Modeling PTSD effects through Stress-Enhanced Fear Learning

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Abstract

This report captures the road map of this semester-long project with Dr. Gergely Turi at New York State Psychiatric Institute/Columbia Psychiatry. The project utilized stress-enhanced fear learning methods to model PTSD in laboratory mice. The mice were of different sex, age as well as genotypes. Various data analysis methods were utilized on data collected from the experiment. Our goal in this project is through finding contributing factors to a prolonged contextual freezing behavior in mice to help us understand better the long-term effects of PTSD.

Introduction

Post-traumatic stress disorder (PTSD) has had tremendous effect on the lives of human beings. Its diversity of symptoms and the long-lasting effect are some of the most researched psychiatric topics (*1*). Past research (Rau et al. 2009) has found that such symptoms can be modeled through stress-enhanced fear learning (SEFL) methods on animals, specifically laboratory mice. SEFL uses an acute stressor, such as electric shocks, to mimic a traumatic event on the mice and consequently observe their behavioral differences while exposed to the traumatic context. Rats were also taken into a different environment after the initial treatment to further observe the SEFL effect on contextual fearing(Conoscenti and Fanselow 2019). In this study, we utilize such SEFL methods to aim to discover the contributing factors to PTSD lasting effects and such model that will accurately predict PTSD effects.

Methods

Experimental design for Stress Enhanced Fear Conditioning. Our study's experimental methods are based on a contextual fear conditioning paradigm designed by Rau et al (2009). The mice were separated by two conditions: SEFL and control groups (Figure 1). The SEFL and control groups' main difference was that during the Day 1 or 'sefla' stage of our experiment mice within the sefl group would endure 1mA electric shocks that each lasted one second 10 times randomized within the experiment hour. On the other hand, the control mice did not receive any treatment, or electric shocks during this hour but were exposed the same environment. This was our SEFL context A (sefla), which induces the traumatic event or experience. Upon the initial stage, all mice from both conditions will be brought to and received a second stage treatment on Day 2, which introduces a one-time 1mA electric shock that lasts one second within a three-minute experiment window in a different contextual environment than the experimental condition on Day 1. Upon these two days, we concluded the treatment inducement stages. In the next five experimental stages, namely recall1 to recall5, no more electric shocks were prescribed but the mice were exposed to the seflb context and their contextual freezing was recorded to further analyze differences between the SEFL and control groups. The mouse behavior in the conditioning box was recorded with an overhead camera using commercial software (FreezFrame). The recorded trials were then loaded in the same software and threshold was set up for freezing (no noticeable movement for at least 0.5 s). Freezing behavior was averaged in 1 min bins then the average value of these bins was used for data analysis. The compiled data was stored on Google drive. Notice that the experiments were done prior to me joining to Dr. Turi's lab in the Spring 2024 semester.

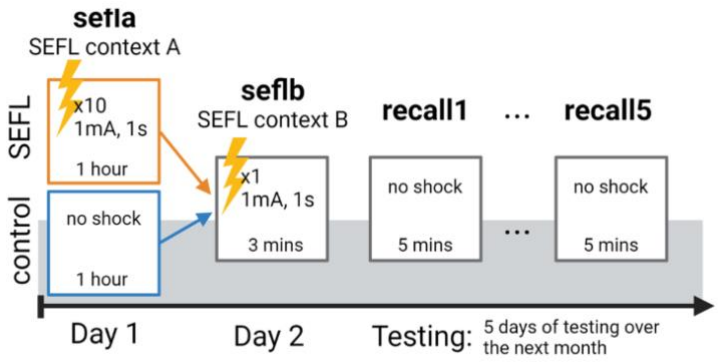


Figure 1 - Experimental design

Experimental design for Early Life Stress. Limited bedding paradigm was implemented as described by Bath et al (*2*). From the date of birth of a litter until four days after birth (P4), the

