Unraveling Aggression: Insights into Aggression through Mouse Brain Activity

Summary

In this study, we conducted a comprehensive analysis of data obtained from a Tube Test experience involving two mice, aiming to unravel the neural correlates of aggressive behavior. Our approach involved an examination of raw data, plotting frame-by-frame details for each trial. Computing statistics on the average values within specific brain areas for both winning and losing mice.

Employing Principal Component Analysis (PCA), which enabled us to visualize and understand the underlying structure of the variables.

Further, we employed a grid search methodology to identify the optimal linear Support Vector Machine (SVM) model for our data. Finally, we assessed the model's validity, we subjected it to permutation tests, ensuring its robustness and reliability in capturing the nuances of aggressive behavior.

To explore each area brain importance, we examined the coefficients of the SVM model associated with each region.

Our findings highlight a significant correlation between the prefrontal cortex (PrL) and cingulate gyrus (Cg) regions and the distinctions between winning and losing in the tube test.

Introduction

Aggression is a normal part of mammalian behavior as animals fight to defend territory, acquire resources, compete for mates, and protect young (Huntingford 1987).

In the study of behavioral neuroscience, understanding the neural mechanisms underpinning aggressive behavior remains a complex and interesting challenge. This study explores the complexities of social dominance interactions by analyzing brain data from a Tube Test involving two mice. The objective is to point specific brain areas associated with the differences between winning and losing outcomes.

To investigate the neural correlates of aggression, we focused on brain areas associated with social behavior and aggression in rodents:

Amygdala: The amygdala plays a crucial role in processing emotions, including fear and aggression. It consists of several subregions, such as the basolateral amygdala (BLA) and central amygdala (CEL), which are involved in emotional responses (LeDoux 2000).

Hippocampus: The hippocampus (Ca) is associated with learning and memory. It also plays a role in the modulation of emotional responses and social behaviors (Dong 2010).

Hypothalamus: The ventromedial hypothalamus (VMH) is known to regulate aggressive behaviors. It integrates sensory information and hormonal signals to modulate social interactions. (Anderson 2012)

Cingulate Cortex: is often associated with emotional processing, cognitive control, and decision-making. It plays a role in detecting and resolving conflict, which is essential for regulating social interactions, including those involving aggression (Shackman 2011).

Striatum: The ventral striatum, including the nucleus accumbens (AcbS), is implicated in reward processing and motivated behaviors, including aggression. (Nathalie Mandairon 1 2009)

This research not only contributes to our understanding of mice behavior but also lays the groundwork for future investigations into the complex interplay between neural dynamics and aggressive social interactions.

Methods

The Tube Test:

We conducted a tube test on 3 different mice of type C57 (wild type).

The Tube Test, a straightforward and robust behavioral assay, has been established as a reliable metric for assessing social hierarchy in mice (Zhengxiao Fan n.d.). This test encompasses three distinct stages: the habituation stage, training stage, and testing stage.

The habituation stage, spanning 3 days, is designed to alleviate stress and anxiety. During this phase, each mouse is introduced to the testing area alongside the tube to familiarize itself with both the apparatus and the surroundings.

The second stage, training, extends over a period of 2 days. In this phase, each mouse is once again introduced to the tube and trained to navigate from one end to the other. The training is aimed at motivating the mouse to traverse the tube, fostering an inclination to successfully navigate it during the subsequent testing stage.

The final and main stage is the testing phase, where mice are paired and subjected to trials, this stage last at least 4 days. Each trial is standardized to last 120-180 seconds. Positioned at opposite ends of a tube, each mouse has been trained to traverse to the other side. However, due to the narrow dimensions of the tube, only one mouse can successfully pass through, prompting the other to retreat. This testing process is repeated with all mice in pairs.

Microfiber Calcium Measures

To investigate the brain activity during the tube test we use multi-fiber photometry of behaviour-related neuronal dynamics in 24 brain regions.

Multi fiber calcium recording is a simple method to investigate brain activity, although its lack of cellular resolution, the temporal resolution, and the ability to record in a living moving mice made to more sustainable method for brain activity measurement. (Yaroslav Sych 2018)

The regions were injured with a virus expressing the GCaMP6m indicator. A 24-fiber array was inserted to the mouse brain. In the tube test, the fibers recorded calcium signals in each fiber by exciting the GCaMP6m fluorescence. The records captured fluorescence changes ($\Delta F/F$).

