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Problems and Progress regarding Sex Bias and Omission in Neuroscience Research

Sex bias and omission in neuroscience research

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Abstract

Neuroscience research has historically ignored female animals. This neglect comes in two general forms. The first is sex bias, defined as favoring one sex over another; in this case, male over female. The second is sex omission, which is the lack of reporting sex. The recognition of this phenomenon has generated fierce debate across the sciences. Here we test whether sex bias and omission are still present in the neuroscience literature, whether studies employing both males and females neglect sex as an experimental variable, and whether sex bias and omission differs between animal models and journals. To accomplish this we analyzed the largest ever number of neuroscience articles for sex bias and omission: 6,636 articles using mice or rats in 6 journals published 2010-2014. Sex omission is declining, as increasing numbers of articles report sex. Sex bias remains present and also intensifies, as increasing numbers of articles report the sole use of males. Articles using both males and females are also increasing, but few report assessing sex as an experimental variable. Sex bias and omission varies substantially by animal model and journal. These findings are essential for understanding the complex status of sex bias and omission in neuroscience research and may inform effective decisions regarding policy action.

Significance Statement

Neuroscience research has historically favored the use of male over female animals, or ignored animal sex. Recognition of this sex bias and omission has spurred fierce debate and study, including new regulatory policies and scientific findings. Here we further probe this phenomenon by conducting the largest ever analysis of neuroscience research articles for sex bias and omission. We show that sex bias is still present and intensifying, and that sex omission is declining. The extent of sex bias and omission varies widely by animal model and journal. These results produce key implications for research conduct, regulatory policies, and scientific culture by revealing the still present but complex nature of sex bias and omission.

Keywords

Neuroscience, sex bias, sex omission, animal models, journals

Introduction

Neuroscience research has historically demonstrated sex bias, in this case favoring the use of male over female research animals, and sex omission, which is the lack of reporting research animal sex (Berkley, 1992; Mogil and Chanda, 2005; Beery and Zucker, 2011; Shansky and Woolley, 2016). While neuroscience is not the only biomedical discipline exhibiting sex bias, Beery and Zucker (2011) demonstrated that neuroscience, pharmacology, physiology, and endocrinology exhibited the largest sex biases in research animal use out of 10 analyzed disciplines. Collectively, this phenomenon of discipline-specific sex bias has generated fierce debate, resulting in awareness campaigns, studies, regulatory policies, and position commentaries (Becker et al., 2005; Clayton and Collins, 2014; Fields, 2014; Johnson et al., 2014; McCullough et al., 2014; Ruigrok et al., 2014; Yoon et al., 2014; Cahill and Aswad, 2015; Klein et al., 2015; McCarthy, 2015; Park et al., 2015; Richardson et al., 2015; Becker et al., 2016; Eliot and Richardson, 2016; Guizzetti et al., 2016; Maney, 2016; Mogil, 2016; Panzica and Melcangi, 2016; Tannenbaum et al., 2016; Zakiniaeiz et al., 2016; Brooks and Clayton, 2017; Duchesne et al., 2017; Joel and McCarthy, 2017; Karp et al., 2017; McEwen and Milner, 2017; Miller et al., 2017). Many authors argue that it is vital to document experimental animal sex, and to thoughtfully select and justify the sex of experimental animals. Important for this discussion, and especially for the implementation and evaluation of regulatory policies, is the evaluation of sex bias and omission in the neuroscience research literature. Here we provide these data by testing the hypotheses that sex bias and omission still persists in the neuroscience literature, that studies employing both males and females neglect sex as an experimental variable, and that sex bias and omission varies by rodent species and journal origin. To accomplish this, our team of 11 trained curators assessed all research articles using rats and/or mice published from 2010-2014 in the following journals: Journal of Neuroscience, Journal of Neurophysiology, Nature Neuroscience, Neuron, Nature and Science. These journals were chosen given their prominence in the neuroscience field, and also to align with previous studies (Beery and Zucker, 2011; Shansky and Woolley, 2016). A comprehensive approach to article selection was undertaken to decrease sampling bias within the analyzed journals, and research articles were analyzed given that this is the final common output of academic neuroscience research.

Materials and Methods

Inclusion Criteria and Coding of Articles

Articles were analyzed from 2010-2014 from the following journals: Journal of Neuroscience, Journal of Neurophysiology, Nature Neuroscience, Neuron, Nature and Science. A team of 11 trained curators (8 females, 3 males; Assessing Rodent Sex in Neuroscience Literature team, ARSiNL team) examined all articles published per year within the targeted journals. Trained curators were used because the divergent and extensive vocabulary used to describe animal sex and its treatment as an experimental variable make automated text mining approaches challenging. Articles were first determined to be primary research articles by the curators. Following previously published studies (Berkley, 1992; Sechzer et al., 1994; Mogil and Chanda, 2005; Beery and Zucker, 2011; Yoon et al., 2014; Shansky and Woolley, 2016), reviews, editorials, and similar non-primary research articles were excluded from analysis. Articles were then analyzed for neuroscience-relevance. Articles from the Journal of Neuroscience, Journal of Neurophysiology, Nature Neuroscience and Neuron were automatically accepted as neuroscience-relevant. A broad inclusion criterion was employed for articles from Nature and Science. Articles in these journals were included for analysis if the article topic encompassed any aspect of the central or peripheral nervous system, ranging from the molecular to behavioral level of analysis. In all journals, articles using fetal animals and primary neuron cultures were included in the overall analysis as in a previous study (Taylor et al., 2011), given that cells express chromosomal sex (XX or XY) and that sex differences have been detected even at the embryonic stage and in primary neuron culture. These inclusion criteria identified 13,857 primary research neuroscience articles. Articles were then coded for species. Species categories were: mouse, rat, and other species. Articles using other species were excluded from further analysis, resulting in a pool of 6,636 neuroscience articles that employed rats and/or mice. 2,611 articles employed rats, and 4,221 articles employed mice. Articles using a rat and/or mouse and another species were included in analysis, with the non-rodent portion of the article excluded from analysis. Articles using both mice and rats were included in analysis (196 articles). Articles using both mice and rats were included in both the mice and rat categories, but only counted once in analyses that combined mice and rat datasets. The reason for focusing the study on the analysis of articles employing mice and/or rats is further explained in the discussion.