offspring and their mothers remained in standard cages, which consisted of cob bedding, a 4x4cm nestlet, and an igloo-shaped enrichment toy as shelter (Fig. 2A). On P4, the pups were weighed and individually marked with an Aramis Laboratory Animal Mircotattoo System and Ketchum 5oz Green Paste following a designated label system. The pups were then randomly separated into control or ELS groups. The control mice and their dams were transferred to another clean, standard cage (Fig. 2B). The ELS mice and their mothers were transferred into a cage with a wire mesh floor, a half-sized (2x4cm) cotton nestlet, and no enrichment toy as shelter (Fig. 2C). The litters and their mothers remained in these modified cage conditions for seven days, until P11. At 11 days old, all of the pups were weighed again and returned to clean, standard cages with their mothers (Fig. 2D). Ten days later, at P21, the pups were weaned, separated by sex, and remained in standard cages in the holding room for the remainder of the study (Fig. 2E).

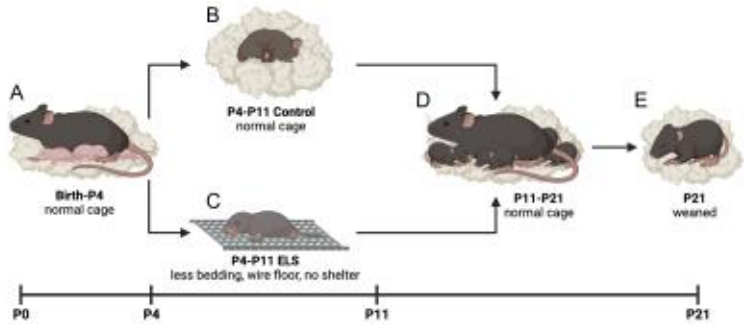


Figure 2 - Experimental design for ELS

Statistical analyses. Analysis of Variance (ANOVA) models were used to compare freezing behavior between treatment groups. Significant difference between groups were considered if it's below the alpha level of 0.05. Effect sizes were determined using eta-squared. Power of the analyses was determined by non-central F-distribution power test taking into the account of our critical value, effect size and degrees of freedom through Pingouin package. The models were set up in a Colab Jupyter Notebook environment (Python 3.11.8) using the following packages: numpy, pandas, datetime, statmodels and sklearn. Plots were generated in the same environment using seaborn and matplotlib.pyplot.

Results

Data were collected from the experiments described above. There were 196 mice in total that were in our experiment, with 121 male mice and 73 female mice, spread across 14 different cohorts. Two of such cohorts were added additional early life stress (els) condition, which was further analyzed in the later rounds. The average age at the start of the study for the mice were 16.0 weeks with a standard deviation of 3.76 weeks.

1. ANOVA Analysis

We filtered out 6 cohorts from our total dataset for the first round of analysis, with the groups being ptsd2, ptsd3, ptsd4, ptsd5, pstsd6 and ptsd9. In this first round of analysis, single factor ANOVA was heavily utilized, I would aggregate experimental stages to easily compare and investigate for potential factors.

(1.) Single Factor ANOVA

I first compared the freezing time between young and old mice where we coded mice that have an age of younger than 12 weeks to be 'young' mice. Comparing across all 6 cohorts and

across both treatment and control conditions, I have found significant effect of age on the freezing time, $p<0.05$, where the younger mice tend to have less freezing time than 12-week and older mice ***[Figure 4]***. This significant effect of age on the freezing time led us to further investigate the age effect within each specific cohort. Of all six cohorts, we have found that ptsd2($p = 0.012$), ptsd3($p = 0.008$) and ptsd6($p = 0.005$) groups to have significant difference in freezing time between the 12-week or less mice and older than 12-week mice. Ptsd9 group did not have any mice that are less than 12 weeks old at the start of our study which we excluded this cohort from this round of the study. Single factor ANOVA were further used to investigate that if younger mice would have a significant different freezing time than older mice within each of the SEFL, or treatment group, and the control group. Results showed that both within SEFL and control groups, the younger mice had significant different freezing time from the older mice, with $p = 5.72e\text{-}24$ and $p = 2.06e\text{-}08$ respectively ***[Figure 5]***. Furthermore, single factor ANOVA discovered that there was significant difference in freezing time across all 6 cohorts within SEFL and control groups, with p-value close to 0 for both of the ANOVA analyses results.

