A systematic review of animal models and sex as a variable in itch research

Joshua J. Wheeler, MSc\textsuperscript{a,b}, Katherine N. Allen-Moyer, PhD\textsuperscript{c}, John M. Davis, MD\textsuperscript{d}, Santosh K. Mishra, PhD\textsuperscript{a,b,e,*}

Introduction: Pruritus (or itch) research has gained momentum in the last decades and use of animal models to study itch behavior are a vital part of the research. Recent studies have found that many fields using animal models, including neuroscience, are predisposed toward using male animals in preclinical research. To address sex bias in animal research, the National Institutes of Health (NIH) began requiring researchers to include sex as a variable beginning in June 2015. Here, we test whether researchers studying itch are biased toward using males in preclinical research.

Methods: The NIH’s PubMed database was searched for primary research articles written between August 2007 and December 2018 using the words “Itch” and “Pruritus.” The following information was extracted from articles fitting our inclusion criteria: type of itch (acute or chronic), the animal model and the sex of the animals used, and whether researchers considered sex as a variable. z-Tests, binomial tests, and the Cochran-Armitage test for trend were used to explore relationships between animal models and the usage of both sexes.

Results: We found 5.3% ± 1.2% of papers in a given year used 1 of our 4 animal models. Mice were the most frequently used animal model, followed by rats, nonhuman primates, and dogs. Overall, researchers used male animals regardless of the animal model used. In preclinical research conducted on both male and female animals, sex was not considered a variable in a majority of these studies. Finally, since 2015, there has not been a change in the usage of male or female mice. Briefly, the incidence of papers utilizing both sexes has not changed.

Discussion: We have found that itch researchers have a bias towards males in animal research. This bias has not changed since the NIH’s mandate to include sex as a variable in preclinical research.

Keywords: Itch, Mice, Rats, Dogs, Nonhuman primates

Historically, sex as a variable has been overlooked despite early calls to report the sex of animals in biomedical research\textsuperscript{[1]}. In 1993, the National Institutes of Health (NIH) began requiring the inclusion of women in clinical studies\textsuperscript{[2]} (Congress, 1993). Even with the NIH’s mandate to include women in clinical studies, animal studies continued to be biased toward using male animals over female animals in preclinical studies\textsuperscript{[3,4]}. Realizing that preclinical studies were biased towards using male mice, the NIH and other researchers set out to generate policy to require the use of both male and female animals in preclinical research\textsuperscript{[3,5]}. As of June 2015, the NIH officially requires researchers to report sex as a variable in preclinical animal studies (NOT-OD-15-102) to reduce sex bias.

Sex bias, preferentially selecting one sex in animal studies, appears to have its roots in a 1923 study that identified a sex difference in rat locomotion due to the rat estrous cycle. However, in 2005, a meta-analysis determined female rodents were not any more variable than male rodents in pain studies\textsuperscript{[6]}. In addition, more recent meta-analyses have shown female rodent variability is no greater than male rodent variability\textsuperscript{[7–9]}. A recent representative look at sex bias in general neuroscience research found that neuroscience continues to prefer male animals in preclinical biomedical studies\textsuperscript{[10]}. These sex biases are unfortunate considering sex differences have been repeatedly found in neuroscience models, especially in the structure and function of the nervous system\textsuperscript{[11–15]}. In humans, sex differences have been observed in the somatosensory cortex\textsuperscript{[16–19]}, peripheral nervous system\textsuperscript{[20,21]}, and somatosensory processing\textsuperscript{[22]}. In humans, chronic itch presents differently in men and women\textsuperscript{[23]}. Further, sex differences in itch perception in humans have been found\textsuperscript{[24]}. Even the skin, the organ most closely associated with itch outside the nervous system, has sex-dependent physiology in humans\textsuperscript{[25,26]}. Unfortunately, use of males and females in animal models to study somatosensory processes, including itch, appears to be inconsistent at best. Knowing whether animal models display sex differences is critical for translation of study results to humans.

