# Reporting and Misreporting of Sex Differences in the Biological Sciences

Yesenia Garcia-Sifuentes[1] and Donna L. Maney[1, 2]

[1]Graduate Program in Neuroscience and [2]Department of Psychology, Emory University

*Abstract*

As part of an initiative to improve rigor and reproducibility in biomedical research, the U. S. National Institutes of Health now requires the consideration of sex as a biological variable in preclinical studies. This new policy has been interpreted by some as a call to compare males and females with each other. Researchers testing for sex differences may not be trained to do so, however, increasing risk for misinterpretation of results. Using a list of recently published articles curated by Woitowich et al. (eLife, 2020; 9:e56344), we examined reports of sex differences and non-differences across nine biological disciplines. Sex differences were claimed in the majority of the 147 articles we analyzed; however, statistical evidence supporting those differences was often missing. For example, when a sex-specific effect of a manipulation was claimed, authors usually had not tested statistically whether females and males responded differently. Thus, sex-specific effects may be over-reported. In contrast, we also encountered practices that could mask sex differences, such as pooling the sexes without first testing for a difference. Our findings support the need for continuing efforts to train researchers how to test for and report sex differences in order to promote rigor and reproducibility in biomedical research.

## *Introduction*

Historically, biomedical research has not considered sex as a biological variable (SABV). Including only one sex in preclinical studies—or not reporting sex at all—is a widespread issue (Sugimoto et al., 2019). In a cross-disciplinary, quantitative assessment of the 2009 biomedical literature, Beery and Zucker (2011) found a concerning bias toward the use of males only. As awareness of this issue increased, in 2016 the National Institutes of Health (NIH) implemented a policy requiring consideration of SABV in the design, analysis, and reporting of all NIH-funded preclinical research (NIH, 2015; Clayton, 2018). By addressing the long-standing over-representation of male non-human animals and cells, the policy was intended not only to ameliorate health inequities but to improve rigor and reproducibility in biomedical research (Clayton & Collins, 2014).

Although the NIH policy does not explicitly require that males and females be compared directly with each other, the fact that more NIH-funded researchers must now study both sexes should lead to an increase in the frequency of such comparisons (Maney, 2016). For example, there should be more testing for sex-specific responses to experimental treatments. However, in a follow-up to Beery and Zucker's 2011 study, Woitowich et al. (2020) showed evidence to the contrary. Their analysis revealed that between 2011 and 2019, although the proportion of articles that included both sexes significantly increased (see also Will et al., 2017), the number that analyzed the data by sex did not. This finding contrasts sharply with expectations, given not only the NIH mandate but also numerous calls over the past decade to disaggregate all preclinical data by sex and to test for sex differences (e.g., Becker et al., 2016; Potluri et al., 2017; Shansky & Murphy, 2021; Tannenbaum, 2019; Woitowich & Woodruff, 2019).

One potential barrier to SABV implementation is a lack of relevant resources; for example, not all researchers have received training in experimental design and data analysis that would allow them to test for sex differences using appropriate statistical approaches. This barrier is quite important not only because it prevents rigorous consideration of sex in the first

48 place, but also because any less-than-rigorous test for sex differences creates risk for
49 misinterpretation of results and dissemination of misinformation to other scientists and to the
50 public (Maney, 2016). In other words, simply calling for the sexes to be compared is not enough
51 if researchers are not trained to do so; if SABV is implemented haphazardly, it has the potential
52 to decrease, rather than increase, rigor and reproducibility.

53 In this study, our goal was to analyze recently published articles to determine how often
54 sex differences are being reported and what statistical evidence is most often used to support
55 findings of difference. To conduct this assessment, we leveraged the collection of articles
56 originally curated by Woitowich et al. (2020) for their analysis of the extent to which SABV is
57 being implemented. Their original list, which was itself generated using criteria developed by
58 Beery & Zucker (2009), included 720 articles published in 2019 across nine biological
59 disciplines and 34 scholarly journals. Of those, Woitowich et al. identified 151 articles that
60 included females and males and that analyzed data disaggregated by sex or with sex as fixed
61 factor or covariate. Working with that list of 151 articles, we asked the following questions for
62 each: First, was a sex difference reported? If so, what statistical approaches were used to
63 support the claim? We focused in particular on studies with factorial designs in which the
64 authors reported that the effect of one factor, for example treatment, depended on sex. Next, we
65 asked whether data from males and females were kept separate throughout the article, and if
66 they were pooled, whether the authors tested for a sex difference before pooling. Finally, we
67 noted whether the authors used the term “sex” or “gender”, particularly in the context of
68 preclinical (non-human animal) studies.
69
70 ***Results***
71 We began with 151 articles, published in 2019, that were determined by Woitowich et al.
72 to have (1) included both males and females and (2) reported data by sex (disaggregated or
73 with sex included in the statistical model). Of those, we identified four that contained data from

74 only one sex (e.g., animals of the other sex had been used only as stimulus animals or to
75 calculate sex ratios). After excluding those articles, our final sample size was 147. See Table 1
76 for the sample sizes of articles from each discipline. More than one-third of the studies were on
77 humans (35%) and a similarly large proportion on rats or mice (31%). The remainder
78 encompassed a wide variety of species including non-human primates, dogs, cats, pigs, sheep,
79 deer, squirrels, racoons, Tasmanian devils, lemur, lions, meerkats, and mongoose. All codes
80 and results of coding are shown in Tables S1-S3.
81
82 **Table 1.** Journals surveyed by discipline.