Articles were then analyzed for research animal sex. Sex categories were: male, female, no sex reported, and male and female. Articles containing both male and females were further subdivided into those wherein biological sex was not considered as an experimental variable and those wherein biological sex was considered an experimental variable. Articles were considered to have addressed sex as a biological variable if any formal statistical comparison or assertion of such a comparison of males and females was performed, including if the data or analysis was not shown, and including whether sex differences were detected or not. Very few articles reported data disaggregated by sex but did not perform or assert to have performed a statistical comparison. These articles were coded as not having addressed sex as an experimental variable since there was no comparison. Intra- and inter-curator error rates were assessed, with the rates being 0% and 7%, respectively. Experimental power was not assessed. When distinct experiments within an article employed different sexes, articles were considered male/female with biological sex not considered an experimental variable, following a previous study (Beery and Zucker, 2011).

Statistics

Experiments were analyzed via linear regressions, and ANCOVAs (Prism version 6.07, GraphPad Software, La Jolla, CA). P values < 0.05 were considered *a priori* as significant. Data are presented as percentages, or absolute proportions. Further statistical information is presented in Table 1.

Results

Our research article inclusion criteria resulted in an initial pool of 13,857 neuroscience research articles. Of these articles, 6,636 used rats or mice, and were further analyzed for sex bias and omission (Figure 1A). The percentage of articles using rats or mice remained fairly constant across years, with a calculated linear regression finding no correlation between the percent of articles using mice and rats and year (Figure 1B; slope −0.61, $r^2 = 0.08$, $P > 0.05$). From these findings we concluded that articles using mice and rats are a significant and stable proportion of the neuroscience literature.

Sex omission is decreasing but sex bias remains present

Articles using rats and mice were then analyzed to determine how animal sex was reported (Figure 2A). Articles were categorized as either not reporting sex, or reporting both males and females, only males, and only females. The percentage of articles not reporting sex decreased from 47% in 2010 to 19% in 2014 (slope −7.24, $r^2 = 0.86$, $P < 0.03$). The percentage of articles reporting both male and female animals increased from 17% in 2010 to 38% in 2013, and plateaued at 35% in 2014 (slope 4.78, $r^2 = 0.86$, $P < 0.03$). Articles reporting only males increased from 31% in 2010 to 40% in 2014 (slope 2.19, $r^2 = 0.89$, $P < 0.02$). The percentage of articles reporting only female animals remained stable and low throughout the assessed period, ranging from 5% in 2010 to 6% in 2014 (slope 0.27, $r^2 = 0.28$, $P > 0.05$). Overall, these results indicate that sex omission is decreasing and that sex bias remains present over the assessed period, with articles reporting the sole use of males not only comprising the largest proportion of published articles, but also continuing to increase across years.

Sex bias and omission vary considerably by animal model

We next tested the hypothesis that sex bias and omission vary by animal model. Many more articles used mice (4,221) than rats (2,611), which could potentially influence a dataset incorporating both species. In both mice (Figure 2B) and rats (Figure 2C), the percentage of articles not reporting sex decreased between 2010 and 2014 (mice: slope −7.65, $r^2 = 0.91$, $P < 0.02$; rats: slope −7.17, $r^2 = 0.79$, $P < 0.05$). In mice, articles reporting both males and females increased over time, and comprised the largest proportion of published articles by 2012, and reached 44% by 2014 (slope 5.42, $r^2 = 0.91$, $P < 0.02$). Articles reporting only males also increased, but at a lesser extent, from 22% in 2010 to 29% in 2014 (slope 1.95, $r^2 = 0.95$, $P < 0.006$). The percentage of articles reporting only females remained low and stable, ranging from 3% in 2010 to 4% in 2014 (slope 0.27, $r^2 = 0.28$, $P > 0.05$). In contrast to mice, for articles using rats the percentage reporting males dominated the distribution, ranging from 42% in 2010 to 58% in 2014 (slope 3.72, $r^2 = 0.88$, $P < 0.02$), and showed a substantially different Y-intercept compared to mice (rats: -7422; mice: -3899) There was also an absolute increase in the percentage of articles reporting both males and females from 8% in 2010 to 20% in 2014, but this did not reach significance because of the relatively stable percentages from 2012-2014 (21%, 24%, and 20%, respectively) (slope 3.10, $r^2 = 0.65$, $P > 0.05$). Similar to mice, the percentage of articles reporting only female rats remained low, ranging from 7% in 2010 to 9% in 2014 (rats:

slope 0.35, $r^2 = 0.66$, $P > 0.05$). These findings demonstrate that articles using different species show important distinctions that diverge across time. Though both species show decreases in sex omission, by 2014 sex is less likely to be reported in mice studies compared to rat studies. Regarding sex bias, by 2014 the majority of rat studies report the use of only males. A substantial and increasing percentage of mice studies also report the use of only males, however, a larger proportion of mice studies report the use of both males and females.

