Models of predicting motion of mice in stress-enhanced fear learning(SEFL) procedure

Mentored Research Report

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Contents

1 Introduction on stress-enhanced fear learning procedure

Post-traumatic stress disorder (PTSD) is a serious condition that many people experience during their lives. It involves a wide variety of symptoms and often occurs alongside other mental health issues. One way to study PTSD in rodents is through a method called stress-enhanced fear learning (SEFL). In SEFL, researchers use a single stressful event. They expose some animals to 15 footshocks and others to a situation with no shocks. Those that receive the footshocks tend to show greater fear responses later on[1].

2 Objective

Our main aim is to create a stable and durable behavioral state that serves as a model to investigate the cognitive and anxiety aspects of SEFL in mice.

Aim1: Validate the Stress-Enhanced Fear Learning (SEFL) protocol and to identify its long-term effects on freezing behavior in a context where fear conditioning occurred.

Aim2: By analyzing the data, we hope to find out which independent variable decides most on the long-term effects on freezing behavior.

Aim3: Observe whether the age and sex of mice can make a big difference to the long-term behavior(freezing-time of recall4).

3 Experiment and data

The experiment is designed as follow: on Day 1, the mice in the stress group receive 10 foot shocks over one hour in context A while the control mice are placed in context A with no foot shock for one hour. On Day 2, all mice, including stressed and control mice, are placed in context B for 5 minutes and receive 1 foot shock. In the following month, all mice are placed in context B for five 5-minute exposures with no foot shock, and their freezing is monitored. Researchers collected each mouse's freezing time of each testing. The testing procedure is showing in the below picture[2]. Notice that the experiments were done prior to me joining to Dr. Turi's lab in the Spring 2024 semester.

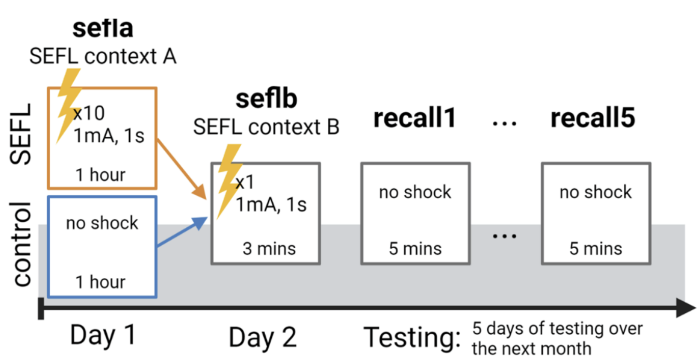

Figure 1: The stress-enhanced fear learning procedure

The collected data contains the freezing time from sefla to recall5, including each mouse age and sex. There were 196 rats in total that were in our experiment, with 121 male rats and 73 female rats, spread across 14 different cohorts. Due to missing data in recall5, we select the age, sex and freezing time from sefla to recall3 as the independent variable, select the freezing time of recall4 as the dependent variable.

4 Methods

The section introduce some machine learning algorithms and their results for analyzing the dataset. Readers can find the code via github[2].

4.1 State estimation of a physical system with unknown governing equation

We first tried to reproduce a method introduced by a paper related to state estimation[3]. It introduces a reparametrization technique for stochastic variational inference using Markov Gaussian processes, facilitating an approximate Bayesian approach for state estimation. This method is particularly useful when the equations that describe system evolution over time are partially or fully unknown. Unlike traditional state estimation methods, the approach simultaneously learns missing terms in the mathematical model and provides a state estimate from an approximate Bayesian viewpoint, which is very useful for observing animal behavior. However, due to our limited data points, it is hard for us to form a reasonable prior equation to solve the SDE and get an accuracy results. In the end, we refuse the model by small amount of dataset.

4.2 Supervised Learning

In this section, supervised learning, including OLS, Random Forest, Boosting and some other machine learning methods. We tend to use these methods to predict the long-term freezing time and analyze the most importance variables. We also seperate the Control group and Experiment Group(SEFL group), in order to better observe how does the electric shock change the freezing motion of mice.

