Assessment of the male sex bias in Parkinson’s research on non-human subjects: a meta-analysis

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Neuroanatomical sex differences identified in both non-human animals and humans suggest sexual dimorphism impacts symptomology and treatment of Parkinson’s disease in humans. Therefore, it is important to investigate the possibility of a sex bias in non-human animal research, because half of the human population may be inadequately represented. We report findings from a meta-analysis in which a Pubmed search was conducted to investigate a sex bias in biomedical research investigating Parkinson’s Disease, and whether or not the gender identity of the first and last author is correlated to the sex of the non-human animal investigated. It was hypothesized that male humans and male non-human animals would be more likely to be used for research, and that the gender identity of the first and last authors would correlate with the sex of the non-human animal investigated. The number of studies conducted on male non-human animals was significantly greater than the number of studies conducted on female non-human animals. Male first authors were significantly more likely to conduct studies on male non-human animals than female non-human animals. Female first authors were not more likely to conduct studies on female non-human animals than male non-human animals. A significant correlation was not found between the gender identities of the last authors and the sex of the non-human animal studied. These findings suggest that the first author’s gender identity does have an influence on subject sex, and female scientists are needed, even as non-principal investigators, to eliminate the sex bias in PD research.

Abbreviations: COMT – Catechol-O-methyl transferase; Parkinson’s disease

Keywords: Sex Bias; Parkinson’s disease; Sexual dimorphism

Introduction

In 1993, the Federal Drug Administration required all human clinical trials to include females (Beery and Zucker, 2010). The implied purpose of the action was to provide a basis for medicines and treatments that help both sexes, while eliminating a male sex bias in scientific research. However, Beery and Zucker, (2010) conducted a that found a male sex bias across eight biological fields. Furthermore, the number of studies conducted on males outnumbered females in the field of neuroscience, with a ratio of 5.5:1. This may be caused by the lack of any enforcement to incorporate female non-human animals into biomedical research.

Unlike Beery and Zucker’s (2010) investigation, this study investigates a possible correlation between the first and last author’s gender identity and the sex of the non-human animal subjects investigated in Parkinson’s disease (PD) research. It was hypothesized that not only will more studies incorporate males over females, but that the gender identity of the authors will have a correlation with the sex of the non-human animal investigated.

As mentioned previously, neuro-anatomical sex differences identified in both non-human animals and humans suggest sexual dimorphism impacts symptomology and treatment of PD in humans. Sexual dimorphism
is the difference in morphology between male and female members of the same species. More specifically, neuroatatomical sex differences have been proposed to form the neural basis for sex-specific behavior, reproductive, and non-reproductive functions (Hofman and Swaab, 1991).

Sexual dimorphism was once thought, and still is by some, to be irrelevant to non-reproductive brain functioning (Cahill, 2012). In particular, it was thought that the costs and time constraints associated with using female non-human animals outweighed any possible sex difference in neurological diseases and disorders. It was believed that the male and female brain were not different in a biomedical context, therefore studying males was sufficient investigation to understand human neurological diseases and disorders. However, recent studies have shown that sexual dimorphism plays a major role in symptomology of post-traumatic stress disorder, sleep disorders, PD and other diseases (Cahill, 2012). As Cahill discusses, even if the role of sexual dimorphism in neurology is unknown, it is even more reason to incorporate more females into clinical trials and female non-human animal studies.

Some studies suggest that sexual dimorphism plays a major role in the development and symptomology of PD (Laatikaineen et al., 2013). PD is a complex progressive disorder of the nervous system that affects movement. A major characteristic of PD is a decrease of dopamine in the striatum.

This is reflected by males and females responding to PD treatment differently, and presenting different symptoms (Laatikaineen et al., 2013). An example of the effect of sexual dimorphism in the treatment of PD is the differences between sexes in catechol-O-methyltransferase (COMT), an enzyme that metabolizes catecholamines (Laatikaineen et al., 2013).

COMT inhibitors are often used in addition to dopamine agonists to treat PD. Tolcapone, an inhibitor of COMT, significantly increases 3,4-dihydroxyphenylacetic acid levels, which is a metabolite of the neurotransmitter dopamine, in the prefrontal cortex and cerebellum of female rats, but not male rats (Laatikaineen et al., 2013). This is significant because it is a difference between male and female rats in PD treatment.

It is important to evaluate the translational properties of the role of sexual dimorphism in PD. However, it has been noted by other scientists that the research of translational properties is inadequate. When Laatikaineen (2013) searched the literature, the only studies found were exclusively conducted on human males. This study investigates if there is a significant gap in the research conducted on human females and males in PD research.

However, the few studies that have investigated possible sex and gender differences in PD have found a few differences. In 2003, Van Den Eeden’s (2008) survey of 588 newly diagnosed patients found that males were more likely to be diagnosed with PD than females. Another survey conducted in 2007 (Haaxm et al., 2007), found that females diagnosed with PD exhibited tremors more often than males, and across all of the ages studied. This suggests that the sexual dimorphism seen in rats in PD research translates to humans, and disregarding females both at the non-human animal level of research and in clinical trials could be potentially hurting males and females.

