PERSPECTIVE

JEAN-SEBASTIEN AUSTIN

Equality need not be painful

Pain researchers' arguments for using only male rodents in preclinical pain research don't hold up to scrutiny, says **Jeffrey S. Mogil**.

Sex differences in pain and analgesia are real and robust. I found this out in graduate school, more or less by accident: a casual reanalysis of data from an experiment performed earlier that day revealed that the drug that my colleagues and I were studying worked completely in the male mice but not at all in the females[1]. I thought that such a glaring yet unexpected difference would whet my colleagues' appetites for further research. I was mistaken. The postdoctoral fellow I was working with dismissed the finding and encouraged me to get back to work and focus on the "real" phenomenon. Fortunately I ignored him, and I have spent much of my career since studying sex differences in pain, which are as real in people as they are in mice. Yet 25 years later, despite a wealth of evidence and even a change in preclinical-research guidelines, many pain researchers still do not include female animals in their studies.

Women, the data show, are more sensitive to, and less tolerant of, pain than men[2]. However, this quantitative sex difference in pain intensity is much less important than the emerging evidence of qualitative sex differences in pain processing. My colleagues and I have demonstrated, for example, that in the spinal cord, male and female rodents process pain through entirely different immune cells: microglia in male rodents and T cells in females[3].

Qualitative sex differences such as these can be seen almost everywhere: stress-induced inhibition of pain, the effects of genes on pain, social modulation of pain and memory of pain. How sensitive a rodent is to pain can even be affected by the sex of the person doing the experiment[4]. These results seem to be the perfect justification for policies that are emerging across the world, including from the US National Institutes of Health[5] (NIH), that mandate the use of animals of both sexes in preclinical research.

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THE 50/50 SOLUTION

Since 2014, the NIH has required the "consideration of sex as a biological variable" in preclinical research. But pain researchers and others still persist with experiments that test only male rodents. Of the 71 research articles that used rodents published in the journal *Pain* in 2015, 56 tested only males, 6 tested only females (4 of these were female-specific studies) and 6 did not disclose the sex. Only 3 papers (4.2%) affirmed the use of both sexes. There is little difference between these percentages and those for the period 1996–2005 (ref. 6).

Why is there so much resistance to using female mice and rats in pain studies? My conversations with many researchers who still use only male animals revealed three major fears.

First, researchers worry that including females in their experiments will increase variability — females have fluctuating gonadal hormone levels — thus necessitating the testing of more subjects. This concern seems superficially plausible, but it is empirically false. Variability in pain data is no higher in female mice than in males[6] — a fact that has been shown to be true for biomedical animal research as a whole[7]. This is probably because male animals have their own source of variability: cage dominance hierarchies. Male rodents fight each other for status. Experiments might be affected by which animal is dominant and which is submissive, and by how long ago the aggression occurred.

Second, many researchers think that the NIH policy forces them to double their sample sizes, greatly increasing the cost of experiments. This belief is also false — the policy simply asks for the adequate consideration of sex in all experiments (see go.nature.com/28icfmw). There is no need to use enough animals to ensure that there is sufficient statistical power to detect all quantitative sex differences, which would indeed increase costs, but rather enough to allow the observation of large sex differences, such as those seen in our studies of different immune cells in pain processing[3]. This simply requires that half of the subjects are female, a constraint that adds nothing to the cost of the study. Although this 50/50 strategy will not detect small sex differences, it will reveal major discrepancies. It is better to discover such low-hanging fruit than to remain oblivious to all sex differences because no one looked for them. What is more, the discovery of a sex difference can make a paper more interesting, or turn one paper into two. It's hard to see the downside.

A third fear, which I do think is justified, is that reviewers might ask scientists to repeat all their studies in every phase of the oestrous cycle. I would note, however, that there are better ways to study the effects of gonadal hormones[8] and that many sex differences in pain are actually due to testosterone.

It is crucial not to lose sight of the bigger issue — researchers have an obligation to attempt to solve the problems that are important to society. Most patients with pain are women. We fail in our duties if we conduct research using only male rodents, producing results that might serve only men. The message to my fellow pain researchers is: start including female mice and rats in all your experiments today. You have nothing to lose, and both men and women with pain have everything to gain. ■

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