Sex Bias in Basic and Preclinical Noise-Induced Hearing Loss Research

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Abstract

Introduction: Sex differences in brain biochemistry, physiology, structure, and function have been gaining increasing attention in the scientific community. Males and females can have different responses to medications, diseases, and environmental variables. A small number of the approximately 7500 studies of noise-induced hearing loss (NIHL) have identified sex differences, but the mechanisms and characterization of these differences have not been thoroughly studied. The National Institutes of Health (NIH) issued a mandate in 2015 to include sex as a biological variable in all NIH-funded research beginning in January 2016. Materials and Methods: In the present study, the representation of sex as a biological variable in preclinical and basic studies of NIHL was quantified for a 5-year period from January 2011 to December 2015 prior to the implementation of the NIH mandate. Results: The analysis of 210 basic and preclinical studies showed that when sex is specified, experiments are predominantly performed on male animals. Discussion: This bias is present in studies completed in the United States and foreign institutions, and the proportion of studies using only male participants has actually increased over the 5-year period examined. Conclusion: These results underscore the need to invest resources in studying NIHL in both sexes to better understand how sex shapes the outcomes and to optimize treatment and prevention strategies.

Keywords: Basic research, noise-induced hearing loss, preclinical research, sex bias

INTRODUCTION

Approximately 26 million Americans aged 20–69 have noise-induced hearing loss (NIHL) (Centers for Disease Control and Prevention). The factors contributing to individual differences in susceptibility to noise exposure and in the perceptual and emotional consequences of NIHL have long been of interest to the research community. Many studies in animal models have explored the genetic basis for susceptibility to NIHL, but the effects of other biological variables such as sex and gender have received less attention. The existence of sex differences in brain biochemistry, physiology, structure, and function is broadly accepted. Men and women have different responses to medications, diseases, and environmental variables such as stress and nutrition.

With regard to the auditory system, men show longer delays in auditory brainstem responses and distortion product otoacoustic emissions than women, a phenomenon typically attributed to the slightly longer length of the male cochlea. The existence of these differences has implications for the function of the auditory system and its susceptibility to NIHL. It is important to understand how sex shapes the outcomes of damaging noise exposure to optimize treatment and prevention strategies for individual patients.

Despite the growing realization that sex often modulates the clinical responses of patients, only a few studies have investigated sex differences in susceptibility to NIHL in humans. The studies of permanent hearing loss, generally caused by long-term occupational exposure, tend to indicate that men are more susceptible than women to hearing loss across all frequencies. However, there is still some question about whether the sex differences typically observed in hearing loss are in fact due mostly to differences in the noise exposure experienced by men and women.

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How to cite this article: Lauer AM, Schrode KM. Sex Bias in Basic and Preclinical Noise-Induced Hearing Loss Research. Noise Health 2017;19:207-12.
The question of sex-specific effects of noise exposure is most likely to be resolved by studies in animals, where noise exposure can be precisely controlled. In chinchillas exposed to loud noise, males exhibited larger threshold shifts at lower frequencies, whereas females exhibited larger threshold shifts at higher frequencies. Other studies in mice have shown sex differences in hearing outcomes after exposure to an augmented acoustic environment (moderate level background sounds) intended to prevent hearing loss. These few studies are just a small fraction of the approximately 8000 NIHL studies referenced in the PubMed database. In 2015, the National Institutes of Health (NIH) issued a mandate to include sex as a biological variable in all NIH-funded research beginning in January 2016. Clinical trials funded by NIH have been required to include women since 1993 when Congress passed the Revitalization Act. Sex bias has been identified in basic and preclinical surgical, neuroscience and biomedical research. In the present study, the representation of sex as a biological variable in preclinical and basic studies of NIHL was quantified for a 5-year period from January 2011 to December 2015 to determine the representation of this variable prior to the implementation of the NIH mandate and to better understand the extent of investigation in to the sex-specific effects of noise exposure.