4.2.1 Ordinary Linear Regression(OLS)

Initially, I used the freezing time from sefla to Recall3, as well as the time interval between sefla and seflb to Recall4, as 11 independent variables in an OLS regression model. The objective was to predict the freezing time for Recall4. We can observe that whether mice are given electric shocks can significantly impact the long-term behavior(freezing time of recall4)

```
                            OLS Regression Results
==============================================================================
Dep. Variable:       freezing_recall4   R-squared:                       0.700
Model:                            OLS   Adj. R-squared:                  0.627
Method:                 Least Squares   F-statistic:                     9.559
Date:                Sun, 21 Apr 2024   Prob (F-statistic):           1.43e-08
Time:                        19:08:31   Log-Likelihood:                -199.80
No. Observations:                  57   AIC:                             423.6
Df Residuals:                      45   BIC:                             448.1
Df Model:                          11
Covariance Type:            nonrobust
====================================================================================
                       coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------------
freezing_sefla       0.3797      0.121      3.148      0.003       0.137       0.623
freezing_seflb      -0.1954      0.153     -1.276      0.209      -0.504       0.113
date_seflb          -2.0642      4.515     -0.457      0.650     -11.157       7.029
freezing_recall1     0.2003      0.105      1.908      0.063      -0.011       0.412
date_recall1         0.5687      3.255      0.175      0.862      -5.988       7.125
freezing_recall2    -0.0979      0.148     -0.661      0.512      -0.396       0.200
date_recall2        -0.4378      5.104     -0.086      0.932     -10.718       9.842
freezing_recall3     0.4986      0.130      3.841      0.000       0.237       0.760
date_recall3        -0.5590      1.281     -0.436      0.665      -3.139       2.021
date_recall4         0.5690      1.128      0.504      0.617      -1.704       2.842
condition          -12.6194      6.000     -2.103      0.041     -24.704      -0.535
...
Notes:
[1] Standard Errors assume that the covariance matrix of the errors is correctly specified.
[2] The smallest eigenvalue is 9.39e-30. This might indicate that there are
strong multicollinearity problems or that the design matrix is singular.
```
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Figure 2: OLS result for both group.

Then I separately analyze the Experiment(SEFL) group and Control group. The OLS result of SEFL group is showing below.

```
                            OLS Regression Results
==============================================================================
Dep. Variable:       freezing_recall4   R-squared:                       0.404
Model:                            OLS   Adj. R-squared:                  0.166
Method:                 Least Squares   F-statistic:                     1.697
Date:                Thu, 11 Apr 2024   Prob (F-statistic):              0.137
Time:                        03:54:54   Log-Likelihood:                -134.64
No. Observations:                  36   AIC:                             291.3
Df Residuals:                      25   BIC:                             308.7
Df Model:                          10
Covariance Type:            nonrobust
====================================================================================
                       coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------------
freezing_sefla       0.1332      0.189      0.704      0.488      -0.256       0.523
freezing_seflb      -0.0906      0.269     -0.337      0.739      -0.644       0.463
date_seflb          -5.2287     16.804     -0.311      0.758     -39.837      29.380
freezing_recall1     0.2901      0.172      1.689      0.104      -0.064       0.644
freezing_recall2    -0.2846      0.256     -1.113      0.276      -0.811       0.242
date_recall2        -1.0433      2.564     -0.407      0.687      -6.323       4.236
freezing_recall3     0.4038      0.260      1.553      0.133      -0.132       0.939
date_recall3         0.7687      1.979      0.388      0.701      -3.307       4.844
date_recall4         0.2367      1.036      0.228      0.821      -1.898       2.371
sex                 -0.2796     10.493     -0.027      0.979     -21.890      21.331
age_selfa            7.8371      6.089      1.287      0.210      -4.703      20.378
==============================================================================
Omnibus:                        1.925   Durbin-Watson:                   2.140
Prob(Omnibus):                  0.382   Jarque-Bera (JB):                1.728
Skew:                           0.510   Prob(JB):                        0.421
Kurtosis:                       2.666   Cond. No.                         847.
==============================================================================
```

Figure 3: OLS result for sefl group.

We can observe that the OLS model pass all the test, including Durbin-Watson test and Jarque-Bera test, indicating that the data is suitable for doing OLS. From the table above, we can observe that the two most important variable is freezing time of recall1 and recall3. Age may do contribute to the long-term behavior, but it is not significant. I didn't observe that the sex is a significant variables in predicting long-term behavior. However, with R square as 0.4 and the testing MSE as 103.75, the result suggesting that the OLS model is not accuracy enough for predicting the freezing recall time of recall4. The model has an R-squared of 0.571 with F-stat significant; freezing-time of sefla and recall1 shows significant.