The targeted areas included: In the hippocampus - Ca, in the cortex - cingulate cortex (Cg), motor cortex (M), primary somatosensory barrel cortex (S1BC), prelimbic (PrL), and infralimbic (IL). In the basal forebrain - Caudate putamen (Cpu), globus pallidus (CPu-GP), claustrum (Cl), nucleus accumbens shell (AcbS), Nucleus Accumbens Core (AcbC), and (AcShv). In the amygdala - basolateral amygdala (BLA), centrolateral amygdala (CEL). In the thalamus - ventrolateral (ThVL), ventral posterior medial (ThVPM), and posterior medial (ThlPo).

The Analysis:

Given the brain data of these mice couple we want to analyze which area is the most dominate to indicate aggression or lack of aggression.

The Data:

The brain data from each trial focused specifically on the time when the mice were inside the tube and active, resulting in an inconsistent period (number of frames) across trials. In all trials, one mouse won by passing through the tube while the other withdrew. The dataset includes 24 different areas, some with duplicates (e.g., cpu, PrL, CA) and a target label indicating whether the mouse won or lost. Be aware that the scale of values changes from one plot to another.

This examination reveals peaks of activity during the trial, suggesting that these occur when the mice meet or during decision-making.

Figure SEQ Figure * ARABIC 1 winner and loser by frame

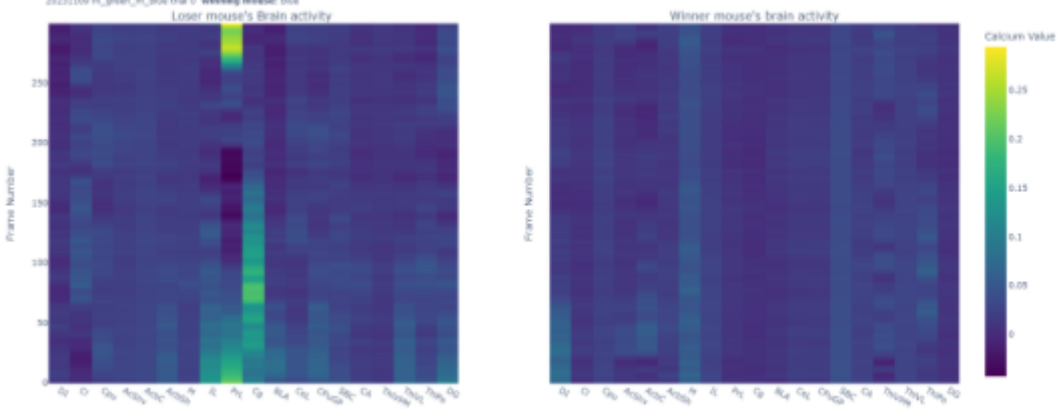


Figure 2 Brain activity difference between winner and loser by frame

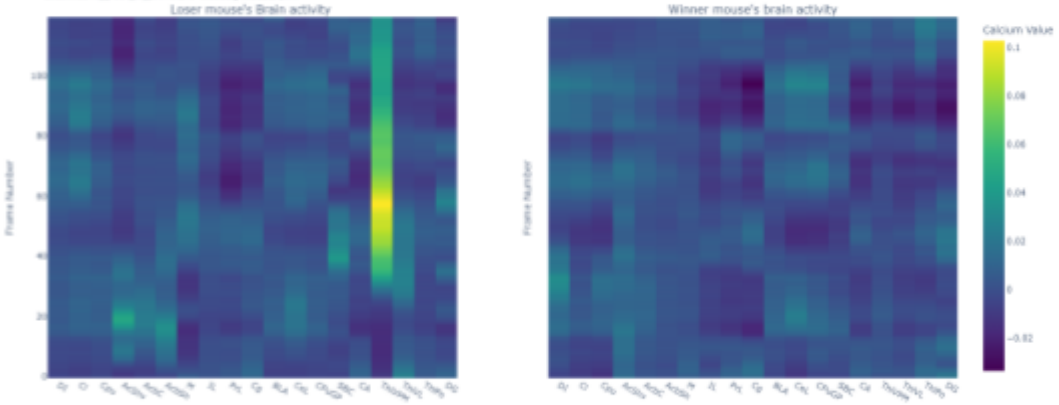
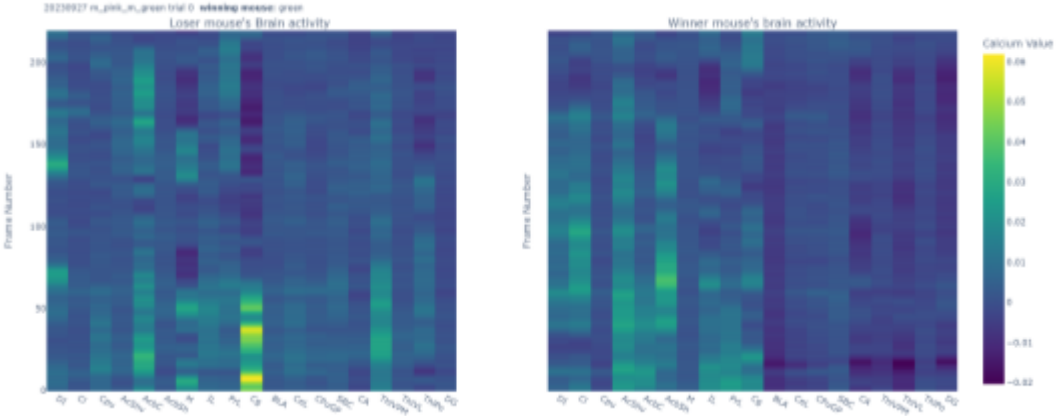