Sex as a factor in freezing time difference were also investigated in our study. However, we have found no effect of sex in freezing time difference in our first round of analysis across the six cohorts selected.

One additional potential factor was looked at in this first round of analysis which the time when the experiment was being conducted during the day. We would like to know whether the experiments conducted in the morning had any significant difference on freezing than their afternoon counterparts. Across all six groups and experiment conditions, we have found significant difference between the mice whose experiment were conducted in the morning compared to those in the afternoon, $p = 0.002$ ***[Figure 6]***. Within each of the six cohorts, significant difference on freezing time between morning and afternoon experiments were found in ptsd2, ptsd6 and ptsd9 cohorts, with $p < 0.0001$, $p = 0.004$ and $p = 0.03$ respectively.

(2.) Repeated Measure ANOVA

We further looked each of these effects during each of the experimental stage across these six cohorts. We specifically filtered out the sefla stage as we considered the difference on freezing time between sefl and control groups in this specific stage will be largely contributed to whether the mice receive the treatment; all other potential factors in making the difference will be hard to notice and in turn disturb the pattern we observe in other stages. We have thus simply focus on stages from seflb to recall5.

We examined across all 6 cohorts through age and sex variables, with 12 weeks again being the old and young variable dividing condition. There were 24 young and 22 old mice in these six cohorts that received sefl treatment; 15 young and 13 old mice in the control groups. Of all mice in the sefl group, 30 of them were male and 16 were female; in control group, there were 20 male and 8 female mice.

Repeated measure ANOVA were utilized in this round of the analysis to test out specific experimental stage difference. Using repeated measure ANOVA, we found that there was a significant difference between the young and old mice in the sefl group on their freezing time, $p = 7.60e\text{-}05$ with an effect size of 0.356 ***[Figure 7]***. Similar significant difference was also found on its control group counterpart indicating that the age did contribute to a difference in freezing time, $p=1.29e\text{-}02$, with an effect size of 0.31.

We also utilized repeated measure ANOVA to look at the difference of freezing time between gender and time of the day across each of the experiment stage. However, similar to

results from single factor ANOVA analysis, we were not able to conclude that sex had a significant effect on freezing time. Repeated measure ANOVA also failed to conclude a significant difference on freezing time between the time of the experiments conducted. We did not see a consistent pattern as to in single factor ANOVA, which suggested that experiments conducted in the morning and afternoon had significant freezing time difference.

(3.) Repeated Measure ANOVA on Early Life Stress Analysis

Our third and final round of ANOVA analysis focused on finding the potential effect of early life stress on the laboratory mice. Early life stress (ELS) was another condition that were tested onto these mice with our assumption that mice with ELS treatment will be more persistent to our SEFL treatment than those without ELS treatment. In this round of analysis, we did not pre-filter through any specific cohorts and used the entire dataset instead. The experimental design here followed a 2 by 2 layout (Figure 12). We first focused on the two cohorts of mice within els1 and els2 cohorts and then expanded comparisons to other cohorts.

ELS and SEFL Condition Table

	SEFL	Control
ELS	ELS+SEFL	ELS
No ELS	SEFL	Control

Figure 12 - Conditions for combining ELS and SEFL experiments

Repeated measure ANOVA showed that there was a significant different between mice with ELS and non-ELS conditions across the two experiment conditions, with a p value of 8.22e-03 and effect size of 0.094. We further looked at ELS and non-ELS mice comparisons within the specific condition groups. We have found that ELS does have a significant effect on freezing time difference for mice who also received SEFL treatment, with p-value of 3.39e-03 and an effect size of 0.23. On the other hand, within control group, ELS did not show such a significant effect. After power analysis was conducted for sample size, this insignificant result could be due to low number of mice in our dataset.

