COMPARISON OF SOCIAL BEHAVIOR AND HOUSING CONDITION EFFECTS ON SOCIABILITY SCORES IN LEWES AND NY3 MOUSE LINES USING A THREE-CHAMBER PARADIGM TEST

Undergraduate Honors Thesis

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Abstract

Studying social behavior in mice is a crucial area of research in neuroscience, providing insights into information transmission between conspecifics and modulators of their behavior in addition to identifying what these social signals may control. However, little research has been done to compare social behaviors amongst strains of wild-derived mice such as NY3 and LEWES, which have been bred from mice caught in the wild compared to the typically utilized laboratory strains bred for generations within the laboratory. The objective of this research project is to differentiate between social behaviors, as measured by sociability scores, in these mouse lines using a three-chamber paradigm test (3CT). The study also aims to assess the effects of housing conditions, specifically between single-housing and pair-housing, on sociability given the implications of isolation as a social stressor, and thus making it important to understand the impact of housing conditions on social behavior in these mouse lines.

The three-chamber paradigm test is a commonly used technique used for studying social behavior in mice which involves placing a test mouse in a chamber with three compartments and giving it the opportunity to interact with either a stranger mouse, the social stimulus, or an inanimate object, the non-social stimulus. The subsequently derived sociability score is a measure of how much time the test mouse spends in the compartment with the social stimulus compared to the time spent with the non-social stimulus, and is ultimately used as a measure of social behavior in mice.

The outcomes of this study may be of significant value in future behavioral studies involving these or genetically similar mouse strains, as they may provide insight into the determinants of sociability and the ways in which housing conditions may affect such social behaviors.

Acknowledgements

I would like to express my deep appreciation to my research supervisor, Dr. Andrew Moeller, for his invaluable guidance and unwavering support throughout my thesis project. His expertise and encouragement have been instrumental in shaping my research skills and academic pursuits.

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Table of Contents

Title Page……...
Abstract..……. 1
Acknowledgements……..3
Table of Contents…….4
Introduction……..5
Materials & Methods…….……..…….8,9

- Methods
    - Pair-Housing vs Single- Housing
    - Three-Chamber Paradigm Behavioral Trials
- Behavioral Trial .AVI Processing
- Sociability Scoring

Results & Discussion…….12

- Mixed Effect Model
- Sociability Scores in NY3 vs. LEWES Lines
- Sociability Scores in Pair vs. Single-Housing
    - Sociability in Lines vs. Housing Condition
- Sociability Scores in Female vs. Male Mice
    - Sociability in Lines vs. Sex
- Comparing Sociability Scores Between Line, Sex, and Housing Condition

Biological Implications and Future Work……..24
Citations…….26

Introduction

Evaluating the behavior of mice has been at the forefront of research in neuroscience for years where significant breakthroughs in behavioral outcomes have provided researchers with ample data for the development of novel therapies, such as the use of astrocytes as a therapeutic target in Alzheimer's Disease (Rodríguez-Giraldo et al., 2022). Social behavior in particular is a crucial area of research as it provides researchers with critical insights regarding information transmitted between conspecifics and the modulators of their behavior as well as what these social signals control (Morozov, 2019). By examining social behavior, researchers can gain a deeper understanding of the mechanisms underlying communication through different modalities such as vocalizations and body language, and how environmental, genetic, and experiential factors shape social behavior. Ultimately, this knowledge can inform a broad range of scientific inquiries, from understanding the neural circuits involved in social behavior to developing novel therapies for disorders that impact social interactions.

While there are a great deal of studies explaining the multitude of factors affecting a mouse's behavioral tendencies in a social setting, little has been done to compare the social behaviors between strains of wild- derived strains such as the NY3 and LEWES lines. Wild-derived mice are captured from the wild and then bred in captivity for use in research and are particularly advantageous to use in behavioral research compared to normal lab strains due to their exhibition of a more complex and wide range of behaviors which are more representative of those observed in natural populations (Harper, 2008). This makes them valuable models for future studies involving genetic and environmental factors that influence behavior in wild populations.

Mice are objectively social animals with tendencies to live in groups. However, mice are often used in laboratory experiments and kept in isolation or pair-housing conditions (Van Loo et al., 2004). Single housing and pair housing have been widely used in animal research, but it is important to understand the impact of these housing conditions on the mice's behavior and sociability.

Single-housing is often used in laboratory experiments due to its reduction in variability of experimental data as well as allowing for better control over environmental factors. However, single housing has been shown to have negative effects on the sociability of mice; For example, mice that are single-housed have been found to show reduced social behavior compared to group-housed mice and have been found to show increased anxiety-like behavior and elevated stress hormone levels (Pais et al., 2019). Pair-housing is thought to better mimic the social environment of mice in the wild (Harper, 2008) where pair-housed mice have even been found to show increased social behavior compared to single-housed mice such as increased social grooming behavior (Green et al., 2010). Additionally, pair-housing has been found to reduce anxiety-like behavior and stress hormone levels in mice (Van Loo et al., 2004).

Variable social behaviors can be identified using a predetermined three- chamber paradigm test (3CT) sociability protocol. The 3CT is a commonly used technique used for studying social behavior in mice which involves placing a test mouse in a chamber with three compartments and giving it the opportunity to interact with either a stranger mouse, the social stimulus, or an inanimate object, the non-social stimulus [*Figure 1*] (Yang et al., 2011). The 3CT test serves as

not only a useful tool in evaluating the variable social behaviors of the mouse strains, but is also one of the most popular social behavior tests (Kaidanovich-Beilin et al., 2011) used in research evaluating multiple mouse strains making the results of this research project even more applicable for future uses. The subsequently derived sociability score is a measure of how much time the test mouse spends in the compartment with the social stimulus compared to the time spent with the non-social stimulus, and is ultimately used as a measure of social behavior in mice (Sankoorikal et al., 2005).

