Harnessing the Power of Sex Differences: What a Difference Ten Years Did Not Make

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Highlights

68% of Neuroscience and Psychiatry papers reported the use of both sexes in 2019

Only 19% of studies in 2019 used sex consistently throughout the study analyses

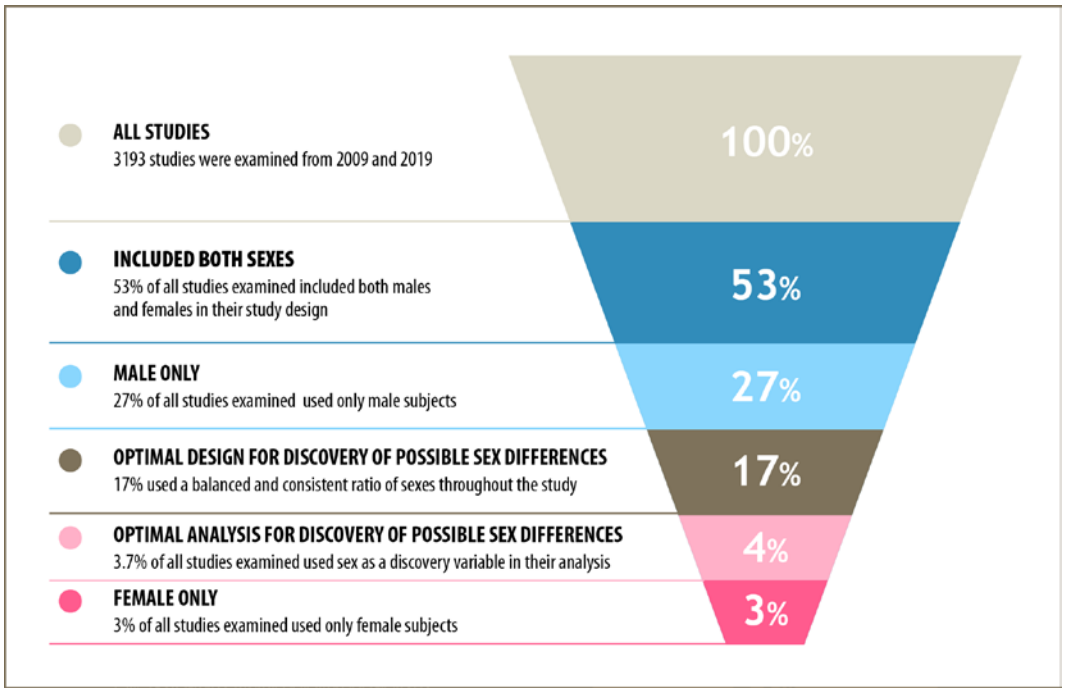
Of the studies that used males and females, 59% did not include sex in the analyses

Only 5% of studies in 2019 used sex as a discovery variable in their analyses

Male only papers were 8.4 times more prevalent than female-only papers

Abstract

Sex differences exist in a variety of neurological and psychiatric diseases but most past research has been conducted in males. Multiple mandates have been initiated across funding agencies and scientific publishers for research to include males and females in research. What has been lacking in the literature is a detailed assessment of how sex is incorporated into the design (e.g. balanced design) and into the analyses (e.g. covariate). Here we investigated papers in 2009 and 2019 in six journals in Neuroscience and Psychiatry. We found a 30% increase in the percentage of papers that included both sexes from 2009 to 2019 to 68% in 2019. Despite this increase, only 19% of all studies used an optimal design for discovery of possible sex differences and only 5% analyzed sex as a discovery variable in 2019. The percentage of single sex papers remains unchanged across the ten years (3% for female-only, 27% for male-only). Neuroscience had fewer papers (20%) that analyzed by sex compared to Psychiatry (61%). Overall, in 2019, only 5% (up from 2% in 2009) of studies used an optimal design and analysis for discovery of possible sex differences. Therefore, little progress has been made across the last ten years in harnessing the power that sex differences can afford in research for discovery and therapeutic potential for neurological and psychiatric disease to improve the health of men, women and gender diverse individuals.



Introduction

It has become clear that the consideration of sex in published reports is essential to our understanding of disease and the biological mechanisms that contribute to the etiology, manifestation and treatment of disease (Mauvais-Jarvis et al., 2020). The study and disaggregation of sex differences are critical to our understanding of precision medicine in finding effective treatments for disease. Sex differences have been observed in the prevalence and manifestation of a number of neurological and psychiatric diseases (Irvine et al., 2012, Eid et al., 2019). Females are more likely to be diagnosed with multiple sclerosis, major depressive disorder, and have a greater lifetime risk of Alzheimer's Disease compared to males, whereas males are more likely to be diagnosed with autism spectrum disorder, attention and hyperactivity disorder, and Parkinson's Disease (Gillies et al. 2014; Mauvais-Jarvis et al., 2020; Gutiérrez-Lobos et al., 2002; Liu et al., 2019; Anstey et al., 2021; Nebel et al., 2018). Even in diseases that do not show strong sex differences in prevalence, age of disease onset or manifestation can be different between the sexes (Häfner et al., 1992; Liu and Mager, 2016). Sex differences in incidence can shift with age as schizophrenia is more likely to emerge in males earlier in life than in females, but later in life in females compared to males (Häfner et al., 1992) and the incidence of stroke increases dramatically postmenopause in females (Reeves et al., 2008). Perhaps more concerning, there are notable differences in time to diagnosis (Westergaard et al., 2019), disease progression (Irvine et al., 2012; Golden and Voskuhl, 2017), vaccine response (Fischinger et al., 2019) and treatment efficacy/drug response (Zucker and Predergast, 2020; Soldin & Mattison, 2009; Sohrabji et al., 2017). Harnessing the knowledge that males and females can differ on a number of disease-related outcomes will be fruitful in not only understanding disease but also in determining whether sex-specific risk factors for disease may warrant further attention. For example, the manifestation of cardiovascular disease can be different between the sexes (Liu and Mager, 2016), prompting calls for changes to the diagnostic guidelines for cardiovascular disease (Trutter et al., 2020). Thus, to make headway for precision medicine and most effective treatment and diagnoses, sex must be taken into consideration.

