BIOMEDICAL RESEARCH

Of Mice and Women: The Bias in Animal Models

Male rodents are cheaper and easier to work with than females, but scientists worry that research done on males alone won't apply across the sexes

In 2008, Rae Silver, a neuroscientist at Columbia University, and her colleagues discovered a remarkable connection between the immune system and anxiety in mice. But something bothered her: They had done the research using only male mice. "Females have far more anxiety responses than males," she says, so she wasn't sure if her research would hold for them. And she wasn't alone in her concern. Others had found a widespread bias toward males in animal studies; almost nobody was using females in basic research.

"It's cuckoo that for diseases such as asthma, stroke, pain, immune diseases, where there are huge sex differences, people are just studying male animals," says behavioral neuroscientist Irving Zucker, a professor emeritus at the University of California, Berkeley. "It just makes no sense."

In 1993, the National Institutes of Health (NIH) Revitalization Act mandated that women and minorities be included in clinical research, because treatments had been shown to have different effects in different populations. A 2001 Institute of Medicine (IOM) report published by the National Academy Press pointed to evidence that the same was true for research using animal models: The sex of the animal can lead to qualitatively different results. And earlier this month, Silver helped organize a workshop in San Francisco, California, on behalf of the IOM's Forum on Neuroscience and Nervous System Disorders, where representatives from academia, journals, funding agencies, and the pharmaceutical industry discussed solutions to this problem of systematic sex bias in animal studies.

This bias compromises the safety and effectiveness of drugs in women, says Silver, although it's not clear exactly how much. She points to the 10 drugs that were withdrawn from the market between 1997 and 2000 because of adverse health effects. According to a 2001 U.S. General Accounting Office report (now the Government Accountability Office), eight of them posed higher risks for women than for men, and in four of those, the risk was likely due to physiological differences. Silver suspects that lack of adequate preclinical testing in female animal models could partly explain that result.

Furthermore, obesity researcher Deborah Clegg of the University of Texas Southwestern Medical Center in Dallas says that increasing the use of female animals in research could accelerate the trend toward personalized medicine for women and ensure they're not left behind. "We'll have our own drugs, we'll have our own dosing. We'll be different, 'cause we know we are," she says.

Bench parity. Deborah Clegg says that using more female lab animals in basic research will lead to better medicines for women.

The trouble with females

Male rats and mice have become the default animal model for many diseases because they are easier and cheaper to work with than females. Female rodents have a 4-day ovarian cycle, so researchers who use them must take daily vaginal swabs in experiments where hormones might play a role. "Otherwise, the data are uninterpretable," says obesity researcher Andrew Greenberg of the Human Nutrition Research Center on Aging at Tufts University in Boston. Scientists may also need to keep as many as four times the number of female animals as male animals to make sure their subjects are cycling in sync. And even with those precautions, the cycle may still lead to less-clear results that are more difficult to publish.

All this can add up to a lot of trouble and a lot more money than the typical funding-agency grant provides. Many researchers say they get turned down for grants to cover the larger cost of using female animals, and many don't even bother to apply.

What is the extent of this bias, and where is it concentrated? To find out, Zucker and postdoctoral researcher Annaliese Beery recently did a survey of journal articles published in 2009 reporting results of research that used mammals. They found that only one field skewed toward females: reproductive biology. But neuroscience, pharmacology, and physiology—the very disciplines whose animal research is most likely to translate into humans—strongly skewed male. What's more, many articles across all fields failed to report subject sex at all (in immunology, 60% omitted that information), and even when both males and females were included, two-thirds of those studies failed to analyze the data by sex.

She-rats. Female rodents can be more expensive and time-consuming to work with.

Yet many studies show that there can be surprising sex-differentiated effects in those fields. Last month, Clegg published a microarray study in the *International Journal of Obesity* that shows major sex-based differences in the gene-expression profiles of fat tissue from mice on a high-fat diet. The research was funded not by NIH, which had turned down Clegg's prior applications for research on sex-based differences, but by the Society for Women's Health Research. The society funded several top researchers to do "anything we wanted," work which "would help a lot of other scientists," Clegg writes by e-mail.

By not studying sex differences, researchers could be missing out on potential new treatments for both men and women, says Rhonda Voskuhl, director of the multiple sclerosis (MS) research and treatment program at the University of California, Los Angeles. Clinicians had observed that in women with MS, pregnancy reduces relapse of the disease by 80%. "This is an invaluable clue," Voskuhl says. "It's better than any drug we have." Based on that clinical observation, she and her colleagues tested estriol, an estrogen produced during pregnancy, in a mouse model for MS, using both male and female animals. Encouraging lab results in the female rodents led to clinical trials testing estriol pills as a therapy for female MS

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patients. The researchers expect to complete the phase II/III trials by 2013.

A similar observation—that younger men are less susceptible to MS—led to a potential therapy for men: testosterone. Voskuhl's group tested it first in mice and then as a gel in men, with promising phase II results.

Funding issues

Whatever encouraging results scientists get in the lab, drugs resulting from animal research are ultimately manufactured and sold by pharmaceutical companies, which want to make a profit. At the workshop, Morgan Sheng, vice president of neuroscience at Genentech, said that companies hesitate to spend the money necessary to test a drug candidate in both male and female animals unless the disease in question meets three rather stringent conditions: It must be known to affect men and women differently, its basic physiological mechanism must be well understood, and it must have a reasonable animal model. Otherwise, "I'm not compelled that it's a great scientific experiment to study the sex difference," he says. For funding agencies, some researchers favor implementing a policy like the Revitalization Act for animal research. "Several recent studies have demonstrated the advantage of using heterogenous, rather than homogenous, populations in animal studies, since such studies enhance the likelihood that results generalize," Charles Mobbs, a neuroscientist at Mount Sinai Medical Center in New York City, writes by e-mail. "The NIH has recognized this regarding clinical studies, and it is certainly time a similar policy was implemented regarding animal studies."