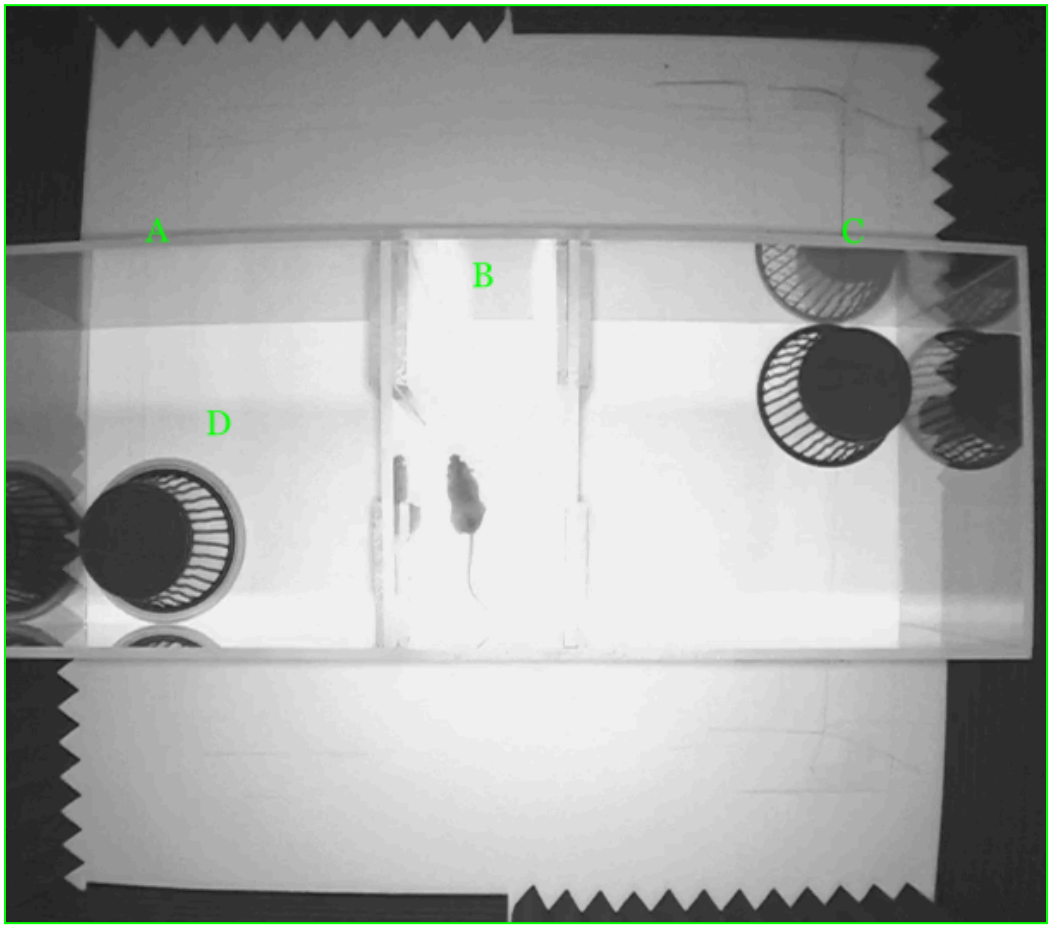

Figure 1. The three- chamber paradigm test is set up using a rectangular, three-chambered box (labeled A, B and C). Dividing walls are made from clear Plexiglass, with small openings that allow access into each chamber. The center chamber (B) is the start location. The empty wire cup in the right chamber is the non-social stimulus while the wire cup with the plastic bottom holding the stranger mouse is the social stimulus (D). A camera will be set up to record time spent engaging with the social and/or non-social stimulus.

The present study hypothesizes that there will be varied social behaviors observed in the 3CT between the NY3 and LEWES strains, with a preference towards sociability from the NY3 strain in comparison to the LEWES. Additionally, it is hypothesized that the pair-housed mice, regardless of strain, will exhibit more sociability than those that were single-housed. The overarching goal of this project would be to facilitate more effective future social behavioral mouse studies by clarifying the inherit social tendencies of certain mouse strains and their efficacy in certain behavioral studies in addition to the ways in which housing conditions may affect such social behaviors.

Materials

The NY3 and LEWES mouse lines used within this study were wild-derived inbred strains commonly used in behavioral studies. NY3s have been shown to have high levels of spatial and social memory (Miller et al., 2023), while the LEWES strain has been found to display tamed social behavior in genome- sequencing tests (Liu et al., 2022).

A total of 26 NY3 mice (13 female, 13 male) and 14 LEWES mice (4 female, 10 male) were used for a total of 40 wild-derived mice used for this study. Mice were selected based on line as well as age; only mice between the ages of 4 and 6 weeks were utilized in this study. These mice were obtained from the Sheehan Laboratory Mouse Colony. All use of animal handling and processing was done in accordance with the Institutional Animal Care and Use Committee (IACUC).

Additionally, a total of 11 stranger mice (8 NY3 and 3 LEWES) were selected for the 3CT based on mouse line, sex, and proximity to the test mice's age. Size and weight were initially factors as well but were eventually deemed insignificant as only breeding cages held mice with the best criteria for stranger mice and are often variably sized.

Methods

Pair-Housing vs. Single Housing

Of the 26 NY3 mice used in the study, 16 were pair-housed (6 female, 10 male) and 10 were single-housed (5 female, 5 male) such that there was a replicate count of greater than three (n>3) for the NY3 line. Similarly, of the 14 LEWES mice used, 8 were pair-housed (2 female, 6 male) and 6 were single-housed 6 were single-housed (3 female, 3 male) such that there was a replicate count of greater than five (n>5) for the LEWES line.

The Litter Effect is a phenomenon referring to the influence of littermates on the behavior of an individual mouse in a social behavioral study. Litter Effect is often known to impact the outcome of behavioral research, similar to this study, and is typically controlled to ensure significant results (Jiménez & Zylka, 2021). Litter Effect was controlled for by singling out and then splitting litters by sex and then into single and/or pair housing.

Three-Chamber Paradigm Behavioral Trials

The three- chamber apparatus for the three- chamber paradigm test is a plexiglass box divided into three compartments by two transparent partitions [Figure 1]. At the floor level of each

partition there is an opening located in the center to allow for access into each chamber. Two small, round wire cages are placed in the diagonal corner of the apparatus for enclosing a stranger mouse or nothing (the social vs non-social stimulus). After a 5 week habituation period to their respective housing condition, the mouse strains begin the 3CT.

On the test day, a stranger mouse matching in line, sex, and age to the test mouse is placed into one of the two wire cages while nothing will be placed into the other and the subject mouse will be placed in the center and allowed to freely explore the center chamber for 5 minutes in order to allow for habitualization. After 5 minutes, the test mouse will be allowed 10 minutes of access to the two side chambers where they will have the choice between a social stimulus (the stranger mouse) and a non-social stimulus (the empty wire cup) in this sociability phase. The location of the stranger was alternated between tests and were not exposed to the test mice before introduction in the 3CT.