We also dived into such comparisons for ELS treatment within each of the els1 and els2 cohort. We have found through repeated measure ANOVA that within els1 cohort, we saw a significant effect of ELS treatment on the freezing time (p = 1.08e-2, effect size = 0.15) ***[Figure 8]*** but not within els2 cohort ***[Figure 9]***.

This finding led us investigate the detailed differences between els1 and els2 cohorts. Age and sex again showed a difference between the two groups. Els1 mice had an average age at the start of the study around 16.8 weeks and a range of 16.3 to 17.7 weeks whereas els2 cohort mice had an average of around 20.8 weeks with a range of 20.5 to 22.4 weeks. Els1 cohort also on average had more female mice than els2 cohort. This led us to the hypothesis of younger age will be more vulnerable ELS treatment than older mice and female mice will be more susceptible to ELS treatment than male mice. We again coded a young variable for the mice of els1 and els2 cohort with the cut-off value being at 18.5 which was the midpoint between the upper threshold of els1 mice and lower threshold of els2 mice. Repeated ANOVA confirmed with our hypothesis that such effect was found on the younger mice (p=1.08e-2, effect size = 0.15) ***[Figure 10]*** but the older mice (p = 4.35e-1, effect size = 0.02) ***[Figure 11]***. Further repeated measure ANOVA were also applied onto the subset of data filtered by the sex, which showed that there was a significant effect of ELS treatment on freezing time on male mice (p = 7.46e-03, effect size = 0.22) but not on female mice (p = 2.71e-1), which contradicted our hypothesis. We further compared the mice that received ELS treatment within the els groups with other non-els mice from other cohorts. After comparing with multiple cohorts as well as filter the data set into combination of age and sex variables, however, we did not find such significant results.

2. Deeplabcut and Keypoint MoSeq

Other non-traditional statistical analysis methods were also utilized. We implemented a software package, DeepLabCut, for animal pose estimation (Nath et al. 2019). This package utilized algorithms to track user-defined animal pose. We utilized this package to track important body parts of mice, such as nose, eyes, ears, neck, back as well as tail in the video that was recorded using depth camera during the experiment. Upon the track was done through DeepLabCut algorithms, we then fed our tracked video into Keypoint MoSeq algorithm which will allow us to further breakdown a series of animal behaviors into trackable movement syllabi (Weinreb et al. 2023). We are currently at the step of understanding the results for movement syllabi from one batch of mice and hope to use this understanding to better utilize these available packages to understand the different contributing factors to PTSD effects.

3. Other methods

We have also studied the possibility of using state-estimation (Course and Nair, 2023) techniques for modeling and predicting our data. However, upon studies during the first three weeks, we ruled out this potential method due to our dataset being on a smaller scale.

My peer, Ruojun Li, a fellow MA Statistics student also worked on this project with Dr. Turi and I. She focused on utilizing regression, random forest and other machine learning techniques as well as other unsupervised methods to further study and explore on this dataset. Her results and findings will be in a separate report.