Most research articles incorporating both males and females do not assess sex as an experimental variable

Though it is promising that more articles are reporting the use of both males and females, these articles do not necessarily consider sex as an experimental variable. This phenomenon was first documented by Beery and Zucker (Beery and Zucker, 2011), who found that only ~20% of neuroscience studies that used both sexes actually analyzed data by sex. We thus tested whether articles using both males and females reported any statistical test or statement indicating that data from males and females were compared, whether a sex difference or similarity was detected. Our analysis found that the vast majority of articles did not report considering sex as an experimental variable, even though both males and females were included in the study (Figure 3). Depending on the year, only 12-25% of assessed studies included any indicator that data from males and females were compared. The overall percentage of articles incorporating sex as an experimental variable remained relatively stable from 2011-2014, after a substantial decrease between the years 2010 (25%) and 2011 (14%) (Figure 3; slope -2.055, $r^2 = 0.38$, $P > 0.05$). These data show that even though there is increased documentation of the use of males and females, most studies still do not report analyzing sex as an experimental variable.

Sex bias and omission varies between journals

An important facet of the analysis presented thus far is that it pooled articles across six different journals. This provides the advantage of a broad sampling of the neuroscience literature. One limitation is that scientific journals may have differing policies and/or customs regarding methods documentation, including the requirement of reporting sex. This may create differences between journals in the percentage of articles reporting varying categories of animal sex. To address this question, articles were analyzed by their journal of origin, including the Journal of

Neurophysiology (J Neurophys; 848 articles), the Journal of Neuroscience (J Neurosci; 4,105 articles), Nature (243 articles), Nature Neuroscience (Nature Neurosci, 582 articles), Neuron (649 articles), and Science (209 articles) (Figure 4). Journals differed in the percentages of articles not reporting sex between 2010-2014 (Figure 5A; $F_{(5,18)}=5.42$, $P<0.004$). In five of the six journals, the percentage of articles not reporting sex decreased between 2010 and 2014, although there were varying degrees of change in magnitude between journals (Figure 5A; J Neurophys: slope -5.92, $r^2 = 0.87$, $P < 0.02$; J Neurosci: slope -8.10, $r^2 = 0.75$, $P = 0.059$; Nature: slope -13.13, $r^2 = 0.96$, $P < 0.004$; Nature Neurosci: slope -11.18, $r^2 = 0.81$, $P < 0.04$; Neuron: slope -3.34, $r^2 = 0.94$, $P < 0.006$). Of this group, Neuron showed the least overall change in magnitude, beginning with 69% of articles not reporting sex in 2010, decreasing to only 55% in 2014. In contrast, one journal, Science, showed a surprising increase in the percentage of articles with undocumented sex in 2014 compared to earlier years, increasing from 51% in 2010 to 58% in 2014 (Figure 5A; slope 1.86, $r^2 = 0.09$, $P > 0.05$).

Journals also differed in the percentages of articles reporting male and female animals (Figure 5B; $F_{(5,18)}=3.78$, $P<0.02$), with most journals showing varying patterns of increased percentages between 2010-2014 (J Neurophys: slope 2.88, $r^2 = 0.78$, $P < 0.05$; J Neurosci: slope 5.57, $r^2 = 0.72$, $P = 0.07$; Nature: slope 12.26, $r^2 = 0.82$, $P < 0.04$; Nature Neuroscience: slope 4.13, $r^2 = 0.66$, $P = 0.09$; Neuron: slope 2.43, $r^2 = 0.70$, $P = 0.07$; Science: slope 0.20, $r^2 = 0.00$, $P > 0.05$). Journals did not differ in the overall change/slope of the percentage of articles reporting only males (Figure 5C; $F_{(5,18)}=1.86$, $P>0.05$). However, elevations between journals significantly differed ($F_{(5,23)}=3.09$, $P<0.03$), and select journals showed changes across time in the percentage of articles reporting only males (J Neurophys: slope 3.18, $r^2 = 0.72$, $P = 0.07$; J Neurosci: slope 2.03, $r^2 = 0.83$, $P < .04$; Nature: slope 1.26, $r^2 = 0.11$, $P > 0.05$; Nature Neuroscience: slope 6.34, $r^2 = 0.74$, $P = 0.06$; Neuron: slope 0.99, $r^2 = 0.33$, $P > 0.05$; Science: slope -1.37, $r^2 = 0.06$, $P > 0.05$). Similarly, journals also did not differ in the overall change in the percentage of articles reporting only females (Figure 5D; $F_{(5,18)}=0.36$, $P>0.05$), but likewise showed a significant difference in elevation ($F_{(5,23)}=5.30$, $P<0.003$). No individual journals showed changes across time in the percentage of articles reporting only females (J Neurophys: slope -0.15, $r^2 = 0.33$, $P > 0.05$; J Neurosci: slope -0.39, $r^2 = 0.09$, $P > 0.05$; Nature: slope -0.39, $r^2 = 0.09$, $P > 0.05$; Nature Neuroscience: slope 0.62, $r^2 = 0.28$, $P > 0.05$; Neuron: slope -0.08, $r^2 = 0.00$, $P > 0.05$; Science: slope -0.69, $r^2 = 0.10$, $P > 0.05$).