Behavioral Trial .AVI Processing

Manual scoring was performed blinded to mouse strain and housing condition. This was done by randomizing the dates and times obtained from the 3CT recordings and matching mouse ID's and line only after engagement with social stimulus and non-social stimulus was recorded. All videos generated within the experiment were scored by the same experimenter to minimize human error.

For manual scoring purposes, behaviors that were counted as interactions [*Figure 2*] included directly interacting with the stimulus mouse or non-social object between the wire bars of the wire cup, sniffing the base of the cup containing the stimulus, interacting with parts of the

stimulus that are protruding from the cup, such as the tail of the stimulus mouse, and actively attending to (sniffing/facing) the stimulus while climbing the cup. Behaviors that were not counted included standing near the cup without attending to (sniffing/facing) the cup or the contained stimulus and self-grooming in the proximity of the cup. Two timers were used to record the time spent with the social stimulus and the time spent with the non-social stimulus separately.

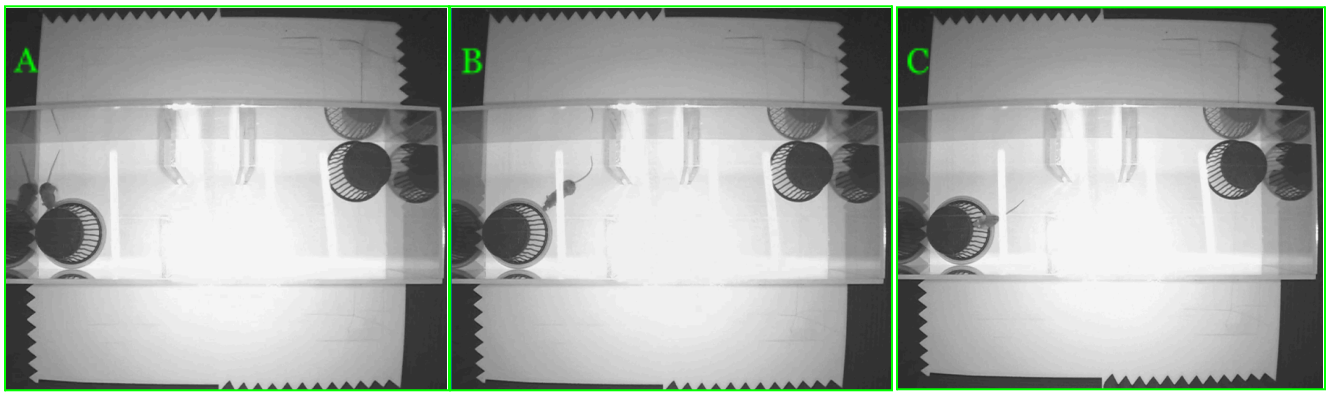

Figure 2. The figure above shows examples of the sociability patterns observed and recorded as engagement. (A) shows direct interaction with the stimulus mouse between the wire bars of the wire cup, (B) shows sniffing of the base of the cup containing the stimulus, and (C) shows active attention to (sniffing/facing) the stimulus while climbing the wire cup.

Sociability Scoring

A sociability score is a measure of the level of social interaction exhibited by a mouse and is calculated based on the mouse's behavior during the three-chamber test. In order to calculate the sociability score, the time spent by the test mouse engaging with the social and non-social stimulus is recorded and utilized; specifically, the amount of time spent interacting with the stranger mouse (the social stimulus) is compared to the amount of time spent interacting with the empty wire cup (the non-social stimulus). A sociability score is then calculated as the difference between these two times, normalized to the total time spent in both chambers, in this case 10 minutes (or 600 seconds). A positive sociability score will indicate a preference for social

interaction, while a negative score will indicate a preference for nonsocial interaction and a score of 0 will indicate no preference for either type of interaction (Sankoorikal et al., 2005). The sociability score from the 3CT is what is ultimately used to determine social behavior and housing condition differences between NY3 and LEWES lines.

Results and Discussion

Mixed Effect Model

Meta-data points (including variables such as mouse ID, line, sex, litter, date of birth, age, housing condition, weight, etc.) were placed into coding-language R for statistical computing and graphics. The meta-data was then merged with the behavioral data and subsequent sociability scores and groups were separated based on their respective subsets: sex, housing condition, line, and the final overarching subset- mouse line, sex, and housing condition). Code was then generated and used to make the bar plots showing the mean of the variables (and their standard errors) with the x-axis value defined as the group variable and the y-axis as the response variable.

A mixed effect model with the function LMER-Test:lmer was then run. A mixed-effect model is a statistical method used to analyze data with both fixed and random effects, and in this study in particular, to analyze data that include repeated measures of behavior across multiple mice while also accounting for individual differences between the mice. In this study, the response variable is defined as the sociability score. The fixed effects are the factors explaining the variability in the sociability score while the random effect defined was the litter in order to address that the

samples are not independent of each other given the Litter Effect. 14 litters were defined for a total of 14 degrees of freedom.