Figure 3 Brain activity difference between winner and loser by frame



The figures above display the brain activity of the winner on the right and the loser on the left. Notably, the loser's brain activity shows distinct peaks particularly in the PrL and ThlVPM areas.

Further analysis reveals interesting patterns; for instance, in figures 1 and figure 2, where the green mouse lost, there were noticeable peaks in ThlVPM, PrL, and Cg.

However, in figure 3 the green mouse won without significant peaks.

In these two figures, a closer examination reveals distinctive peaks in the winner's brain activity of the blue mouse. Notably, both peaks are localized within the Cg area, suggesting a specific neural involvement in the success of the blue mouse during the Tube Test. This observation underscores the potential significance of the cortex gyrus region in influencing the winning outcome, justifying further exploration into the precise role it plays in mediating social dominance interactions among mice.

Figure 4 Brain activity difference between winner and loser by frame

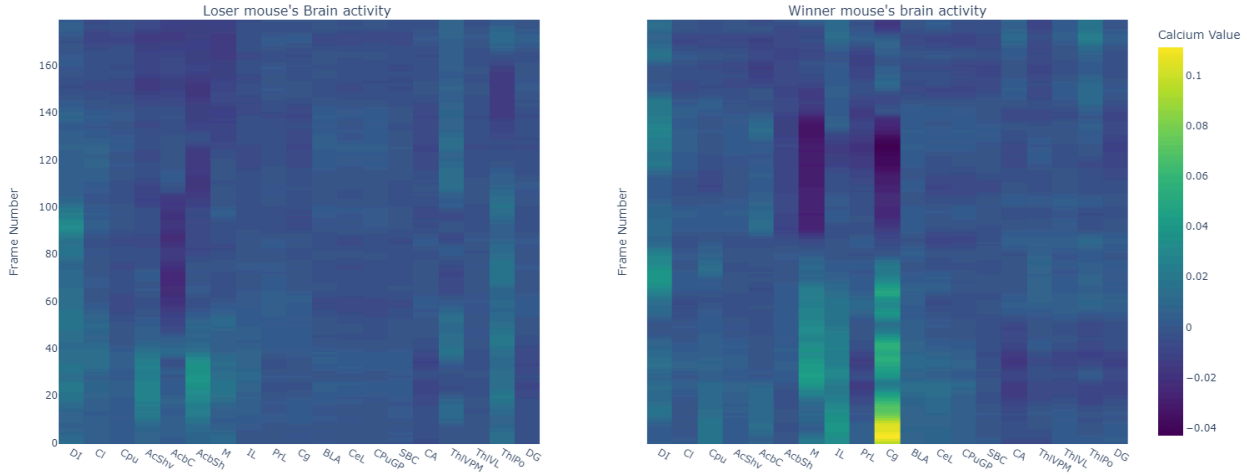
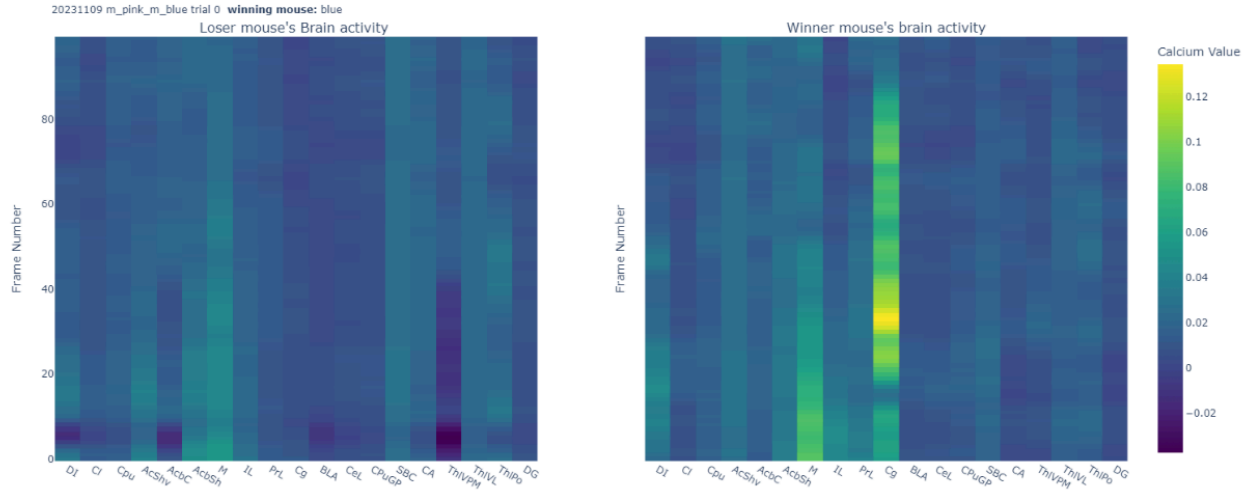


