NEUROSCIENCE

Are hormones a "female problem" for animal research?

Outdated gender stereotypes are influencing experimental design in laboratory animals

By **Rebecca M. Shansky**

One of the most deep-seated misconceptions about the human psyche is that men are simple and women are complicated (*1*). Gender psychology scholars trace this belief back to at least the 19th century, when the long-standing view that women were inferior versions of men started to fall out of favor (*2*). In response, biological theories on the sexes were restructured into a narrative that characterized the emergent psychological properties of the female brain—"sensitivity, perceptual acumen, and emotionality"—as not lesser than, but complementary to, those of men's brains (*1*). This framed women as a disordered, unstable yin to men's rational, orderly yang, thus preserving the patriarchy. So-called scientific explanations of why women's mental proclivities deviated from men's relied heavily on the purported influence of reproductive physiology on the female mind (*3*). More than 100 years later, this idea still shapes not just how society perceives women but also how biomedical scientists approach animal research.

The notion that a woman's disposition is a direct product of the activity in her ovaries persists today. Women, but not men, are still pejoratively described as hormonal or emotional, which curiously neglects the well-documented fact that men also possess both hormones and emotions (*4*). On a societal and cultural level, this stereotype feeds implicit (and explicit) biases in policymaking, hiring practices, and education (*5*), doing a disservice to people of all genders. But because the idea has also infiltrated the very practice of preclinical animal research, it also poses a public health problem.

In the field of neuroscience, animal models are used to investigate the mechanisms that link brain structure, function, and behavior, with a broad goal of helping people who suffer from mental illness and neurological disease. But knowingly or not, the field has largely regarded as unequal the inherent value of studying the female versus male brain. This imbalance is rooted in the erroneous belief that circulating ovarian hormones make data from female animals messier and more variable than data from males (*6*). This biases not just the subjects chosen, but also experimental design, data interpretation, and the peer review of grants and manuscripts when female animals are included in experiments. And because basic science informs clinical developments, the current understanding of how to most effectively treat disease in humans is similarly unbalanced. Researchers in neuroscience and other biomedical disciplines should consider whether long-standing, culturally derived beliefs about gender have shaped attitudes and ideologies about scientific rigor in laboratory experiments.

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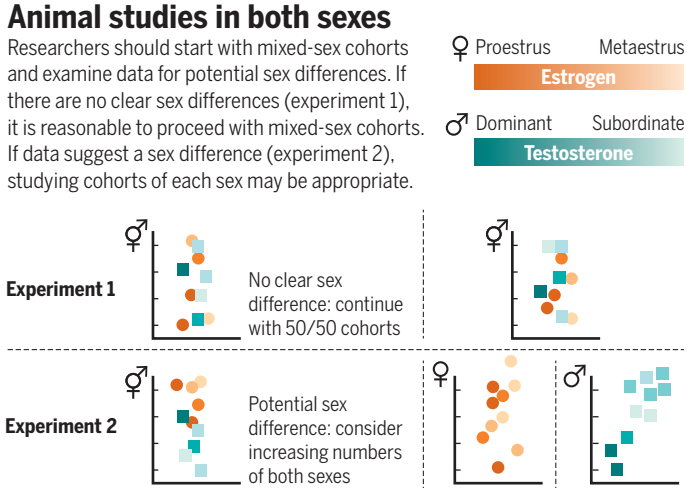
Animal studies in both sexes

Researchers should start with mixed-sex cohorts and examine data for potential sex differences. If there are no clear sex differences (experiment 1), it is reasonable to proceed with mixed-sex cohorts. If data suggest a sex difference (experiment 2), studying cohorts of each sex may be appropriate.



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Until just a few years ago, no major funding agencies in North America or Europe required grant recipients to use both sexes in animal studies—including the largest biomedical funding source in the world, the U.S. National Institutes of Health (NIH). The result was a body of preclinical literature that represented more than half a century of scientific inquiry but was mostly conducted in male animals. A survey of publications using primarily rodent and nonhuman primate subjects found that this imbalance was true for physiology, pharmacology, and even endocrinology research, but the most egregiously lopsided field was neuroscience, which in 2009 included male animals almost six times as often as females (*7*).

How did our understanding of the brain become so skewed? There is an old proverb—"a little knowledge is a dangerous thing"—that might be applicable here. In this case, the "little knowledge" was that circulating levels of ovarian hormones in female rodents, as in women, regularly fluctuated. Although much shorter than the human menstrual cycle, the 4- to 5-day estrous cycle in rodents is similarly characterized by phases of low and high concentrations of estrogen and progesterone (*8*). This meant that among an experimental cohort of female rats or mice, individual animals could have up to fourfold differences in the amounts of circulating ovarian hormones. Presuming that these hormonal differences would lead to complicated, uninterpretable data, neuroscientists chose to avoid the issue altogether and exclude female animals from their research (*6*). And so what may have begun as a seemingly practical choice became dogma—the default way to study the brain was to study it in male animals, because females had a distinctive and problematic source of variability.