Our systemic review was designed to study the use of male and female animals in itch-related research (August 2007 to December 2018). It was also intended to find what, if any, progress has been made toward reducing male animal bias in preclinical biomedical research since the NIH’s mandate in June 2015. We also explored the usage of 4 common animal models in itch research: mice, rats, nonhuman primates, and dogs.
Methods

Inclusion criteria and article coding

Utilizing the PRISMA guidelines\(^{27}\) (Fig. 1), we searched for articles indexed in the National Institutes of Health PubMed database for articles containing the words “ITCH or PRURITUS” from August 1, 2007 to December 31, 2018. The first paper identifying a central molecular mechanism for itch was published in August 2007; therefore, we selected August 2007 as our start date for our PubMed query\(^{28}\). Similar to other neuroscience based meta-analyses\(^{4,6,10,29}\), we excluded review articles, editorials, case studies, clinical trials, and other nonprimary research literature. Papers studying the ubiquitin ligase ITCH\(^{32}\) were also excluded. Other exclusion criteria can be found in the PRISMA flow diagram in Figure 1. One author (J.J.W.) read the material and method sections to identify the following: whether it fit the inclusion criteria, animal model used, sex of the animal model used, and whether sex was considered a variable.

Articles fitting our inclusion criteria were assessed to determine which species was (or were) used in the study. The species categories are mouse, rat, dog, and nonhuman primate. We included nonhuman primates because they have been used for itch research\(^{31,34}\) as well as models for human diseases in other areas of research, including neuroscience\(^{33–38}\), due to the homology of the nervous system\(^{39}\).

Dogs have been used as models for many nocifensive processes in humans including: atopic dermatitis (AD)\(^{40–43}\), allergic responses\(^{43–45}\), asthma\(^{46–48}\), osteoarthritis\(^{49,50}\), food allergies\(^{51,52}\), and spinal cord injury\(^{53}\). In addition, results from dogs are frequently more translatable to humans largely due to the fact that dogs and humans share the same environments\(^{53,54}\) and that some diseases, like AD, have similar etiologies\(^{42,55–58}\). Further, dogs are one of the more frequently used nonrodent models for human disease\(^{59,60}\) besides nonhuman primates and pigs.

The type of itch (acute, chronic, or both) was coded. Acute itch was determined to be studied when the behavior responses to a compound were measured after injection or in models that lasted fewer than 3 days. Chronic itch was determined to be studied when the paper used NC/Nga mice, any atopic dermatitis model, any psoriasis model, and acetone-ether-water model, neonatal injections of capsaicin, or allergic contact dermatitis. Both types of itch were determined to be studied when an acute behavior and a chronic itch model were used.

Statistical analysis

For each year, values were recorded as absolute percentage of articles where the variable of interest was reported. Some studies used multiple animal models or mouse strains in a given year; therefore, these years have total percentages > 100%. Values in the text are presented as mean ± SD, where mean is the average number of articles across years. Most statistical tests featured in this paper explore the animal, species, or sex appearing most frequently in studies conducted across the entire time period considered. These total percentages are reported in Table 1.

When analysis objectives were to discover which animal, species, or sex was most frequently studied, \(\chi^2\) goodness of fit tests were used when sample sizes allowed (expected counts for each group \(\geq 5\)) to explore whether each group was reported equally often in studies across all considered years. If sample size requirements to conduct the \(\chi^2\) goodness of fit test were not met, groups with small sample sizes would be tested in aggregate or a binomial test would be run comparing the two largest categories or the largest category against all other categories combined. If the preliminary test found a significant difference between groups, the group appearing the largest proportion of times was then compared via 1-sided \(z\)-tests when assumptions of normality were met or binomial tests otherwise against all other groups in aggregate, or against the second most commonly occurring group to see if the group reported most often was studied most frequently. Assumptions of normality were met when, for \(n\) the number of articles across years. Most statistical tests featured in the text are presented as mean ± SD, where mean is the average number of articles across years. Some studies used multiple animal models or mouse strains in a given year; therefore, these years have total percentages > 100%. Values in the text are presented as mean ± SD, where mean is the average number of articles across years. Most statistical tests featured in this paper explore the animal, species, or sex appearing most frequently in studies conducted across the entire time period considered. These total percentages are reported in Table 1.