Discussion

The key finding of this study is that substantial progress has been made in the reduction of sex omission, but that male sex bias remains a persistent and perhaps even intensifying phenomenon in the neuroscience literature. Complementing this general finding, we find that sex omission and bias vary considerably between journal and animal model. This indicates that though it is accurate to state that sex omission and bias is a generalizable phenomenon across neuroscience research, the extent and nature of omission and bias should be carefully documented and defined to achieve maximum practical utility. For example, levels of sex bias and omission differ markedly between studies employing rats than those employing mice. This finding explains a discrepancy between a prior study that detected weaker sex bias and omission but limited its automated text mining analysis to biomedical studies that employed mice (Florez-Vargas et al., 2016), compared to studies that employed trained curators but analyzed biomedical and neuroscience studies that employed multiple model animals (Berkley, 1992; Sechzer et al., 1994; Mogil and Chanda, 2005; Beery and Zucker, 2011; Yoon et al., 2014; Shansky and Woolley, 2016).

This study detected a distinct shift in sex omission and bias across time. During the years 2010-2011, we detected similar levels of sex omission and male sex bias in neuroscience articles as reported by previous studies analyzing smaller data sets, providing important validation (Beery and Zucker, 2011; Shansky and Woolley, 2016). Sex omission and sex bias then markedly change during 2011-14. During this time period, sex omission dramatically decreased, indicating significant progress in documenting research animal sex. However, as of 2014 over 20% of all research articles still failed to report animal sex, which we consider an unacceptably high number for an essential experimental component. From a broader perspective, if such a basic detail as animal sex is omitted, other methods that may or may not seem obscure but are necessary for successful replication may also not be included in the methods section of manuscripts (Thigpen et al., 2013; Freedman et al., 2017).

Regarding male sex bias, reports of the sole use of males increased, most predominantly in rats, but also in mice. Furthermore, even when studies used both males and females, few reported incorporating sex as an experimental variable. Collectively our data indicates that sex bias remained present and perhaps even intensified during 2010-2014, despite awareness campaigns and other efforts. Remarkably, these measured decreases in sex omission and increases in male sex bias occurred *before* the implementation of the National Institute of Health (NIH) Sex as a Biological Variable (SABV) (NOT-OD-15-102) regulatory policy, which went into effect on January 25, 2016 (Clayton and Collins, 2014). Thus, the dataset produced by our study may prove useful for empirically evaluating the general success of SABV and similar efforts, though our study was not explicitly designed to assess article compliance with specific aspects of the SABV or any other funding agency mandate. Future studies intending to assess the impact of SABV should evaluate the success of specific aspects of SABV requirements. For instance, one subtle but relevant aspect of SABV is the requirement to prospectively develop a research design that, at a minimum, reports data disaggregated by sex without requiring a statistical test evaluating sex as an experimental variable (NIH Guide Notice NOT-OD-15-102). The design of the current study does not differentiate between studies that report data disaggregated by sex with no comparison versus studies that report aggregate sex data with no comparison. Anecdotally, our curators found very few articles that reported data disaggregated by sex but that did not perform or assert to have performed a statistical comparison by sex. Other aspects of SABV may also be relevant to the design of future studies assessing the effect of SABV. These aspects may include the presence of justification for single sex studies, or if both sexes are used whether the experimental design/analysis is sufficiently powered to detect robust sex differences. Importantly, SABV is not the only relevant funding agency policy that may impact sex omission and bias in the neuroscience literature. For example, the Canadian Institutes of Health Research is a signature on the Government of Canada's Health Portfolio Sex- and Gender-Based Analysis Policy and has detailed criteria for how to evaluate sex and gender that differs from that outlined by SABV. Since the exact policy requirements regarding biological sex vary by funding agency, future studies will need to be *a priori* designed to either directly assess specific funding agency policies (and whether these policies even apply to a particular research study), or generally assess sex omission and bias in the neuroscience literature regardless of research article funding source.

One aspect of the current study is that analysis was restricted to research articles using mice and/or rats. Articles using mice and rats were analyzed in the current work for the following four reasons. First, the wide availability of rats and mice concomitant with an abundance of research protocols and external secondary sex characteristics more easily enables the analysis of both male and female animals. Second, rats and mice have many documented sex differences in brain and behavior. Third, to align the findings of the current study with previous work which analyzed mice and/or rats (Mogil and Chanda, 2005; Florez-Vargas et al., 2016; Shansky and Woolley, 2016) and non-human mammals (Beery and Zucker, 2011). Fourth, because a previous study indicated that mice and rats were by far the predominant species reported in neuroscience research articles (Beery and Zucker 2011). Beery and Zucker reported that over 85% of neuroscience research articles employed mice or rats, a much higher percentage than that detected by the current study (~48%; Figure 1A). Three possibilities may contribute to this large difference between studies in the measured proportion of research articles using mice and rats. The first possibility is differences in journal selection. Compared to the current study, Beery and Zucker, 2011, analyzed an overlapping but different suite of journals representing the Neuroscience discipline: Journal of Neuroscience, Neuroscience, The Journal of Comparative Neurology, and Nature Neuroscience. Given that two of these journals were included in the analysis of the current study, we believe that journal selection is not likely a major influence. The second possibility regards article sampling, in that the current study analyzed a much larger number of research articles than Beery and Zucker. The third possibility may be how the percentage of rat and mouse studies was calculated. Beery and Zucker, 2011, used only non-human studies to calculate the percentage of mice and rat studies in the neuroscience literature, while the current study included both non-human and human studies. We favor this last possibility as the most likely explanation. We note that the exclusion of animals other than rats and mice from the current study was not because we consider these species (including humans) unimportant for neuroscience research. Given the finding of this study that the majority of neuroscience research articles involves work in species other than mice and rats (Figure 1A), scientists from both contemporary and earlier generations likely also share this assessment (Beach, 1950; Krebs, 1975; Brenowitz and Zakon, 2015; Remage-Healey et al., 2017). Indeed, our study is the first to detect that sex bias and omission varies across any species of research animal. Based on this critical finding, future studies should address the intersection of species and sex by directly testing whether sex bias and omission vary across research animals beyond mice and rats.