[*Table 1*] The random effect was then used to produce an intercept showing that the litter was not significant. Estimates, standard errors, degrees of freedom, T-values for the test statistic, and then the p-values were subsequently calculated.

```
Sociability_score ~ Mouse_Line * Housing * Sex + (1 | Litter)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']
Formula: formula
   Data: sd

REML criterion at convergence: 261.7

Scaled residuals:
     Min       1Q   Median       3Q      Max
-1.78016 -0.69895  0.00995  0.51440  1.77347

Random effects:
 Groups   Name        Variance Std.Dev.
 Litter   (Intercept)  68.85    8.297
 Residual             103.80   10.188
Number of obs: 40, groups:  Litter, 14

Fixed effects:
                                   Estimate Std. Error      df t value Pr(>|t|)
(Intercept)                          -8.978     10.088  22.695  -0.890   0.3828
Mouse_LineNY3                        24.832     11.393  20.843   2.180   0.0409 *
HousingSingle                        11.408     10.610  30.131   1.075   0.2908
SexMale                              12.768     11.442  26.674   1.116   0.2744
Mouse_LineNY3:HousingSingle         -23.691     13.049  32.000  -1.816   0.0788 .
Mouse_LineNY3:SexMale               -23.149     12.525  30.197  -1.848   0.0744 .
HousingSingle:SexMale               -22.519     12.929  28.598  -1.742   0.0923 .
Mouse_LineNY3:HousingSingle:SexMale  32.266     15.565  27.479   2.073   0.0477 *
-
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 1. The table above shows the results of a linear mixed model fit by REML. Random effects included litter (Variance: 68.85; St.Dev.: 8.297) and residual data (Variance: 103.8; St.Dev.: 10.188). Fixed effects were additionally evaluated. The NY3 line was comparable to the LEWES line by an estimate value of +24.832 with a Std. Error of 11.393; df of 22.695; t-value of 2.180; and a p-value of 0.0409. The values single vs. pair-housing, male vs. female, single vs. pair housing by line, male vs. female by line, male vs. female by housing condition, and an overarching analysis of line vs. housing condition vs. sex were subsequently analyzed using the same pattern as was described above for the mouse line.

Statistical data of importance to note are housing conditions and sex factors, which display p-values of 0.2908 and 0.2744. Observing the extremely significant estimate value of +24.832 for the mouse line factor, we hypothesize this stark difference in mouse lines affects the housing

and sex effects. These results justify looking within each mouse line, specifically between the females and males between the lines and analyzing the interacting between mouse line and housing condition, especially given their borderline statistical significance. All other fixed effects excluding the line vs. housing, line vs. sex, and housing vs. sex were statistically significant, producing a p-value within the statistical threshold of 0.05. The excluding factors are still considered borderline significant being within a range of 0.02-0.04 from the statistical threshold. Additionally, the last fixed effect looking at mouse line, housing condition, and sex displays a p-value of 0.0477, making each factor statistically significant from one another in their comparison to each other. This makes this comparison in particular intriguing to look at in its graphical representation.

Sociability Scores in NY3 vs. LEWES Lines

Following the 3CT and subsequent .AVI processing, sociability scores were calculated in order to determine overarching differences in social behavior between the two wild-type strains, irrespective of housing condition, or sex. *Figure 3A* shows the raw graphical representation of the engagement time with the social and non-social stimulus between the mouse lines while *3B* displays these findings in terms of sociability between the NY3 and LEWES lines.

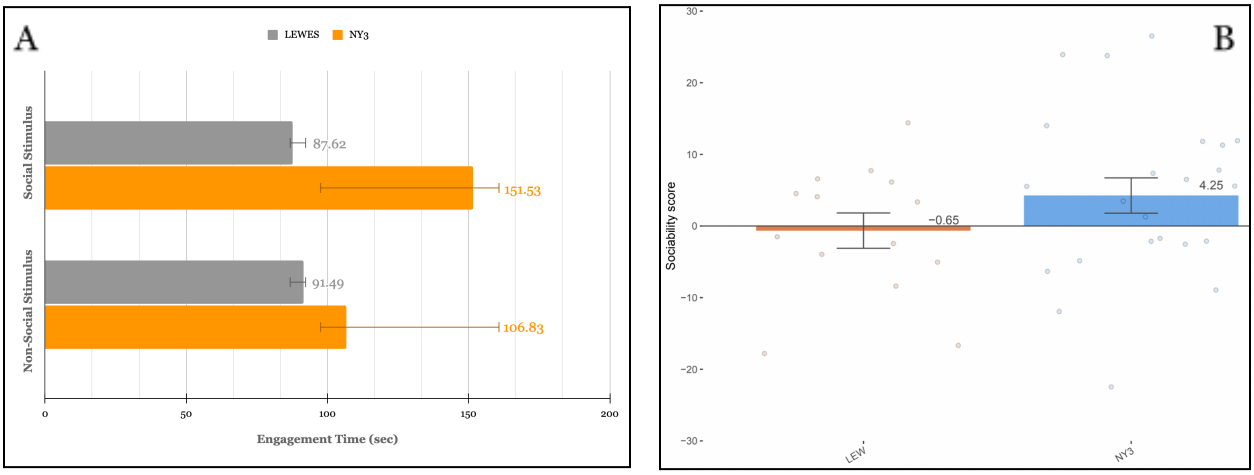