| DISCIPLINE | JOURNAL 1 | JOURNAL 2 | JOURNAL 3 | JOURNAL 4 | NO. ARTICLES |
|---|---|---|---|---|---|
| **BEHAVIOR** | Behavioral Ecology & Sociobiology | Animal Behavior | Animal Cognition | Behavioral Ecology | 40 |
| **BEHAVIORAL PHYSIOLOGY** | Journal of Comparative Psychology | Behavioral Neuroscience | Physiology and Behavior | Hormones and Behavior | 20 |
| **ENDOCRINOLOGY** | European Journal of Endocrinology | Journal of Neuroendocrinology | Endocrinology | American Journal of Physiology – Endocrinology & Metabolism | 27 |
| **GENERAL BIOLOGY** | PLoS Biology | Proceedings of the Royal Society B: Biological Sciences | Nature | Science | 9 |
| **IMMUNOLOGY** | Journal of immunology | Infection and Immunity | Immunity | Vaccine | 10 |
| **NEUROSCIENCE** | Journal of Neuroscience | Neuroscience | Journal of Comparative Neurology | Nature Neuroscience | 9 |
| **PHARMACOLOGY** | Neuropsycho-pharmacology | Journal of Psychopharmacology | Journal of Pharmacology and Experimental Therapeutics | British Journal of Pharmacology | 11 |
| **PHYSIOLOGY** | Journal of Physiology (London) | American Journal of Physiology – Renal Physiology | American Journal of Physiology – Gastrointestinal and Liver Physiology | American Journal of Physiology – Heart and Circulatory Physiology | 12 |
| **REPRODUCTION** | Biology of Reproduction | Reproduction | | | 9 |

83
84

*Question 1: Was a sex difference reported?*

Results pertaining to Question 1 are shown in Fig. 1A. Comparing the sexes, either statistically or by assertion, was common, occurring in 80% of the articles. A positive finding of a sex difference was reported in 83 articles, or 56%. Of the articles reporting a sex difference, 41 (49%) mentioned that result in the title or the abstract. Thus, in our sample of articles in which data were reported by sex, a sex difference was reported in more than half of the articles and in half of those, the difference was treated as a major finding. In 44% of articles, a sex difference was neither stated nor implied.

These results are broken down by discipline in Fig. 1B. The sexes were most commonly compared in the field of Endocrinology (93%) and least often in the field of Neuroscience (33%). When sex differences were found in the field of Endocrinology, however, they were reported in the title or abstract only 32% of the time. In the field of Reproduction, the sexes were compared 89% of the time and in 100% of those cases, a sex difference was mentioned in the title or abstract. Sex differences were least likely to be emphasized in the title or abstract in the fields of General Biology and Neuroscience (11% each).

Although a sex difference was claimed in a majority of articles (57%), not all of these differences were supported with statistical evidence. In nearly a third of the articles reporting a sex difference, or 24/83 articles, the sexes were never actually compared statistically. In these cases, the authors claimed that the sexes responded differentially to a treatment when the effect of treatment was not statistically compared across sex. This issue is explored in more detail under *Question 2*, below. Finally, we noted at least five articles in which the authors claimed that there was no sex difference, but did not appear to have tested statistically for one.

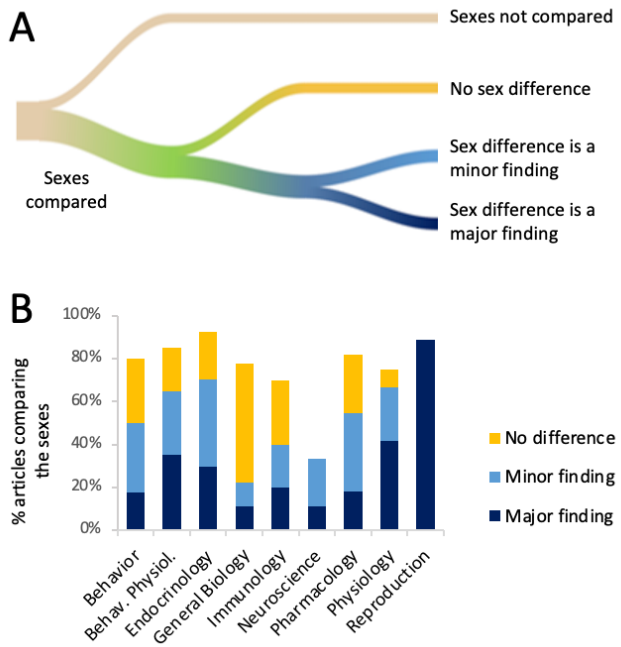
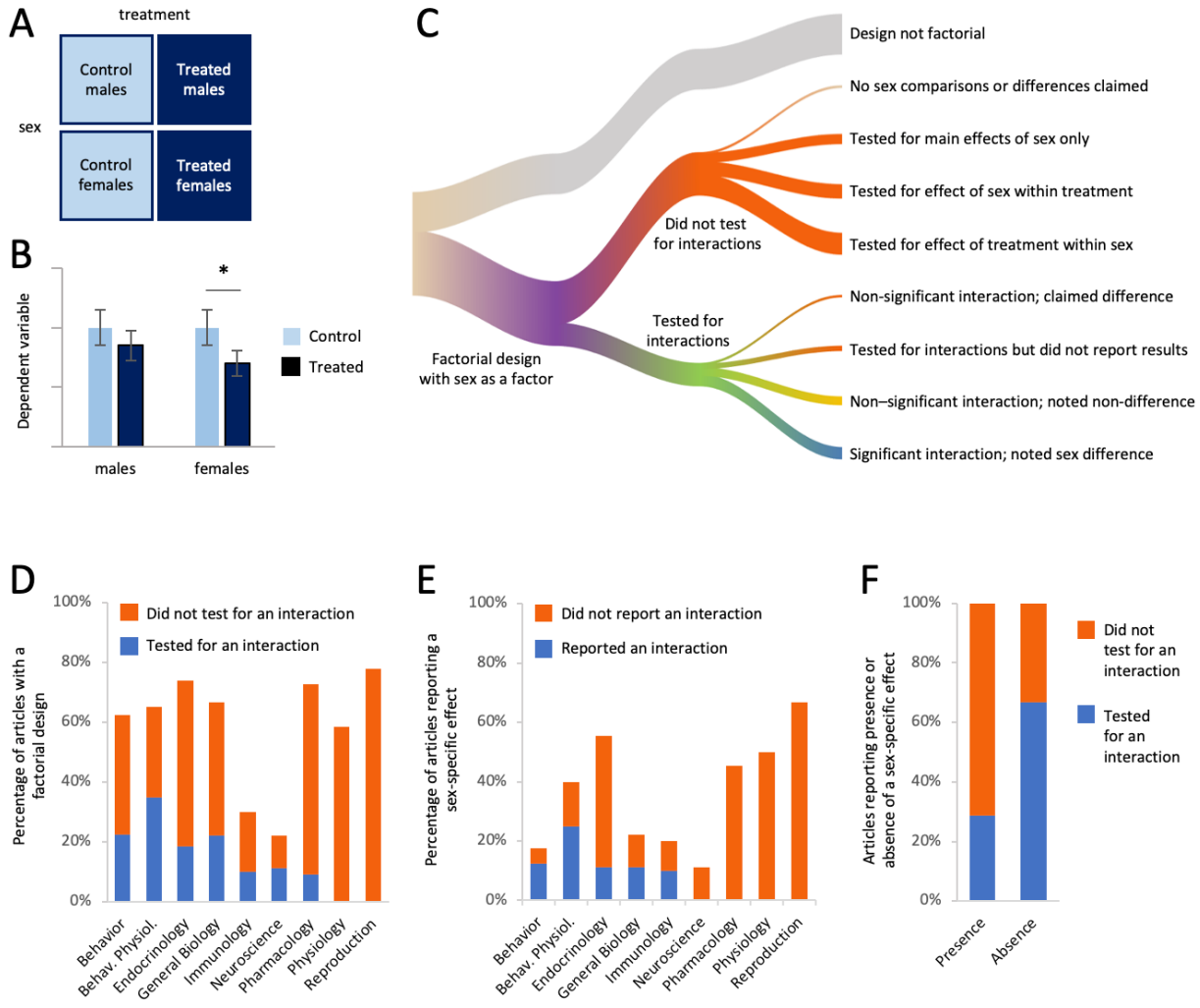