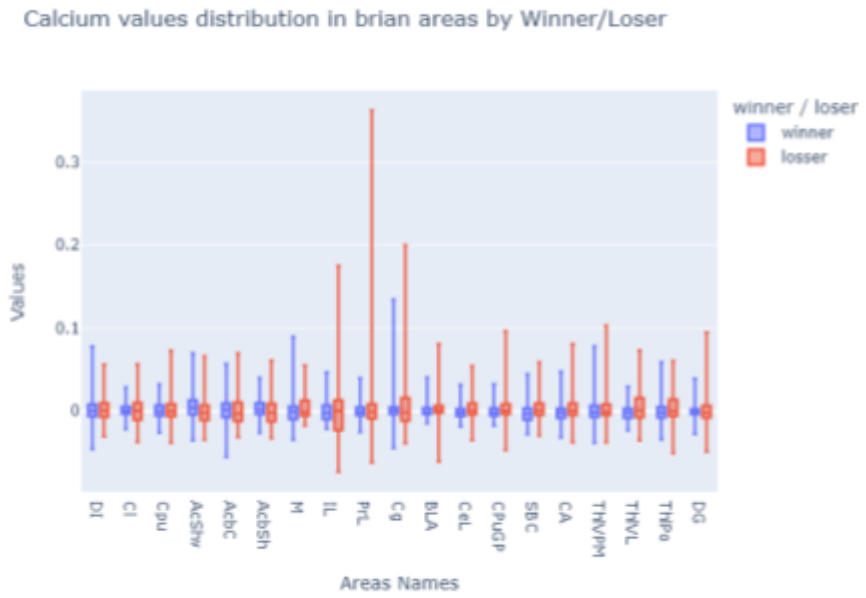
Figure 5 Brain activity difference between winner and loser by frame



Data Exploration:

Upon examining the raw data, we preformed statistical measures such as mean, median, and quantiles within each group of winners and losers.

Figure 6



Observable variations surfaced among some areas for both winners and losers, showcasing differences in average values and deviations within each area.

To enhance our comprehension of the distinctions between the mice, we specifically plotted the