When analysis objectives were to discover which animal, species, or sex was most frequently studied, \(\chi^2\) goodness of fit tests were used when sample sizes allowed (expected counts for each group \(\geq 5\)) to explore whether each group was reported equally often in studies across all considered years. If sample size requirements to conduct the \(\chi^2\) goodness of fit test were not met, groups with small sample sizes would be tested in aggregate or a binomial test would be run comparing the two largest categories or the largest category against all other categories combined. If the preliminary test found a significant difference between groups, the group appearing the largest proportion of times was then compared via 1-sided \(z\)-tests when assumptions of normality were met or binomial tests otherwise against all other groups in aggregate, or against the second most commonly occurring group to see if the group reported most often was studied most frequently. Assumptions of normality were met when, for \(n\) the number of articles considered for the test, \(p\) the proportion of articles about the variable of interest, \(np\) \(\geq 10\) and \(n (1 − p) \geq 10\).

The Cochran-Armitage test for trend was used to evaluate changes in proportions of articles written on variables of interest over time. The Cochran-Armitage test evaluates linear trends with both positive and negative slopes against the null hypothesis that proportions remain constant over time\(^{61}\). With one proportion recorded per year and 12 years’ worth of data, more data would be required to check for quadratic trends or seasonality. In addition, with few data points, it is always possible any linear trend found is spurious.

To test if the mean number of a particular sex category changed from before the NIH’s mandate (Pre-2015) to after (Post-2015), used multiple unpaired t-tests using Prism’s GraphPad version 8.1. Significance claims were made at the level of \(\alpha = 0.05\). Note that the symbol “≪” denotes \(P\)-values smaller than 0.0001. Numerical \(P\)-values are listed in text.
| Variables                  | No. Studies | Average ± SD Percentage Across Years | Percentage Across all Years | Test Statistic | P     |
|---------------------------|-------------|--------------------------------------|----------------------------|----------------|-------|
| Itch type                 |             |                                      |                            |                |       |
| Acute                     | 335         | 62.7% ± 10.9%                         | 58.98%                     | z-test: acute itch more likely than other types | <0.0001 |
| Chronic                   | 211         | 34.0% ± 9.3%                          | 37.15%                     |                |       |
| Both                      | 22          | 3.3% ± 3.4%                           | 3.87%                      |                |       |
| Demographics*             | 572 (568)   |                                      |                            |                |       |
| Mice                      | 476         | 82.6% ± 4.5%                          | 83.22%                     | z-test: mice more likely than other species | <0.0001 |
| Rats                      | 54          | 10.3% ± 4.0%                          | 9.44%                      |                |       |
| Nonhuman primates         | 19          | 4.3% ± 3.6%                           | 3.32%                      |                |       |
| Dogs                      | 23          | 3.6% ± 2.9%                           | 4.02%                      |                |       |
| Sex*                      | 572 (568)   |                                      |                            |                |       |
| Male                      | 322         | 56.1% ± 8.9%                          | 56.29%                     | z-test: males more likely than other sexes | 0.001  |
| Female                    | 74          | 13.7% ± 4.2%                          | 12.94%                     |                |       |
| Both                      | 59          | 10.2% ± 2.9%                          | 10.32%                     |                |       |
| Not specified             | 117         | 20.5% ± 5.3%                          | 20.45%                     |                |       |
| Sex differences           | 59          |                                      |                            | Binomial test: more likely not to test for sex differences across all animal models | <0.0001 |
| Mice*                     | 503 (476)   |                                      |                            |                |       |
| C57Bl6 background         | 195         | 34.7% ± 20.4%                         | 38.77%                     | z-test: C57Bl6 background mice more likely than ICR | <0.0001 |
| CD-1 or ICR               | 121         | 30.4% ± 16.9%                         | 24.06%                     |                |       |
| BALB/c                    | 75          | 15.1% ± 8.0%                          | 14.91%                     |                |       |
| Swiss Webster             | 22          | 4.3% ± 2.9%                           | 4.37%                      |                |       |
| NC/Nga                    | 44          | 10.2% ± 5.2%                          | 8.75%                      |                |       |
| Other                     | 46          | 10.0% ± 5.2%                          | 9.14%                      |                |       |
| Mouse sex                 | 476         |                                      |                            |                |       |
| Male                      | 274         | 59.3% ± 11.9%                         | 57.56%                     | z-test: male mice more likely than other sexes | 0.0005 |
| Female                    | 66          | 13.6% ± 6.5%                          | 13.87%                     |                |       |
| Both                      | 36          | 6.6% ± 3.8%                           | 7.57%                      |                |       |
| Not specified             | 100         | 20.5% ± 6.4%                          | 21.00%                     |                |       |
| Sex differences           | 36          |                                      |                            | Binomial test: more likely not to test for sex differences | <0.0001 |
| Rats                      | 54          |                                      |                            |                |       |
| Sprague-Dawley            | 35          | 60.5% ± 31.0%                         | 64.81%                     | z-test: Sprague-Dawley rats more likely than other species | 0.0206 |
| Wistar                    | 9           | 15.2% ± 20.6%                         | 16.67%                     |                |       |
| Other                     | 10          | 23.5% ± 31.3%                         | 18.52%                     |                |       |
| Rat sex                   | 54          |                                      |                            | Binomial test: male rats more likely than other sexes | <0.0001 |
| Male                      | 44          | 82.4% ± 19.9%                         | 81.48%                     |                |       |
| Female                    | 3           | 3.4% ± 8.3%                           | 5.56%                      |                |       |
| Both                      | 0           | 0                                      |                            |                |       |
| Not specified             | 7           | 14.2% ± 19.6%                         | 12.96%                     |                |       |
| Nonhuman primates         | 19          |                                      |                            | Binomial test: no evidence to assume Macaca mulatta more common than Macaca fascicularis | 0.4073 |
| Macaca mulatta            | 10          | 43.8% ± 42.8%                         | 52.63%                     |                |       |
| Macaca fascicularis       | 8           | 39.6% ± 41.9%                         | 42.11%                     |                |       |
| Macaca fuscata            | 1           | 8.3% ± 28.9%                          | 5.26%                      |                |       |
| Nonhuman primate sex      | 19          |                                      |                            | Binomial test: no evidence to assume both sexes reported more often than other sex categories | 0.8204 |
| Male                      | 4           | 20.8% ± 39.6%                         | 21.05%                     |                |       |
| Female                    | 4           | 20.8% ± 39.6%                         | 21.05%                     |                |       |
| Both                      | 8           | 31.3% ± 41.5%                         | 42.11%                     |                |       |
| Not specified             | 3           | 18.8% ± 32.2%                         | 15.79%                     |                |       |
The R-code used for statistical analysis in this paper has been hosted on GitHub and can be accessed at the following URL: https://github.com/knallen4/ItchCode.

