuits, and between neural activity and behavior, comes from basic research in the mouse, and mouse models are likely going to be really central tools in our fight against a broad array of neurological and psychological diseases," Datta said.

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— Sandeep Robert Datta, professor of neurobiology in the Blavatnik Institute

For more than 50 years, researchers have preferentially used male mice in experiments, and nowhere has this practice been more prominent than in neuroscience. In fact, a 2011 analysis found that there were over five times as many single-sex neuroscience studies of male mice than of female mice. Over time, this practice has resulted in a poorer understanding of the female brain, likely contributing to the misdiagnosis of mental and neurological conditions in women, as well as the development of drugs that have more side effects for women — issues outlined by Shansky in a 2021 perspective in Nature Neuroscience.

The disparity in sex representation common in animal research has also been historically mirrored in research involving human subjects.

"This bias starts in basic science, but the repercussions are rolled into drug development, and lead to bias in drugs being produced, and how drugs are suited for the different sexes," said lead author Dana Levy, a research fellow in neurobiology at HMS. For example, Levy noted that conditions such as anxiety, depression, and pain are known to manifest differently in female mice and women than in the male mice that are more often used in early-stage drug testing.

To address the problem of sex bias in scientific research, the National Institutes of Health published a policy in 2016 requiring researchers to include male and female subjects and samples in experiments. However, follow-up studies that look across scientific disciplines and examine neuroscience specifically indicate that progress has been slow.

The reasons for such a long-standing bias in neuroscience are complicated, Datta said: "Part of it is just plain old sexism, and part of it is conservatism in the sense that people have studied male mice for so long that they don't want to make a change."

Yet perhaps the biggest reason for excluding female mice, Datta said, stems from a widespread assumption that their behavior is broadly affected by cyclic variations in hormones such as estrogen and progesterone — the rodent version of a menstrual cycle, known as the estrous cycle. According to Datta and Levy, estrous status is known to have a strong effect on certain social and sexual behaviors in mice. However, data on the influence of estrous status in other behavioral contexts have been mixed, resulting in what Datta calls "a genuine disagreement in the literature."

"We wanted to measure how much the estrous cycle seemed to influence basic patterns of exploration," Datta said. "Our question was whether these ongoing changes in the hormonal state of the mouse affect other neural circuits in a way that's confusing for researchers."

"We assumed, like everybody else, that adding females was just going to complicate our experiments," Levy added, "And so we said, 'why not test this.'"

Testing assumptions

The researchers studied genetically identical males and females from a common strain of lab mouse in a circular open field — a standard lab setup for behavioral neuroscience experiments. In practice, the test involved placing a mouse in a 5-gallon Home Depot bucket for 20 minutes and using a camera to record the mouse's movements and behaviors in 3D as it freely explored the space. The researchers swabbed each female mouse to determine its estrous status and repeated the bucket test with the same individual multiple times.

"This is a very interesting example of how assumptions that affect the way that we conduct and design our science are sometimes just assumptions — and it is important to directly test them, because sometimes they're not true."

— Dana Levy, a research fellow at Harvard Medical School

The team analyzed the videos with MoSeq, an artificial intelligence technology previously developed by the lab. The technology uses machine learning algorithms to break down a mouse's movements into around 50 different "syllables," or components of body language: short, single motions such as rearing up, pausing, stepping, or turning. With MoSeq, the researchers gathered in-depth, high-resolution data about the structure and pattern of mouse behavior during each session.

The researchers found that estrous status had very little effect on exploratory behavior in female mice. Instead, patterns of behavior tended to vary much more across female mice than they did throughout the estrous cycle.

"If you give me any random video from our pile, I can tell you which mouse it is. That's how individualized the pattern of behavior is," Datta said, which suggests that in behavioral studies, "a dominant aspect of variation in the data is the fact that individuals have subtly different life histories."

When the researchers compared female and male mice, they found something that surprised them: Males also exhibited individuality of behavior, but they had more behavioral variation within a single mouse and between mice than females.

"People have been making this assumption that we can use male mice to reliably make comparisons within and across experiments, but our data suggest that female mice are more stable in terms of behavior despite the fact that they have the estrous cycle," Datta said.

A case for change

Scientists generally agree that including female mice is important from a fairness perspective, Datta noted, yet some have remained concerned that it could complicate their research. For him, the new findings make a strong scientific case for using female mice in experiments.