The possible underlying cause of such a difference is estrogen and its potential use to slow progression or reduce the risk of PD. Shulman (2007) mentions that this possibility needs to be further investigated, but that a mildly significant sex different in disability and quality-of-life reporting between the sexes has been noted, with women citing greater disability and reduced quality of life. In the study of PD and estrogen therapy replacement in the menopause years, participants were found to have improved scores on the Unified Parkinson’s Disease Rating Scale. This suggests that not only do estrogen levels have an effect on dopamine metabolism, but that estrogen replacement therapy may lead to improvement in PD symptoms, and that it would be beneficial to incorporate females into PD research to find mechanisms of reducing required PD medication for treatment of symptoms, possibly. Given these findings, here we present results of a meta-analysis that investigates a possible sex bias in PD research and propose possible causes for such bias.
Material and Methods

Keyword Search
PubMed was searched for studies, published from 1970 to 2014 that were investigating PD using the following key phrases: “female clinical Parkinson’s,” “male clinical Parkinson’s,” “female rat Parkinson’s,” and “male rat Parkinson’s.” The range of 1970 to 2014 was chosen because it encompasses publications before the FDA requirement of females to be investigated in human clinical trials, and publications after the requirement. PubMed was used because of familiarity and accessibility. Each article was searched to confirm use of female or male subjects. For example, if a search result under “female clinical Parkinson’s” did not investigate females; it was subtracted from the search result total. The PubMed keyword search was conducted three times for each category, two months apart, in order to obtain an accurate representation of the publications surveyed.

Analysis of Search Results
In order to determine whether or not the results fit each keyword search, three-hundred studies were randomly selected out of the search results from the key phrases “female rat Parkinson’s” and “male rat Parkinson’s,” e.g., n=600 for the combined categories. Only articles that were in full text on PubMed were chosen. Each study was identified and categorized based on the sex of the non-human animal investigated and the gender of the first author. In order to be categorized as a study that incorporated female non-human animals, the female non-human animals had to be investigated and not just used for reproduction of male non-human animals. Studies that investigated non-human animals of the same sex used in the key phrase were categorized as “same-sex study,” and studies that investigated non-human animals of the opposite sex used in the key phrase, it was categorized as “opposite sex study.” For example, if the key phrase search was “male rat Parkinson’s,” and a study only utilized male rats, it was categorized as “same-sex.” If the sex of the non-human animal was unknown it was categorized as “unspecified,” and studies that incorporated both sexes was categorized as “both sexes.” Cell culture or embryonic investigations were excluded from the data because the focus was on sex biases on a whole organism level. Rhesus monkeys and various species of rats and mice were encountered when categorizing the search results.

In order to determine the gender identity of the first and last authors, web searches were conducted to retrieve the authors’ biographies and/or profiles that stated their preferred gender pronouns in reference to the scientist. From their preferred gender pronoun, their gender identity was categorized as male or female. If the biography and/or profile of the authors could not be retrieved, the gender of the author was categorized as unknown.

Statistical Methods
Microsoft Excel was used to carry out statistical analysis and generate figures. The number of results from all three trials of a keyword search were averaged. The average number of results for “female clinical Parkinson’s” and “male clinical Parkinson’s” were compared using a 2-tailed unpaired t-test with an alpha level of 0.05. The average number of results for “female rat Parkinson’s” and “male rat Parkinson’s” were compared using a 2-tailed unpaired t-test, with an alpha level of 0.05.

A one-way ANOVA, with an alpha value of 0.05, was used to compare the number of studies conducted on male non-human animals and female non-human animals from the 300 sample of each set of search results “male rat Parkinson’s” and “female rat Parkinson’s.”

Out of the 600 search results, a chi-square test was used to compare the number of female and male first authors who conducted studies on male or female non-human animals. A chi-square test was repeated to compare the number of female and male last authors who conducted studies on male or female non-human animals.
Results

A 2-tailed unpaired t-test was performed on the average number search results from each trial of each key phrase. The average number of search results for “male rat Parkinson’s” was 777 studies, which was significantly different from the average of 307 found with the term “female rat Parkinson’s” (p<0.05), as shown in Figure 1. The average number of search results for “male clinical Parkinson’s” was 1,797 studies, which was significantly greater than the 1,647 search results produced by “female clinical Parkinson’s” (p<0.05), as shown in Figure 1.