Figure 3. (A) The figure above shows the average engagement time with social and non-social stimulus between NY3 and LEWES mouse lines. During the 600sec behavior trial, the LEWES line engaged with the social stimulus for 87.62sec and the non-social stimulus for 91.49sec. The NY3 line engaged with the social stimulus for 151.53sec and the non-social stimulus for 106.83sec. St.Dev. lines are calculated and graphed respectively to the strain. (B) The figure above shows average sociability scores between NY3 and LEWES mouse lines. LEWES was calculated to have a sociability score of -0.65%. NY3 was calculated to have a sociability score of 4.25%. Dot values are made visible for individual mouse sociability values depending on strain. Standard deviations are calculated respectively to the strain.

The sociability scores for the LEWES line was calculated and averaged to a score of -0.65% while the sociability scores for the NY3 line was calculated and averaged to a score of 4.25%, indicating a preference for social interaction for the NY3 strain and a preference for nonsocial interaction for the LEWES strain. These scores match the raw graphical representation of time spent with social vs. non-social stimulus, where the LEWES strain spent on average 3.87sec longer with the non-social stimulus than the social stimulus during the 600sec behavioral trial period, whereas the NY3 strain spent an average of 44.7sec longer with the social stimulus than the non-social stimulus. Additionally, these stark differences in sociability between lines is supported by *Table 1* with an estimate value of +24.382.

Sociability Scores in Pair vs. Single-Housing

Following the 3CT and subsequent .AVI processing, the sociability scores previously calculated were then used to determine differences in social behavior based on housing conditions irrespective of the two wild-type strains. *Figure 4* shows the sociability scores between the pair and single-housed conditions.

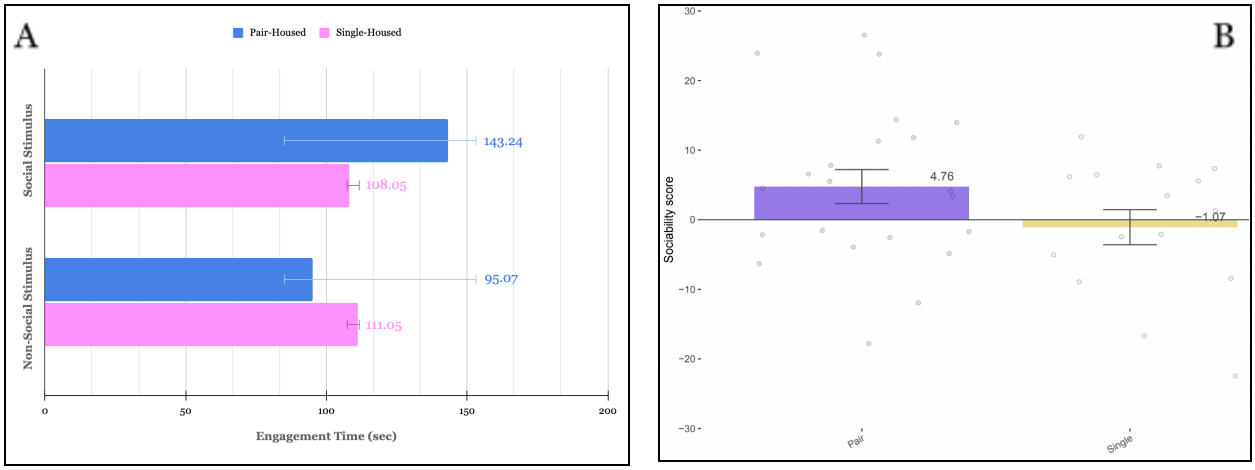

Figure 4. (A) The figure above shows the average engagement time with social and non-social stimulus between pair and single-housed conditions. During the 600sec behavior trial, the pair-housed mice engaged with the social stimulus for 143.24sec and the non-social stimulus for 95.07sec. The single-housed mice engaged with the social stimulus for 108.05sec and the non-social stimulus for 111.05sec. St.Dev. lines are calculated and graphed respectively to the housing condition. (B) The figure above shows average sociability scores between pair and single-housed conditions. Pair-housing sociability score was calculated to an average of 4.76%. Single-housing variable was calculated to have a sociability score of -1.07%. Dot values are made visible for individual mouse sociability values depending on housing condition. Standard deviations are calculated respectively to the housing condition.

As was previously noted, due to the significant differences between the mouse lines (estimate value of +24.832) the similarly stark differences in the housing conditions may have been affected as a result. More reliable results could be obtained with a larger data pool. With this preface given, the sociability scores for the pair-housed mice was calculated and averaged to a score of 4.76% while the sociability scores for the single-housed mice was calculated and averaged to a score of -1.07%, indicating a preference for social interaction for the pair-housed mice and a preference for nonsocial interaction for the single-housed mice. These scores match the raw graphical representation of time spent with social vs. non-social stimulus, where the pair-housed mice spent on average 48.17sec longer with the social stimulus than the non-social stimulus during the 600sec behavioral trial period, whereas the single-housed mice spent an average of 3.0sec longer with the non-social stimulus than the social stimulus. Additionally,

these differences in sociability between housing conditions is supported by *Table 1* with an estimate value of +11.408.

Sociability in Lines vs. Housing Condition

