Machine Learning Analysis for Animal Behavior in Predator-Exposed Rodents

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THE BAKER LAB

Neurocircuitry of Decision-Making

Abstract

- With the rise of artificial intelligence (AI), machine learning analysis will likely become standard use within behavioral laboratory settings.
- Using machine learning analysis, we analyzed behavior expressed by both rats and mice of different strain, sex, age, and housing in order to more closely evaluate behavioral differences in exposure to a predator stimulus.
- The predator stimulus was a 3D printed owl predator that surges towards the center of an arena.
- A training network was taught to base its evaluations of behavior on specific points that correspond with body parts of the rodent.
- As the rodent responded to the stimulus, we were able to evaluate behavioral patterns, such as time spent in hiding, freezing, fleeing, or near the walls of the arena.
- This will allow us to look at the overall movement trajectories prior to and during predator exposure.
- Based upon the early stages of behavioral analysis, we found that rats and mice seem to have different behavioral reactions to predator exposures, and these seem to be dependent on rodent strain, sex, and housing condition.
- We hope to further investigate the differences between species, strain, and sex using machine learning analysis.

Methodology

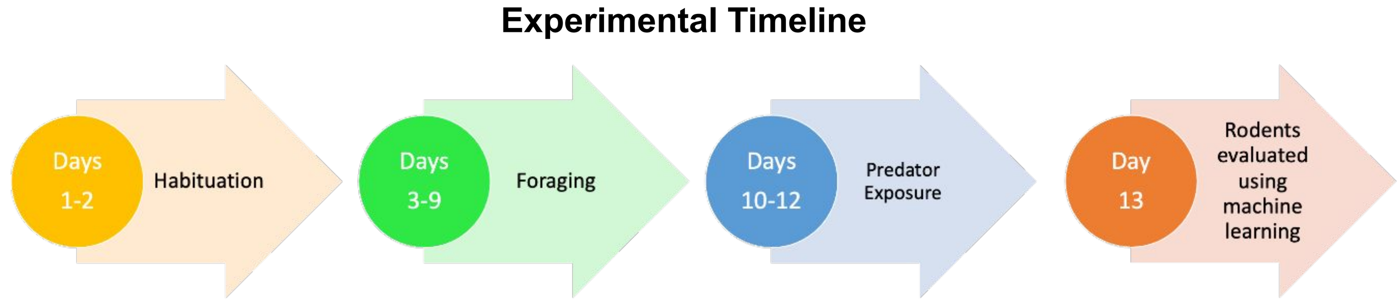
Subjects

- 35 mice
  - 6 CD1 single-housed females
  - 5 CD1 co-housed old males
  - 8 C57 co-housed old males
  - 5 C57 co-housed females
  - 6 C57 young males
  - 5 C57 young females
- 8 rats
  - 4 Sprague Dawley co-housed males
  - 4 Sprague Dawley co-housed females

Measures

- Habituation
  - 20 mins in the hide with 10 sugar pellets once a day for 2 days
- Foraging
  - 5 mins with increasing distance of pellet from hide
  - 6 days
- Predator Exposure
  - When the rodent gets within about 30 cm of the pellet in the arena, the owl surges approximately 100 cm over the food pellet toward the hide at about 70 cm/s.
  - This process is run for 3 minutes, regardless of the number of surges.
  - 2 days
- Machine Learning
  - Simple Behavioral Analysis (SimBA)
    - SimBA is a program that uses machine learning to describe animal behavior in real time.
    - It was used to crop the videos to adjust the area space, accounting for the arena.
    - Open Source code can be found here: https://github.com/sgoldenlab/simba
  - DeepLabCut
    - DeepLabCut is a program that allows us to train a machine learning model in order to predict rodent movement and behavior.
    - After cropping, 20 frames were extracted from each video in order to label body parts by hand on the rodent, owl, and owl shadow, as seen in *Figures 1 and 2*.
    - These frames were then used to train the model on rodent movement and the location of the owl and the owl shadow.
    - Once the model is fully trained, it can be used to analyze rodent movement and behavior (such as grooming and foraging) before, during, and after predator exposure.
    - Open Source code can be found here: https://github.com/DeepLabCut/DeepLabCut