![Figure 1: The average number of search results for each query. Males under “rat Parkinson’s” was significantly more than for females, p=2.56x10^-6. The average number of search results including males under “clinical Parkinson’s” was significantly more than females p=3.72x10^-9. *Notes p<0.05.](image)

The number of studies conducted on male, female, both or unspecified non-human animals are shown in Figure 2. “Single sex studies” and “single sex studies on opposite sex” were categorized based on the criteria outlined in Analysis of Search Results. A one-way ANOVA showed there were significantly more studies conducted on male non-human animals than female non-human animals, p<0.05, as shown in Figure 2. The number of studies conducted on male non-human animals was 276 and the total number of studies conducted on female non-human animals was 188. In addition to significantly more studies investigating male non-human animals, the search results for “female rat Parkinson’s” were significantly more likely to be unspecified or investigate both male and female non-human animals (p<0.05), as shown in Figure 2.

![Figure 2: The number of studies from the 300 sample of each category (“male rat Parkinson’s” and “female rat Parkinson’s”). The average number of studies conducted on males under “single sex studies” was significantly greater than the average number of studies conducted on females, p=7.27x10^-6. The average number of studies unspecified was significantly greater for the key phrase “female rat Parkinson’s” than for the key phrase “male rat Parkinson’s,” p=2.73x10^-5. The average number of studies conducted on both sexes was significantly more for the key phrase “female rat Parkinson’s” than for the key phrase “male rat Parkinson’s”, p=0.006. *Notes p<0.05.](image)

The number of male and female first authors who investigated non-human animals of the same sex as themselves and different sex were compared by performing a chi-square test, as shown in Table 1. A significant difference was not found between female first authors who investigated female non-human animals and who investigated male non-human animals. However, 137 male first authors conducted studies on male non-human animals, which was significantly greater than the 67 male first authors who conducted studies on female non-human animals, \(x^2=9.26\ p<0.05\).

A chi-square test was also used to compare the number of male and female last authors who investigated non-human animals of
same, or opposite, sex as themselves. As shown in Table 2, there was no significant difference found between male and female last authors, $x^2=3.5$, $p>0.05$.

### Table 1: The number of studies from the 300 samples of each category “male rat Parkinson’s” and “female rat Parkinson’s” categorized by the sex of the non-human animal studied and the gender of the first author. The number of male first authors that did research on female non-human animal subjects was significantly less than male first authors that did research on male non-human animal subjects, $p=1.88\times10^{-6}$. *Notes $p<0.05$

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<thead>
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<tr>
<td>Female</td>
<td>70</td>
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### Table 2: The number of studies from the 300 sample of each category (“male rat Parkinson’s” and “female rat Parkinson’s”) categorized by the sex of the non-human animal studied and the gender of the last author.

<table>
<thead>
<tr>
<th>Gender Identity of last author</th>
<th>Sex of the non-human animal studied</th>
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<tr>
<td>Male</td>
<td>Female</td>
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<td>Female</td>
<td>71</td>
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### Discussion

As mentioned previously, there is some evidence that suggests sexual dimorphism plays a role in PD (Haaxma et al., 2007). Furthermore, there is evidence suggesting there is a translation between sexual dimorphism in non-human animals and humans, in regards to PD symptomology and treatment (Van Den Eeden, 2003). It was hypothesized that male humans and male non-human animals are more likely to be used for research. It was also hypothesized that the gender identity of the first and last authors would correlate to the sex of the non-human animal studied. This study confirmed the first part of our hypothesis; there are significantly more studies conducted on male humans and non-human animals in PD research.

There are several possible reasons as to why female humans and female non-human animals are left out of PD research. As mentioned by Cahill, many scientists still believe that it is simply not important to include females in biomedical research (Cahill, 2012). Many of the arguments against using female non-human animals are the cost. One of the common arguments against using female non-human animals often involves time and money constraints. Therefore, future directions should investigate a possible correlation between sources of funding and the sex of the non-human animal investigated.

The second part of the hypothesis was rejected. Although male first authors were significantly more likely to conduct research on male non-human animals, female first authors were not more likely to conduct research on female non-human animals. A correlation between the gender identity of last authors and the sex of the non-human animal studied was insignificant. A confound to the study is that most non-principal investigator researchers do not order the non-human animals themselves, and often use already available resources. However, a possible explanation of male first authors showing a bias is that they may be less likely to request new resources to be ordered by the principal investigator or to apply for funding to obtain female non-human animals. As mentioned previously, further investigations should determine if there is a correlation between sources of funding and the sex of the non-human animal investigated.

As mentioned by Cahill, if scientists were only to study one sex when sexual dimorphism is prevalent in the brain, then questions about neurological diseases, such as PD, are inadequately answered. In other words, we are drawing inaccurate conclusions (Cahill, 2012). If the male sex-bias in PD research continues, there could be negative consequences. If female non-human animals are not included in treatment research, the first time the treatment is being used on females would be in human clinical trials. This could result in inadequate treatment for female patients, possible detriments to their health, or a waste of...
resources, if treatment does not work as intended. These consequences are unknown simply because non-human females are not incorporated into PD research at the same rate as males.

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**References**