Discussion and Conclusion

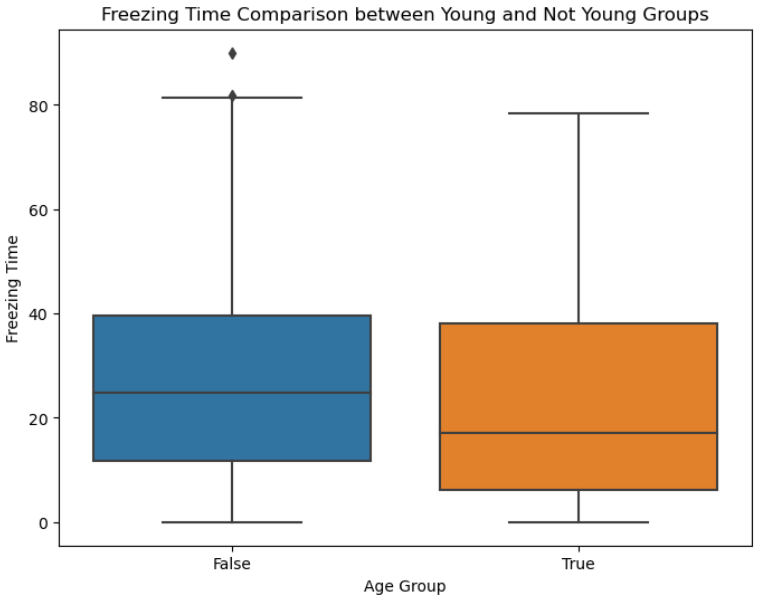
From our results and findings, we found that age does play a significant factor in the effects of SEFL treatment in general. Sex has not appeared to be a significant factor. Furthermore, whether the experiments conducted in the morning or afternoon appeared not to have a significant effect on freezing time when repeated measure ANOVA was applied. Finally, we were able to conclude that younger mice were more susceptible to early life stress compared with older mice.

Future Plan

This study will be further continued through the DeepLabCut and Keypoint MoSeq methods to further study the pose estimation and the movement syllabi of our laboratory mice. I will work with Dr. Turi as a summer intern to further this study. We are hoping to polish our results and publish it as a paper.

Personal Significance

This mentor research project gave me a great opportunity to understand how to work with an experimental dataset. I have previously only worked with survey/opinion data but not on a causal inference data before. Upon receiving such dataset, I was able to initially analyze the dataset to understand the variables as well as meaning of the values. This also gave me an experience working on a field that was new. I spent a lot of time reading the literature behind the dataset, to understand the underlying theories as well as the methods behind. I was able to practice my programming skill in Python and used methods such as linear regression, ANOVA as well as other methods in applied situations outside of the classroom. This was also a unique opportunity to my personal goal, which is to work in the quantitative psychology field.



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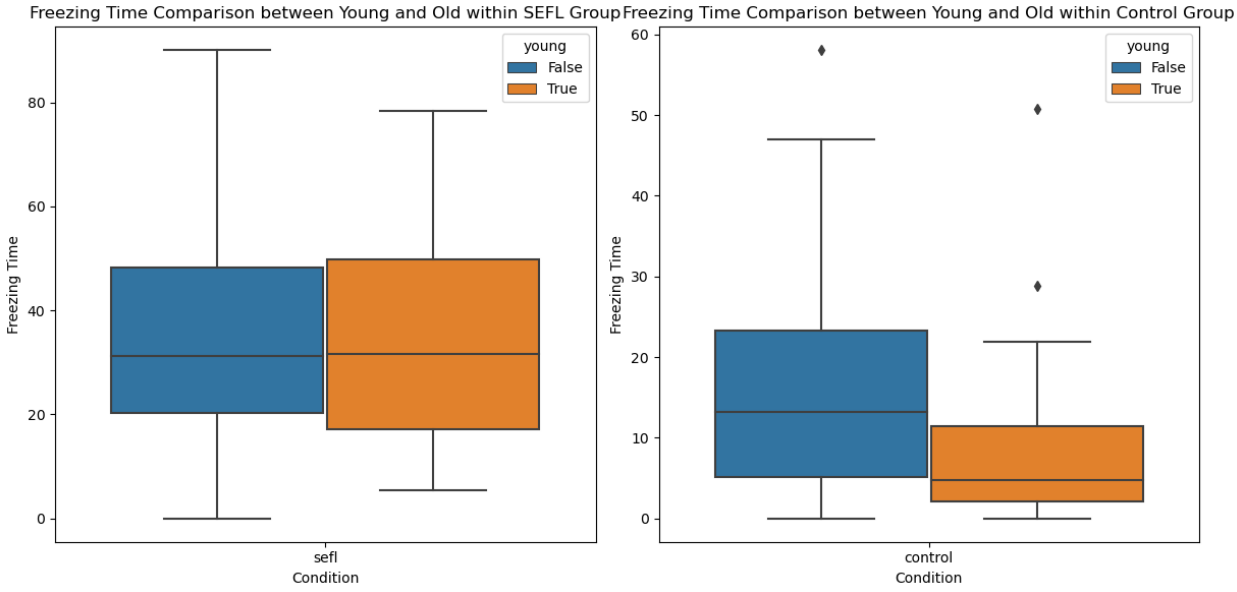

