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Original Paper

The Social Relationship of Mice: The Influence of Sex and Status of Mice to the Approach Behavior toward Their Cage Mate

Zeyu Cai[1*]

[1] University of Toronto Mississauga, Mississauga, Canada

[*] Zeyu Cai, E-mail: abrycecai2000@gmail.com

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Abstract

Based on the statistics given by health report that interpersonal violence is among the leading causes of death for people aged 15-44 years worldwide. In order to explore the reasons of violent behavior occurrence, this article uses rodent as experiment subject and resident-intruder paradigm to conduct a 2x2 experiment. The experiment will measure whether sex or status will be significant on the effect of aggression occurrence and affiliative duration and number of cells count on their medial amygdala. The result of the experiment shows that male exhibit a higher level of aggression toward intruders compared to females. But for females, more afflilative behavior shown in the test toward their conspecifics. And male do have a larger cell counts in their medial amygdala, there is no evidence that these variables are caused driven by sex, status and sex: status interaction.

Keywords

status of mice, resident-intruder paradigm, aggression occurrence, affiliative duration

1. Introduction

Different social animals display different approach behavior toward their conspecifics. A report from world health organization showed that interpersonal violence is among the leading causes of death for people aged 1544 years worldwide, accounting for about 14% of deaths among males and 7% of deaths among females. What’s more, aggression is not only a major source of death worldwide but also a major source of serious health problems in the surviving victims of aggression. Therefore, there is a need to understand the mechanism of violence and aggression to prevent more casualties in the human society. In order to figure out the reason of aggression occurrence and study approach behavior in humans neurologically, rodents have been used for such research purposes due to it's anatomical, physiological are genetically similarity to human, in addition to their small size and easy maintenance cost. For mice, their social behavior is affected by distinct factors including reproduction, territorial conflict, and environmental context—such as punishment and reward. From the previous studies, it has shown that adult male rat will establish a territory when given sufficient living space, and territoriality is strongly enhanced in the presence of females and/or sexual experience. That means the residents will attack other unfamiliar intruders in their territory, and the intruder would show defensive aggression in response to the resident's offence. Thus, aggression can be studied by measuring the frequency, duration, and patterns of the subject's interactions by using such resident-intruder paradigm in a semi-natural environment. In addition, eurological studies in aggression are also critical in studying aggression. Studies shown that medial neucleos in the amygdala plays a key role in emotion behaviors by relaying olfactory information to hypothalamic nuclei involved in reproduction and defense. However, there isn't abundant research and study about the neuro-component of this region or it's role in the olfactory circulatory of the amygdala. Another study have shown that oxytocin in the medial amygadala is critical in social recognition in mice, as Ferguson, J. N. and colleges discovered oxytocin knocked out mice failed to recognize their conspecifics after repeated social exposure. This finding is critical in the context of this study, since mice requires recognition of intruders for the resident-intruder paradigm to have effect.

2. The Process of Experiment

2.1 Experimental Materials

Laboratory animal: 6 male C57Bl/6j mice as breeder; 6 male C57Bl/6j mice as subordinate; 6 female C57Bl/6j mice as breeder; 6 female C57Bl/6j mice as subordinate.

Experimental drug: Avertin anestheia; 20 mL of 0.5% triton-x in Phosphate Buffer Saline; 10%NHS in PBS-T; 1:2000 dilution of NHS secondary antibody

Experimental device: freezing microtome; 40X objective magnification microscope;

2.2 Experimental Method

Step one: At the begining of the test, each rat is placed in their respective cages, after 2~3 min where the mice have accustomed with the environment, another mouse is placed into their cages, the interaction between the 2 subjects are recorded (aggression level and affiliative behavior). Step two: Then after the resident-intruder paradigm, the mice are given avertin anestheia for 90 minutes and transcardially perfused to extract their brains tissues.