Experimental Timeline



Machine Learning Analysis

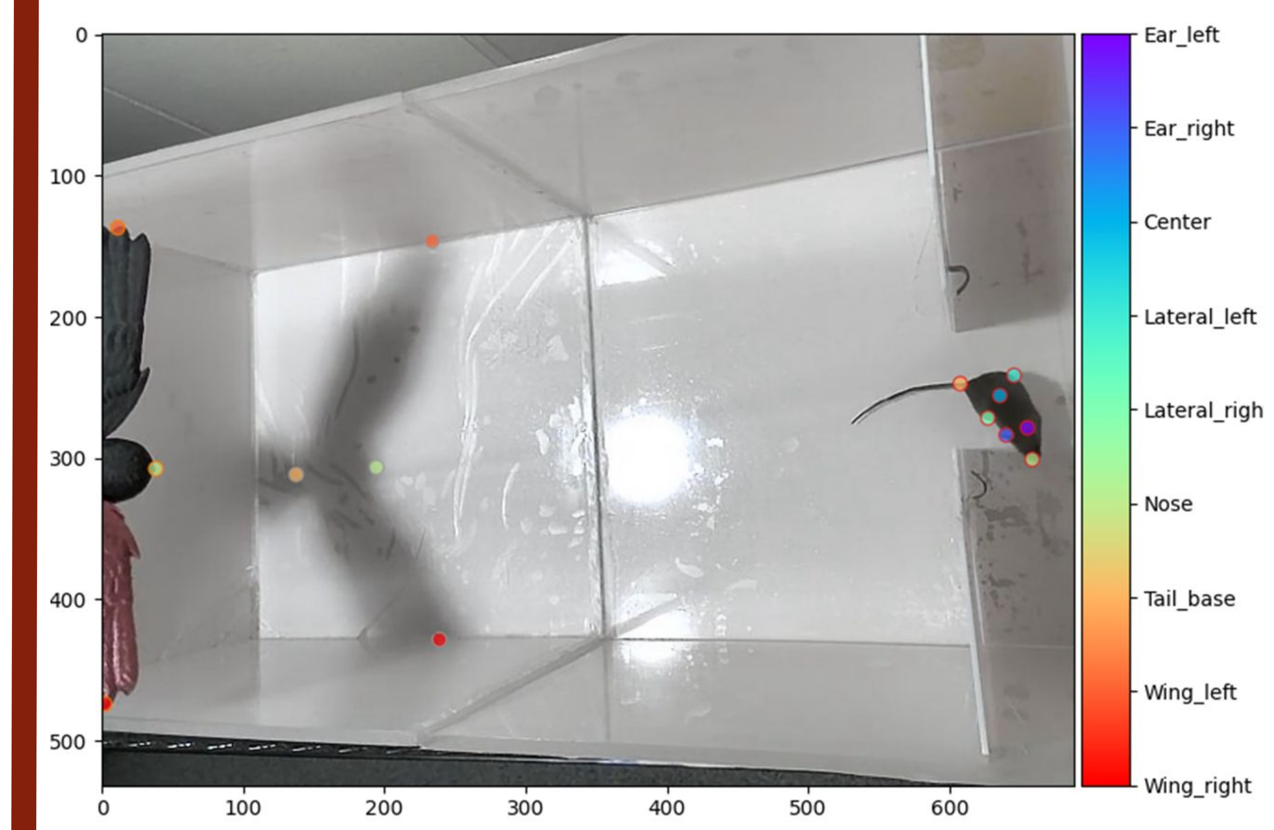


Figure 1. Predator exposure arena pre-owl surge. This is during the pose-estimation tracking section of the machine learning process.