Figure 7

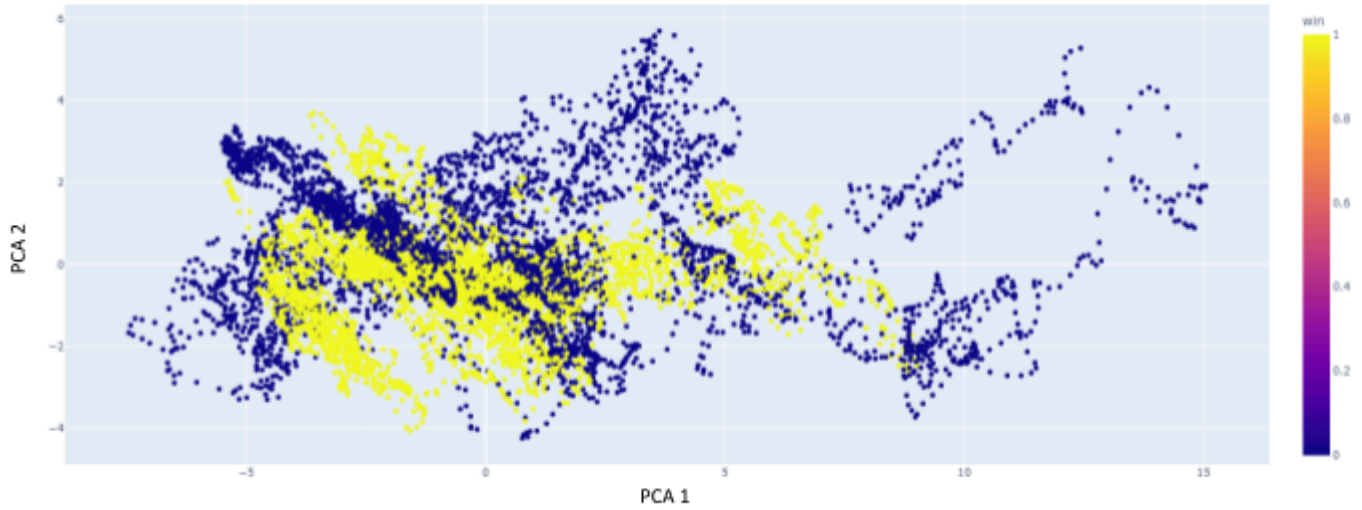
differences between each area. Unsurprisingly, upon a detailed examination of the raw data, a clear pattern emerged: the loser mouse exhibited heightened activity in the prelimbic area (prL) and hippocampus (CA), while the winner mouse demonstrated increased activity in the Acbsh and AcShv areas.

Overall, we can see that the losing mouse has much more active areas than the winning mouse.

PCA Analysis:

PCA Analysis identifies the most significant features in the data that explain the highest variance. Following a PCA, we can examine the most crucial components to examine the importance of different areas.

Figure 8 Total Explained Variance: 63.62%



The two first components can explain 62% of the data variance.

Figure 9

In the PCA coefficients, there are no noticeable variations between the areas. This finding is surprising, considering the distinctions observed in the raw data. One possible explanation is that, during most of the trial time, the mice exhibit relatively low brain activity, and the differences only emerge during specific peaks in certain areas.

SVM Analysis:

Support Vector Machines (SVM) are a type of supervised machine learning algorithm used for classification and regression tasks. The algorithm works by finding a hyperplane that best separates different classes in the input feature space, maximizing the margin between them. SVMs are versatile and can handle linear and non-linear relationships using kernel functions, allowing them to capture complex patterns in the data.

Finding the best model:

Initially, our goal was to fine-tune SVM parameters to identify the most suitable model for the data. Additionally, we aimed to assess whether broadening the data frame through resampling could enhance model performance. For each resample size ranging from 1 to 50 frames, a grid search was conducted to the C parameter for the algorithm.

The C parameter, referred to as the regularization parameter, controls the trade-off between achieving a smooth decision boundary and classifying the training points correctly.

A small C value encourages a wider margin but may allow for some misclassifications, making the model more tolerant to errors and potentially leading to a simpler decision boundary. A large C value, on the other hand, results in a narrower margin and aims to correctly classify more training points, potentially leading to a more complex decision boundary.

The kernel function remained linear since our objective was to investigate each area's values for future feature importance analysis.

The optimal model was identified with a C parameter set to 1, and no data resampling.

Utilizing cross-validation, the algorithm yielded a mean accuracy of 0.755.

Model Evaluating:

We performed a permutation test to assess whether the observed accuracy of the model is significantly different from what would be expected by random chance.

The labels of the dataset are randomly shuffled multiple 100 times.

For each permutation, the model is re-evaluated, and a new accuracy is calculated based on the shuffled labels. The distribution of accuracies obtained from the permuted labels is compared to the original model accuracy.

We checked if the original accuracy is significantly different from what would be expected by random chance, it suggests that the model is learning meaningful patterns in the data.