Step three: Use a freezing microtome to slice brain to 30 mircons, and processed by 1) 20 mL of 0.5% triton-x in PBS as a permilableization solution, 2) 10%NHS in PBS-T as a blocking solution 3) 1:1000 dilution of the C-Fos primary antibody 4) 1:2000 dilution of NHS secondary antibody. Step four: Samples are mounted on the microscope slide for the observation of the medial amygdala. Step five: Sections were mounted on the microscope slide for the observation of the medial amygdala. Step six:

The samples were placed on a 40X objective magnification microscope, stained cells on the sample were counted and recorded.

2.3 Data Collection and Analysis

In the study, the experiment will measure whether sex (independent variable 1) or status (independent variable 2) will be significant on the effect of aggression occurrence (dependent variable 1) affiliative duration (dependent variable 2) and number of cells count on their medial amygdala (dependent variable 3). All the experiment data will be collected by Boris, FIJI and R studio. The data of the subject's aggression occurence, affiliative duration, and number of cell counts on their median amygdala are analyzed on a ANOVA table. Their P value of sex, status, sex: status interaction is compared to significant value of alpha = 0.05.

If their P value is higher than 0.05, the paper cannot conclude the significance of the data is due to the corresponding variables; if P vale is lower than 0.05, the paper can conclude the significance of the data is due to the corresponding variable.

3. Results and Analysis

Based on the 2 way ANOVA Table 1, the effect on the subject's aggression occurrence is significant ($p=0.000897>0.05$) compared to the influence of status ($P=0.105552<0.05$) and sex: status interaction ($P=0.105552<0.05$). From the box plot (Figure 1) and bar plot (Figure 2), it is observed that males have a higher mean aggression occurrence than female, which means the effect of sex on aggression is driven by males. In addition, male breeders show mean aggression distinctly than male breeders according to the plot (Figure 1). But the conclusion can't be drawn as the higher aggression level of male breeders is caused by sex: status interaction due to the significant higher P value.

Table 1: ANOVA table of aggression level

```
nova_output<-aov(formula = aggression_occurences ~ sex + status + sex:status, data
=mydata)> summary(anova_output)
             Df  Sum Sq Mean Sq F value Pr(>F)
sex           1  715.0   715.0  15.179 0.000897 ***
status        1  135.4   135.4   2.874 0.105552
sex:status    1  135.4   135.4   2.874 0.105552
Residuals          20  942.2    47.1
```