Figure 2. Predator exposure arena post-owl surge

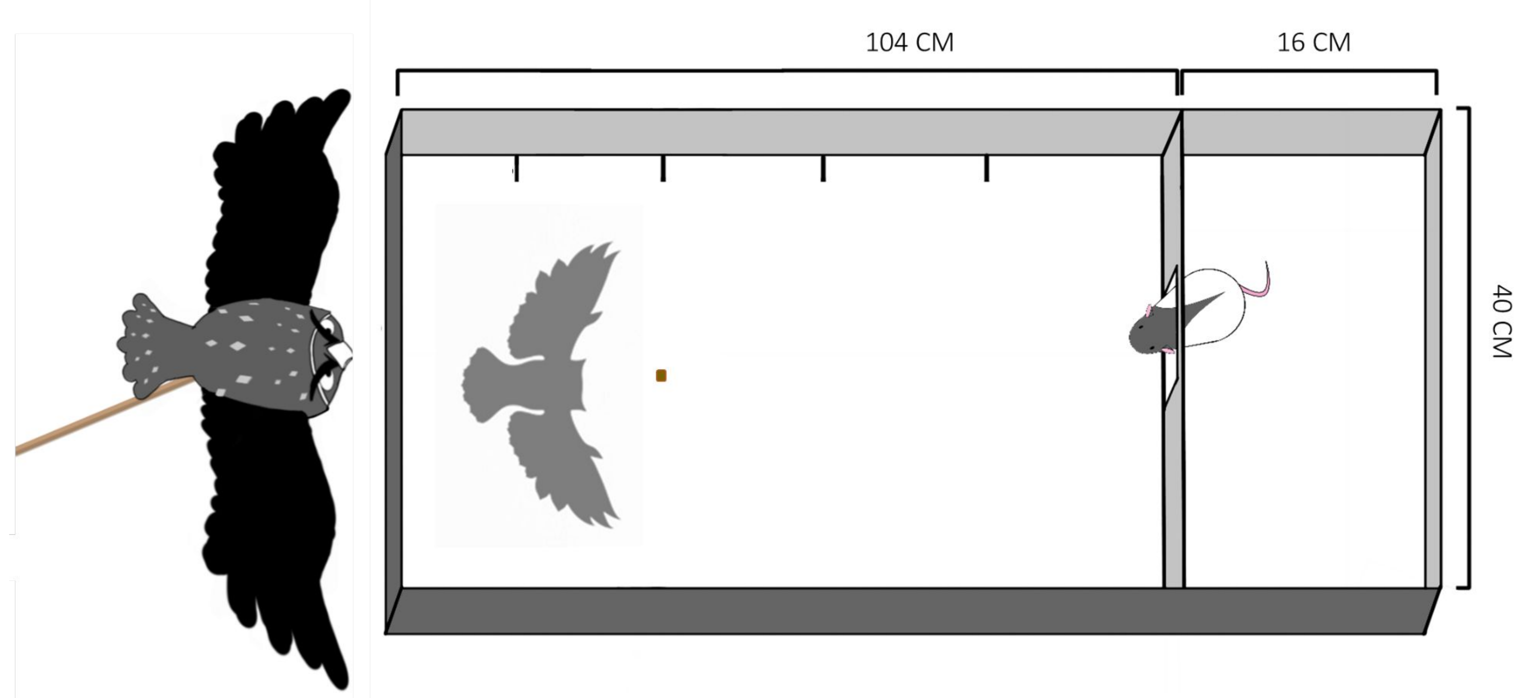


Figure 3. Model of predator exposure arena

```
individuals:
- mouse
- owl
- shadow
uniquebodyparts: []
multianimalbodyparts:
- Ear_left
- Ear_right
- Center
- Lateral_left
- Lateral_right
- Nose
- Tail_base
- Wing_left
- Wing_right
skeleton:
- - bodypart1
  - bodypart2
- - bodypart2
  - bodypart3
- - bodypart1
  - bodypart3
bodyparts: MULTI!
start: 0
stop: 1
numframes2pick: 20

    # Plotting configuration
skeleton_color: black
pcutoff: 0.6
dotsize: 12
alphavalue: 0.7
colormap: rainbow
```