Another novel and central finding of this study was the considerable variability in sex omission across journals. Since our study was not designed to elucidate the etiology of differences in sex omission between journals, it will be an important next step to understand why some journals exhibit relatively low sex omission and others do not. One possibility is differences in culture and practice between neuroscience subfields. A second possibility regards journal adoption and enforcement of relevant editorial policies, which were in flux during the assessed time period. Consistent with this possibility, beginning in 2012, the Journal of Neurophysiology, and more broadly all journals published by the American Physiological Society (Miller, 2012), asked authors to include the sex of research animals, cells, and other biological materials. Journals published by the American Physiological Society also recommend that authors apply the relevant portions of the “Animals in Research: Reporting In Vivo Experiments” (ARRIVE) guidelines (Kilkenny et al., 2010). ARRIVE guidelines cover many aspects of experimental methodology, including biological sex, in an attempt to enhance reproducibility.

The time period of 2013-2014 may prove to be a pivotal point for the reporting of not only animal sex, but other methodological details as well. Building upon earlier workshops such as the “Sex-Specific Reporting of Scientific Research” hosted by the Office of Research on Women's Health of the National Institutes of Health (Wizemann, 2012), in June of 2014, a conference including representatives of the United State National Institute of Health, the American Association for the Advancement of Science, and editors representing more than 30 scientific journals, established the Principles and Guidelines in Reporting Preclinical Research (McNutt, 2014; Nature, 2014; Moher et al., 2015). Dozens of journals have endorsed these guidelines, including the Nature publishing group (which publishes Nature and Nature Neuroscience), Cell Press (which publishes the journal Neuron), Science, and the Journal of Neuroscience and eNeuro. Interestingly, the Journal of Neuroscience showed substantial decreases in sex omission even before the convening of the workshop that resulted in the NIH Principles and Guidelines in Reporting Preclinical Research (Figure 5A). This may reflect internal editorial policy, enforcement, and methods presentation. Given that the Journal of Neuroscience has one of the lowest rates of sex omission during the assessed time period, even when compared to other journals that successfully decreased sex omission, suggests that the mechanisms by which editorial policies are enforced by an individual journal plays an important role. Studies of the effectiveness of the ARRIVE and other guidelines seem to support this speculation (Smidt et al., 2006; Moher et al., 2010; Turner et al., 2012; Baker et al., 2014; Sekula et al., 2017). Thus, the effectiveness of different enforcement techniques across journals should be directly assessed by future studies, especially comparing journals that mandate the inclusion of sex in both the title and methods of manuscripts (Blaustein, 2012), journals that include animal sex is reported on author, reviewer or editor checklists (Han et al., 2017), journals with statements in the author guidelines, and journals with no relevant policies at all. A significant challenge of understanding the etiology of differences between journals is the temporal lag between the implementation of journal policies and its effects on individual research articles. Given the lengthy time required for manuscript preparation, peer review and manuscript revision, it may take months or perhaps years for manifestation of changes at the level of editorial or granting agency policy to be reflected in individual research articles. Nevertheless, future studies should continue to monitor sex omission, sex bias, and potentially other critical experimental details across years. This would allow for the evaluation of relevant scientific journal policies, but also to help remove the potential barriers to scientific reproducibility generated by erratic reporting of animal sex. This will be particularly important given the emerging recognition that sex can play a significant and complex role in influencing specific neural substrates.

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Figure Legends

Figure 1. Articles using mice and rats are a significant and stable proportion of the neuroscience literature. A) From 2010-2014, 13,857 neuroscience research articles were published by the Journal of Neuroscience, Journal of Neurophysiology, Nature Neuroscience, Neuron, Science and Nature (grey bar). Of these articles, 6,636 used rats or mice, and were further analyzed (purple bar). The total number of articles using mice and rats was consistently distributed across years. B) The percentage of articles using rats or mice remained fairly constant across years.

Figure 2. Sex omission is decreasing but sex bias remains present, with different patterns observed in articles using mice versus those using rats. Articles were categorized as either not reporting sex (orange), reporting both males and females (red), only males (green), and only females (blue). A) All articles, using both Mice and Rats. Articles not reporting animal sex decreased 2010-2014. Articles using only male animals increased 2010-2014, comprising the largest proportion of articles by 2011. Articles reporting the use of both male and female animals also increased over time, nearing but not overtaking the percentage of articles using only males by 2013.Articles using only female animals remained stable and low. B) Mice. Articles not reporting mice sex decreased 2010-2014. Articles reporting the use of both male and female mice increased over time, and comprised the largest proportion of articles by 2012. Articles using only male mice increased 2010-2014. Articles using only female mice remained stable and low. C) Rats. Articles not reporting rat sex decreased 2010-2014. Article using only male rats increased 2010-2014, and comprised the largest proportion of articles by 2011. Articles reporting the use of both male and female rats increased 2010-2014, but were a much smaller proportion of the dataset than articles using only male rats. Articles using only female rats remained stable and low.

