

SCIENCE FACT OR SCIENCE FICTION: DOES SEX MAKE A DIFFERENCE IN PRECLINICAL RESEARCH?

ANIMAL RESEARCH: WHAT'S SEX GOT TO DO WITH IT?

For health scientists conducting animal research, studying female animals may seem like more trouble than it's worth. Many researchers prefer to study male animals because they are concerned that hormonal fluctuations in females will make data too variable. Others believe males and females are similar enough to make sex irrelevant as a variable, so they leave it out of their study designs. Taken together, sex bias toward male animals and the belief that sex is more-or-less irrelevant means that female animals are being excluded from preclinical research by default.¹

Is there a convincing case for using male animals exclusively? Or does sex make a significant difference in animal research?

SEX BIAS & SEX BLINDNESS

If a preclinical study is looking at a health concern that mostly or only affects men, it seems pretty reasonable to consider male animals exclusively. But researchers tend to prefer male animals even when studying conditions that affect more women than men. For example, women are diagnosed with chronic pain more frequently than men and respond differently to some pain medications; yet researchers involved in developing chronic pain drugs tend to study male animals almost exclusively.^{1,2}

We now know, despite longstanding assumptions, that sex differences exist beyond reproductive functions—right down to the cellular level—and that health research is much more reliable when women are equally represented in clinical trials.^{3,4,5} But this knowledge has yet to fully translate to research involving animal subjects.

An analysis of successful CIHR operating grant applications found that Canadian researchers often assume sex doesn't matter if sex differences haven't been found in their particular research area.⁶ However, the enduring practice of excluding both women and female animals from health research means there are many areas where sex differences have never even been investigated.

In fields where researchers are considering sex differences, like preclinical pain research, significant advances are being made.

CASE STUDY: SEX DIFFERENCES IN CHRONIC PAIN RESEARCH

Researchers at McGill's Pain Genetics Lab argue that meaningful sex differences are just waiting to be discovered by researchers who include both male and female animals in their research—and they've uncovered some important findings thanks to their commitment to studying both sexes.⁷

“There are huge, striking sex differences in pain—big stuff, not just a little more or a little less of something.”

Dr. Jeffrey Mogil,
McGill Lab Principal Investigator⁸

The Hospital for Sick Children (SickKids) in Toronto is also interested in sex differences in health research and how they might even lead to more effective treatments for patients. “Understanding the pathways of pain and sex differences is absolutely essential as we design the next generation of more sophisticated, targeted pain medications,” says Dr. Michael Salter, Chief of Research at SickKids.

In a new study, conducted by researchers from the McGill Lab, SickKids and Duke University, researchers looking at sex difference and pain recently made a big discovery.

The generally accepted notion in pain research has been that immune cells called microglia are responsible for transmitting pain through the nervous system; however, this theory is based on experiments carried out overwhelmingly on male rodents.¹



SCIENCE FACT OR SCIENCE FICTION: DOES SEX MAKE A DIFFERENCE IN PRECLINICAL RESEARCH?

In the new study, published in *Nature Neuroscience*, researchers interfered with microglia functioning and found that, while pain was blocked in male mice, it had no effect on pain transmission in female mice. An entirely different type of immune cell, the T cell, appears to carry out this function in females.⁹

This sexual dimorphism suggests that
**MALE MICE CANNOT BE USED AS PROXIES
FOR FEMALES IN PAIN RESEARCH.**⁹



While this finding is impressive on its own, Mogil argues that for sex differences to have a clinical impact, preclinical researchers must investigate both quantitative and qualitative sex differences. In a separate experiment, researchers at the McGill lab found that a particular pain response was dependent on testosterone levels—with lowered testosterone male mice experienced a different pain response.⁹

BETTER SCIENCE, BUT AT WHAT COST?

Considering sex differences clearly leads to better science and provides opportunities for new health breakthroughs. But for scientists conducting small studies with limited budgets, the million-dollar question is: could male animals still be the better choice because they are easier and cheaper to work with?