**Results**

Our initial PubMed query identified 10,531 articles in our search window. Of these 10,531 articles, 568 primary research articles remained after removing articles not fitting our inclusion criteria across all years. Figure 1 shows the PRISMA flow chart outlining coding criteria for articles included in this study. We found that, on average, 5.3% ± 1.2% of papers within a given year used an animal model. Figure 2A shows the yearly breakdown of articles using animal models by percentage from August 2007 to December 2018, while Figure 2B shows the distribution of the percentage of articles using animal models during that time period.

To determine the type of itch studied we categorized whether an acute itch or chronic itch model was used. Figure 3A shows the average breakdown of the type of itch studied from August 2007 to December 2018. Figure 3B shows the distribution of the type of itch studied from this time period. Overall acute itch was most likely to be studied (62.7% ± 10.9%, \( P \ll 0.0001 \)), followed by research considering chronic itch (34.0% ± 9.3%). Research observing both acute and chronic itch was the least frequently performed. On average only 3.3% ± 3.4% of itch research during this time period studied both acute and chronic itch.

| Variables | No. Studies | Average ± SD Percentage Across Years | Percentage Across all Years | Test Statistic | \( P \) |
|-----------|-------------|---------------------------------------|-----------------------------|---------------|-------|
| Sex differences | 8 | 8.3% ± 28.9% | 12.5% | Binomial test: more likely not to test for sex differences | 0.0351 |
| Tested | 1 | 33.3% ± 49.2% | 87.50% |
| Not tested | 7 | |
| Dogs | | |
| Client owned | 14 | 40.3% ± 50.0% | 60.87% | Binomial test: client owned dogs featured in more articles than Beagles | 0.0318 |
| Beagle | 5 | 16.7% ± 31.0% | 21.74% |
| Maltese-Beagle | 4 | 18.1% ± 34.4% | 17.39% |
| Dog sex | 23 | 0 | 0 | Binomial test: no evidence to assume both dog sexes reported more frequently over other categories | 0.1050 |
| Male | 0 | 4.2% ± 14.4% | 4.35% |
| Female | 1 | 57.8% ± 33.6% | 65.22% |
| Both | 15 | 21.4% ± 23.0% | 30.43% |
| Not specified | 7 | |
| Sex differences | 15 | 2.1% ± 7.2% | 6.67% | Binomial test: more likely not to test for sex differences | 0.0005 |
| Tested | 1 | 81.25% ± 38.6% | 93.33% |
| Not tested | 14 | |