Figure 3. The vast majority of articles using both male and female animals do not report analyzing sex as an experimental variable. Articles using both male and female animals were evaluated for any formal statistical test or statement that data from males and females were compared, regardless of outcome and whether or not data were reported. The overall percentage of articles incorporating sex as an experimental variable remained low and relatively stable from 2011-2014 (~14%), after a noticeable decrease from the year 2010 (25%).

Figure 4. Sex omission and bias differ by journal and change from 2010 to 2014. Articles were analyzed from the following journals: Journal of Neuroscience, Journal of Neurophysiology, Nature Neuroscience, Neuron, Science and Nature. Four of the six journals showed large decreases in sex omission. Of this group, Neuron showed the smallest decrease, beginning with 69% of articles not reporting sex in 2010, decreasing to 55% in 2014. In contrast, one journal, Science, showed an increase in the percentage of articles not reporting sex, rising from 51% in 2010 to 58% in 2014.

Figure 5. Patterns of sex omission and bias markedly differ across years by journal. A) Articles not reporting sex. The percentage of articles not reporting sex decreased in 5 of 6 journals. The percentage of articles not reporting sex increased in the journal Science. The journals Science and Neuron showed high percentages of articles not reporting sex. B) Articles reporting both males and females. Most journals show increased percentages of articles reporting both males and females, although different patterns occur across time. C) Articles reporting only males. D) Articles reporting only females. The percentage of articles reporting the sole use of female animals remained stable and low in all journals. Legend: Journal of Neuroscience (green), Journal of Neurophysiology (black), Nature Neuroscience (blue), Neuron (red), Science (orange) and Nature (purple).

Tables

Table 1. Details of Statistical Analysis

Figure	Data Structure	Type of Test	Confidence Intervals
1B	Normal Distribution	Linear Regression	-4.389 to 3.178
2A	Normal Distribution	Linear Regression	Male Only: 0.8086 to 3.575; Female Only: -0.5272 to 1.069; Male and Female: 1.277 to 8.273; Unspecified Sex: -12.64 to -1.834
2B	Normal Distribution	Linear Regression	Male Only: 1.105 to 2.797; Female Only: -0.5271 to 1.073; Male and Female: 2.294 to 8.554; Unspecified Sex: -12.16 to -3.138
2C	Normal Distribution	Linear Regression	Male Only: 1.197 to 6.233; Female Only: -0.1067 to 0.8067; Male and Female: -1.040 to 7.242; Unspecified Sex: -13.97 to -0.3654
3	Normal Distribution	Linear Regression	-6.877 to 2.767
5A	Normal Distribution	Linear Regression, ANCOVA	J. Neurophysiology: -10.05 to -1.779; J. Neuroscience: -16.76 to 0.5671; Nature: -18.14 to -8.113; Nature Neuroscience: -21.17 to -1.192; Neuron: -4.831 to -1.845; Science: -8.771 to 12.49
5B	Normal Distribution	Linear Regression, ANCOVA	J. Neurophysiology: 0.03390 to 5.726; J. Neuroscience: -0.8596 to 11.99; Nature: 1.781 to 22.73; Nature Neuroscience: -1.290 to 9.552; Neuron: -0.5016 to 5.360; Science: -8.859 to 9.249
5C	Normal Distribution	Linear Regression, ANCOVA	J. Neurophysiology: -0.4495 to 6.815; J. Neuroscience: 0.3678 to 3.688; Nature: -5.311 to 7.824; Nature Neuroscience: -

			0.6518 to 13.34; Neuron: -1.601 to 3.571; Science: -11.46 to 8.715
5D	Normal Distribution	Linear Regression, ANCOVA	J. Neurophysiology: -0.5273 to 0.2373; J. Neuroscience: -2.637 to 1.863; Nature: -2.637 to 1.862; Nature Neuroscience: -1.188 to 2.426; Neuron: -2.402 to 2.252; Science: -4.550 to 3.180

Confidence intervals for linear regressions indicate the 95% confidence interval surrounding the slope. R^2 and other relevant statistics are reported in the Results section. Acronyms: J: Journal; ANCOVA: Analysis of Covariance.

Figure 1

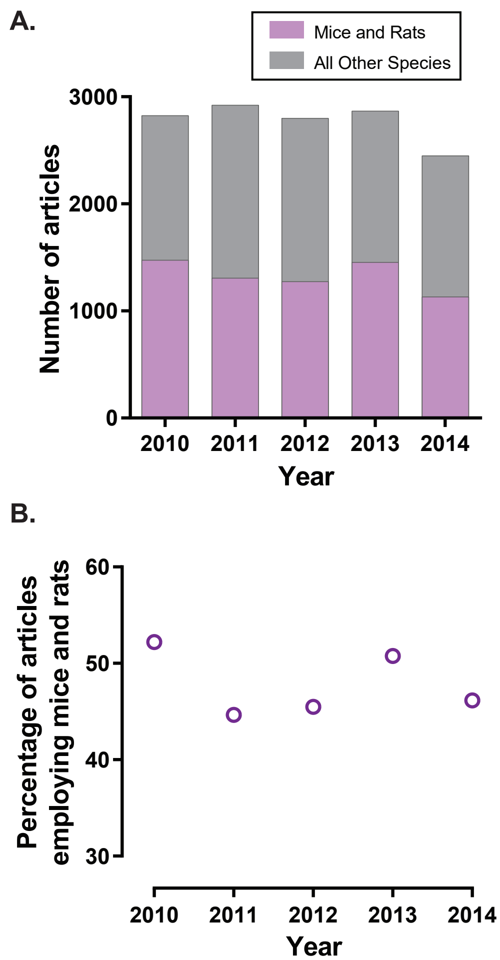

A.
Mice and Rats
All Other Species
Number of articles
3000
2000
1000
0
2010
2011
2012
2013
2014
Year
B.
Percentage of articles employing mice and rats
60
50
40
30
2010
2011
2012
2013
2014
Year