In 2014, a group of researchers from the Universities of Chicago and Berkeley published a meta-analysis of some 300 articles that measured nearly 10,000 traits in male and randomly-cycling female mice. The analysis showed not only that female mice are no more variable than males but that males are actually considerably more variable for several traits.¹¹ Given these findings, the authors suggest that experiments don't necessarily need to monitor the estrous cycle. This is good news given that the process is time-consuming and could be a barrier to researchers choosing to use female animals.

REFERENCES

1. Mogil, J.S. and M.L. Chanda, The case for the inclusion of female subjects in basic science studies of pain, in *Pain*. 2005. p. 1-5.
2. Whitley, H. and W. Lindsey, Sex-based differences in drug activity, in *Am Fam Physician*. 2009. p. 1254-8.
3. Rosenfeld, J.A., *Handbook of women's health (Excerpt)*. 2nd ed. Cambridge medicine. 2009, Cambridge, UK : New York: Cambridge University Press. x, 341 p.
4. Straface, E., et al., Sex differences at cellular level: "cells have a sex". *Handb Exp Pharmacol*, 2012(214): p. 49-65.
5. Shah, K., C.E. McCormack, and N.A. Bradbury, Do you know the sex of your cells? *Am J Physiol Cell Physiol*, 2014. 306(1): p. C3-18.
6. Johnson J, S.Z., Vissandjee B, Stewart DE, Does a change in health research funding policy related to the integration of sex and gender have an impact? , in *PLoS One*. 2014.
7. Mogil, J.S. Sex Differences. [cited 2015 August 12, 2015]; Available from: <http://www.psych.mcgill.ca/labs/mogillab/paingenetics/research/sex-differences/>.
8. Sutherland, S., A Move Toward Sex Equality in Preclinical Research: NEW NIH policy requires equal representation of female and male animals, cells in studies. 2014, Pain Research Forum.
9. Sorge, R.E., et al., Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat Neurosci*, 2015. 18(8): p. 1081-3.
10. Mogil, J.S., Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. *Nat Rev Neurosci*, 2012. 13(12): p. 859-66.
11. Prendergast, B.J., K.G. Onishi, and I. Zucker, Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev*, 2014. 40: p. 1-5.

“ These observations indicate that, contrary to widespread preconceptions, females are no more variable than males across diverse physiological, morphological and behavioral traits.” ”

These findings could also mean that, in animal research examining just one sex, female animals may be the better choice.

As for costs, thanks to the power of supply and demand, male mice *are* often cheaper than females (though not always!) However, as demand for female animals increases, so will the supply and prices will likely drop.

CONCLUSION

For years, male animals have been the default research subject. But preclinical research on pain, like the studies mentioned above, indicate that there are important sex differences, which merit consideration.

Findings will be more reliable when researchers make a conscious decision about whether to use male or female animals, to report on the sex of the animals studied, and avoid assuming that findings in male animals can be generalized to females.

ABOUT THE RESEARCH

Dr. Jeffrey Mogil, Ph.D., Co-Senior Author of the study and head of McGill's Pain Genetics Lab, is an authority on preclinical pain research and sex differences in pain and analgesia. Dr Mogil is the E.P. Taylor Professor of Pain Studies at McGill University and holds the Canada Research Chair in the Genetics of Pain. He is the Director of the Alan Edwards Centre for Research on Pain at McGill University.

Dr. Michael Salter, M.D., Ph.D., Co-Senior Author of the study and Chief of Research at the Hospital for Sick Children (SickKids), is a world-leading neuroscientist who has made pioneering contributions to our understanding of the fundamental molecular and cellular mechanisms of normal and pathological neuroplasticity. Dr. Salter holds a Canada Research Chair in Neuroplasticity and Pain and is the Anne and Max Tanenbaum Chair in Molecular Medicine at SickKids.