Many of the health disparities in treatment and diagnosis have been attributed to the lack of research in females and inclusion of women in clinical trials (Feldman et al., 2019; Lee, 2018; Yakerson, 2019). In an effort to increase the enrolment of women in clinical research, the United States Congress passed The Revitalization Act of 1993. This Act, stated that women and minorities must be included as subjects in clinical research funded by the National Institutes of Health (NIH). However, implementation of the requirement of women and minorities has not translated into analysis by sex or race/ethnicity (Geller et al., 2018). The importance of sex consideration in research led the NIH to further mandate the inclusion of sex as a biological variable (SABV) in biomedical research in 2016 (Clayton and Collins, 2014). However, this mandate, much like the one for clinical trials in 1993, did not include specifications as to the analysis of the data by sex (Mazure and Jones, 2015) nor did it specify sample size requirements (Miller et al.,2017; McCullough et al., 2014; Tannenbaum et al., 2016). Other countries have followed suit with some notable differences as the Canadian Institutes of Health Research (CIHR) mandated Sex and Gender-Based Analysis (SGBA) policy in 2019, and Horizon Europe has indicated the need for inclusive intersectionality analyses of gender and sex as of December 2020. Despite the act of prescriptive guidelines from NIH there are a number of reviews with suggestions on the appropriate incorporation of SABV and SGBA in the literature (Miller et al., 2017; McCarthy, 2015; McCarthy et al., 2012, Shansky and Woolley, 2016). However, despite the mandates and recommendations there have been implementation issues of the mandate as reviewers and authors of papers may be applying SABV and SGBA inconsistently perhaps given the lack of official guidelines (Galea et al., 2020; Woitowich and Woodruff, 2019).

It is important to note that the biomedical and clinical research community is beginning to make corrections for a long standing bias of using males predominately in research. With the publication in 2011 by Beery and Zucker it became clear that, although there was considerable variation by research field, the majority of studies were not using both sexes (Beery and Zucker, 2011). Studies in human populations were more likely to use both males and females across the ten disciplines examined compared to studies using animals (Beery and Zucker, 2011). Recently, a ten year follow up was done comparing the these data from 2009 across the ten various biological disciplines with publications from 2019, demonstrating there has been an increase in the inclusion of both sexes in research from 20% in 2009 to 49% in 2019 in articles, with Neuroscience having one of the largest increases in sex inclusion (Woitowich et al. 2020). Even though a greater proportion of neuroscience studies are including both sexes in recent years, there are issues in how these sexes have been included, as approximately one third of sex-inclusive studies surveyed by Woitowich and colleagues in 2020 did not specify sex in the sample size descriptions. Perhaps less known is that the majority of studies that used both males and females failed to analyze the data by sex with Neuroscience at approximately 80% in 2009 (Beery and Zucker, 2011) and 44% of Neuroscience papers in 2017 (Mamlouk et al., 2020) failing to analyze by sex. Furthermore, it was disappointing to see that there has been lack of an increase in analyses by sex as only one discipline (Pharmacology) improved in analyses of sex in their papers over the ten years, as overall there was in fact a decrease by 8% in the papers that indicated they used sex in their analyses (Woitowich et al., 2020). Furthermore, sex bias favouring males is still prevalent in neuroscience research (Will et al., 2017; Woitowich et al., 2020). In fact a study from Will and colleagues (2017) indicated that the use of solely males in studies increased from 2010 to 2014, while the number of female studies remained at a constant low value, approximately 5% of Neuroscience papers. Thus, across the 10 years, studies indicate that although the sex omission rate is decreasing across disciplines, the use of sex in the analyses and the large differential in single-sex studies favoring males have not appreciably changed over the years (Woitowich et al., 2020).

What has been lacking in the literature is a detailed assessment of not only how sex is reported in papers (sample sizes disclosed, whether the study design is balanced, sex used consistently throughout the studies within the papers) but also how males and females are included in any analyses. Often in clinical studies, sex is used as a covariate which removes the statistical linear association between two variables and does not inform on the effect of sex. Therefore, in the present study, we examined not only whether a statistical analysis was done in the studies but what type of analysis was done to determine whether sex was controlled for, via a covariate analyses, or explicitly examined as a discovery variable. We were also interested in how many papers used an experimental design that was optimal for discovery of potential sex differences (balanced design, sex used through throughout the experiments).

Given that the original studies (Beery and Zucker, 2011; Woitowich et al., 2020) were a sampling of the first 10-20 papers in the year, we wanted to do an exhaustive analyses of two disciplines by looking in depth at the various ways that sex was used in analyses. Given the prominent sex differences in neurological and psychiatry disorders, we chose to do a detailed examination of journals that targeted Neuroscience and Psychiatry. As the mandates for inclusion of males and females in biomedical research were in place in 2016, we examined two years over the ten-year period of 2009 to 2019 as was done by Woitowich and colleagues and as these were dates before and after the mandates. Therefore, we assessed sex bias/omission, design of experiment (balanced ratios, consistent use of sex) and determined how papers were analyzing by sex. We hypothesized that there would be an increase in the number of papers that included both sexes from 2009 to 2019 in Neuroscience and Psychiatry papers, but also that there would be an increase in experimental design that was not optimized to examine sex as a biological variable. We also expected that the

majority of studies that analyzed sex as a factor would do so without using sex as a primary discovery variable across both disciplines, irrespective of year.

Methods

We exhaustively examined research papers within three journals in Neuroscience and Psychiatry across two years. We choose journals based on the high ISI Clarivate rankings that published primary research papers. Three Neuroscience journals (*Nature Neuroscience, Neuron, Journal of Neuroscience*) and three Psychiatry journals (*Molecular Psychiatry, Biological Psychiatry, Neuropsychopharmacology*) were chosen. We assessed papers published in the year 2009 and in 2019 to assess whether there has been an increase in the inclusion of sexes, improvements to experimental design and analyses to examine potential differences between the sexes.

Studies included

All primary research articles from 2009 and 2019 were analyzed if the papers used rats, mice, human subjects, or if fetal cells/cell lines were included. Brief communications, reviews, viewpoints etc. were excluded. This resulted in a review of a total of 3191 publications (see Figure 1). Assessments were done by two trained curators who had >99% interrater reliability (RKR, TFLS). When the categorization of analyses within the paper (see below) was questioned, these were confirmed by AYA, a biostatistician - who was consulted on 0.5% of the papers reviewed or 16 times in total.

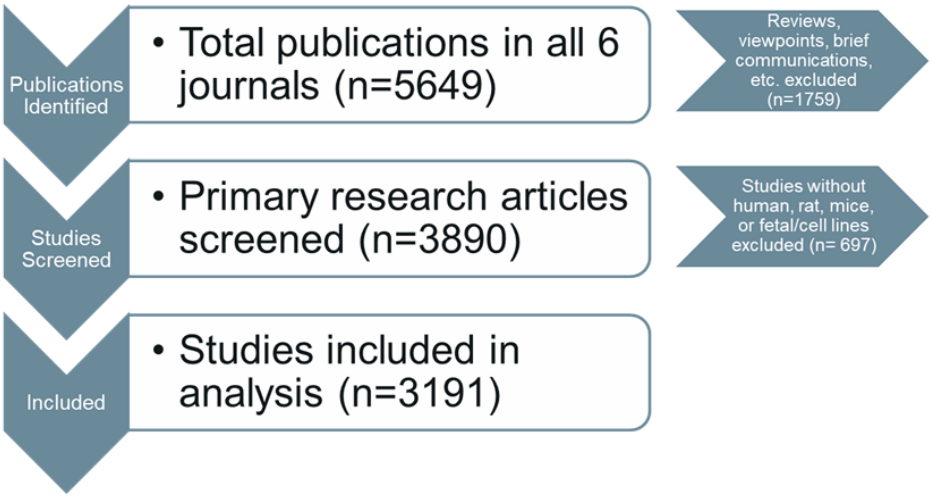


Figure 1. Inclusion of studies from all 6 journals. Reviews, viewpoints, brief communications and any other non- primary research articles were excluded. A total of 2456 studies did not match the inclusion criteria and were excluded. Only primary research articles containing human, rat, mice or fetal/cell lines were analyzed further (n=3191).