**Materials and Methods**

**Literature search**

Publications indexed in PubMed were reviewed from the 5-year period between January 1, 2011 and December 31, 2015. The search term “noise induced hearing loss” yielded 1209 results from this time period. When filters were applied to include publications written in English, with an abstract available, performed on other animals (nonhuman), and within the stated publication dates, the search yielded 366 results. The search records were exported to a data file and manually reviewed in Microsoft Excel spreadsheet. Further criteria for exclusion from the study included: not performed in animals, no full text was available online via PubMed or Johns Hopkins Libraries, the study included ototoxicity experiments with no acoustic exposure conditions, reviews (not original research), inclusion of marine mammals or nonmammals. The final dataset included a total of 210 manuscripts.

**Variables coded**

The final PubMed search results were manually reviewed for the statement of sex in methods or results sections. Variables coded included: year of publication, international or United States (US) study site, total number of subjects included, sex of subjects (male, female, both, not specified), total number of male and female subjects, data reported separately by sex, effects of ovarian hormones or cycles, sex-specific data reported when both sexes were tested. No studies were found to include the effects of ovarian hormones or cycles, so this variable is not discussed further. Only two studies reported sex-specific results, so this variable was not analyzed further.

**Data analysis**

Data were summarized as frequencies and percentages and were calculated using Microsoft Excel spreadsheet. Calculations included the total number and percentage of foreign and US studies, the total number and percentage of studies reporting the total number of animals used, the total number and percentage of studies reporting the sex of the animals, the total number and percentage of studies using both sexes, the total number of studies reporting using only males or females, and the total number and percentage of male and female participants from studies reporting the sex of the animals and the number of each sex used. Reporting and the use of sex were compared for international and US study sites. The use of males, females, or both sexes over time was also considered.

**Statistical analysis**

Chi-square tests were performed to test for significant differences in the proportions of observations across groups, with a $P < 0.05$ significance criterion. A two-way analysis of variance was performed on the data for sex bias over time. Power calculations for main effects were performed with an alpha criterion of 0.05.

**Results**

**Overall sex bias**

Out of 210 manuscripts, 140 (67%) reported the total number of animals included in the study, while 70 (33%) manuscripts did not [Figure 1A]. One hundred and fifty-four studies (73%) stated the sex of the animals used, whereas 56 (27%) did not [Figure 1B]. Of the 154 studies reporting sex, 94 (61%) studies used only males, 22 (14%) studies used only females, and 38 (25%) used both sexes [Figure 1C]. The proportion of studies using only males, only females, or both sexes was significantly different across groups ($\chi^2(2)=114.94, P < 0.001$). In all cases using both sexes, the number of male and female participants was equal or nearly equal when reported, but this information was only available for five studies. The total number of male or female subjects was reported in 81 studies, including 67 studies reporting the total number of males and 14 studies reporting the total number of females. In those studies, a total of 4241 subjects were used, with 3436 (81%) males and 805 (19%) females [Figure 1D].

The proportion of male participants was significantly different than the proportion of female participants ($\chi^2(2)=1632.20, P < 0.001$).

**Sex bias in international and United States studies**

Of the final 210 manuscripts, 130 (62%) were performed at international sites and 80 (38%) were performed at US sites.
Ninety-two (60%) of the 154 studies reporting sex were performed at international sites, and 62 (40%) were performed at US sites. The proportions of studies reporting sex were not different in international and US studies ($\chi^2(2) = 0.829, P = 0.363$).

Fifty-nine (64%) of the international studies reported using only males, 13 (14%) reported using only females, and 20 (22%) reported using both sexes. Thirty-five (56%) of the US studies reported using only males, 9 (15%) reported using only females, and 18 (29%) reported using both sexes. The proportions of studies using only males, only females, or both sexes were not significantly different between international and US sites ($\chi^2(2) = 1.160, P = 0.560$).

**Sex bias over time**

The total number of basic and preclinical NIHL studies was highest in 2011, dropped in 2012, increased in 2013 and 2014, and then dropped slightly again in 2015 [Figure 3]. The total number of studies reporting use of only male participants followed a similar pattern, and the percentage of male-only studies actually increased from 2011 (37%) to 2015 (56%). The total number of studies reporting the use of only females or both sexes remained at 11 or fewer in all years examined, in contrast to the 31–54 studies reporting the use of only males. The total number and percentage of female-only studies remained relatively stable from 2011 (13%) to 2015 (15%). The total number and percentage of studies reporting the use of both sexes dropped in half from 2011 (20%) to 2015 (10%). The main effect of publication year was not statistically significant ($F(4) = 1.495$, $P = 0.291$, power = 0.116), but the main effect of sex included in the studies (male, female, or both) was significant ($F(2) = 20.33$, $P < 0.001$, power = 0.997).