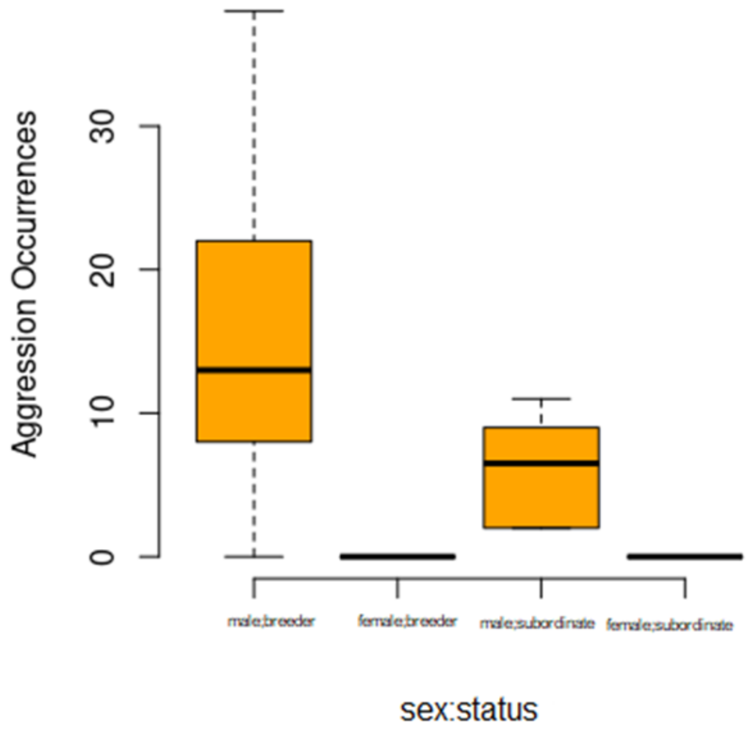

Figure 1. Box Plot of Aggression Occurrences

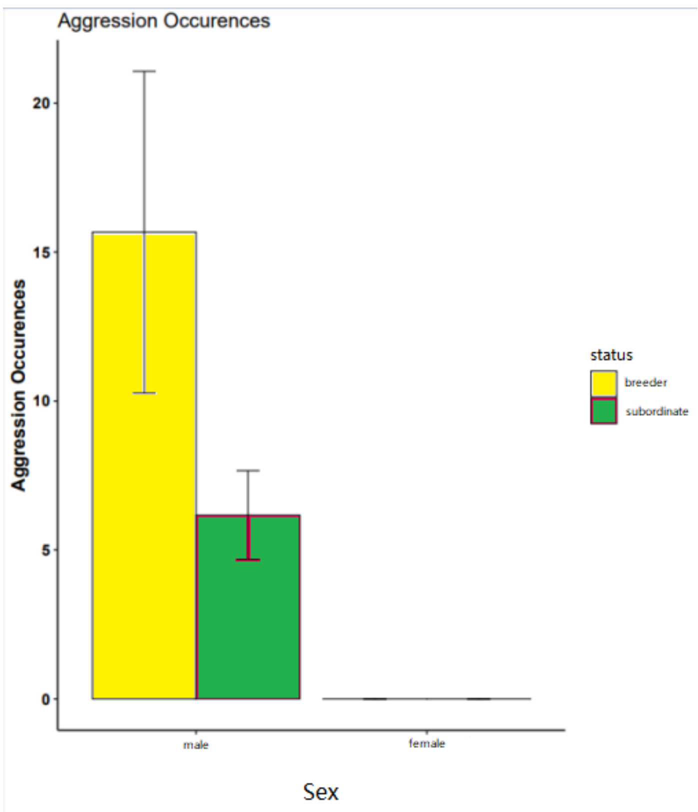

Figure 2. Bar Plot of Aggression Occurrence

From the 2 way ANOVA table (Table 2), nether sex (P=0.1190>0.05), status (P=0.4240>0.05), or sex: status interaction (P=0.4780>0.05) have significant effect on the subject's affiliation duration. From the box plot (Figure 3) and bar plot (Figure 4), it is observed that female have a higher mean affiliative duration than male, but since the P vale of sex is higher than the significance level, this paper cannot arrive at the conclusion that sex differences attribute to the higher affiliative duration of female. Expect this, Although the plot (Figure 4) clearly demonstrates higher affiliative duration occur to male subordinates rather than male breeders, the paper can't draw a conclusion that sex: status interaction could lead to the higher affiliative duration of male subordinate because the P value of sex: status interaction is higher than the significant level.

Table 2. ANOVA Table of Affiliative Duration

```
anova_output<-aov(formula = affiliative_duration ~ sex + status + sex:status, data=mydata)> summary(anova_output)
Df Sum Sq Mean Sq F value Pr(>F)
sex 1 84029 84029 2.647 0.119
status 1 21139 21139 0.666 0.424
sex:status 1 16575 16575 0.522 0.478
Residuals 20 634895 31745
```