**Fig. 1. The sexes were compared in the majority of the articles analyzed.** (A) The river plot shows the proportions of articles comparing the sexes, either statistically or qualitatively, and the outcomes of those comparisons. The width of each stream is proportional to the number of articles represented in that stream. If a sex difference was mentioned in the title or abstract, the article was coded as "major finding." For a larger river plot showing how (A) fits into the larger context of the study by Woitowich et al. (2020), please see Fig. S1. (B) The percentage of articles in which sexes were compared is plotted for each discipline. All data are shown in Table S3.

*Question 2: Did the study have a factorial design with sex as a factor, and if so, did the authors test statistically whether the effect of other factors depended on sex?*

For each article, we asked whether it contained a study with a factorial design in which sex was one of the factors. This design is common when researchers are interested in testing whether the sexes respond differently to a manipulation such as a drug treatment (Fig 2A). Below, we use the term "treatment" to refer to any non-sex factor in a factorial design. Such factors were not limited to treatment, however; they also included variables such as genotype, season, age, exposure to stimuli, etc. Hypothetical results of a study with such a design are shown in Fig. 2B. In order to draw a conclusion about whether responses to treatment differed between females and males, the effect of the treatment must be compared across sex. Although there are several ways of making such a comparison (see Cumming, 2012; Gelman & Stern, 2006), it is typically done by testing for an interaction between sex and treatment in a two-way analysis of variance (ANOVA). If the interaction is significant, then a claim can be made that the sexes responded differently to the treatment. Comparing the treated and control groups within each sex, in other words disaggregating the data by sex and testing for effects of treatment

123 separately in females and males, does not test whether the sexes responded differently; that is,
124 it does not test whether the magnitude of the response differs between females and males
125 (Gelman & Stern, 2006; Makin & de Xivry, 2019; Maney, 2016; Nieuwenhuis et al., 2011; Radke
126 et al., 2021).




127

**Fig. 2. Factorial designs and sex-specific effects.** For each article, we noted whether it contained a study with a factorial design with sex as a factor (A), for example males and females nested inside treated and control groups. (B) In this hypothetical dataset, there was a significant effect of treatment only in females. Some authors would claim that the treatment had a "sex-specific" effect without testing statistically whether the response to treatment depended on sex. In this example, it does not (see Maney, 2016; Nieuwenhuis et al., 2011). (C) The river plot shows the proportion of articles with a factorial design and the analysis strategy for those. The width of each stream is proportional to the number of articles represented in that stream. (D) The percentage of articles with a factorial design is plotted for each discipline. Only a minority tested for an interaction. (E) The percentage of articles reporting a sex-specific effect is plotted for each discipline. Only a minority reported a significant interaction. (F) Testing for an interaction was less common in articles claiming the presence of a sex-specific effect than in articles claiming the absence of such an effect.

128

The results pertaining to Question 2 are shown in Fig 2C-2F. Out of the 147 articles we analyzed, 91 (62%) contained at least one study with a factorial design in which sex was a factor (Fig. 2C). Regardless of whether a sex difference was claimed, we found that the authors explicitly tested for interactions between sex and other factors in only 26 of the 91 articles (29%). Testing for interactions varied by discipline (Fig. 2D). Authors were most likely to test for and report the results of interactions in the field of Behavioral Physiology (54% of relevant articles) and least likely in the fields of Physiology (0%) and Reproduction (0%).