The sociability scores previously calculated were used to determine differences in social behavior based on housing conditions as a factor of the two wild-type strains. *Figure 5* shows the sociability scores between the pair and single-housed conditions as a factor of the NY3 and LEWES mouse lines.

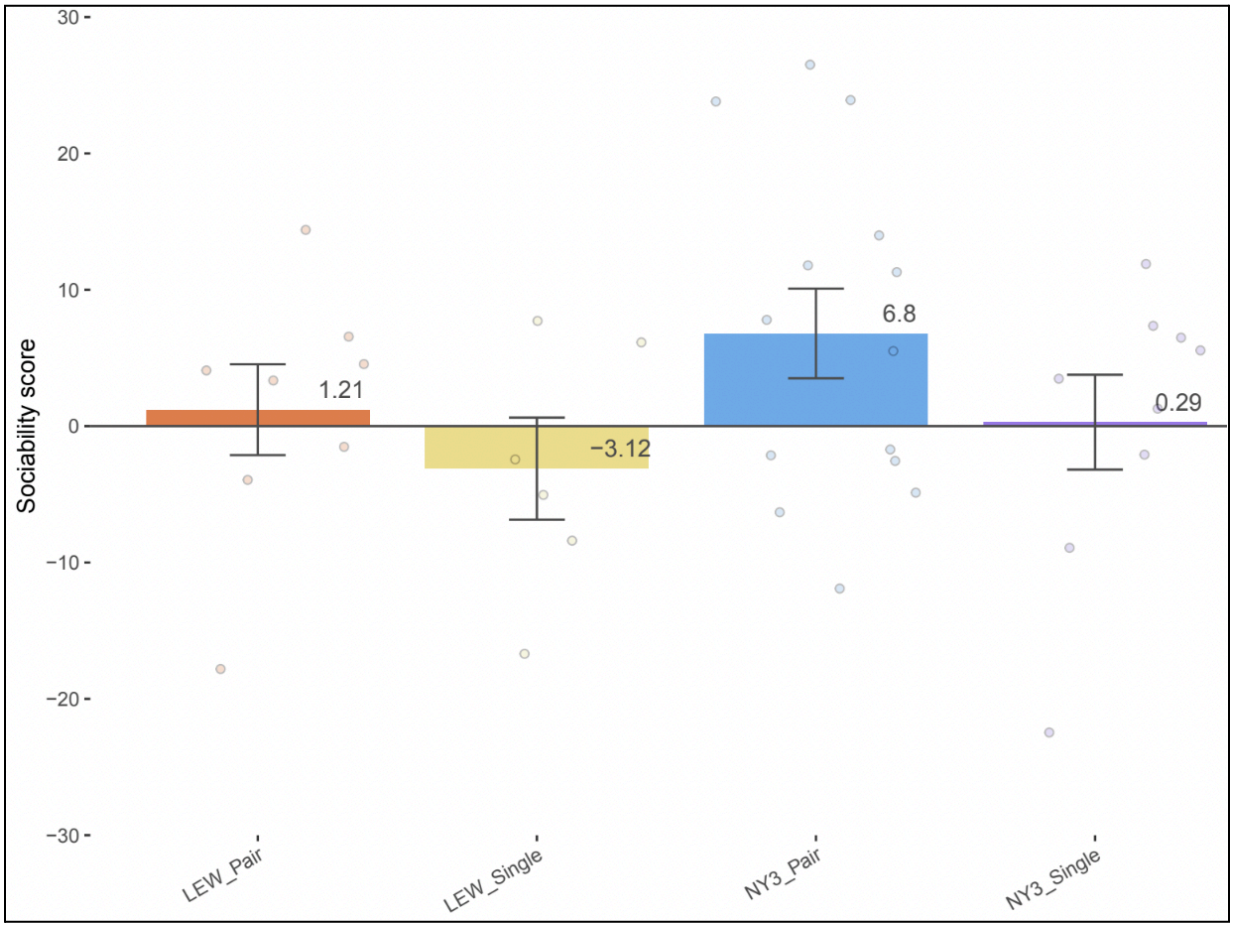

Figure 5. The figure above shows the averaged sociability scores between the pair and single-housed conditions as a factor of the NY3 and LEWES mouse lines. During the 600sec behavior trial, the LEWES pair-housed mice's sociability score was calculated to an average of 1.21%, and -3.12% for the LEWES single-housed mice. Comparatively, the NY3 pair-housed mice's sociability score was calculated to an average of 6.80%, and 0.29% for the NY3 single-housed mice.

The results in *Figure 5* display what is expected given the calculated individual trends from the sociability scores between mouse lines and the sociability scores between housing conditions. The NY3 line scored an average of 5.59% higher than the LEWES for the pair-housed mice and 3.41% higher than the LEWES for the single-housed mice. These results support the hypothesis that not only do the NY3 mice have a higher preference for social interaction compared to the LEWES mice, but the hypothesis holds true to for the housing conditions as well, where the single and pair-housed NY3 mice display more sociability than the single and pair-housed LEWES mice.

Sociability Scores in Female vs. Male Mice

The sociability scores previously calculated were then used to determine differences in social behavior based on sex irrespective of the mouse line. *Figure 6A,B* shows the sociability scores between the female and male mice tested.

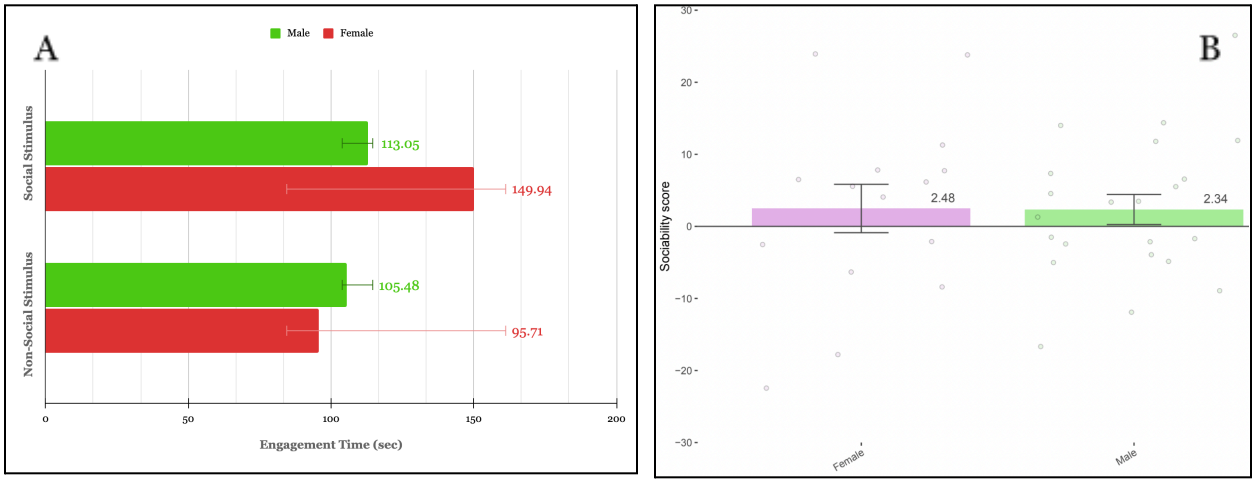

Figure 6. (A) The figure above shows the average engagement time with social and non-social stimulus between female and male test mice. During the 600sec behavior trial, the male mice engaged with the social stimulus for 113.05sec and the non-social stimulus for 105.48sec. The female mice engaged with the social stimulus for 149.94sec and the non-social stimulus for 95.71sec. St.Dev. lines are calculated and graphed respectively to the sex. (B) The figure above shows average sociability scores between male and female test mice. Male sociability score was calculated to an average of 2.34%. The female sociability score was calculated to an average of 2.48%. Dot

values are made visible for individual mouse sociability values depending on sex. St.Dev. lines are calculated and graphed respectively to the sex.

As was previously noted, due to the significant differences between the mouse lines (estimate value of +24.832) the similarities between the female and male test mice may have been affected as a result. More reliable results could be obtained with more data. With this preface given, the sociability scores for the male mice was calculated and averaged to a score of 2.34% while the sociability scores for the female mice was calculated and averaged to a score of 2.48%, indicating a preference for social interaction for both female and male test mice. These scores contrast slightly to the raw graphical representation of time spent with social vs. non-social stimulus, where the male mice spent on average 7.65sec longer with the social stimulus than the non-social stimulus during the 600sec behavioral trial period, and the female mice spent an average of 54.23sec longer with the non-social stimulus than the social stimulus. Though the difference in times between stimuli is significantly larger than the female test mice, the sociability scores between male and female mice are nearly identical, indicating that while there are few differences in sociability between sexes, the female test mice have more of a preference for social interaction than the male test mice. Additionally, these differences in preference to social vs. non-social interaction between sexes is supported by *Table 1* with an estimate value of +12.768.

Sociability in Lines vs. Sex