Categorization of Inclusion of males and females and Sex-Based Analyses:

Studies that matched the inclusion criteria were first examined to determine whether they included males and females, males only, females only, did not report sex, or were inconsistent throughout (i.e. used males in one experiment, and both sexes in another). If the study looked at both sexes, we determined whether there was balanced design (an equal ratio of male to female subjects). An unbalanced design was defined as one sex accounting for more than 60% of the total sample size.

Studies that included both sexes were then examined to determine whether they included any form of analysis using sex as a factor. Studies that did any type of sex analysis were then broken down into 6 categories: complete analysis by sex, sex as a covariate, main effect of sex only, analyzed sexes separately, statistics not given, and “mixed analysis”. Studies which analyzed the main and interaction effects of sex were classified as “complete analysis by sex”. Studies that only tested for a main effect of sex and did not do further analyses were classified as “main effect”. Some papers stated that there was or was not an effect of sex but provided no statistical evidence to back up the

statement and these papers were classified as “statistics not given”. A “mixed analysis” category was also included which consisted of studies which were inconsistent in their analyses throughout the study (i.e. analyzed sex in one experiment but did not analyze by sex in subsequent experiments). Any studies that used both sexes but did not fit into any of these “analyzed” categories were classified as “not analyzed”. When sex information and analyses were only reported in the supplementary section of the studies, these studies were put into a “supplementary only” category.

Statistical Analyses:
Data were reported and analyzed as percentages of total papers per journal per year. We used proportional data to run general linear analysis of variance (ANOVA) across year (2009, 2019) and discipline (Neuroscience, Psychiatry), with method of analyses (complete analysis by sex, covariate, main effect, statistics not given, analyzed separately, mixed), single sex (male, female) as within-subjects factors. Post-hoc comparisons used Newman-Keuls comparisons. Significance was set at α=0.05 and effect sizes are provided.

RESULTS

Most neuroscience papers used rodents, whereas most psychiatry papers used human subjects.

The total number of papers published differed widely by journal and year (see Table 1) from a low of 55 (2019, *Molecular Psychiatry*) to a high of 1067 (2009, *Journal of Neuroscience*), thus we used proportional variables within each analysis.

Table 1. The number of papers examined that were published in 2009 or 2019 in the six journals investigated.

Journal	Number of Papers
Neuron 2009	159
Neuron 2019	207
Nature Neuroscience 2009	118
Nature Neuroscience 2019	143
Journal of Neuroscience 2009	1067
Journal of Neuroscience 2019	588
Molecular Psychiatry 2009	70
Molecular Psychiatry 2019	55
Biological Psychiatry 2009	245
Biological Psychiatry 2019	136
Neuropsychopharmacology 2009	209
Neuropsychopharmacology 2019	196
Total	**3193**

We also categorized the papers reviewed by subject species or tissue: rat, mouse, human or cell lines and as can be seen in Figure 2. The majority of studies in the three Psychiatry journals used human subjects (54.3%) compared to 41.1% of the studies that were in rodents whereas the majority of studies in the Neuroscience journals primarily used rodents (65.3%) compared to humans (20.9%). Neuroscience published more studies using cell lines at 13.7% compared to Psychiatry at 4.7%.

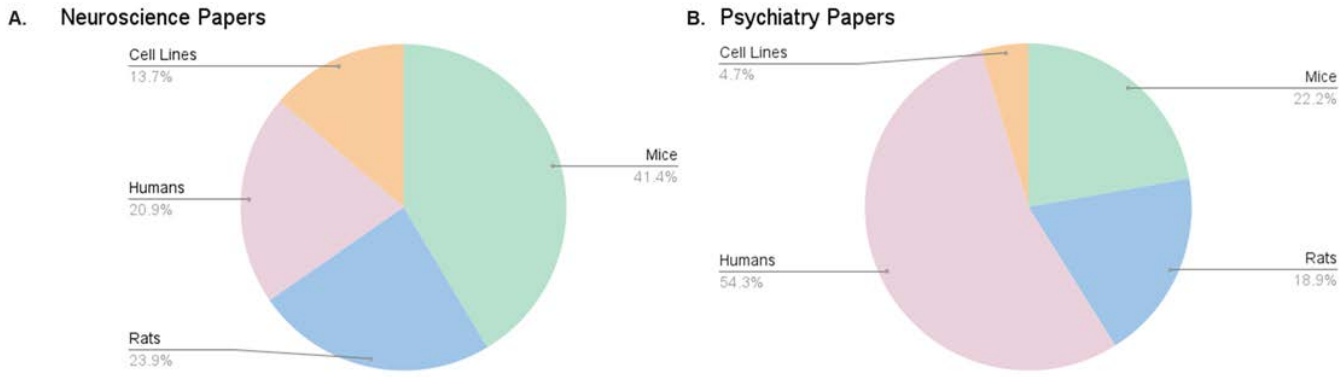


Figure 2. Reported species model used from each study. (A) Rodents (mice (41.4%) and rats (23.9%)) were the most common species by studies in the Neuroscience discipline. (B) Human subjects (54.3%) were the most common species used in Psychiatry studies.

The percentage of papers including males and females doubled from 2009 to 2019 in Neuroscience but was relatively stable in Psychiatry.

Each paper was examined to determine whether any part of the paper mentioned the use of both sexes in the study, even if the data were not shown. Across all years and disciplines, 52.93% of all papers mentioned using both sexes, which increased from 37.84% in 2009 to 68.01% in 2019. Overall, 45.28% of all Neuroscience publications mentioned using both sexes, while 60.58% of all Psychiatry publications mentioned using both sexes. Neuroscience publications with the mention of both sexes significantly increased over the ten years from 20.17% to an astonishing 70.39% (p=0.003). Psychiatry publications changed less dramatically over the years but did increase from 55.52% in 2009 to 65.63% in 2019 (p=0.32; interaction effect of year by discipline: F(1,8)=8.844, p=0.017, n_p^2= 0.525 ; see Figure 3A). There were also significant main effects of year (F(1,8)=20.02, p=0.002, n_p^2= 0.714) and discipline (F(1,8)=5.14, p=0.05, n_p^2= 0.39).