*Individual counts sum to more than the total number of articles as some papers reported more than 1 species, strain, or breed. Numbers are reported as total sums (number of unique articles).

Figure 2. Quantification of the number of papers utilizing animal models in itch studies from August 2007 to December 2018. A, Distribution of the percentage articles using animal models from August 2007 to December 2018. B, Yearly breakdown of the percentage of articles using animal models in itch research. Bars represent percentage of total for that year. Box plots show minimum to maximum values with Q1, Median, and Q3 values used to generate the box.
We next considered the demographics of the animal models used in itch research from August 2007 to December 2018 (Fig. 4A). The overwhelming majority of animal studies used mice (82.6% ± 4.5%), followed by rats (10.3% ± 4.0%), nonhuman primates (4.3% ± 3.6%), and dogs (3.6% ± 2.9%) (Fig. 4B). Overall, researchers were most likely to use mice during this period (P < 0.0001). Since mice make up the majority of animal models used during this time period, we looked into which mice were most commonly used and found that mice on a C57Bl/6 background were most commonly used (34.7% ± 20.4%), closely followed by ICR (30.4% ± 16.9%). Other mouse strains frequently used include BALB/c mice (15.1% ± 8.0%), NC/Nga mice (10.2% ± 5.2%), and Swiss Webster mice (4.3% ± 2.9%). Overall, mice on the C57 background were more likely to be used over ICR, the second most commonly used mouse strain (P < 0.0001). Other (C3H/HeN, APOC1, DBA/1, 129S6, Hairless, SPF, SKH-1, Kunning, CBA/2, ddY, HR1, 129SvJ, B6129PF2/J, or unspecified) mouse strains were used in 10.01% ± 5.2% of research performed during this time period.

Interestingly, there was a linear increase over time in the percentage of research using mice on the C57Bl/6 background (P < 0.0001). This increase in the use of C57Bl/6 was concomitant with a significant linear decrease in the use of ICR mice in this same time period (P < 0.0001) (Fig. 4E). The use of other mouse strains remained constant over this time period. Occasionally, papers utilized more than 1 animal model or mouse strain, resulting in percentages > 100% in a given year. We also found that Sprague-Dawley rats were the rat strain most frequently used during this time period (60.5% ± 31.0%, P = 0.0206), followed by Wistar rats (15.2% ± 20.6%) and other (Lister Hooded, Long-Evans, CDR, or unspecified) rat strains (23.5% ± 31.3%) (Figs. 4F, G). There is no statistical evidence of a trend in the use of Sprague-Dawley rats between August 2007 and December 2018 (Fig. 4H).

Researchers studying nonhuman primates most commonly used Macaca mulatta (43.8% ± 42.8%) and Macaca fascicularis (39.6% ± 41.9%) (Figs. 4I, J). Researchers were just as likely to use these 2 species of macaques in their studies. In a unique study, researchers observed group dynamics of itch in a wild population of Macaca fuscata fuscata. The study observing itch behavior in wild mice accounted for the remaining percentage of nonhuman primate research (8.3% ± 28.9%). Overall researchers were equally likely to use either M. fascicularis or M. mulatta, with neither species increasing in use between August 2007 and December 2018 (Fig. 4K).

Researchers most frequently used client owned dogs (40.3% ± 50.0%), Beagle (16.7% ± 31.0%) and maltse-beagle crosses (18.1% ± 34.4%) purpose bred for laboratory research accounted for the remainder of dog animal models (Figs. 4L, M). Overall, client owned dogs were more likely to be used (P = 0.0318) during this time period. Similar to nonhuman primates, researchers were just as likely to use beagles, maltse-beagle crosses, or client owned dogs, with none of these options increasing in usage between August 2007 and December 2018 (Fig. 4N).