The sociability scores previously calculated were used to determine differences in social behavior based on sexes as a factor of the two wild-type strains. *Figure 7* shows the sociability scores between the pair and single-housed conditions as a factor of the NY3 and LEWES mouse lines.

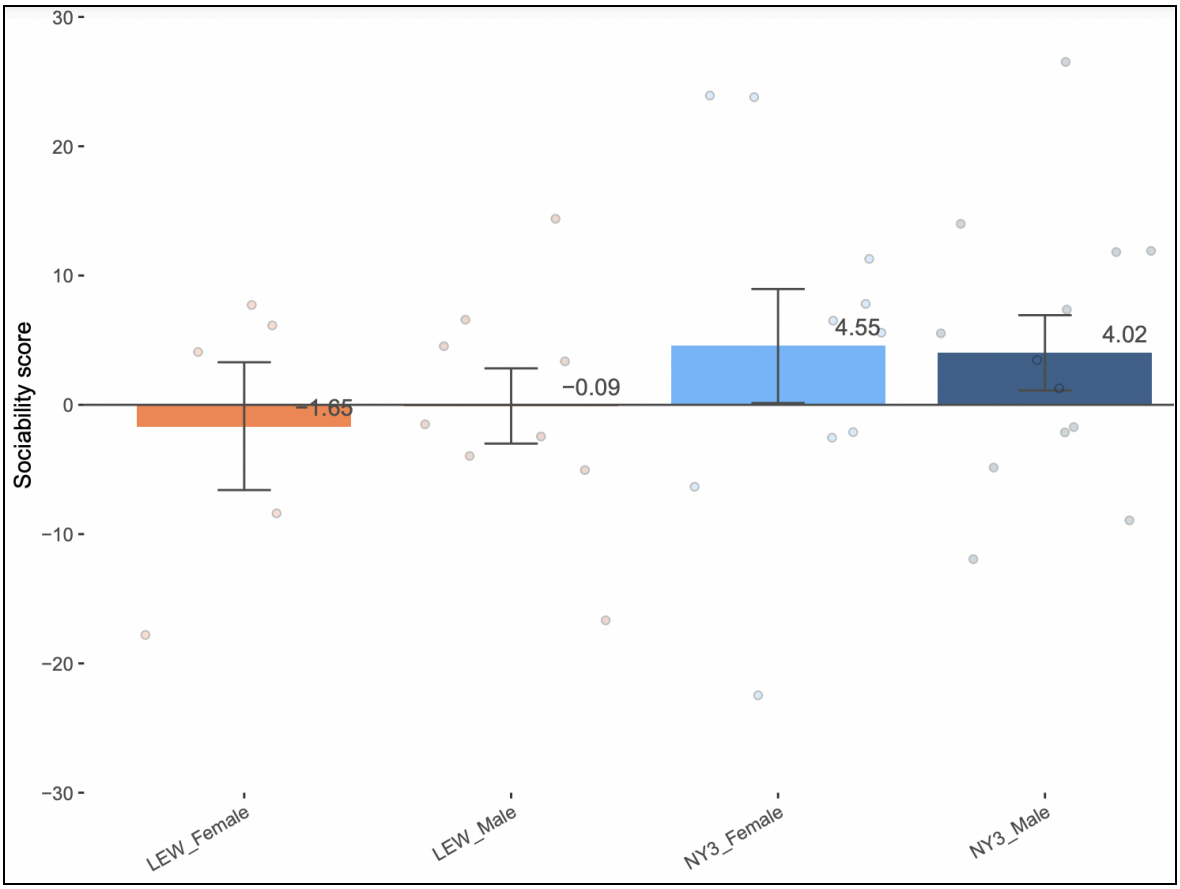

Figure 7. The figure above shows the averaged sociability scores between the female and male test mice as a factor of the NY3 and LEWES mouse lines. During the 600sec behavior trial, the LEWES female mice's sociability score was calculated to an average of -1.65%, and -0.09% for the LEWES male mice. Comparatively, the NY3 female mice's sociability score was calculated to an average of 4.55%, and 4.02% for the NY3 male mice. St.Dev. lines are calculated and graphed respectively to the sex.

The results in *Figure 7*, for the most part, display what is expected given the calculated individual trends from the sociability scores between mouse lines and the sociability scores between female and male test mice. Given the sociability scores between female and male test mice displayed in *Figure 6*, it would be expected that the LEWES female sociability score be slightly higher than the male. Results showed that the LEWES female test mice scored an average of 1.56% lower than the LEWES male test mice. These unexpected results could be a function of lack of sufficient data, specifically a large enough sized data pool of female and male

test mice mixed between lines. As expected, however, when comparing sex between the NY3 and LEWES lines, the NY3 line scored higher for both sexes with an averaged difference in sociability of 6.20% for females and 4.11% for males. Additionally, the NY3 female mice scored slightly higher in their sociability than the NY3 males by an averaged sociability of 0.53%. These results support the hypothesis that not only do the NY3 mice have a higher preference for social interaction compared to the LEWES mice, but the hypothesis holds true to for differences in sex as well, where the female and male NY3 mice display more sociability than the female and male LEWES mice.

Comparing Sociability Scores Between Line, Sex, and Housing Condition

The sociability scores previously calculated were used to determine differences in social behavior based on sexes and housing conditions as a factor of the two wild-type strains. *Figure 8* shows the sociability scores between the female and male test mice and the pair and single-housed conditions as a factor of the NY3 and LEWES mouse lines.

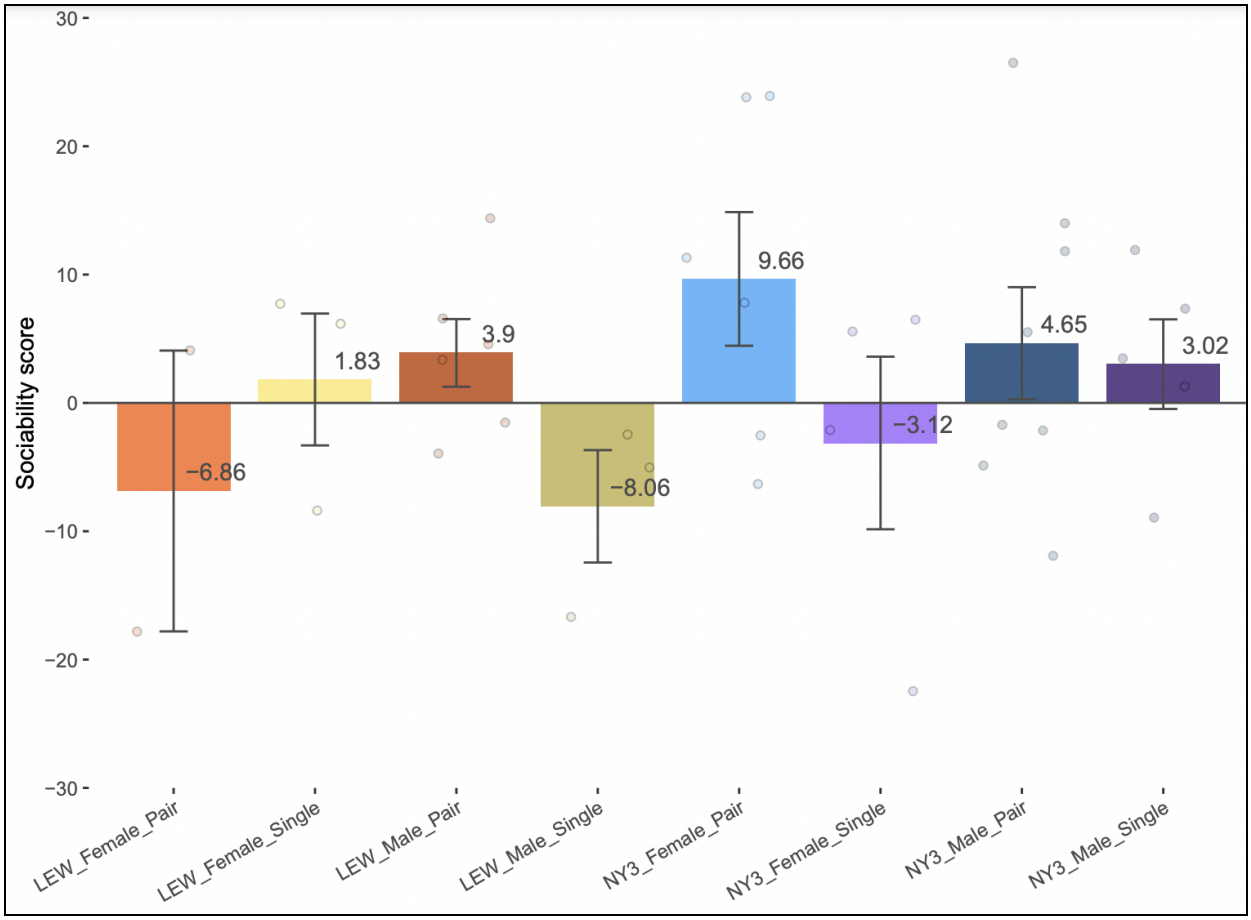

Figure 8. The figure above shows the sociability scores between the female and male test mice and the pair and single-housed conditions as a factor of the NY3 and LEWES mouse lines. During the 600sec behavior trial, the LEWES female, pair-housed mice (n=2) were calculated to have an averaged sociability score of -6.86%; the LEWES female, single-housed mice (n=3) were calculated to have an averaged sociability score of 1.83%; the LEWES male, pair-housed mice (n=6) were calculated to have an averaged sociability score of 3.90%; the LEWES male, single-housed mice (n=3) were calculated to have an averaged sociability score of -8.06%; the NY3 female, pair-housed mice (n=6) were calculated to have an averaged sociability score of 9.66%; the NY3 female, single-housed mice (n=4) were calculated to have an averaged sociability score of -3.12%; the NY3 male, pair-housed mice (n=8) were calculated to have an averaged sociability score of 4.65%; the NY3 male, single-housed mice (n=5) were calculated to have an averaged sociability score of 3.02%. St.Dev. lines are calculated and graphed respectively to the sex and housing condition.

The results presented in *Figure 8* provide sufficient evidence supporting the hypothesis the NY3 mouse line tends to exhibit higher sociability than the LEWES mouse line and that pair-housed mice generally displayed higher sociability than single-housed mice, although there is some variability in the results depending on sex and mouse line. The LEWES female, pair-housed