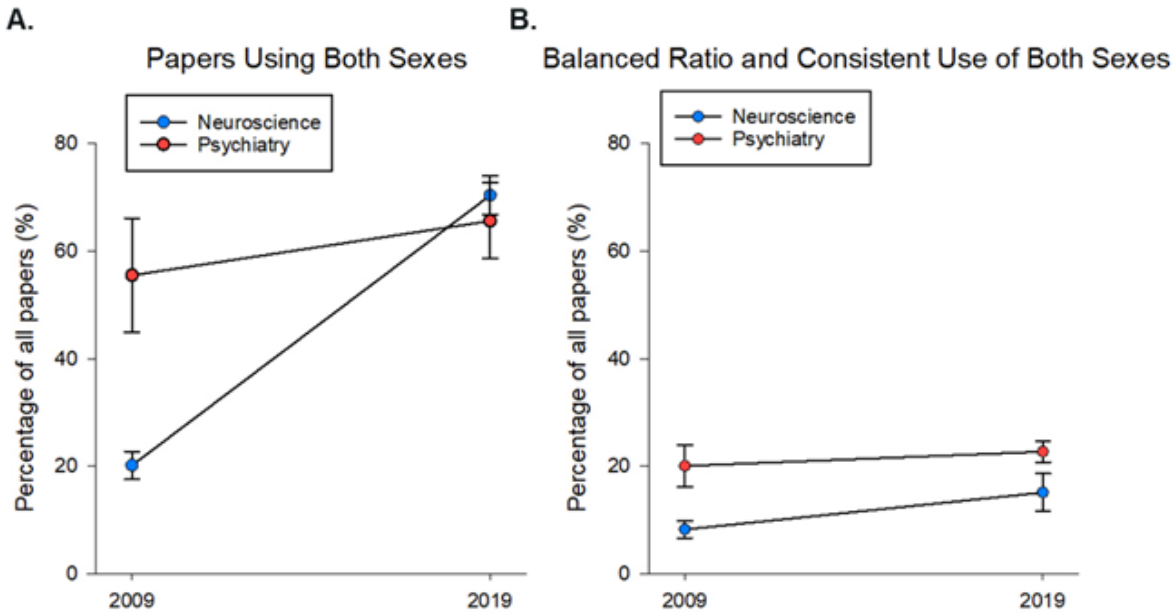


Figure 3. The percentage of papers including both sexes. (A) Percentage of papers using both sexes in any aspect of the study, regardless of consistency or balanced ratios. (B) Percentage of papers using both sexes consistently throughout the study with balanced ratios of the sexes. Means ∓ standard error of the mean.

However, as noted above, the papers that included males and females included studies that mentioned the inclusion of both sexes but did not necessarily show these data. We then calculated a more rigorous count of the inclusion of sexes by including only studies that examined sexes in a balanced design and consistently used males and females throughout all the experiments in the paper. When including a more stringent criteria of inclusion of both sexes, the overall percentage of studies including both sexes dropped to 16.54% overall, 14.15% in 2009 to 18.93% in 2019. When examining this rigorous count of the inclusion of sexes in the studies, Psychiatry publications (21.40%) more often used both sexes compared to Neuroscience publications (11.69%; main effect of discipline, ($F(1,8)=11.19$, $p=0.01$, $n_p^2= 0.583$). There was no main effect of year ($F(1,8)=2.715$, $p=0.137$, $n_p^2= 0.253$) or interaction ($F(1,8)=0.532$, $p=0.48$, $n_p^2= 0.062$; see Figure 3B).

How is SABV incorporated into the experimental design in the research papers? 65% of studies that used males and females did not use an optimal experimental design to elucidate possible sex differences in their findings.

As noted above, although the percentage of studies using both sexes has increased, there are changes in the way that sex is being reported or used. What is driving the large discrepancy between the 52.93% of all studies using both sexes but only 16.54% of studies using sex optimally for discovery (consistently throughout the study, in a balanced design)? There were several scenarios we encountered in studies that used males and females which included 1) sample sizes were not given (25%), 2) the proportions of the sexes were dramatically different (34%), or 3) the use of sex was not used consistently throughout the studies (15%, see Figure 4).

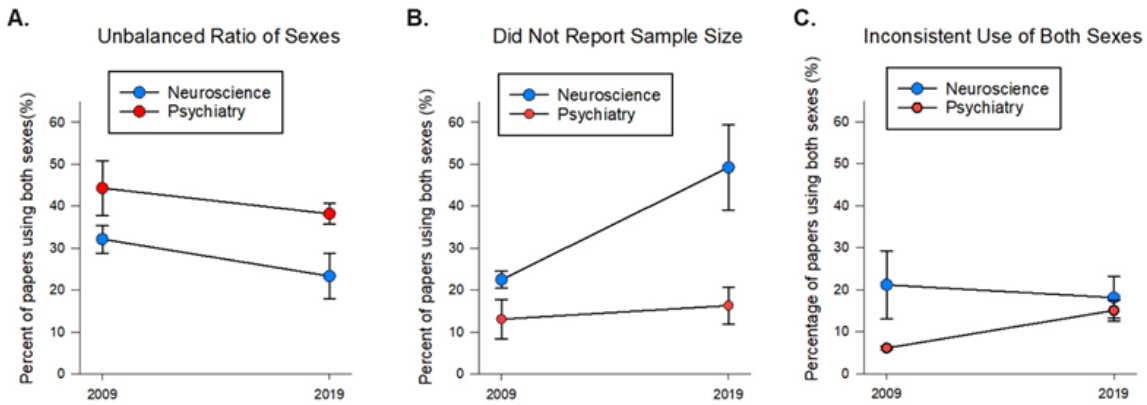


Figure 4. Breakdown of how studies that reported using both sexes are actually using them. (A) Unbalanced design (i.e. more than 60% of the subjects were one sex) was 34.52% of all papers including both sexes. (B) Papers using both sexes but not disclosing sample sizes, are increasing in Neuroscience papers but not Psychiatry papers. (C) Inconsistent use of sex (i.e. using a balanced ratio in one aspect of the design, and an unbalanced ratio or one sex only in another aspect) accounted for 15.11% of studies that used males and females. Means ∓ standard error of the mean.

Of the papers that used both sexes, 34.52% did not use a balanced design, with more Psychiatry papers employing this practice (main effect of discipline: $F(1,8)=8.189$, $p=0.021$, $n_p^2= 0.505$, See Figure 4A). There were no other main or interaction effects (all p's > 0.153).

Just over quarter (25.29%) of the papers that used both sexes did not identify sample sizes. This percentage has effectively doubled across the years from 17.79% in 2009 to 32.79% in 2019 and this practice is twice as high in Neuroscience (35.87%) compared to Psychiatry (14.71%; Figure 4B; main effects: year ($F(1,8)=6.06$, $p=0.039$, $n_p^2= 0.431$) discipline: ($F(1,8)=12.08$, $p=0.008$, $n_p^2= 0.602$). Inspection of the graph indicates the increase is driven by journals in Neuroscience as the

percentage increased from 22.49% in 2009 to 49.25% in 2019 (*a priori* p=0.014), while the percentage did not change across the ten years in Psychiatry (from 13.09% to 16.32%, p=0.72; interaction ($F(1,8)=3.73$, $p=0.089$, $n_p^2= 0.318$).