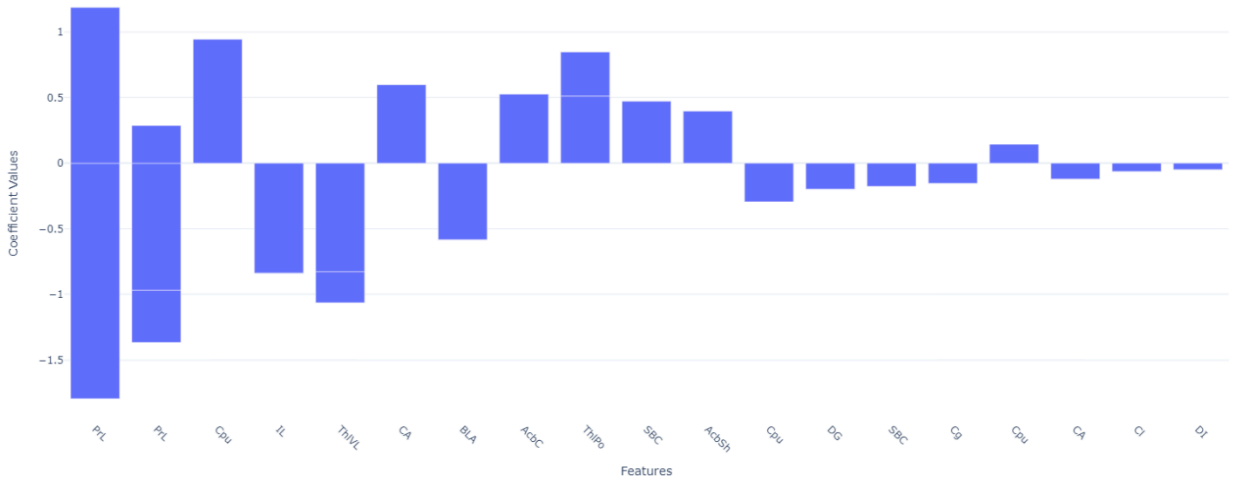
A p-value is calculated, representing the probability of observing an accuracy greater than the observed accuracy under the assumption that the model's performance is due to random chance.

The P-value is 0.0099, then we can conclude that the model accuracy is significantly different from random chance.

Feature Importance:

Following the identification of the optimal model for the data and its evaluation through cross-validation and permutation testing, our focus shifts to examining the model's coefficients. These coefficients, linked to each brain area, offer insights into the contribution of each feature to the decision boundary. Features with larger absolute coefficients have a more substantial impact on the decision boundary.

Figure 10 Sorted Coefficients of SVM with Linear Kernel



Notably, the SVM model highlights the importance of the PrL, Cpu, IL, and ThIVL areas. This aligns with our observations during the plotting of raw data and average values for losers and winners, confirming the expected significance of these specific brain regions in influencing outcomes.

Results

The analysis found that the most significant areas for indicating losing and winning are PrL, Cpu, IL and ThVL. The prelimbic (PL) and infralimbic (IL) regions of the medial prefrontal cortex (mPFC) have been implicated in different aspects of avoidance and reward-seeking (Floresco 2020) and allows rodents to adapt their responding under changing experimental circumstances (Melissa J. Sharpe 2015). This information adds that the losing mice might be overthinking about the outcome of the test and are not focusing on their goal to cross the tube.

The ventrolateral is dedicated to the relay and feedback of motor information, particularly motor control (BRUMBERG n.d.).

These findings emphasize the crucial roles of PrL in influencing social dominance interactions among mice. This correlation provides valuable insights into the neural basis of aggressive behavior in this context, contributing to our understanding of mice behavior and offering potential implications for broader neurobiological mechanisms related to social dominance.

Discussion

There are additional steps and unanswered questions that could enhance the study in the future:

Adding variables like mouse ID, trial number, and date could help isolate individual differences and account for potential time effects.

Running the model separately for each mouse pair might decrease biases, providing a more nuanced analysis. Enriching the dataset with details on mice actions, such as touching or stopping during the trial, could offer a more comprehensive understanding of the observed behaviors.

Exploring diverse algorithms, such as Decision Tree, Linear Regression, and Gradient Boosting Regressor, could contribute to a more thorough evaluation of the data.

An interesting observation that justifies further exploration is the features' importance analysis, revealing both negative and positive influences of the Prelimbic cortex (PrL) on the model. This inconsistency challenges the expectation of more uniform behavior from the same brain region, prompting deeper investigation into potential underlying factors.

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