But do they really? The idea that the estrous cycle would make data from female animals more variable than that from males seemed like such a reasonable assumption that it was not examined scientifically until 2014, in a meta-analysis of nearly 300 published neuroscience articles that used mice as research subjects (*9*). Evaluation of variability in an array of physiological, cellular, hormonal, and behavioral measures revealed that data collected from female mice—regardless of the estrous cycle—did not vary more than that from males, and in some instances data from males varied more than female data. A complementary

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meta-analysis of neuroscience studies in rats came to the same conclusion (*10*). One factor that affected variability in mice of both sexes was whether animals were housed in groups rather than alone. This is particularly noteworthy because group-housed males, but not group-housed females, will establish a dominance hierarchy. Circulating testosterone levels in dominant males are, on average, five times as high as in subordinates (*11*), and so data variability in males could be due to their dominance status and corresponding hormone levels.

Both testosterone and estrogen are powerful neuromodulators (*12*), and so if the potential for intracohort variation in ovarian hormones is concerning, then the same concerns should apply to hormone-associated variability in male subjects. But when male animals made up the majority of experimental subjects, basic scientists mostly considered hormonal variability a nonissue. No one lamented that data would be uninterpretable without careful tracking of dominant versus subordinate cage mates or testosterone assays—any variability in data was largely dismissed as natural randomness. However, when the NIH began to explore requiring its grantees to include both sexes in animal experiments, concerns regarding data variability resulting from ovarian hormones were central to the discussion (*6*).

Mandates to consider sex as a biological variable (SABV) were introduced by the NIH and the Canadian Institutes of Health Research (CIHR) in 2016, and although these policies received a great deal of praise, the most common objection was that animal researchers would now have to quadruple the number of animals in their experiments to assess estrus-associated effects, which would be costly and time consuming. But because data from male and female rodents are equally variable (*9*, *10*), there is no scientific justification for requiring estrous cycle assessment (or statistical power to evaluate estrous effects) in female animals without also demanding evaluation of testosterone (or statistical power to account for social dominance effects) in male subjects. Conversely, if the degree of variability normally observed in a cohort of male animals is acceptable from a scientific rigor standpoint (as it historically has been), then the same degree of variability should be acceptable in a cohort of females, and the estrous cycle should not be a primary concern.

Does this mean that researchers should never account for the estrous cycle when they study female rodents? Not at all. To the contrary, gonadal hormones in both sexes deserve the same experimental focus and rigor that are applied to answering any other scientific question—if the scientific question is about gonadal hormones. The field of neuroendocrinology is vast, and decades of research show that circulating hormones play critical roles in brain development, gene expression, neurotransmission, plasticity, and behavior (*12*)—in females and males. Therefore, the potential for gonadal hormones to influence experimental outcomes should be approached in the same manner as any other neuromodulatory system. It is important to note that gonadal hormones do not serve identical purposes in males and females and that the scientific question under investigation may depend on hormones in one sex but not the other.

A reasonable way to study both sexes is to use cohorts comprising half males and half females (*13*) (see the figure). Although such an approach may not allow enough statistical power to detect significant sex-associated differences, examination of data will allow the observation of potential trends. Decisions can then be made whether to follow up with a study explicitly designed to detect sex-associated differences. If a follow-up study is warranted, then both the female estrous cycle and male cage dominance should be considered as potential sources of variability. If experimental data from males and females do not differ, then it is reasonable to continue to use mixed cohorts in future experiments without controlling for these factors.

"When females are studied through a male lens, the true crux of the research question for females can be missed."

Because the SABV mandate does not explicitly dictate how to incorporate both sexes into experimental designs, one compromise upon which some neuroscientists have landed is to conduct experiments in males first, then, armed with their findings, tackle the same question in females. This strategy is flawed, however, because it requires the demonstrably false assumption that what is discovered in males is how the brain really works, whereas in females, the same neurobiological processes are probably more complicated. More problematically, it perpetuates the scientifically inaccurate (*13*) idea that male brains are a standard from which female brains deviate.

When females are studied through a male lens, the true crux of the research question for females can be missed. This issue is most evidently troublesome in neuroscience studies related to mood and anxiety disorders. Illnesses such as major depressive disorder and posttraumatic stress disorder are twice as prevalent in women, but common behavioral tests designed to model their symptoms in rodents were developed and validated in males. The result has been an unclear picture of the neural mechanisms that may underlie disease susceptibility in women, because animal behavioral tests such as the elevated plus maze, forced swim, and fear conditioning do not reliably produce greater effects in female rodents than they do in males (*14*). In addition, there is no clear pattern of how ovarian hormones affect outcomes in these tests, complicating preclinical research on mood disorders that are clearly hormonally linked, such as premenstrual dysphoric disorder or postpartum depression. One possible explanation for these inconsistencies is the use of male-defined animal behaviors in these tests to evaluate the emotional and motivational states of females. Standard outcome measures in models of psychiatric disease may need to be recalibrated to incorporate sex-specific behavioral strategies.

SABV will help rectify the current imbalance in knowledge about the brain and ensure that in the future, more of the data collected in the laboratory represents both sexes. Having this information is a key first step in advancing personalized medicine for both men and women. The policy is therefore a laudable move for NIH and CIHR, and hopefully other funding bodies will soon follow. But it is imperative that, in adhering to the mandate, researchers do not allow antiquated gender stereotypes to bias standards for scientific rigor. Women are not more complicated than men, and hormones are not a "female problem" for animal research. When these assumptions are finally abandoned and male and female animal brains viewed as equally informative to the field, the potential for neuroscience research to advance mental and neurological health for everyone is likely to improve. ■

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