The percentage of inconsistent use of both sexes across the studies within a paper was 15.11% of all those that indicated they used both sexes. This percentage did not significantly change by year or by discipline (see Figure 4C; all p's >0.10).

Just over 4.04% of all papers examined referred to the sex effects in the supplemental section but there were no significant differences across year (although this did double from 2.54% in 2009 to 5.56% in 2019) or by discipline (4.05% in Neuroscience, 4.03% in Psychiatry; all p's >0.28; see Table 2).

Discipline	Mean ± SEM 2009	Mean ± SEM 2019
Neuroscience	3.9 ± 2.0	4.2 ± 4.2
Psychiatry	1.2 ± 1.2	6.9 ± 2.1

Table 2. The proportional percent of times that male and female data was found in the supplemental section. There were no significant differences by year or discipline. Overall four percent of papers referred to data on males and females in the supplemental section, not in the main body of the paper.

Male-only papers disproportionally outnumbered female-only papers and the percent of single-sex studies has remained constant over the years

The percentage of studies across disciplines that were male-only was 26.96%, versus those that were female-only studies at 3.29%. Thus, male-only papers were 8.4 times more common than female only papers, regardless of the year (main effect of sex: $F(1,8)=66.42$, $p<0.0001$, $n_p^2= 0.89$; Figure 5A). The percentage of studies that only included one sex remained constant across years (27% in 2009 to 26.78% in 2019; p=0.82) and did not differ across disciplines (22.75% in Neuroscience, 31.16% in Psychiatry, p=0.17).

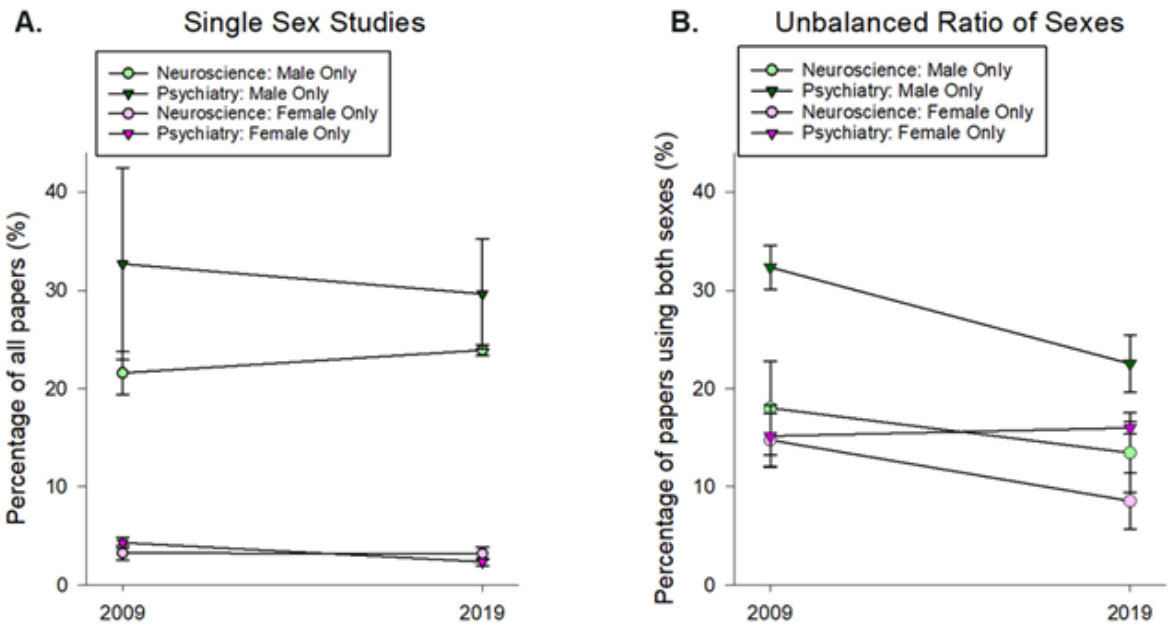


Figure 5. (A) Percentage of single sex studies across years and disciplines. Male only studies were 8.4 times higher than female only studies. (B) Of the studies using both sexes and used an unbalanced ratio of sex, there were more studies with greater proportion of males compared to females. Means ∓ standard error of the mean.

Of the papers that used males and females in an unbalanced designs, more skewed towards males (21.58% more males versus 13.61% more females: main effect of sex skew: $F(1,8)=20.23$, $p=0.002$, $n_p^2= 0.717$) and there were more sex-skewed papers in Psychiatry journals compared to Neuroscience (21.51% compared to 13.69%: main effect of discipline $F(1,8)=9.017$, $p=0.017$, $n_p^2= 0.531$). There were no other main effects or interactions (all p's >0.121); see Figure 5B.

We also did a thematic analysis on the responses that were given as to why single sex studies were used. In total there were 51 documented responses (Table 3). The reasons noted were driven mainly by references to reducing variability or confounds in the data (50.98%, see Table 3).

Reason	Proportion
To Reduce Confounds/Variability/Hormones	50.98%
Behaviour (i.e. aggression/fighting)	15.69%
To Avoid Sex Differences	11.76%
Disease Prevalence	11.76%
Lack of Previously Observed Sex Difference	5.88%
Insufficient Offspring	3.92%

Table 3. Thematic themes given for only including one sex in study design. 50.98% of single sex studies that gave a reason for the use of one sex referred to the reason to reduce confounds or variability mainly due to fluctuating hormones.

The majority of papers did not analyze by sex (58%), but of those that did, more Psychiatry publications compared Neuroscience publications analyzed their data by sex and this percentage has not changed over ten years

Overall, while 52.93% papers indicated they used both sexes, the majority of these studies did not analyze by sex (58.89%). Neuroscience papers using both sexes were much more likely to not analyze by sex (78.24%) compared to Psychiatry papers using both sexes (39.53%; F(1,8)= 61.01, p<0.001, n_p^2= 0.884). Over the years the percentage of papers not analyzing by sex has significantly decreased from 64.56% of papers using both sexes in 2009 to 53.22% of papers using both sexes in 2019, but unfortunately still remains above 50% (F(1,8)=5.24, p=0.05, n_p^2= 0.3955; see Figure 6A).