Of the studies with a factorial design, 58% reported that the sexes responded differently to one or more other factors. The language used to state these conclusions often included the phrase “sex difference” but could also include “sex-specific effect” or that a treatment had an effect “in males but not females” or vice-versa. Of the 52 articles containing such conclusions, the authors presented statistics showing a significant interaction, in other words appropriate evidence that females and males responded differently, in only 15 (29%). In one of those articles, the authors presented statistical evidence that the interaction was non-significant, yet claimed a sex-specific effect nonetheless. In an additional five articles, the authors mentioned testing for interactions but presented no results or statistics (e.g., *p* values) for those interactions. In the remainder of articles containing claims of sex-specific effects, the authors took one of two approaches; neither approach included a two-way ANOVA. Instead, authors proceeded to what would normally be the post-hoc tests conducted after finding a significant interaction in the ANOVA. In 24 articles (46% of articles with claims of sex-specific effects) authors reported the effect of treatment within each sex and, reaching different conclusions for each sex (e.g., finding a *p* value below 0.05 in one sex but not the other), inappropriately argued that the response to treatment differed between females and males (see Fig. 2B). In seven other articles claiming a sex-specific effect (14%), the sexes were compared within treatment; for example authors compared the treated males with the treated females, not considering the control animals. Neither approach tests whether the treatment had different effects in females

and males. Thus, a substantial majority of articles containing claims of sex-specific effects (71%) did not present statistical evidence to support those claims; in the majority of those articles (24/37), the sexes were never compared statistically at all.

The prevalence of reporting sex-specific effects varied by discipline (Fig. 2E). Articles in the field of Reproduction were most likely to contain claims of sex-specific effects (67%) and in Neuroscience least likely (10%). Such claims were most likely to be backed up with statistical evidence in the field of Behavioral Physiology (63%). None of the articles in the fields of Reproduction, Physiology, Pharmacology, or Neuroscience contained statistical evidence for the sex-specific effects that were claimed.

The omission of tests for interactions was related to whether researchers were claiming sex differences or not. Among the articles that were missing tests for interactions and yet contained conclusions about the presence or absence of sex-specific effects (40 articles), those claims were in favor of sex differences 88% of the time, compared with only 12% claiming that the responses in females and males were similar. Of all of the articles claiming similar responses to treatment, authors tested for interactions in the majority of cases (68%; Fig. 2F).

*Question 3: Were the data from males and females pooled for any of the analyses?*

In this study we included only articles in which data were reported by sex as previously determined by Woitowich et al. (2020). Thus, any articles in which the sexes were pooled for all analyses were not included here. We assigned each of the 147 articles to one of three categories, as follows (Fig. 3A). In 34 (23%) of the articles, data from males and females were analyzed separately throughout. In 60 (41%) of the articles, males and females were analyzed in the same statistical models, but in those cases sex was included as a fixed factor or a covariate. In most cases when sex was a covariate, authors reported the results of the effect of sex rather than simply controlling for sex. In the remaining 53 (36%) articles, the sexes were pooled for at least some of the analyses.

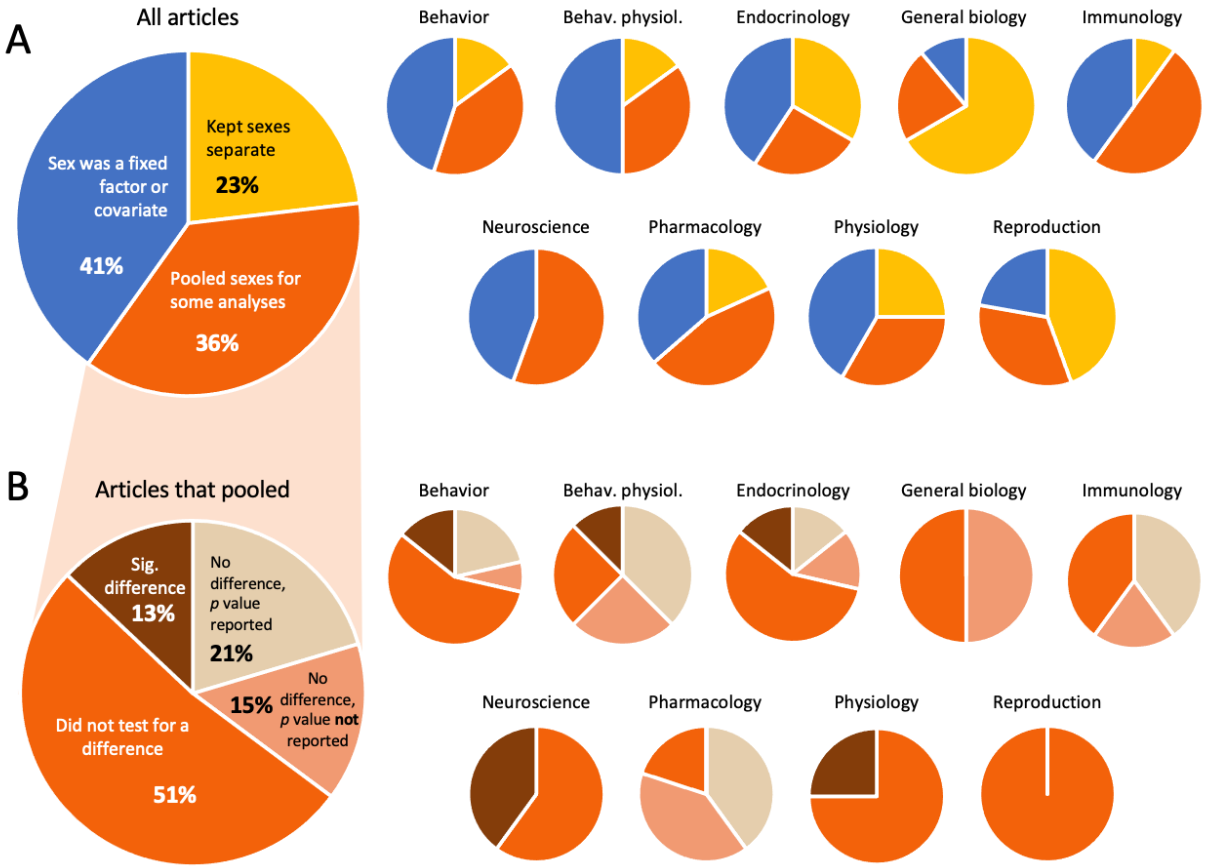



**Fig. 3. Proportion of articles in which the sexes were pooled**. (A) In our sample, roughly one-third of the articles pooled the sexes for at least some analyses. (B) Among the articles that pooled, more than half did not test for a sex difference before pooling. In both (A) and (B), the smaller pie charts to show the proportions within discipline.