mice (n=2) in particular had an averaged sociability score of -6.86%, which was the lowest score among all the groups. These results were particularly unexpected given the results in *Figure 6B*, where female test mice were slightly more sociable than males. As was noted in *Figure 7*, these unexpected results could be a function of lack of sufficient data, specifically a large enough sized data pool of female and male test mice mixed between lines. In contrast, the NY3 female, pair-housed mice (n=6) had the highest average score of 9.66%. This difference between the two mouse lines is also apparent when comparing the male groups, where the NY3 male, pair-housed mice (n=8) had an averaged sociability score of 4.65%, which was higher than the LEWES male, pair-housed mice (n=6) score of 3.90%. Additionally, though the results generally support the hypothesis that pair-housed mice are more sociable than single-housed mice, the effect is not consistent across all groups. The LEWES male, single-housed mice (n=3) had an averaged sociability score of -8.06%, which was the lowest score among all the male groups. Comparably, the NY3 male, single-housed mice (n=5) had a relatively high average score of 3.02%. The data also shows that sex may have a slight effect on sociability scores, with females generally exhibiting higher scores than males. The difference is not substantial however when analyzing the female, pair-housed mice, which had an averaged sociability score of 9.66%, while the male, pair-housed mice had a score of 4.65%.

Additionally, these results support the hypothesis regarding *Table 1* where it was hypothesized that the housing conditions and sex factors, which have calculated p-values of 0.2908 and 0.2744 respectively, would be affected by the significant estimate value of +24.832 for the mouse line factor.

Biological Implications and Future Work

The results of this study indicate that the hypothesis was supported and that the NY3 line is more social than the LEWES line as evidenced by the significantly higher sociability scores and longer engagement time with social stimuli. Additionally, pair-housed mice, irrespective of mouse line, also displayed significantly higher sociability scores than single-housed mice. While there was a slight and variable difference in sociability scores between male and female mice, these results and their differences are not statistically significant.

These findings have the potential for important biological implications in that they may suggest that genetic differences between mouse strains have the potential to play a role in social behavior tendencies and sociability. The differences in sociability scores between the NY3 and LEWES lines may be due to differences in gene expression or neural pathways related to their presenting social behaviors. Future studies could utilize the clinical specimens (fecal, blood, and tissue samples) obtained during this study but left unexplored, in order to identify underlying genetic and neural mechanisms responsible for these differences. Potential environmental factors that may influence sociability in these mouse strains could also be investigated. Additionally, the significant differences in sociability scores between pair-housed and single-housed mice suggest that housing conditions could play a role in the development and expression of social behavior, which is consistent with previous research showing the negative effects of social isolation on behavior and health outcomes (Cacioppo et al., 2015). Future studies could further explore the mechanisms by which housing conditions influence sociability and potential mediators to improve welfare for single-housed animals.

Overall, the findings of this study provide important insights into the genetic and environmental factors that influence social behavior in mice. Further research in this area could have implications for the development of treatments for social deficits in humans as well.

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