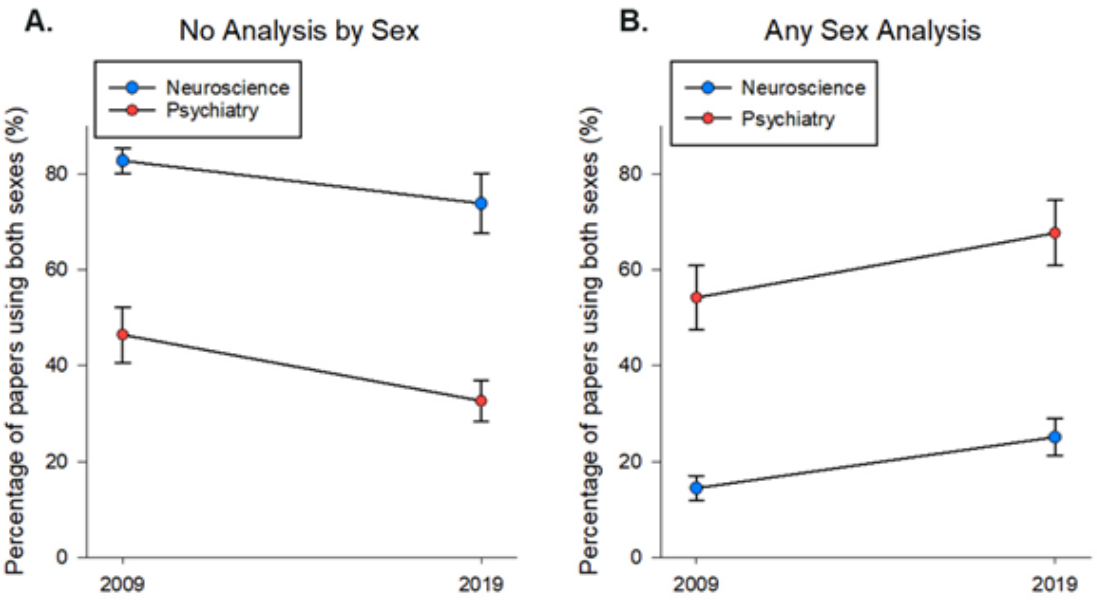


Figure 6. (A) The majority of papers using both sexes did not analyze by sex, but this decreased slightly over 10 years. (B) Any analysis of sex in studies using both sexes. Psychiatry papers were more likely to perform any type of sex analysis than neuroscience papers. Means ∓ standard error of the mean.

Of the studies that indicated they used both sexes, 40.34% said they analyzed their data by sex. The percentage of papers that indicated they did an analysis by sex increased from 34.32% in 2009 to 46.36% in 2019 irrespective of discipline (main effect of year: F(1,8)=5.17, p=0.05, n_p^2= 0.39). However, more Psychiatry papers analyzed by sex at 60.89% compared to 19.78% of Neuroscience papers (main effect of discipline: F(1,8)=60.27, p<0.0001, n_p^2= 0.88). There was no significant interaction (p=0.79; see Figure 6B).

How are papers analyzing by sex? Most papers use sex as a covariate in their analyses and only 6% of papers that used both sexes analyzed sex as a discovery variable

We further broke down how the papers analyzed by sex, as indicated in the Methods section. We had 6 categories: complete analysis by sex (analyzed as a discovery variable), stats not given, covariate, main effect, analyzed separately, and mixed. Overall of the 52.93% of papers that used both sexes, only 6.00% used sex as a discovery variable which translates into to 3.68% of papers overall. The largest percentage of studies (14.36%) used sex as a covariate, while 3.96% of studies did not give any statistics (despite indicating they had analyzed), 1.74% of studies used main effects, 5.78% analyzed males and females separately, and 8.49% of studies used mixed analyses (see Figure 7).

Psychiatry papers were more likely to analyze using sex as a covariate (24.51% (Psychiatry) to 4.21% (Neuroscience); p=0.0001) or a mixed analyses (13.66% (Psychiatry) to 3.31% (Neuroscience); p=0.003) compared to Neuroscience papers, regardless of the year (Analyses Type by Discipline: F(5,40)=10.23, p<0.001 or p<0.0001, n_p^2= 0.56). There was a main effect of Analysis Type (F(5,40)=13.14, p<0.0001, n_p^2= 0.62), showing that the covariate analyses were more often used than any other analysis (all p's< 0.001). There was also a main effect of Discipline (F(1,8)=60.27, p<0.0001 or p=0.00005, n_p^2= 0.88) and a main effect of year with 2019 being higher than 2009 (F(1,8)=5.17, p=0.05, n_p^2= 0.39), but no other significant main or interaction effects (all p's> 0.43,n_p^2 <0.11).

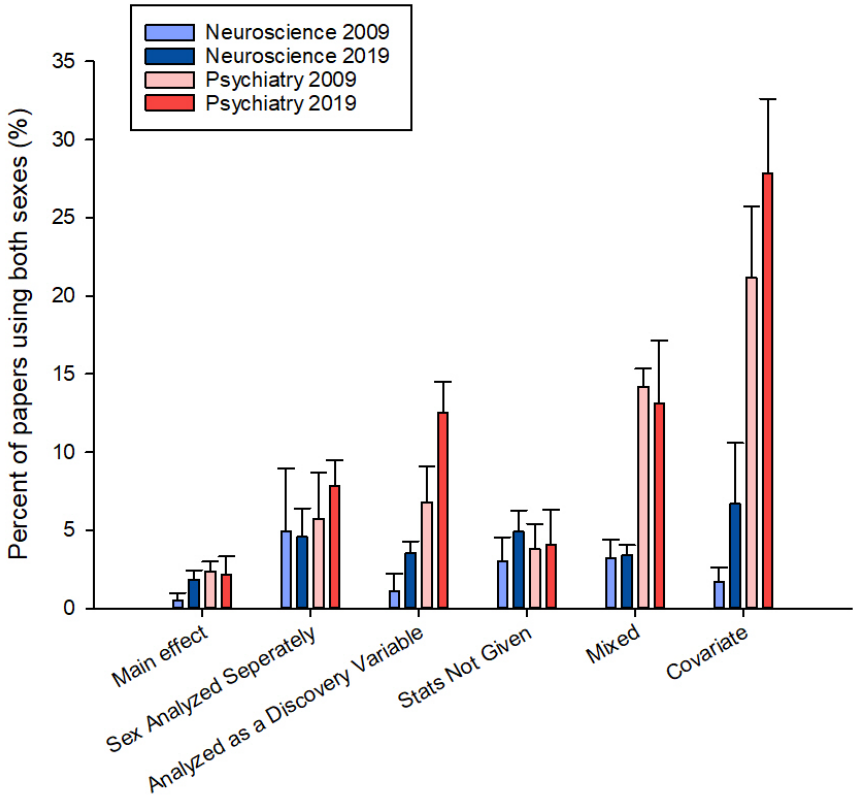


Figure 7. Breakdown of the type of analyses used by papers that used both males and females. Categories of sex analysis include: main effect of sex, sexes analyzed separately, sex analyzed as a discovery variable, stats not given (i.e. state some analysis was done but did not provide any statistics for it), mixed (i.e. any combination of analyses which may or may not be consistent throughout the study), and sex as a covariate. Psychiatry papers were more likely to use sex as a covariate. Only 6% of the studies that used both sexes used sex as a discovery variable. Covariate analysis was used more frequently than any other analysis. Means ∓ standard error of the mean.