Thus, I add the learning curve data into our original dataset. Learning curve data capture the motion of every shock in sefla, and represent the motion in data. I do a lot of experiment in how to add the learning curve data in original dataset. I try to add every detail of the learning curve data, it cause overfitting problem: it did well on training dataset but did bad on test dataset; I also add the mean of learning curve data, it cause underfitting problem. The final solution is to compute the coefficient of OLS that capture the the increase in learning curve data, then add it into the original dataset. For the new dataset, the model has an R-squared of 0.571 with F-stat significant; freezing-time of sefla and recall1 shows significance. Age may do contribute to the long-term behavior, but it is not significant. Sex still not shows significance in model.

I do similar procedure in control group. The control group results are very different from the sefl group. The OLS regression model accounted for a substantial portion of the variance, evidenced by an R-squared value of 0.842. Also, the F-statistic of the model is 5.334, with a corresponding p-value of 0.00706. The freezing time of recall3 is the most significant important feature in this model; besides, sex shows significance in this model. Age doesn't show significance in this model. The test MSE is 18, indicating the accuracy of prediction of this model.

I have also tested the control group with learning curve coefficient, the result didn't change a lot. Due to limited space, I will show the result in the conclusion part

```
                            OLS Regression Results
==============================================================================
Dep. Variable:       freezing_recall4   R-squared:                       0.842
Model:                            OLS   Adj. R-squared:                  0.684
Method:                 Least Squares   F-statistic:                     5.334
Date:                Thu, 11 Apr 2024   Prob (F-statistic):            0.00706
Time:                        03:55:07   Log-Likelihood:                -60.606
No. Observations:                  21   AIC:                             143.2
Df Residuals:                      10   BIC:                             154.7
Df Model:                          10
Covariance Type:            nonrobust
====================================================================================
                       coef    std err          t      P>|t|      [0.025      0.975]
------------------------------------------------------------------------------------
freezing_sefla       0.3524      0.197      1.788      0.104      -0.087       0.791
freezing_seflb      -1.2194      0.578     -2.108      0.061      -2.508       0.069
date_seflb          23.8375     11.644      2.047      0.068      -2.106      49.781
freezing_recall1    -0.0066      0.214     -0.031      0.976      -0.483       0.470
freezing_recall2    -0.1418      0.218     -0.651      0.530      -0.627       0.344
date_recall2        -2.6092      1.583     -1.648      0.130      -6.136       0.918
freezing_recall3     0.5128      0.177      2.895      0.016       0.118       0.908
date_recall3         0.3521      1.439      0.245      0.812      -2.853       3.557
date_recall4         1.0076      0.828      1.217      0.251      -0.837       2.852
sex                -25.9943      9.145     -2.842      0.017     -46.371      -5.617
age_selfa           -1.9634      5.059     -0.388      0.706     -13.236       9.309
==============================================================================
Omnibus:                        1.188   Durbin-Watson:                   1.777
Prob(Omnibus):                  0.552   Jarque-Bera (JB):                0.896
Skew:                           0.201   Prob(JB):                        0.639
Kurtosis:                       2.071   Cond. No.                         489.
==============================================================================
```

Figure 4: OLS result for control group

Regarding why the control group performed better, if this is in terms of lower variance or more predictive power in the regression model, it could be due to:

1.The absence of stressors or interventions, leading to more predictable and stable outcomes.

2.The possibility that the treatment has an adverse effect or introduces complexity that the regression model cannot adequately capture.

However, both two assumption haven't been tested. It need further study and experiment.

4.2.2 Random Forest and other Machine Learning methods

In this analysis, I assessed the predictive performance of three regression models: Decision Tree, Random Forest, and Gradient Boosting. We utilized Mean Squared Error (MSE) as our evaluation metric, preferring models with lower MSE values as they indicate better predictive accuracy. Cross-validation was conducted using a 5-fold approach to evaluate model robustness. The models were then fitted to the training data and predictions were generated for both training and test datasets to calculate train and test MSEs. The results were two-fold: firstly, I obtained an average MSE for each model through cross-validation, ensuring an unbiased performance metric. Secondly, I determined the training and testing MSE for each model, providing insight into their performance on both seen and unseen data. I also extracted feature importances, highlighting which predictors were most influential for each model.