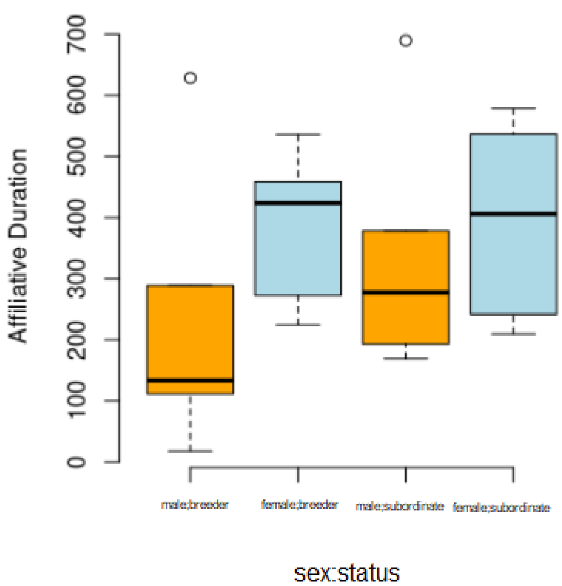

Figure 3. Box Plot of Affiliative Duration

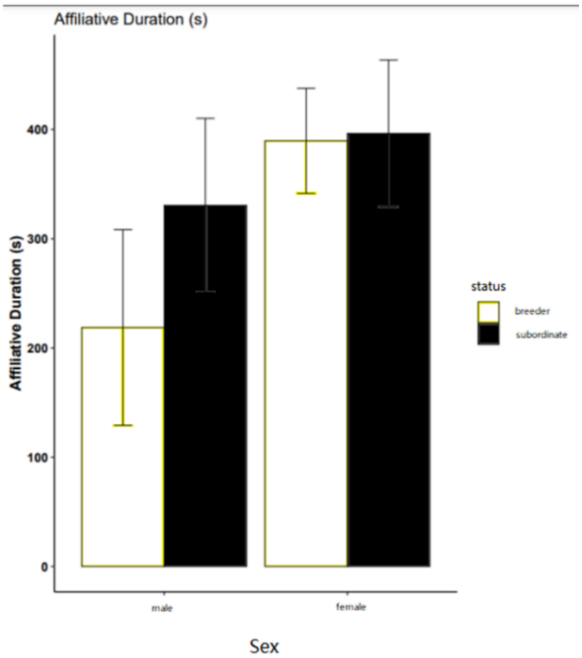

Figure 4. Bar Plot of Affiliative Duration

From the 2 way ANOVA Table (Table 3), it is clearly evident that nether status (P=0.5490>0.05) or sex: status interaction (P=0.5114>0.05) have more significant effect on the subject's cell count in their median amygdala than sex (P=0.1190>0.05), which is near to the significance but not achieve it. From the box plot (Figure 5) and bar plot (Figure 6), it is clear that male have higher cell counts compared to females, but since the P value of sex is higher than the significant value, this paper can't achieve the conclusion that the higher cell counts of males is caused by sex differences.

Table 3. ANOVA Table of Cell Counts

```
anova_output<-aov(formula = cellcounts ~ sex + status + sex:status, data=mydata)> summary (anova_output)
Df Sum Sq Mean Sq F value Pr(>F)
sex 1 32487 32487 3.469 0.0773 .
status 1 3480 3480 0.372 0.5490
sex: status 1 4187 4187 0.447 0.5114
Residuals 20 187299 9365
```