Further, we considered the use of sex in itch research from August 2007 to December 2018 (Fig. 5A). Overall, researchers were most likely to use males in animal research (56.1% ± 8.9%, P = 0.0001) over female animals (13.7% ± 4.2%) or both sexes (10.2% ± 2.8%). Approximately, one-fifth of articles published in a given year (20.5% ± 5.9%) between August 2007 and December 2018 did not report the sex of their animal models.

Unsurprisingly, researchers using mice followed the same trends as the general sex usage with male mice most likely to be used (59.3% ± 11.9%, P = 0.0005), followed by the use of female mice (13.6% ± 6.5%) and the use of both sexes (6.6% ± 3.8%) (Figs. 5C, E). In addition, nearly one fifth (20.5% ± 6.4%) of mouse research did not list the sex of the mice used. Researchers using rats were more likely to use male rats (82.4% ± 19.9%, P < 0.0001) over female rats and rats of unspecified sexes (3.5% ± 8.3%) (Fig. 5F). No studies written between August 2007 and December 2018 used both male and female rats. The sex of the rats used were unspecified in 14.2% ± 19.6% of articles during this time period. Researchers studying nonhuman primates were most likely to use both sexes in itch research during this time period (31.2% ± 41.4%) (Fig. 5G). Nonhuman primate researchers were equally likely to use only male (20.8% ± 39.6%) or only female animals (20.8% ± 39.6%) in itch research during this time period. Like mice and the overall trend, roughly one fifth of primate researchers did not specify the sex of the animal models used (18.8% ± 32.2%). Researchers using dogs were most likely to use both sexes in research (57.8% ± 33.6%, P < 0.0001) (Fig. 5H). Similar to other animal models, roughly one fifth of researchers using dogs did not specify the sex of the animals used (21.4% ± 23.0%). Dog researchers least frequently studied only female dogs (4.2% ± 14.4%). There is no statistical evidence that researchers report using both male and female dogs more frequently than they fail to report the sex of the dogs (P = 0.0669). No research articles using only male dogs were published during this time period.

In instances where researchers used male and female animals investigators were far more likely to not run tests to determine if there are any sex differences (89.1% ± 17.6%) than running tests to determine if there are any sex differences (10.0% ± 15.2%, P < 0.0001) in the experimental outcomes (Figs. 5B, D). When using mice, researchers were also unlikely to test for sex differences (P < 0.0001). Researchers examined for sex differences in 9.0% ± 15.7% of studies, and did not evaluate for sex differences in 74.4% ± 37.9% of studies (Fig. 5I). During this time period no papers were published using both male and female rats. Nonhuman primate researchers also did not test for sex differences in their studies (P = 0.0351) (Fig. 5J). Similarly, researchers using dogs were unlikely to test for sex differences (P = 0.0005) (Fig. 5K).
Figure 4. Animal study demographics for August 2007 to December 2018. A, Pie chart showing the average percentage of articles using mice, rats, non-human primates, and dogs in animal research. B, Distribution of each animal model over the August 2007 to December 2018 time period. C, Pie chart showing the average percentage of articles using mice in animal research. D, Distribution of the percentage of each mouse model over the August 2007 to December 2018 time period. E, Change in use of the different mouse strains in used animal research from August 2007 to December 2008. F, Pie chart showing the average percentage of articles using rats in animal research. G, Distribution of the percentage of rat strains used from August 2007 to December 2018. H, Change in use of the different rat strains in animal research from August 2007 to December 2008. I, Pie chart showing the average percentage of articles using non-human primates in animal research. J, Distribution of the percentage of non-human primate species used between August 2007 and December 2008. K, Change in use of the different nonhuman primate species used in animal research from August 2007 to December 2008. L, Pie chart showing the average percentage of articles using dogs in animal research. M, Distribution of the percentage of dog breeds used between August 2007 and December 2018. N, Change in use of the different dog breeds used in animal research from August 2007 to December 2008. Box plots show minimum to maximum values with Q1, median, and Q3 values.
We were also interested in seeing if the NIH’s mandate to include sex as a variable had begun to appear in published research. We found that in both Pre-NIH (before 2015) and Post-NIH (after 2015) data sets male animals were more likely to be used in preclinical research and that there has not yet been a change in the usage of females, both sexes, or in articles that do not report the sex of the animals used (Fig. 6A). In research using both sexes, there has not been a change in the number of articles that test for sex as one of their variables (Fig. 6B). In mice, males are still most likely to be used both before and after the NIH’s 2015 mandate (Fig. 6C). In studies using rats, male animals were more likely to be used both before and after the NIH’s 2015 mandate (Fig. 6E). No studies used both male and female rats in our data set, so we were not able to determine if there was a change in the consideration of sex as a variable in rat studies (Fig. 6F). Researchers using non-human primates were equally likely to use males, females, both sexes, or not report sex both before and after the NIH’s 2015 mandate (Fig. 6G). The consideration of sex as a variable in non-human primate research did not change from before to after the NIH’s 2015 mandate (Fig. 6H). In dogs, there was also not a
Figure 6. Changes in sex as a variable reporting before and after the NIH’s 2015 mandate to report sex in preclinical research. A, Distribution of the aggregate use of male and female animals in preclinical research. B, Distribution of aggregate consideration of sex as a variable in preclinical research using both sexes. C, Distribution of the use of male and female animals in research using mice. D, Distribution of aggregate consideration of sex as a variable in preclinical research using mice of both sexes. E, Distribution of the use of male and female animals in research using rats. F, Distribution of aggregate consideration of sex as a variable in preclinical research using rats of both sexes. G, Distribution of the use of male and female animals in research using nonhuman primates. H, Distribution of aggregate consideration of sex as a variable in preclinical research using nonhuman primates of both sexes. I, Distribution of the use of male and female animals in research using dogs. J, Distribution of aggregate consideration of sex as a variable in preclinical research using dogs of both sexes. Box plots show minimum to maximum values with Q1, median, and Q3 values, ns = not significant based on an unpaired multiple t test comparison.
change in use of males, females, or both sexes from before to after 2015 (Fig. 6I), nor in the consideration of sex as a variable (Fig. 6J).