**DISCUSSION**

The present study finds evidence of sex bias in preclinical and basic research on NIHL in research published from 2011
through 2015. Nearly one-third of the studies examined did not report the sex of animals used in the research. In addition, one-third of the studies did not report the total number of animals used in the studies. Of those studies that did report the sex of the animals, over four times as many studies used only male participants compared to those using only female participants. Both sexes were used in only one quarter of the studies. Similarly, approximately four times as many male participants were used compared to the female participants in those studies reporting the number of participants of each sex used.

Nearly two-thirds of the manuscripts reviewed were performed at international study sites outside the US, and a proportional number of international and US studies reported the sex of the animals used. The pattern of studies using only males, only females, or both sexes was similar for international and US sites. Male-only studies were the most prevalent type in both international and US sites, with a somewhat higher proportion in international studies.

Interestingly, the overall number of NIHL studies published was highest in 2011. The number of studies fell by over 40% in 2012, then recovered to near 2011 levels over the following 2 years. A reduction in the total number of studies was again observed in 2015. This general pattern was also observed in studies using only male participants,
and the relative percentage of studies using only male participants increased by nearly 20% from 2011 to 2015. The overall number and percentage of studies using only females did not change substantially between 2011 and 2015. A decrease followed by a recovery in the number of female-only studies was observed between 2012 and 2014. Interestingly, the number of studies using both sexes decreased each year from 2011 to 2015. Consequently, the proportion of studies using only male participants compared to only females of both sexes was larger in 2014 and 2015 compared to the earlier years. The proportion of studies using only males, only females, or both sexes was most similar in 2012, the year with the fewest published studies. These data indicate that sex bias in basic and preclinical research worsened in the years prior to the NIH mandate to consider sex as a biological variable.

Sex bias has been reported in preclinical and basic surgical research,[28] biological research,[29] and general biomedical research.[27] Consistent with the present study, these studies showed a predominant use of male participants and also a low rate of reporting the results by sex. Of 10 biological fields investigated, sex bias was most prominent in neuroscience,[29] of which auditory neuroscience can be considered a subfield. Beery and Zuker,[29] actually reported increased male bias in neuroscience and biomedical research over a 50-year period. The present results are in line with trends observed in neuroscience research.

A common argument for the preferential use of male participants in basic and preclinical research is that female participants are more variable than males due to hormonal fluctuations occurring during different phases of the reproductive cycle. A recent meta-analysis found that female rats were not more variable at any stage of the estrous cycle than male participants in neuroscience research, even when sex differences in the outcome measures were observed.[32] However, male rats of different strains showed different levels of variability.[32] Gene expression also shows similar levels of variability in male and female mice and humans.[33]

Future studies considering sex as a biological variable will be useful for basic and preclinical NIHL research and neuroscience research as a whole.[34-36] Inclusion of both male and female participants has been required for NIH-funded clinical trials for over 20 years. Improving the translation of basic and preclinical research to benefit human hearing health requires a more complete understanding of the sex differences and similarities in the effects of variables affecting hearing status. This objective can be achieved through the use of several research designs,[37] and McCarthy et al. have developed a framework for interpreting sex differences.[6,38] Additional variables that may differ between sexes, such as body weight, should also be taken into account when interpreting sex-specific pharmaceutical effects.[39] Consideration of sex as a biological variable in basic and preclinical NIHL research at the molecular, cellular, and systems levels will inform future research and increase rigor and reproducibility as currently mandated for NIH-funded studies.[35,40-42]

Financial support and sponsorship
This work was supported by NIH grants DC000023, DC012352, and the David M. Rubenstein Fund for Research.

Conflicts of interest
There are no conflicts of interest.

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