"The fact that female behavior is more reliable suggests that including females might actually decrease the overall variability in your data under many circumstances," Datta said.

Based on their findings, researchers in the Datta lab have already switched from male mice to mixed groups or female mice in their other experiments that involve circular, open-field testing.

Datta cautioned that the study looks at only one mouse strain in one lab setup, and so the results cannot be generalized to other strains and setups without further testing. However, he noted that the strain and setup are commonly used in neuroscience research, including in early-stage drug development to test how a potential drug affects mouse locomotion.

Datta said that the findings "should encourage folks who are interested in drug development in this context to include both sexes in their analysis."

Now, Datta and Levy are interested in exploring how internal states beyond hormonal status, such as hunger, thirst, pain, and illness, affect exploratory behavior in mice.

"The question is, who wins in this tug-of-war between your current internal state and your individual identity," Levy explained.

They also want to delve deeper into the neural basis of the individuality of mouse behavior that they saw in the study.

"I was shocked by how much stable variation between individuals we were observing — it's like these mice really are individuals," Datta said. "We're used to thinking of lab mice as interchangeable widgets, but they're not at all. So, what is controlling these individualized patterns of behavior?"

"We want to understand the mechanisms of individuality: how variability between individuals comes about, how it affects behavior, what can alter it, and what brain regions support it," Levy added.

To this end, the Datta lab is examining mouse behavior from birth until death to understand how individualized patterns of behavior emerge and crystallize during development, and how they change throughout life.

The researchers also hope that their work will open the door for more rigorous, quantitative research on whether and how the estrous cycle affects mouse behavior in other contexts, such as completing complex tasks.
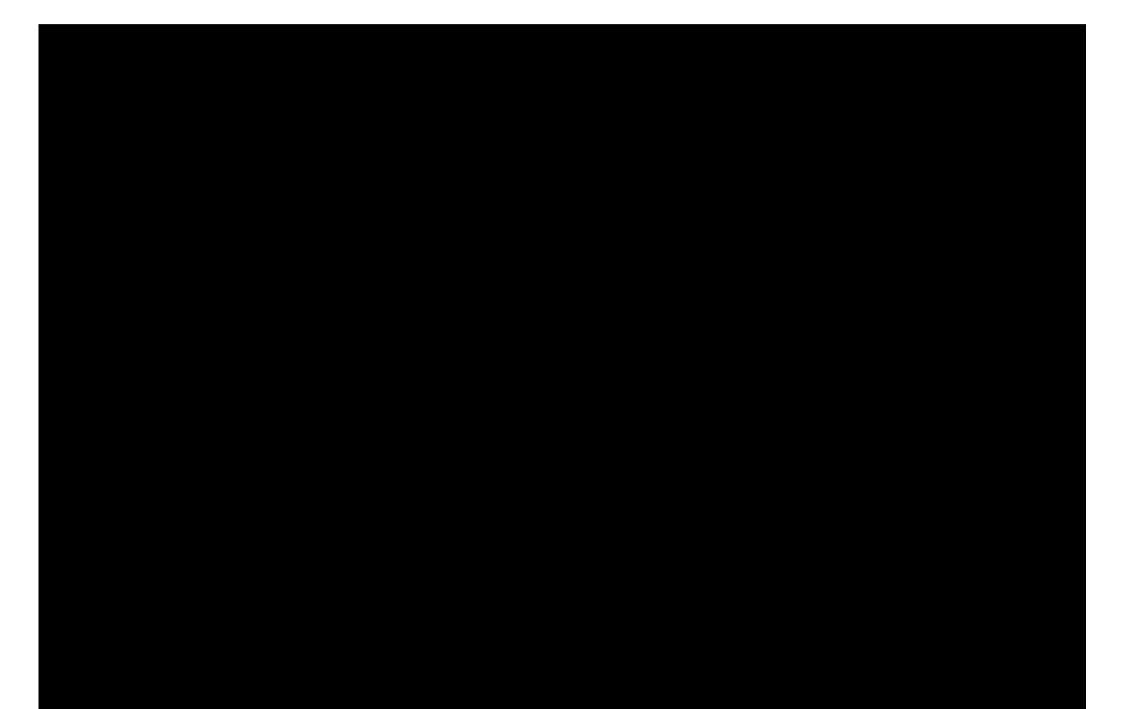
"This is a very interesting example of how assumptions that affect the way that we conduct and design our science are sometimes just assumptions — and it is important to directly test them, because sometimes they're not true," Levy said.

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