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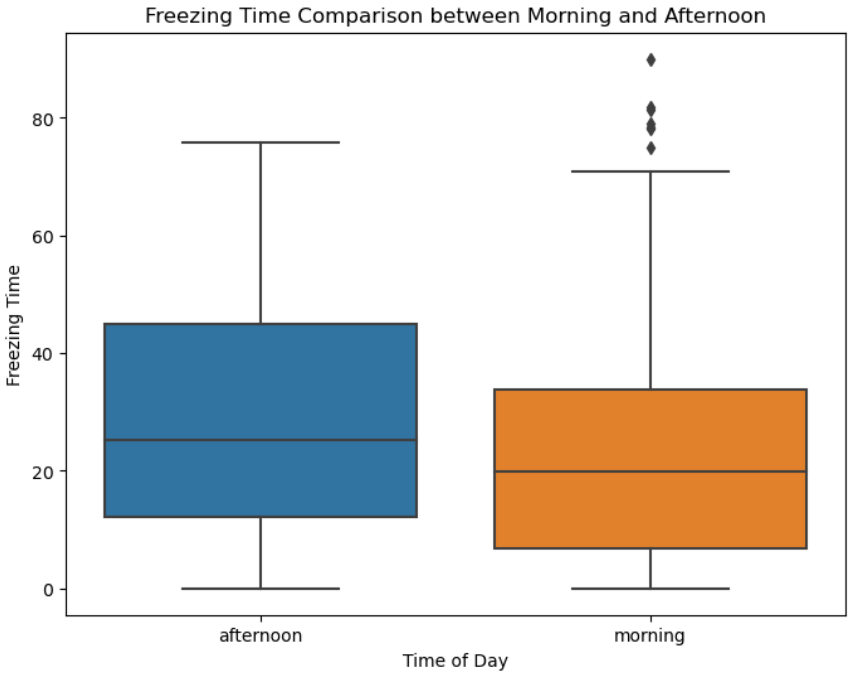

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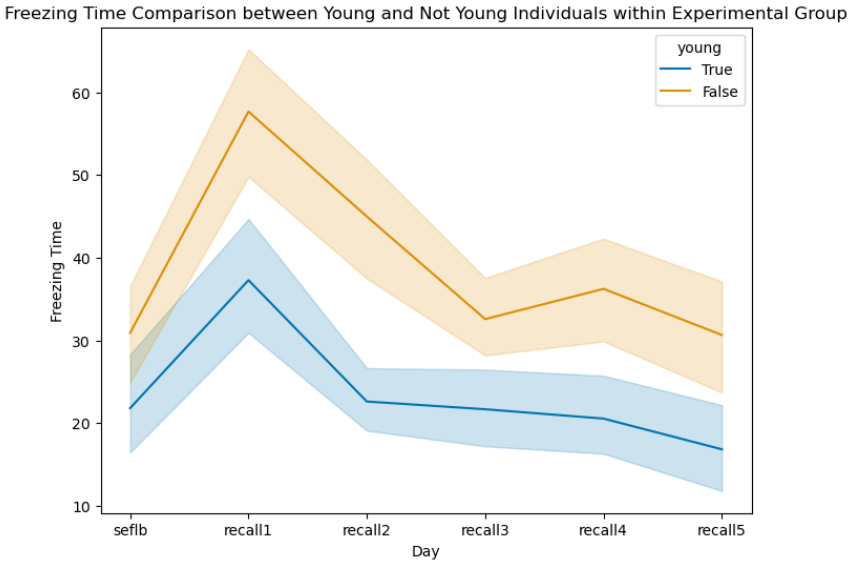