Figure 2

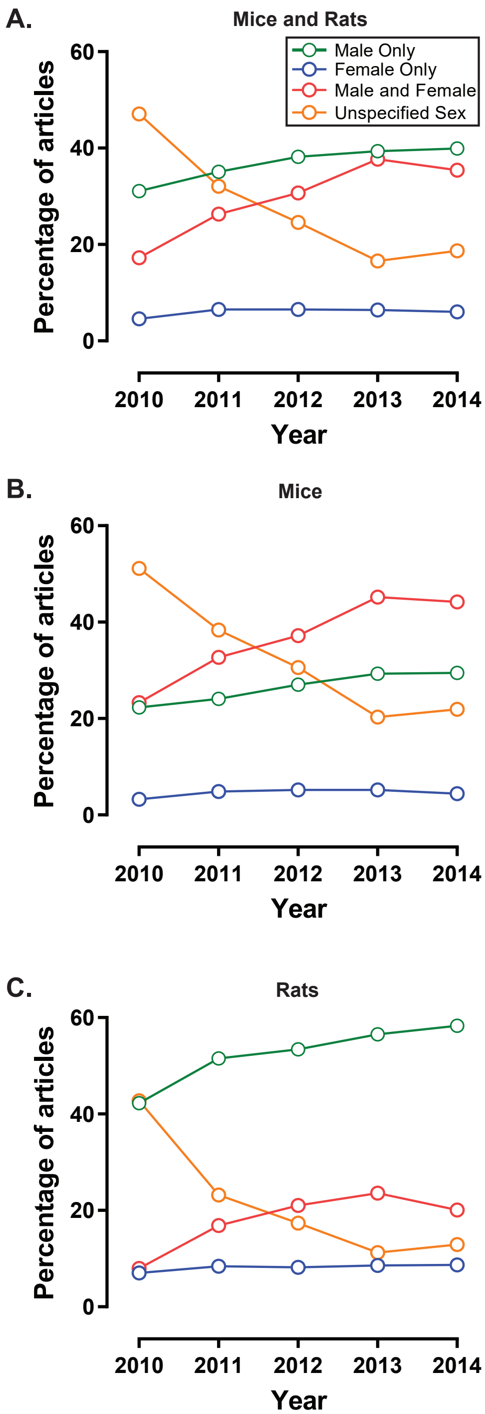
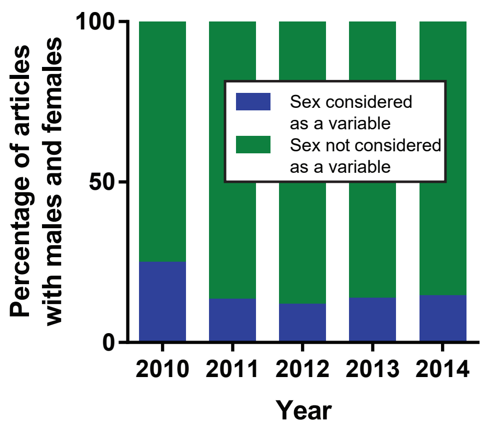

A.
Mice and Rats
Male Only
Female Only
Male and Female
Unspecified Sex
Percentage of articles
0
20
40
60
2010
2011
2012
2013
2014
Year
B.
Mice
Percentage of articles
Year
C.
Rats
Percentage of articles
Year

Figure 3



100
50
0
Percentage of articles
with males and females
Sex considered
as a variable
Sex not considered
as a variable
2010
2011
2012
2013
2014
Year