Overall, there has not been a significant change in testing for sex differences in animal studies by researchers.

**Discussion**

To best of our knowledge, this is the first systemic review reported on preclinical itch research. The major finding of this systemic review sheds light on most widely used mammalian species and their genetic background that have been the major arsenal of itch research since 2007. While we found a substantial number of male rodents have been used in nonclinical studies, both sexes were commonly used for nonhuman primates and dogs. Surprisingly, even after NIH’s 2015 mandate to report sex as a variable, we found no change in the usage of both sex in preclinical itch research.

Comparable to other meta-analyses, we found that mice constituted the majority of animal models used[60,62,63]. Overall, mice on the C57Bl/6 background were most commonly used, which is unsurprising given that it is the frequently studied mouse strain across biomedical disciplines[64]. Our mouse data has also revealed a shift toward using mice on the inbred C57Bl/6 background and away from the outbred CD-1 background. This shift is unsurprising given the NIH’s choice to use the C57Bl/6 to develop the mouse reference genome[65]. However, the use of other strains of mice might still be valuable given that there are strain differences in immune response to chronic pain conditions[66]. Further, outbred mouse strains do not appear to have higher variation in behavioral outcomes when compared to inbred mouse strains[67].

Sprague-Dawley rats were most likely to be used during this period (August 2007 and December 2018). This result was unsurprising, since Sprague-Dawley rats are one of the more commonly used rat strains in nocifensive research especially when trying to discern strain differences[68–72]. We also found that rat animals were the most commonly used sex in mouse and rat studies.

Unsurprisingly, macaques were the only nonhuman primate model used in itch research during this time period (August 2007 and December 2018), which is expected given that macaques are the most frequently used nonhuman primate used in biomedical research[73]. Meanwhile, nonhuman primate studies use both male and female animals equally, which is likely due to the following reasons; (a) the cost of housing these animals, (b) the relative scarcity of facilities to house nonhuman primates for research[73] (USDA Animal Health), and (c) issues with procuring nonhuman primates[74].

Researchers using dogs were most likely to use both sexes in their research and were unlikely to consider sex as one of their variables. Surprisingly, we found that researchers were most likely to use client-owned dogs in preclinical research studies. Similar to nonhuman primates, this is most likely due to the cost of housing dogs and the scarcity of facilities capable of housing research dogs (USDA Animal Health).