Among the articles in which the sexes were pooled, the authors did so without testing for a sex difference more than half of the time (51%; Fig. 3B). When authors did test for a sex difference before pooling, they sometimes found a significant difference yet pooled the sexes anyway; this occurred in 13% of the articles that pooled. When the sexes were pooled after finding no significant difference (36% of the articles that pooled), authors presented *p* values for the sex difference about two-thirds of the time (11 out of 18 articles). Those *p* values ranged from 0.15 to >0.999. Effect sizes were never reported before pooling.

Across disciplines, pooling was most prevalent in Neuroscience (56%) and least prevalent in General Biology (22%). Males and females were most likely to be kept separate in General Biology (67%) and most likely to be included in statistical models in the field of Behavior (50%). When females and males were pooled, authors in the field of Reproduction were least likely to have tested for a sex difference before pooling (0%) and most likely to do so in Pharmacology (80%). Pooling after finding a significant difference was most common in the field of Neuroscience (40% of articles that pooled).

*Question 4: Was the term "gender" used for non-human animals?*

To refer to the categorical variable comprising male/female or man/woman (all were binary), the term "sex" was used exclusively in 69% of the articles (Fig. 4). "Gender" was used exclusively in 9%, and both "sex" and "gender" were used in 19%. When both terms were used, they usually seemed to be used interchangeably. In 4% of the articles, neither term was used.

Of the articles in which the term "gender" was used, 20% of the time it referred to non-human animals, such as mice, rats, and pigs. In one case, both "sex" and "gender" were used to refer to non-human animals in the title. In another case, "gender" was used to refer to human cells.

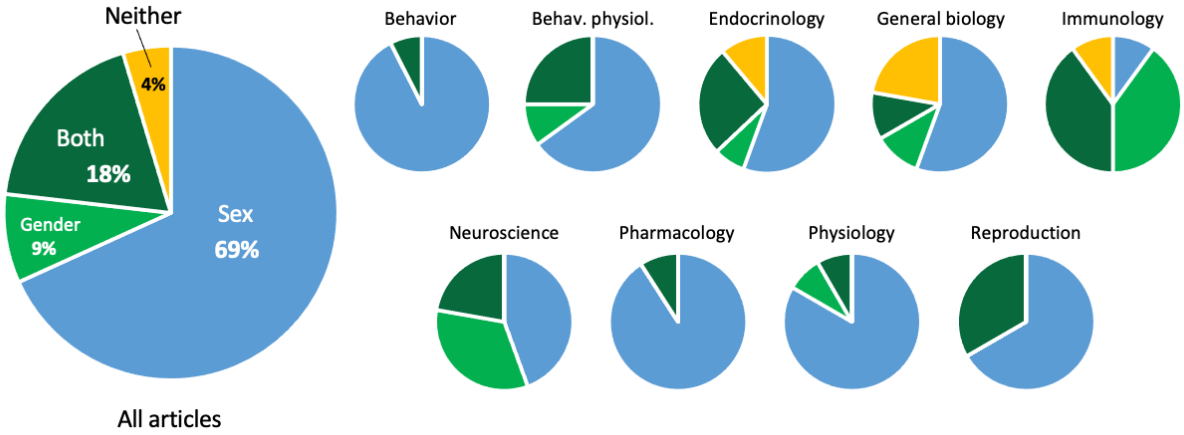



**Fig. 4. Proportions of articles using the terms "sex" and "gender".** The smaller pie charts show the proportions within discipline.

## *Discussion*

### *Finding sex differences*

Woitowich et al. (2020) found that over the past decade, the proportion of biological studies that included both females and males has increased, but the proportion reporting data by sex has not. Here, we have taken a closer look at the studies determined by those authors to have reported data by sex, that is, to have conformed to NIH guidelines on SABV. We found that in this subset of studies, authors typically also compared the sexes either statistically or qualitatively (80% of cases). Thus, the authors that complied with NIH guidelines to disaggregate data usually went *beyond* NIH guidelines to explicitly compare the sexes with each other. This finding is consistent with a larger analysis of articles in the field of Neuroscience from 2010 to 2014; when authors disaggregated data by sex, they usually proceeded to compare the sexes as well (Will et al., 2017). It is important to note, however, that both Will et al. (2017) and Woitowich et al. (2020) found that data were not analyzed by sex in the majority of articles that included both sexes (see Fig. S1). Thus, our current finding that the sexes were usually compared should be interpreted in the context of the subset of articles following NIH guidelines. In the set of articles analyzed here, sex differences were claimed in a majority and were often highlighted in the title or abstract. We therefore found little evidence that researchers—at least those who comply with NIH guidelines—are uninterested in sex differences. Conversely, our finding could indicate that researchers interested in sex differences are primarily the ones following NIH guidelines.

### *Testing for interactions in a factorial design*

Testing whether the sexes respond differently to a treatment requires statistical comparison between the two effects, which is typically done by testing for a sex × treatment interaction. In our analysis, however, tests for interactions were missing 71% of the time (Fig. 2C, D). In these cases, the most common method for detecting differential effects of treatment

was to compare qualitatively the conclusions drawn for each sex; that is, to assert that a *p* value below 0.05 for one sex but not the other (Fig. 2B) represents a meaningful difference between the effects. But null hypothesis significance testing does not allow for such conclusions (Cumming, 2012). This error, and the frequency with which it is made, has been covered in multiple publications; for example Gelman & Stern (2006) titled their commentary "The difference between 'significant' and 'not significant' is not itself statistically significant." Makin & de Xivry (2019) included the error in their "Top ten list of common statistical mistakes". In an analysis of 520 articles in the field of neuroscience, Nieuwenhuis et al. (2011) found that the error was committed in about half of articles containing a factorial design. The current analysis showed that, even a decade later, the frequency of this error in the field of neuroscience has not changed (Fig. 2D), at least when sex is one of the factors under consideration. The frequency of the error was even higher in most of the other disciplines, particularly Physiology and Reproduction, for which we found that authors never tested for interactions.