Figure 4

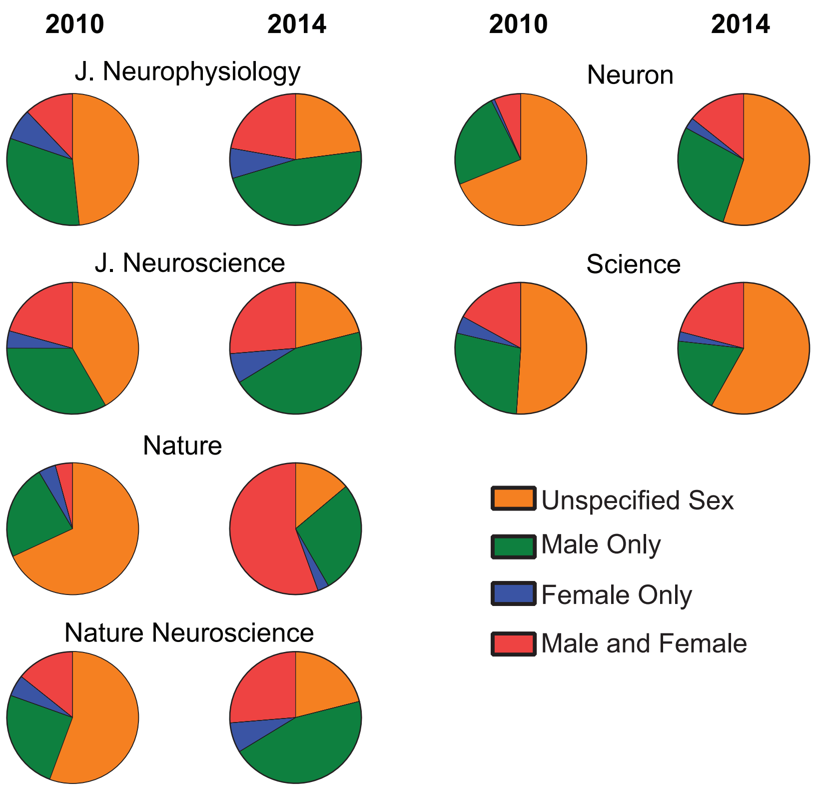
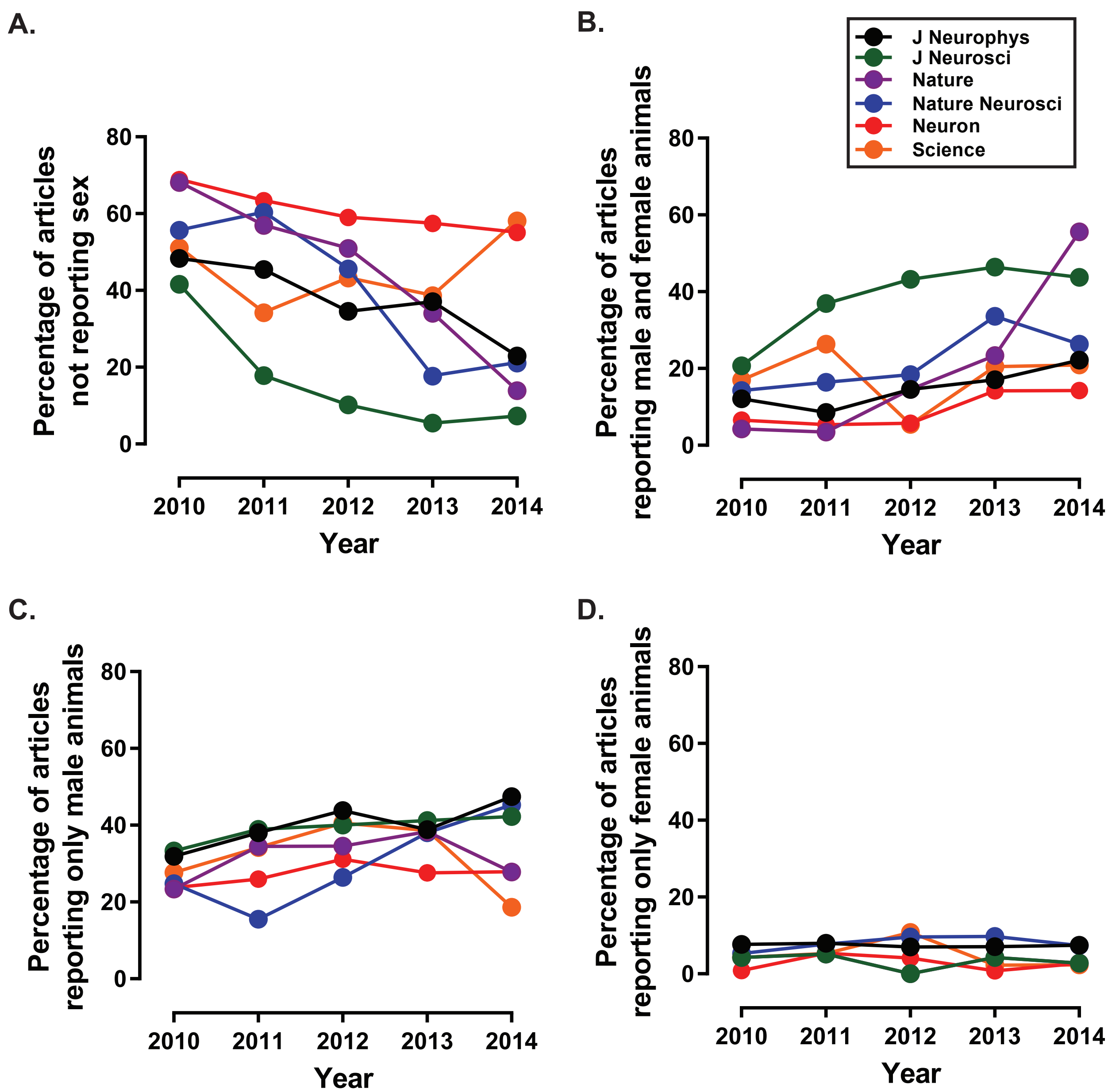

2010
2014
2010
2014
J. Neurophysiology
Neuron
J. Neuroscience
Science
Nature
Nature Neuroscience
Unspecified Sex
Male Only
Female Only
Male and Female

Figure 5


A.
B.
C.
D.
J Neurophys
J Neurosci
Nature
Nature Neurosci
Neuron
Science
Percentage of articles not reporting sex
Percentage of articles reporting male and female animals
Percentage of articles reporting only male animals
Percentage of articles reporting only female animals
0
20
40
60
80
2010
2011
2012
2013
2014
Year