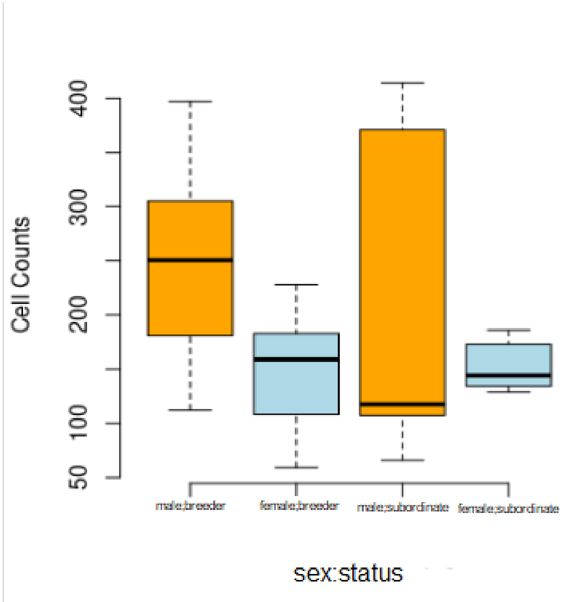

Figure 5. Box Plot of Cell Counts

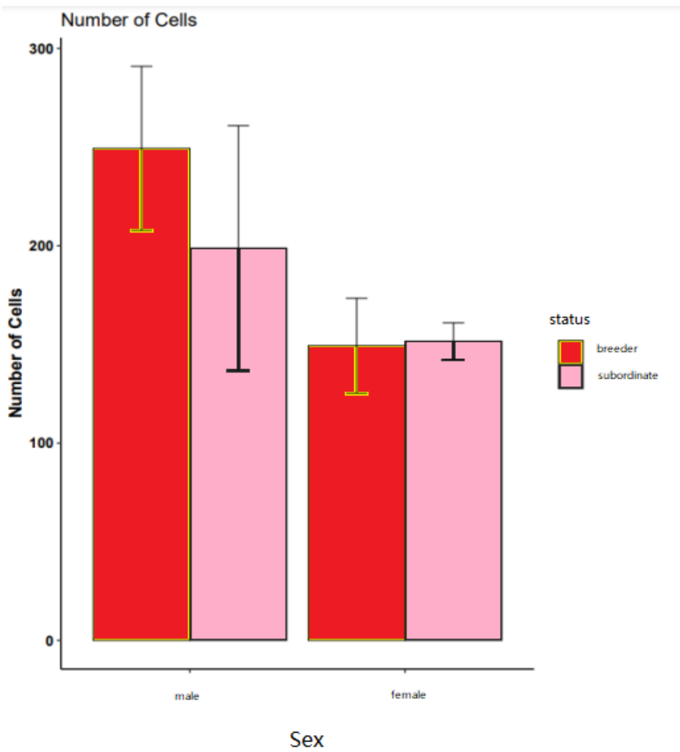

Figure 6. Bar Plot of Cell Counts

4. Discussion

From the results of past studies, males exhibit a higher aggression level since male rats exhibit a higher level of territorial awareness, and is further enhanced in the presence of females and/or sexual experience. Previous research has shown that the higher aggression level of male rats, supported by the territorial awareness in male rats, can be further enhanced in the presence of females and/or by having sexual experience. Since medial amygdala is critical for conspecifics recognition and plays a key role in emotion behaviors by relaying olfactory information to hypothalamic nuclei involved in reproduction and defense aggression, higher aggressive subject have more activity in their medial amygdala. For statistical analysis indicates a not significant result, this study shows results similar to the previous studies. But some differences occur in the experiment, which can be contributed to the method of data recording and the judgement toward mice behavior. For the way of data recording, since this is an online lab project, data is collected through a top down viewing camera video, which may cause errors and reduced accuracy when the two subjects are very close together. For the judgement of mice behavior, mice display some sniffing behaviors, and it poses a challenge for the author to judge the whether the sniffing behavior is toward the other mice or toward the environment. For the former, it can be viewed as the affiliative interaction. For the latter, it shows no interaction happen between mice.

5. Conclusion

In conclusion, the findings from previous studies and the current research support the notion that male rats generally display a higher level of aggression, which is attributed to their heightened territorial awareness. The presence of females and sexual experience further intensifies this aggression. This elevated aggression in males is associated with increased activity in the medial amygdala, a brain region crucial for conspecific recognition and emotional behaviors. Although the statistical analysis did not yield significant results in this study, it aligns with previous findings. However, certain differences in experimental procedures, such as data recording methods and the interpretation of mouse behavior, may have contributed to variations in the results. Online lab projects utilizing top-down camera views for data collection may introduce errors and reduced accuracy, especially when subjects are in close proximity. Additionally, differentiating between sniffing behaviors directed towards other mice versus the environment poses challenges in accurately assessing mouse interactions. Nonetheless, the study concludes that male mice exhibit higher aggression towards intruders compared to females, while females tend to display more affiliative behaviors towards conspecifics. Furthermore, the presence of a larger cell count in the medial amygdala of male mice does not appear to be influenced by sex, status, or their interaction.

Furthermore, the study primarily focused on the influence of sex and sexual experience on aggression levels and medial amygdala activity. However, other factors, such as genetic variations, hormonal fluctuations, and environmental influences, may also contribute to individual differences in aggression. Future studies could consider exploring these factors and their interplay with sex and social experiences to gain a more comprehensive understanding of the underlying mechanisms of aggression.

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