Statements such as the following, usually made without statistical evidence, were common: "The treatment increased expression of gene X in a sex-dependent manner"; "Our results demonstrate that deletion of gene X produces a male-specific increase in the behavior"; "Our findings indicate that females are more sensitive to the drug than males". In some of these cases, the terms "sex-specific", "sex-dependent" or "sexual dimorphism" were used in the title of the article despite a lack of statistical evidence supporting the claim. In many of these articles, some of which stated that finding a sex difference was the major goal of the study, the sexes were not statistically compared at all. Thus, a lack of statistical evidence for sex-specific effects did not prevent authors from asserting such effects. In fact, we found that authors failing to test for interactions were far more likely to claim sex-specific effects than not (88% vs. 12%; Fig. 2F); they were also more likely to do so than were authors that did test for interactions (88% vs. 62%; Table S3). Together, these results suggest a bias toward finding sex differences. In the absence of evidence, differences were claimed more often than not. A bias toward finding sex

differences, where there are none, could artificially inflate the importance of sex in the reporting of biological data. Given that findings of sex × treatment interactions are rare in the human clinical literature, with false positives outnumbering false negatives (Wallach et al., 2016), and given also that sex differences are often misrepresented to the public (Maney, 2014), it is especially important to base conclusions from preclinical research on solid statistical evidence.

*Pooling across sex*

The set of articles we analyzed was pre-screened by Woitowich (2020) to include only studies in which sex was considered as a variable. Nonetheless, even in this sample, data were often pooled across sex for some of the analyses (Fig. 3A). In a majority of these articles, authors did not test for a sex difference before pooling (Fig. 3B). Thus, for at least some analyses represented here, the data were not disaggregated by sex, sex was not a factor in those analyses, and we do not know whether there might have been a sex difference. Even when authors did test for a sex difference before pooling, the relevant statistics were often not presented. Finding and reporting a significant sex difference did not seem to reduce the likelihood that the sexes would be pooled. Note that the original sample of 720 articles in the study by Woitowich et al. included 251 articles in which sex was either not specified or the sexes were pooled for all analyses (Fig. S1). Thus, the issue is more widespread than could be represented in the current study. Pooling is not consistent with the NIH mandate to disaggregate data by sex, and can prevent detection of meaningful differences. We note further that effect sizes were not reported before pooling; in addition to *p* values, effect sizes would be valuable for any assessment of whether data from males and females can be pooled without masking a potentially important difference (Beltz et al., 2019; Diester et al., 2019).

*Correcting for multiple comparisons*

In their article on "Ten statistical mistakes…," Makin and de Xivry (2019) list another issue that we found to be prevalent, although we did not collect systematic data on it. Many authors compared a large number of dependent variables across sex without correcting for multiple comparisons. The omission of the correction increases the risk of false positives, that is, making a type I error, which would result in over-reporting of significant effects. This problem is particularly important for researchers trying to comply with SABV, who may feel compelled to test for sex differences in every measured variable. For example, we noted articles in which researchers measured expression of multiple genes in multiple tissues at multiple time points, resulting in a large number of comparisons across sex. In one such study, authors made 90 separate comparisons in the same set of animals and found 5 significant differences, which is exactly the number one would expect to find by chance. The prevalence of this issue is difficult to estimate because opinions vary about when corrections are necessary; nonetheless, omission of such corrections, when they are clearly needed, is likely contributing to over-reporting of sex differences broadly across disciplines.

*Usage of "sex" and "gender"*

We found that a large majority of studies on non-human animals used "sex" to refer to the categorical variable comprising females and males (Fig. 4). In eight articles, we noted usage of the word "gender" for non-human animals. This usage appears to conflict with current recommendations regarding usage of "gender", that is, gender should refer to socially constructed identities or behaviors rather than biological attributes (Clayton & Tannenbaum, 2016; Holmes & Monks, 2019; Woitowich & Woodruff, 2019). We did not, however, investigate the authors' intended meaning of either term. Although definitions of "gender" vary, the term might be appropriate for non-human animals under certain circumstances, such as when the influence of social interactions is a main point of interest (Cortes et al., 2019). Operational

definitions, even for the term “sex”, are important and, in our experience conducting this study, almost never included in publications. As others have done (e.g., Duchesne et al., 2020; Cortes et al., 2019; Holmes & Monks, 2019; Johnson et al., 2009), we emphasize the importance of clear operational definitions while recognizing the limitations of binary categories.

*Limitations of this study*

This study was underpowered for examining these issues within any particular discipline. For most disciplines, fewer than a dozen articles were in our starting sample; for Neuroscience and Reproduction, only nine. As a result, after we coded the articles, some categories contained few or no articles in a given discipline (see Table S3). The within-discipline analyses, particularly the pie charts in Fig. 3B, should therefore be interpreted with caution. Firm conclusions about whether a particular practice is more prevalent in one discipline than another cannot be drawn from the data presented here.

As is the case for any analysis, qualitative or otherwise, our coding was based on our interpretation of the data presentation and wording in the articles. Details of the statistical approach were sometimes left out, leaving the author’s intentions ambiguous. Although our approach was as systematic as possible, a small number of articles may have been coded in a way that did not completely capture those intentions. We believe our sample size, particularly in the overall analyses across disciplines, was sufficient to reveal the important trends.

*Conclusion*

SABV has been hailed as a game-changing policy that is already bringing previously ignored sex-specific factors to light, particularly for females. In this study, we have shown that a substantial proportion of claimed sex differences, particularly sex-specific effects of experimental manipulations, are not supported by statistical evidence. Although only a minority of studies that include both sexes actually report data by sex (Woitowich et al., 2020), our

findings suggest that when data *are* reported by sex, critical statistical analyses are often missing and the findings likely to be interpreted in misleading ways. Note that in most cases, our findings do *not* indicate that the conclusions were incorrect; they may have been supported by appropriate statistical analyses. Our results emphasize the need for resources and training, particularly those relevant to the study designs and analyses that are commonly used to test for sex differences. Such training would benefit not only the researchers doing the work, but also the peer reviewers, journal editors, and program officers who have the power to hold researchers to a higher standard. Without better awareness of what can and cannot be concluded from separate analysis of males and females, SABV may have the undesired effect of reducing, rather than enhancing, rigor and reproducibility.