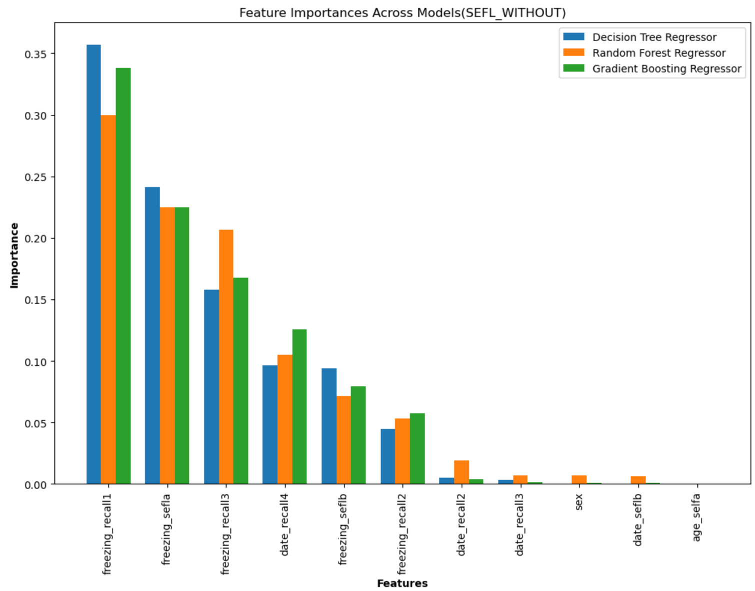

Figure 5: Feature Importance across models(SEFL Group)

The Mean Squared Error (MSE) for the three models—Decision Tree, Random Forest, and Gradient Boosting—were 248.62, 252.84, and 276.16. Across all models, freezing time of sefla, recall1 and recall3 consistently emerged as significant features, indicating their strong predictive value in the context of this analysis. The Decision Tree and Gradient Boosting models displayed a tendency to overfit the training data, a common trait of these algorithms when not properly regularized or tuned. The Random Forest Regressor, while also showing some signs of overfitting, proved to be slightly more robust on the unseen test data.

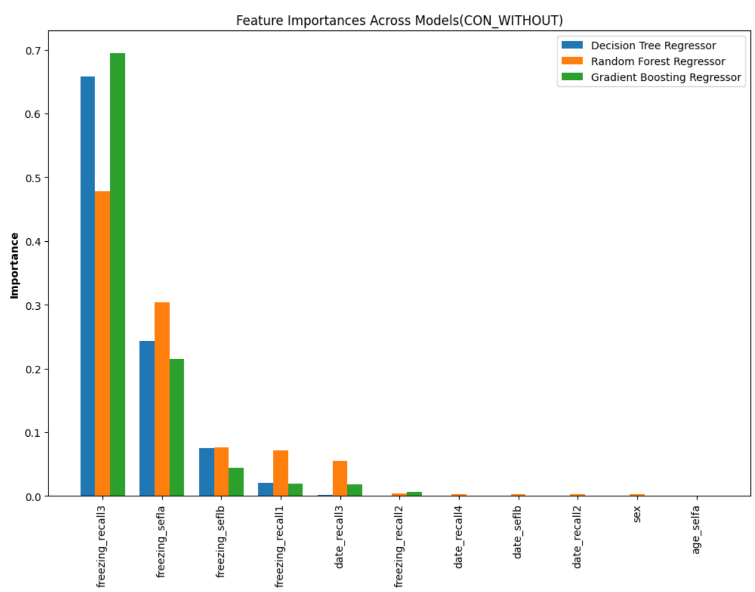

Figure 6: Feature Importance across models(Control Group)

The Decision Tree, Random Forest, and Gradient Boosting Regressors yielded Test MSEs of 103.16, 69.88, and 60.76 respectively. In all models, freezing time of recall3 and sefla were identified as the most important features for prediction. The Gradient Boosting Regressor emerged as the best model with the lowest Test MSE, indicating superior predictive accuracy on the test data.

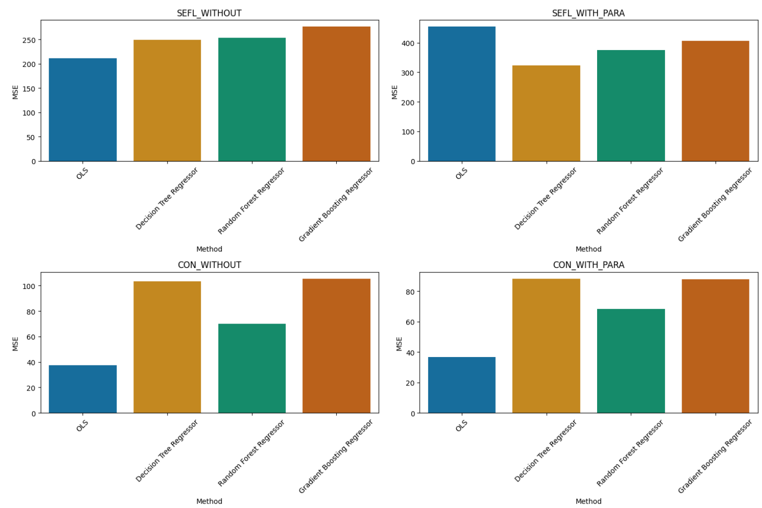

Figure 7: Comparison of supervised learning(test MSE)