Discussion

Our exhaustive survey of 3191 papers across six journals in Neuroscience and Psychiatry revealed some interesting insights into the adoption of SABV over the ten year period from 2009 to 2019. Sixty-eight percent of studies used males and females in 2019, a 30% increase from 38% in 2009, irrespective of discipline. On the face of it, this is a dramatic positive benefit arising for greater knowledge and awareness on the importance of sex and gender as variables in research given the mandates and guidelines for grant applications and publishing through NIH, CIHR, Horizon Europe, and SAGER. However, the way researchers are reporting the use and analyses of males and females is not optimal. This is troubling as collectively science will lose out on valuable information if researchers are neglecting to embrace the power of studying potential sex differences. When we

determined the percentage of studies that used an optimal design for discovery of sex differences, the percentage of studies fell to 16.5%, a far cry from 52.3% of papers that report the use of both males and females in the papers over both years. Further, of the papers that reported using both sexes, 76% of these either did not specify sample size (25%), used unequal proportions of the sexes (36%) or used the sexes inconsistently within the paper (15%). Perhaps even more concerning, the majority of papers that describe using both sexes, did not analyze by sex (58%), and only 6% (4% of all papers) of studies used sex as a discovery variable across years and disciplines. Worse yet, the percentage of papers using optimal designs or analyses for discovery of sex differences has not meaningfully shifted in ten years across either discipline, despite the number of initiatives such as SABV, SGBA and SAGER. These findings should serve as a wake up call to researchers, funders and journals, that if we are to harness the wealth of knowledge from studying both sexes, more needs to be done to improve the appropriate application of sex in reporting and analyses for discovery.

More publications are using both sexes but are they?

As noted, there has been a vast increase in the reporting of both sexes in both Psychiatry and Neuroscience papers from 2009 to 2019. The percentage of all publications that included sex in any experiments across all journals was 52.9% which increased by over 30% over the ten year period from 38% in 2009 to 68% in 2019. Neuroscience showed a 50% increase in reporting the use of both sexes culminating in over 70% of articles using both sexes in 2019. This dramatic increase was not seen in Psychiatry which increased by 10% over the same ten year period to 66%, this is likely driven by the majority of papers using humans in Psychiatry journals, as Beery and Zucker (2011) showed studies using humans are more likely to use males and females in their studies. The great majority of Neuroscience and Psychiatry articles are using both sexes in 2019, which is encouraging. Our finding of a 50% increase across ten years is also higher than the almost 20% increase seen from 2010-1014 in Neuroscience (6 journals) reported by Will and colleagues (2017) and the 34% increase seen by Woitowich and colleagues (2020) across the same ten year period. In addition, the 68% of studies that included males and females in 2019 in our study is notably higher than the 52% of Neuroscience papers reporting the use of both sexes in 2017 (Mamlouk et al., 2020), likely reflecting an upward trend across years. The large progress made in Neuroscience across the 10 years was also noted by Woitowich (2020) who, as noted, saw a 34% increase to 63% in 2019 using a sampling of 20 articles from 4 journals, two of which overlapped with ours (Journal of Neuroscience and Nature Neuroscience). In the present paper we exhaustively sampled from 3 journals in Neuroscience, much like the work by Meitzen and colleagues (Will et al., 2017, Mamlouk et al., 2020) who exhaustively searched for Neuroscience papers in 6 journals, 3 of which overlapped with the journals we chose (Nature Neuroscience, Neuron, Journal of Neuroscience). Thus, collectively, multiple studies, using different journals and methods of sampling, consistently indicate that there is an increasing trend in articles that include males and females in their work.

Although the use of both males and females in research has been steadily increasing to include a majority of studies, research highlighting or mentioning sex differences is scarce. Why might this be? We examined whether or not papers were using optimal designs for discovery of possible sex differences by noting if studies reported sample sizes, whether or not they used a balanced design, and whether they were using males and females consistently throughout the study. Finally we examined how they analyzed the data by sex. When we accounted for studies that did not disclose sample size of the sexes, used unbalanced design or only used both sexes in a portion of the study, we found that only 16% of studies used a design that was optimal for discovery of sex differences. Some researchers will argue that investigating both males and females is only important in the first step and thus the use of both sexes in further experiments, beyond the initial study is not required. However, there are plenty of examples where a trait may not have sex differences but the

mechanisms underlying that trait do show significant differences between males and females (Oberlander and Woolley, 2016; Sorge et al., 2015; Wickens et al., 2021; Yagi et al., 2020). Thus, it is important for researchers to understand that using males and females in one experiment does not preclude the fact that they may show differences in further experiments, as others have discovered. Unfortunately, the use of the most advantageous design for discovery of sex differences was only employed in 16% of the studies overall, and in 2019, this reduced the percentage of studies by 50% from 68% to 19%. Thus, although it appears on the face of it that the majority of studies are using males and females, the majority of these studies do not incorporate sex in their design that is optimal for discovery.

Our findings also demonstrated that 25% of studies using both males and females do not report sample size. This is consistent with the findings from Woitowich et al. (2020) who found overall that 27% failed to provide sample size information when including both sexes. Perhaps more concerning is that particularly in Neuroscience, this trend is increasing over the ten years with almost 50% of studies not reporting the sample size of males and females used. This trend is troubling as the reader is unable to judge how effectively males and females were used in the study.

The majority of papers do not analyze by sex and the type of analyses used is sub-optimal for discovery of any differences between males and females

As many other researchers have reported, the majority of publications do not analyze by sex. In our study we found 58% of studies did not analyze for sex as a factor. Perhaps more concerning is that only 6% of studies that used males and females used sex as a discovery variable, which increased marginally to 8% of studies in 2019. This relates to 4% in all the publications examined, an increase from 2% in 2009 to 5% in 2019. Fourteen percent of all papers that used males and females used sex as a covariate, with the majoriy of papers that using this statistical approach in Psychiatry. A covariate removes the linear association of the factor of sex against the dependent variable, thus in essence it is removing any linear variation due to sex. In our minds this is in opposition to the intention of SABV or SGBA. The point is not to remove the variation due to sex but to determine whether or not sex is a variable that could be causing differences in variation. Others have shown that the use of sex as a covariate can result in the reduction of power and the loss of important information when a sex difference is present (Mersha et al., 2015). Mersha and colleagues (2015) show that 26 more single nucleotide polymorphisms were identified in a sex stratified analysis compared to when sex was used as a covariate. They also found that effect sizes were larger when a sex-stratified analysis was used, contrary to popular opinion that power would be negatively affected with the addition of sex as a discovery variable. Some argue that design and sample sizes are not powered to consider sex-stratified analyses, but if the sex effects are large, or in opposing directions, the resulting power with the inclusion of sex, may improve as others have demonstrated (Buch et al., 2019; Mersha et al., 2015; Hyatt et al., 2020; Galea et al., 2020). Taken together, our survey of the literature suggests that researchers are underestimating the power of using sex as a discovery variable in their research.