*Materials and Methods*

We conducted our analysis using journal articles from a list published by Woitowich et al. (2020). In their study, which was itself based on a study by Beery and Zucker (2011), the authors selected 720 articles from 34 journals in nine biological disciplines. Each discipline was represented by four journals, with the exception of Reproduction, which was represented by two (Table 1). To be included, articles needed to be primary research articles not part of a special issue, describe studies conducted on mammals, and be published in English. For each journal, Woitowich et al. selected the first 20 articles meeting these criteria published in 2019 (40 articles for Reproduction). For most disciplines, all articles were published between January and April, 2019; for others, articles could have been published as late as June, August, or October for Endocrinology, Behavioral Physiology, and Behavior, respectively.

Woitowich et al. (2020) coded each article with respect to whether it contained data analyzed by sex, defined as either that the sexes were kept separate throughout the analysis or that sex was included as a fixed factor or covariate. Of the original 720 articles analyzed, 151 met this criterion. We began our study with this list of 151 articles. Four articles were excluded

because they contained data from only one sex, with animals of the other sex used as stimulus animals or to calculate sex ratios.

All articles were initially scanned by the first author (YGS) to ascertain the experimental designs in each, and a subset of the articles was discussed between the authors to develop an analysis strategy. All articles were then coded by the second author (DLM). The final strategy consisted of four decision trees (Table S1) used to assign articles to hierarchical categories pertaining to each of four central questions (see below). Each article was assigned to only one final category per question (Table S2). A subset of the articles was independently coded by YGS and any discrepancies discussed between the authors until agreement was reached.

*Question 1: Was a sex difference reported?* Because we were interested in the frequency with which sex differences were found, we first identified articles in which the sexes were explicitly compared. We counted as a comparison any of the following: (1) sex was a fixed factor in a statistical model; (2) sex was included as a covariate in a statistical model *and* a p value for the effect of sex was reported; (3) a *p* value for a comparison of means between males and females was presented; (4) the article contained wording suggestive of a comparison, e.g. “males were larger than females”. We also included articles with wording suggestive of a sex difference in response to a treatment, for example “the treatment affected males *but not* females” or “the males responded to treatment, *whereas* the females did not”, or “the treatment had a *sex-specific* effect”. Similarly, we included here articles with language referring to a non-difference, for example “we detected no sex differences in size” or “the response to treatment was similar in males and females.” Articles in which sex was included as a covariate for the purposes of controlling for sex, rather than comparing the sexes, were not coded as having compared the sexes (see Beltz et al., 2019). When the sexes were compared but no results of those comparisons, e.g., *p* values, were reported, that omission was noted and the article was coded accordingly. Each article in which the sexes were compared was then further coded as

395 either reporting a sex difference or not, and if so, whether a sex difference was mentioned in the
396 title or abstract.

397 *Question 2: Did the article contain a study with a factorial design?* We looked for studies
398 with a 2X2 factorial design (Fig. 2A) in which sex was one of the factors. Sex did not need to be
399 explicitly identified as a fixed factor; we included here all studies comparing across levels of one
400 factor that comprised females and males with each of those levels. In some cases that factor
401 was a manipulation, such as a drug treatment or a gene knockout; these factors also included
402 variables such as age, season, presentation of a stimulus, etc. For simplicity, we refer in this
403 article to the other factor as "treatment". Any article containing at least one such study was
404 coded as having a factorial design. The other articles were coded as containing no comparisons
405 across sex or as containing group comparisons across sex. The latter category included studies
406 with sex as a covariate of interest in a model such as a multiple regression, if the authors were
407 not making any claims about potential interactions between sex and other variables.

408 For studies with a factorial design, we further coded the authors' strategy of data
409 analysis. First, we noted whether authors tested for an interaction between sex and treatment.
410 We included one study in which the effect of treatment was explicitly compared across sex
411 using a method other than a classic ANOVA (the magnitude of the differences between treated
412 and control groups were compared across sex). If authors tested statistically whether the effect
413 of treatment depended on sex, we noted the outcome of that test and the interpretation. Articles
414 containing no tests for interactions were assigned to one of several sub-categories in the
415 following order (coded as the first category on this list for which the description was met for any
416 analysis in the article): tested for effects treatment within sex, tested for effects of sex within at
417 least one level of treatment, or tested for main effects of sex only. Within each of those
418 categories we further coded the outcome/interpretation, e.g., sex difference or no sex
419 difference. Any articles containing statements that the sexes responded differently to treatment
420 or that the response was "sex-specific" were coded as reporting a sex-specific effect. We also

noted when authors reported an absence of such a result. Articles not comparing across sex at all, with statistical evidence or by assertion, were coded accordingly.

*Question 3: Did the authors pool males and females?* We assigned articles to one of three categories: analyzed males and females separately throughout, included sex in the statistical model for at least some analyses (with the rest analyzed separately), or pooled for at least some analyses. The second category, included sex in the model, included articles in which AIC or similar statistic was used to choose among models that included sex, although sex may not have been in the model ultimately chosen. This category did not distinguish between analyses including sex as a fixed factor vs. a covariate; this distinction is noted where relevant in Table S2. Any article containing pooled data was coded as pooled, even if some analyses were conducted separately or with sex in the model. For articles that pooled, we further noted whether the authors tested for a sex difference before pooling and, if so, whether *p* values or effect sizes were reported.

*Question 4: Did the authors use the term "sex" or "gender"?* We searched the articles for the terms "sex" and "gender" and noted whether the authors used one or the other, both, or neither. Terms such as "sex hormones" or "gender role", which did not refer to sex/gender variables in the study, were excluded from this assessment. For the articles using "gender" we further noted when the term was used for non-human animals.