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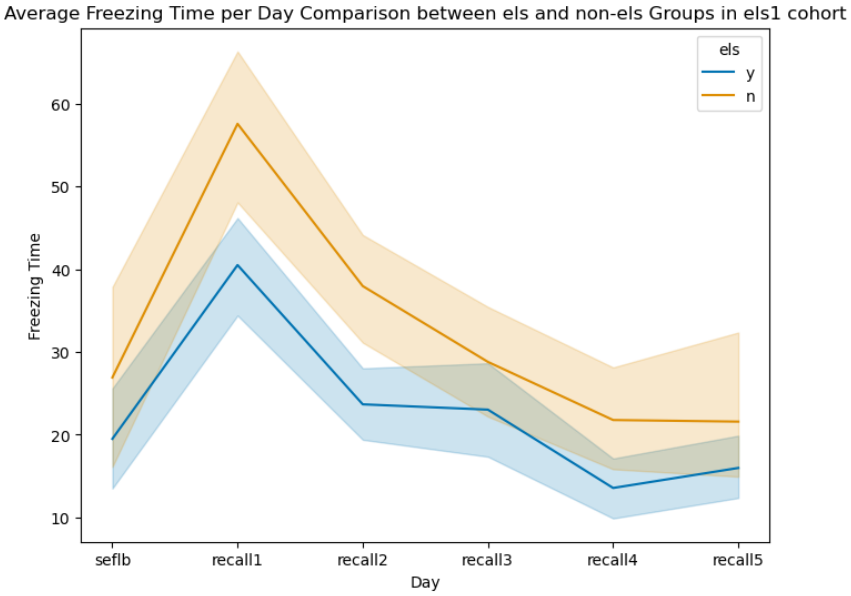

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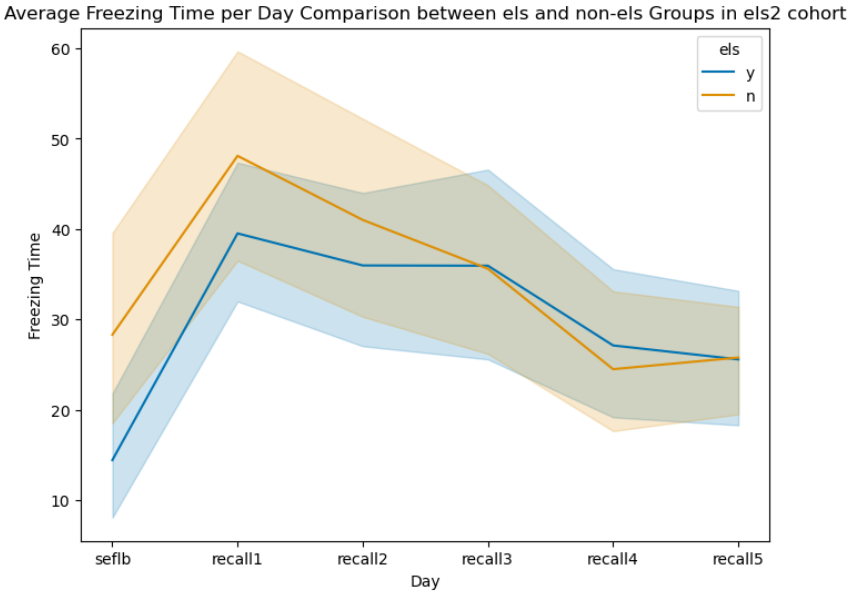