4.3 Unsupervised Learning

I also did some work on Unsupervised Learning. After observing the data, I separated freezing time of recall 4 in to two group: above 30 and below 30. I used three clustering algorithms: Kmeans, EM algorithm and hierarchical clustering to predict the data. I also use PCA with two principle components to reduce the data dimension.

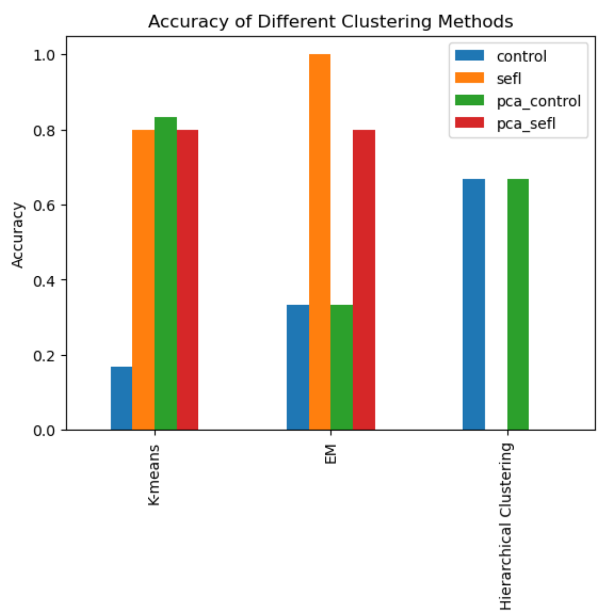

Figure 8: Model accuracy of unsupervised learning

The Expectation-Maximization (EM) clustering algorithm achieves the highest accuracy when applied to PCA-transformed SEFL data, indicating it as the best-performing model among those tested. PCA transformation appears to improve clustering accuracy across all methods, most notably for the SEFL dataset in both K-means and EM algorithms, and for the control dataset in hierarchical clustering.

5 Conclusion

Conclusion1: From the OLS result, we can observe that the SEFL has long-term effects on the mice freezing behavior, which means that SEFL may cause the freezing time in following period last longer.

Conclusion2: From all the results above, we can observe that freezing time of recall1 and recall3 is the two most significant important features among all the independent variables.

Conclusion3: For SEFL group, Age may positively contribute to the long-term behavior, sex is not a significant feature; oppositely, for control group, sex is a significant feature; however, age is not a significant important feature.

6 Future Plan

Plan1: We aim to find out what cause big difference in control group and sefl group. Due to limited data points, my peer Haoyu Chen will analyze mice moving track in the Keypoint MoSeq images.

Plan2: Due to the flexibility of the data, we aim to dig more on unsupervised learning, find a more suitable model to predict the long-term effects on freezing behavior.

7 Training and experience acquired

I've really stepped up my game with machine learning lately. Digging into how it all works and getting my hands dirty with actual data has made me way better at picking out the right models and tweaking them to get good results. I've spent a lot of time working with Decision Trees, Random Forests, and Gradient Boosting, and now I get what makes them tick and how to make them work best. On top of that, I've got a lot better at turning numbers and data into visuals that tell a story. It's been pretty fun figuring out how to make all those charts and graphs that make sense of the details and help everyone else get it easy to understand too.

Big shoutout to Dr. Gergely Turi for all the help along the way. He walks me through the tricky parts and gives me feedback that's spot on. His open book approach to teaching has really encouraged me to keep on learning and exploring.

References

[1] Michael A. Conoscenti and Michael S. Fanselow. Dissociation in effective treatment and behavioral phenotype between stress-enhanced fear learning and learned helplessness. *Frontiers in Behavioral Neuroscience*, 13, 2019.

[2] Gergely Turi, Novak Chen, and Ruojun Li. Quanitative assessment of stress enhanced fear learning in mice, 2024.

[3] K. Course and P.B. Nair. State estimation of a physical system with unknown governing equations. *Nature*, 622:261–267, 2023.