Figure 4. This section of code for the training program is the only section of code that must be manually changed. It corresponds to the body parts in the labeling process. Because this is a multi-animal model, it is evaluating the movements of three individuals: the rodent, the owl, and the owl shadow. This way, the program can track how changes in the owl cause behavioral changes in the rodent (e.g., as the owl surges to the center of the arena, the rodent runs to the hide).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	scorer	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Baker	Phil Bake
2																
3	individuals	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse	mouse
4																
5	bodyparts	Ear_left	Ear_left	Ear_right	Ear_right	Center	Center	Lateral_lei	Lateral_lei	Lateral_rig	Lateral_rig	Nose	Nose	Tail_base	Tail_base	Wing_lef
6																
7	coords	x	y	x	y	x	y	x	y	x	y	x	y	x	y	x
8																
9	labeled-da	595.797	155.447	602.759	134.151	621.001	161.515	596.678	172.808	634.9	154.565	578.002	150.222	621.001	194.959	
10																
11	labeled-da	601.529	177.41	619.859	177.41	603.234	215.35	584.903	218.761	623.27	227.713	613.039	163.768	588.313	253.717	
12																
13	labeled-data\WIN_20230403_10_31_03_Pro_cropp					618.339	216.502	612.982	200.878	633.963	230.34	578.162	211.591	640.659	217.841	
14																
15	labeled-da	592.274	306.889	573.589	299.984	571.152	267.083	586.994	263.427	548.406	279.268			548.812	249.21	
16																
17	labeled-da	530.817	304.826	540.771	307.215	529.225	348.224	513.299	351.409	547.141	362.557	542.762	288.104	525.641	384.455	
18																
19	labeled-da	631.718	432.331	616.154	437.951	616.154	386.936	636.041	383.478	597.131	386.936	638.635	460.865	615.289	355.376	
20																
21	labeled-data\WIN_20230403_1			628.792	326.18	596.764	342.195	594.216	321.813	598.22	364.396	637.527	306.89	573.834	333.46	
22																
23	labeled-data\WIN_20230403_10_31_03_Pro_cropped\img3261.png											576.871	196.854			
24																
25	labeled-da	594.622	231.644			609.148	192.119	629.755	202.93	588.879	190.768			618.945	176.917	

Figure 5. Section of the Excel sheet with recorded pose-estimation tracking data after frames were labeled. This shows the coordinates of the body parts labeled on the mouse, owl, and owl shadow. Empty spaces indicate that no data was recorded for that body part.

Next Steps

- After the videos have gone through pre-processing (cropping, editing, etc.) and pose-estimation tracking (DeepLabCut), they are then imported into SimBA.
- Once the project has been imported into SimBA, the tracking results are imported into SimBA's classification projects.
- Within SimBA, the project will undergo pose-estimation tracking corrections, annotation of behavioral events and optimizing the machine learning hyperparameters and discrimination thresholds.
- There are a number of validation checkpoints to increase classifier explainability and tunability.
- At the end, there will be visual outcomes for the machine learning analysis.
- The outcomes we are interested in is the characterization of fleeing, freezing, and general movement.
  - Open field
  - Time by wall
  - Time spent in hide
  - Velocity and trajectory when leaving the hide
  - Grooming
- Based upon the early stages of behavioral analysis, rats had an initial fear response to the predator, but it appeared that most mice did not.
- However, there seems to be a change in behavior in mice compared to before versus after the predator exposure, indicating that the stimulus had an effect that will be tracked by the machine learning analysis.
  - Further analysis needs to be done to determine exact differences in fear responses when looking at species, strain, sex, and housing condition.

Discussion & Future Applications

- SimBA and other machine learning algorithms will completely revolutionize the way in which scientists conduct research and collect data on animal behavior, as it will increase efficacy and accuracy.
- Prior to machine learning, video analysis was done by hand, requiring a large amount of time, and introduced error into studies.
- However, machine learning removes the need to analyze all videos and cuts down on the required time.
- By automating the analysis process, machine learning allows researchers to focus on the interpretation and application of the results rather than spending time on the tedious task of manual analysis.
- Using a training network ensures that all videos are analyzed accurately and reliably.
- With machine learning, we are able to collect data about subtle behaviors and nuanced measures of trajectories and fleeing velocities.
- Machine learning can recognize patterns in the data that humans may not be aware of
- Machine learning can be trained on large datasets.
- Machine learning can help identify new behavioral patterns and phenomena that were previously unknown or overlooked.
- This data allows us to compare differences within and between groups.
- Training the machine learning network provides data for the program to determine rodent movement in the arena at various points in time.
- These machine learning programs could provide us with critical information about rodent movement and behavior that could be missed by humans.
- Machine learning analysis can determine behavior frame by frame in a way that would be unrealistic to expect of human judgement.
- This model could also be used for future rodent behavior analysis projects.
- Machine learning analysis can be used in any area that requires detailed tracking of the movements of humans or animals
  - For example, testing of new therapies can use machine learning to evaluate the change in facial emotion of people undergoing therapy
  - Another use is tracking the movements of other animals such asfruit flies, zebrafish, and monkeys

References

Nilsson, S. R., Goodwin, N. L., Choong, J. J., Hwang, S., Wright, H. R., Norville, Z. C., ... & Golden, S. A. (2020). Simple Behavioral Analysis (SimBA)–an open source toolkit for computer classification of complex social behaviors in experimental animals. *BioRxiv*, 2020-04.

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