To visualize the data, we used river plots (Weiner, 2017), stacked bar graphs, and pie charts based on formulae and data presented in Table S3.

*Acknowledgments:* We thank Nicole Baran, Isabel Fraccaroli, and Naomi Green for assistance and suggestions in the initial stages of this project, and Chris Goode for making the river plots. We are grateful to Lise Eliot, Chris Goode, and Niki Woitowich for providing comments on the manuscript.

447 *Competing interests*: The authors have no competing interests.

448

449 *References*

450 Becker, J. B., Prendergast, B. J., & Liang, J. W. (2016). Female rats are not more variable than
451 male rats: a meta-analysis of neuroscience studies. Biology of Sex Differences, 7(1), 1-
452 7.

453 Beltz, A. M., Beery, A. K., & Becker, J. B. (2019). Analysis of sex differences in pre-clinical and
454 clinical data sets. Neuropsychopharmacology, 44(13), 2155-2158.

455 Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research.
456 Neuroscience & Biobehavioral Reviews, 35(3), 565-572.

457 Clayton, J. A. (2018). Applying the new SABV (sex as a biological variable) policy to research
458 and clinical care. Physiology & Behavior, 187, 2-5.

459 Clayton, J. A., & Collins, F. S. (2014). Policy: NIH to balance sex in cell and animal studies.
460 Nature News, 509(7500), 282.

461 Clayton, J. A., and Tannenbaum, C. (2016). Reporting sex, gender, or both in clinical research?
462 JAMA 316, 1863–1864.

463 Cortes, L. R., Cisternas, C. D., & Forger, N. G. (2019). Does gender leave an epigenetic imprint
464 on the brain?. Frontiers in Neuroscience, 13, 173.

465 Cumming, G. (2012) Understanding the new statistics: effect sizes, confidence intervals, and
466 meta-analyses. New York, NY: Routledge.

467 Diester, C. M., Banks, M. L., Neigh, G. N., & Negus, S. S. (2019). Experimental design and
468 analysis for consideration of sex as a biological variable. Neuropsychopharmacology,
469 44(13), 2159-2162.

470 Duchesne, A., Pletzer, B., Pavlova, M. A., Lai, M. C., & Einstein, G. (2020). Bridging Gaps
471 Between Sex and Gender in Neurosciences. Frontiers in Neuroscience, 14, 561.

472 Gelman, A., & Stern, H. (2006). The difference between “significant” and “not significant” is not
473 itself statistically significant. The American Statistician, 60(4), 328-331.

474 Holmes, M. M., & Monks, D. A. (2019). Bridging sex and gender in neuroscience by shedding a
475 priori assumptions of causality. Frontiers in Neuroscience, 13, 475.

476 Johnson, J. L., Greaves, L., and Repta, R. (2009). Better science with sex and gender:
477 facilitating the use of a sex and gender-based analysis in health research. International
478 Journal for Equity in Health, 8, 14.

479 Makin, T. R., & de Xivry, J. J. O. (2019). Science Forum: Ten common statistical mistakes to
480 watch out for when writing or reviewing a manuscript. eLife, 8, e48175.

481 Maney, D. L. (2016). Perils and pitfalls of reporting sex differences. Philosophical Transactions
482 of the Royal Society B: Biological Sciences, 371(1688), 20150119.

483 Maney, D. L. (2014). Just like a circus: The public consumption of sex differences. Current
484 Topics in Behavioral Neuroscience, 19, 279-296.

485 Nieuwenhuis, S., Forstmann, B. U., & Wagenmakers, E. J. (2011). Erroneous analyses of
486 interactions in neuroscience: a problem of significance. Nature Neuroscience, 14(9),
487 1105.

488 NIH, Consideration of sex as a biological variable in NIH-funded research. National Institutes of
489 Health Notice Number: NOT-OD-15–102, issued 6-9-2015.

490 Potluri, T., Engle, K., Fink, A. L., Vom Steeg, L. G., & Klein, S. L. (2017). Sex reporting in
491 preclinical microbiological and immunological research. mBio, 8(6), e01868-17.

492 Radke, A. K., Sneddon, E. A., & Monroe, S. C. (2021). Studying Sex Differences in Rodent
493 Models of Addictive Behavior. Current Protocols, 1(4), e119.

494 Shansky, R. M., & Murphy, A. Z. (2021). Considering sex as a biological variable will require a
495 global shift in science culture. Nature Neuroscience, 24(4), 457-464.

496 Sugimoto, C. R., Ahn, Y. Y., Smith, E., Macaluso, B., & Larivière, V. (2019). Factors affecting
497 sex-related reporting in medical research: a cross-disciplinary bibliometric analysis. The
498 Lancet, 393(10171), 550-559.

499 Tannenbaum, C., Ellis, R. P., Eyssel, F., Zou, J. & Schiebinger, L. Sex and gender analysis
500 improves science and engineering. Nature 575, 137–146 (2019).

501 Wallach, J. D., Sullivan, P. G., Trepanowski, J. F., Steyerberg, E. W., & Ioannidis, J. P. (2016).
502 Sex based subgroup differences in randomized controlled trials: empirical evidence from
503 Cochrane meta-analyses. BMJ, 355, i5826.

504 Weiner, J. (2017). Riverplot: Sankey or Ribbon Plots. https://CRAN.R-
505 project.org/package=riverplot.

506 Will, T. R., Proaño, S. B., Thomas, A. M., Kunz, L. M., Thompson, K. C., Ginnari, L. A., ... &
507 Meitzen, J. (2017). Problems and progress regarding sex bias and omission in
508 neuroscience research. eNeuro, 4(6), e0278.

509 Woitowich, N. C., & Woodruff, T. K. (2019). Opinion: Research community needs to better
510 appreciate the value of sex-based research. Proceedings of the National Academy of
511 Sciences, 116(15), 7154-7156.

512 Woitowich, N. C., Beery, A., & Woodruff, T. (2020). Meta-Research: A 10-year follow-up study
513 of sex inclusion in the biological sciences. eLife, 9, e56344.