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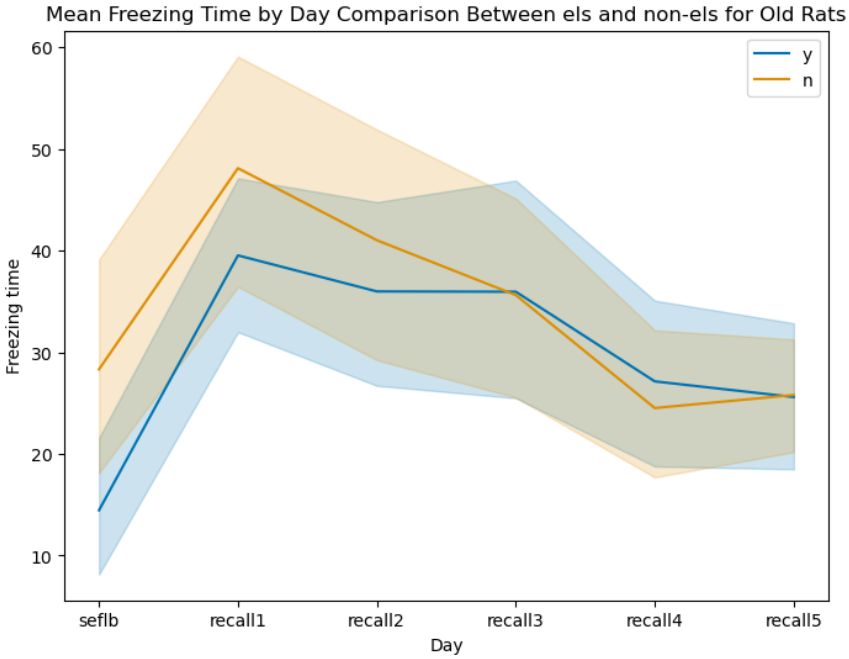

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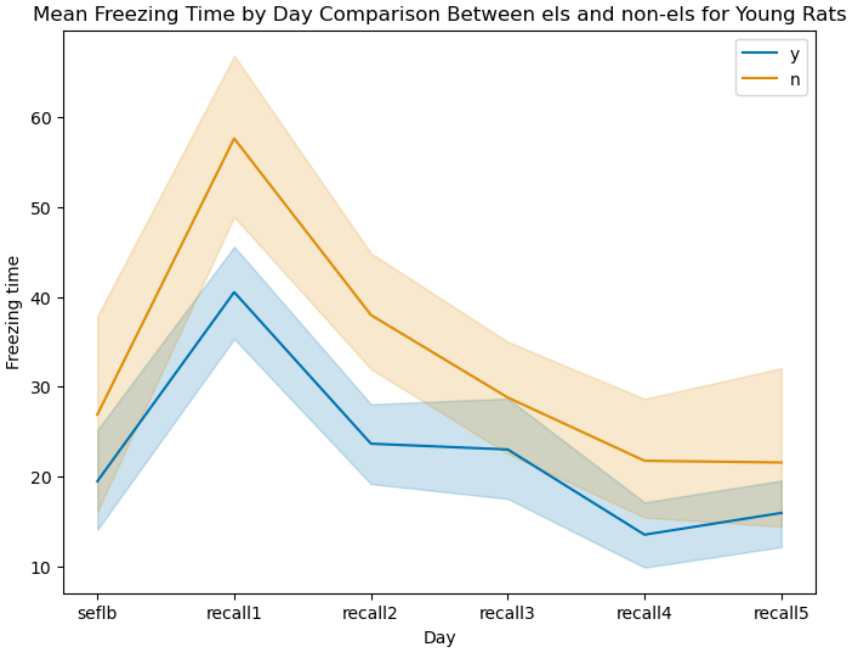

Figure 10

Reference:

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