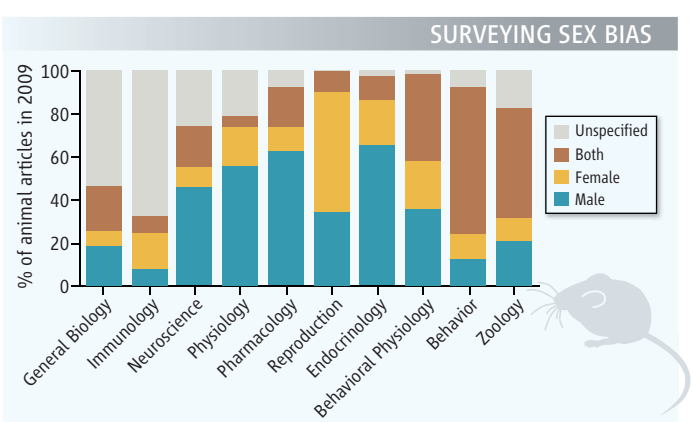
But that probably won't happen. "I cannot foresee how a blanket policy requiring the use of male and female animals could be implemented or would work," says Vivian Pinn, director of the NIH Office of Research on Women's Health (ORWH) in Bethesda, Maryland. "The research and how it's designed has to be based on the science of what is being studied and the availability of models."

Silver advocates channeling limited resources to areas that show clear sex differences, such as pain. "If the NIH says in the guidelines that when I study pain I have to include male and female subjects, you can bet your booties I'm going to study male and female subjects," she says—and she'll be able to ask for the extra money to do it. Without those guidelines, such studies will fall victim to the same fate as her anxiety experiment. If she wanted to rerun it in females, she thinks she would have little chance of convincing the NIH—a consistent supporter of her lab—to give her the funding.

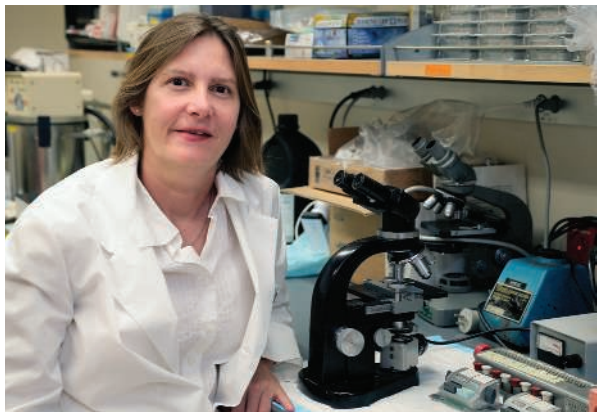
Online
sciencemag.org
Podcast interview with author Corinna Wu.

Mining NIH data from large patient trials could help identify sex differences in people that would be worth studying in animals, says Richard Nakamura, scientific director of the division of intramural research programs at the National Institute of Mental Health in Bethesda. "It's unlikely that we'll find much in relatively small trials," he says. But in areas where NIH is collecting a lot of information, "we should be focusing much more on analyzing that data, and making available funds to analyze that data, to understand sex differences and outcomes."

Even if NIH does not adopt an agency-wide policy, targeted funding opportunities for studying sex differences in animal models can help a great deal, Pinn says. Even relatively small requests for applications can stimulate researchers to pursue areas they didn't before, says neuroscientist Karen Berkley of Florida State University in Tallahassee. ORWH has two such initiatives, Pinn says. It awards 5-year, $5 million grants to establish Specialized Centers of Interdisciplinary Research in Sex and Gender Factors Affecting Women's Health, as well as 2-year Advancing Novel Science in Women's Health Research grants for the study of sex differences.



Skewed by sex. A survey of journal articles from 2009 found the strongest bias toward male animals in fields most likely to translate into humans.



A vindication of the rats of women. Studying rodents of both sexes pointed Rhonda Voskuhl to potential multiple sclerosis treatments.

Guidelines for journals

The quickest action may come from the academic journals, which are moving toward adopting a common set of guidelines for studies using animals, says Sean Murphy of the University of Washington, Seattle, chief editor of the *Journal of Neurochemistry*. The checklist of 20 items, developed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research in London, would require scientists submitting manuscripts to provide details including the sex of the animals used in their experiments. A manuscript describing the recommendations has itself been submitted for publication, and from there, "it's going to be straightforward for journals to adopt the guidelines," he says. "They are sensible and will improve the quality of the papers."

Still, Murphy feels that the guidelines don't go far enough to really shine a light on the importance of sex differences. At the IOM workshop, he wondered whether journal editors should also require authors to give their rationale for studying only one sex and describe the potential implications for not studying the other.

That would at least get scientists thinking about the issue of sex bias. Currently, few graduate or medical students are trained in sex differences, and many don't know how to work with female animals. But Clegg says she answers enthusiastic questions about working with females every time she gives a talk. She says, "People go back thinking, I wonder if I should pick the females up off the shelf and actually look at them."

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