More female only studies are needed

Similar to other reports in Neuroscience and other biological disciplines (Will et al., 2017; Mamlouk et al., 2020; Beery and Zucker, 2011; Beery, 2018), we found female only studies were a small percentage of studies. Our survey of Neuroscience and Psychiatry papers indicated that the percentage of female only studies is very low at 3% and indeed the percentage of studies decreased across the ten-year period in Psychiatry from 4 to 2% of studies. Our findings are comparable with others showing that 5% of Neuroscience studies were female only in 2009 (Beery, 2018) and in 2017 (Mamlouk et al., 2020). On the other hand, we found that male only studies were

over 25% of all studies in the Neuroscience and Psychiatry literature. Although, the use of sex/gender in studies is important, single-sex studies are still needed. Given the dearth of information on women's health and disparities in diagnosis (Westengaard et al., 2019), and continued underrepresentation in clinical trials (Geller et al., 2018), one could argue that we need female only studies even more so than male only studies - or that at least the single sex studies should be in equivalent proportions. Indeed the impetus for SABV and SGBA was instigated in part because of the lack of knowledge of how females differed in their response to treatments and disease (Tingen et al., 2013; White et al., 2021). There are female-specific experiences that affect female health, such as menstruation, hormonal contraceptives, pregnancy and menopause that need to be studied (Galea et al., 2018; Duarte-Guterman et al., 2019; Grandi et al., 2019; Cooke and Davidge, 2019; Champaloux et al., 2017; Lewis et al., 2019; Roeder and Leira, 2021; Peterson et al., 2014). Unfortunately, as highlighted by the current study, the percentage of studies that use only females is devastatingly low and has not improved over ten years. Funders and researchers should work to correct this imbalance.

The rationale for excluding females was often to "avoid effects of the estrous cycle" or "reduce variability" or "for consistency purposes". The result of these exclusions is a profound lack of data or study on females, and lack of understanding of hormonal variation in data. To exclude females based on greater variation than males is not valid, as two studies have found that the variability between males and females is not different in mice (Prendergast et al., 2014) or in rats (Becker et al., 2016). Although it is common to think that females will have more variability due to their hormones, males (rodents and primates), like females, have diurnal fluctuations in cortisol/corticosterone (Harden et al., 2016; Verma et al., 2010). Furthermore, unlike the monthly menstrual or weekly estrous cycles in females, human males have diurnal fluctuations in testosterone levels that vary significantly with age (Harden et al., 2016). Researchers are encouraged to consider that there are a variety of steroid (and peptide) hormones that can vary with diet, age, housing conditions and experience across both sexes (Westenbroek et al. 2004; Grégoire et al., 2014; Dagytė et al. 2009; Pham et al 2003; Duarte-Guterman et al. 2019; Moser et a., 2019; Cui et al., 2013; González et al., 2007). Thus, variability between males and females should not be a limiting factor in the use of males and females in research.

There have been calls in the literature to ensure that editors and reviewers of manuscripts ensure that published reports use both males and females and report on outcomes (Shansky and Murphy, 2021). SAGER guidelines were developed by the European Association of Science Editors to improve sex and gender in research reporting in 2016 (Heidari et al., 2016), and indeed, some journals have adopted SAGER guidelines including over 500 Elsevier journals (Miles, 2020). These guidelines include common sense reporting for journal authors and editors, and arguably could be adapted for funding agencies as well. Among the guidelines for all sections, it is recommended that authors include the sex in the title and abstract, background information on sex/gender effects on the variables of interest in the paper and in the results to disaggregate and analyze the data by sex/gender. However, the percentage of journals that have adopted SAGER are still low with one study finding under 10% of journals in Psychology and in those journals the guidelines were only adopted for the title, abstract and methods but not on reporting of analyses or data by sex/gender (Cavanaugh and Abu Hussein, 2020). However, as can be seen from the present data, the publishing of this information, particularly with respect to the analyses of sex as a discovery variable is limited and a more concerted effort needs to be adopted.

Limitations

We only examined three journals for each of the two disciplines, however we did an exhaustive search of eligible research papers within each journal, culminating in over 3000 articles reviewed.

Contrast this to other papers that surveyed 841 articles across 2 years (Woitowich et al., 2020) to over 6000 articles across 4 years (Will et al., 2017). We, as others (Will et al., 2017), selected journals based on ranking by ISI, with some overlap in journals chosen. However, our exhaustive search of these 6 journals gave values that were not appreciably different from those that used fewer papers within more journals, or exhaustive searching in a greater number of years, suggests either survey method yields similar results. Often the terms sex and gender were used incorrectly. Others have shown that in the fish literature, gender was used incorrectly 99% of the time (Ogle and Schanning, 2012). Often gender is conflated with gender identity, and it is important to understand that gendered effects can be realised when considering a number of intersectional variables with sex/gender identity (reviewed in Rich-Edwards et al., 2018). Others have pointed out that the term sex/gender may be a more appropriate nomenclature given that it is often difficult to disentangle the effect of the two on outcomes (Jäncke, 2018; Franconi et al., 2019).

Conclusions

We hope these data are a wake up call to the research community to not only include males and females in their research but to ensure appropriate methods of integration and analyses. If researchers are merely sprinkling in a few animals of the opposite sex in one of many experiments this will not allow for discovery of the impact of sex as a biological variable. Nor will the non-robust adoption of sex in experiments harness the additional power that the analyses of sex can afford (i.e. Mersha et al., 2015). Time and time again studies show us that the use of sex as a discovery variable can lead not only to fruitful knowledge, but at times, concludes that there are very different mechanisms at play between males and females that will require differential treatment (Cahill and Hall, 2017; Rilling et al., 2014). Indeed, inclusion of sex/gender in analyses and in design will improve not only the health of females but of males, as is shown in the example of lazaroids (Cahill and Hall, 2017). We lose collectively not only as a research community, but to the detriment of individuals in search of more effective treatment when sex is not considered in the design and analyses of our studies. We call on funders, reviewers and researchers to recognise that sex and gender matter across all disciplines and on the importance of intersectionality (Hankivsky, 2012; Wilson et al., 2019). The community needs to be aware that there are many types of sex differences that can occur (Becker and Koob, 2016; McCarthy et al., 2012) and some sex differences are revealed due to perturbations in environment, genotype, and disease (for review see: Gobinath et al., 2017; McCarthy et al., 2012; Rubinow and Schmidt, 2019) and so it is important to continually examine both sexes throughout the studies. Thus, it is imperative that more attention is paid to the appropriate design and analyses of sex and gender in the literature. We need to study how mandates can improve adherence in both study design and dissemination. In order to ensure a healthier future and for our best chance at precision medicine, we need the community of funders, researchers and publishers to embrace the addition of SABV, SGBA and SAGER to improve the health of women, men and gender-diverse individuals.

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