Itch is a major manifestation in cutaneous diseases like AD, commonly known as eczema as well as psoriasis. Approximately, 30% of the world’s population is affected by AD and almost 90% of them reported itch as a major symptom[75]. In animals, for instance like dogs, almost 25% of veterinary visits are related to cutaneous disease where itch is a major symptom[75]. Although in this review we have not explored explicitly whether itch associated with AD is sex-dependent or not; several reports suggest that females have more incidence (1.5 times) of itch than males[23]. A recent study found a notifiable difference between males and females regarding pruritus and wheal size after intracutaneous histamine test. Another study reported brain activation patterns during itch and found that women presenting higher itch intensities and desire to scratch compared to men by using functional magnetic resonance imaging (fMRI)[24]. Similarly, an incidence of itch among psoriasis individuals is almost 85% and frequency of itch is almost daily; however, they did not find a significant correlation between sex and severity of psoriasis according to psoriasis area and severity index score[24]. Another study reported pruritus is a salient feature of psoriasis and affects quality of life and they found stress is a major contributor in the exacerbation of itch[76]. Interestingly, stress has been shown to be sex-dependent, but if that influence itch is unclear and need further attention. Overall, these studies suggest sex-specific differences exist in the physiology of pruritus.

Clearly, we observe single sex use in rodent research. However, dogs and nonhuman primates do not follow the same trend. Overall, we summarize, based on our systemic analysis, that single sex studies of males still predominate in the nonclinical research despite the fact that NIH administered explicitly on the use of both sexes as a variable to be employed in future biological research.

**Sources of funding**

This work was funded through start-up funds provided to SKM by NC State University.

**Conflict of interest disclosures**

The authors declare that they have no financial conflict of interest with regard to the content of this report.

**Acknowledgments**

The authors would like to thank Laura Minnema for facilitating the collaboration between K.N.A. and J.J.W. and S.K.M.

**References**

[1] Berkley KJ. Vive la difference! Trends Neurosci 1992;15:331–2.
[2] Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. Nature 2014;509:282–3.
[3] Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 2011;35:565–72.
[4] Zucker I, Beery AK. Males still dominate animal studies. Nature 2010;465:690.
[5] Landis SC, Amara SG, Asadullah K, et al. A call for transparent reporting to optimize the predictive value of preclinical research. Nature 2012;490:187–91.
[6] Mogil JS, Chanda ML. The case for the inclusion of female subjects in basic science studies of pain. Pain 2005;117:1–5.
[7] Becker JB, Prendergast BJ, Liang JW. Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. Biol Sex Differ 2016;7:34.
[8] Beery AK. Inclusion of females does not increase variability in rodent research studies. Curr Opin Behav Sci 2018;23:143–9.
[9] Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev 2014;40:1–5.
implications for target validation efforts in pain drug discovery. Eur J Pain 2019;23:539–4.

[69] Klune CB, Larkin AE, Leung VSY, et al. Comparing the Rat Grimace Scale and a composite behaviour score in rats. PLoS One 2019;14:e0209467.

[70] LaCroix-Fralish ML, Austin JS, Zheng FY, et al. Patterns of pain: meta-analysis of microarray studies of pain. Pain 2011;152:1888–98.

[71] Mills CD, Hains BC, Johnson KM, et al. Strain and model differences in behavioral outcomes after spinal cord injury in rat. J Neurotrauma 2001;18:743–56.

[72] Mogil JS. The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proc Natl Acad Sci U S A 1999;96:7744–51.

[73] Lankau EW, Turner PV, Mullan RJ, et al. Use of nonhuman primates in research in North America. J Am Assoc Lab Anim Sci 2014;53:278–82.

[74] Prescott MJ, Jennings M. Ethical and welfare implications of the acquisition and transport of non-human primates for use in research and testing. Altern Lab Anim 2004;32 (suppl 1A):323–7.

[75] Wang X, Li L, Shi X, et al. Itching and its related factors in subtypes of eczema: a cross-sectional multicenter study in tertiary hospitals of China. Sci Rep 2018;8:10754.

[76] Yosipovitch G, Goon A, Wee J, et al. The prevalence and clinical characteristics of pruritus among patients with extensive psoriasis. Br J Dermatol 2000;143:969–73.