Summary of Research Progress Since the Last Report:

Homeostatic behaviors:

Since the last report, I have worked to finalize the analysis of the drinking project I was working on. I mentored a student (Cayson Hamilton from BYU) over the summer and have worked with him to add feeding behavior to the project to round it out into a manuscript about homeostatic behaviors. To this end, I have:

- Finished running the Lickometer validation experiments to validate the drinking classifier
- Included feeding behaviors into subsequent analysis
- Looked into diurnal patterns of homeostatic behavior
- Run a genetic validation with Lepr db/db mice (obesity and diabetes mouse model)
- Given a talk at the Complex Trait Community conference meeting (virtual)
- Presented my work internally
    - Tufts retreat poster presentation (won 2nd place)
    - JAX trainee talks
- Presented a poster of my work at the JAX Machine Learning Short Course for rodent behavioral quantification
- Began drafting the manuscript for this project, with plans to finish a draft by the end of the calendar year

Maze assay to measure cognitive decline:

I have also progressed in my maze project to measure cognitive decline. I have:

- Run reversal learning experiments of young adult C57BL/6J mice in my maze assay
- Begun to analyze reversal learning of C57BL/6J mice
- Run reversal learning experiments of 5XFAD mice
- Worked on improving maze model for 5XFAD mice (still in-progress)

Grants:

I have worked on grant-writing. My initial F31 proposal in April was rejected, and I have been working on a resubmission. The biggest critiques aligned with comments that I received from the Qual exam, and I have been making changes to pull my focus towards the timing of cognitive decline in mice, rather than just the tool generation itself. Also, I applied for and received a T32 training grant for the T32 Training Program in Precision Genetics of Aging, Alzheimer's Disease and Related Dementias.

- Resubmitting F31 for December 8th deadline
- Applied and received T32 training grant

Other writing:

I wrote a commentary on a review for the journal of Neuroscience & Biobehavioral Reviews. It is currently under review. I also worked with my undergrad PI to submit my first-author

manuscript on dog SINEs in 3'UTRs to the journal Mobile DNA. Also, we have a paper entitled, Feature representations for lab animal behavior modeling, that we submitted to the Conference on Computer Vision and Pattern Recognition (CVPR) that I am second-author on. I am happy to expand on any details of this paper if anyone is interested! I can also share it if it gets accepted.

Summary of Research Plans for 2024 Spring TAC meeting:

Before the next TAC meeting in the Spring, I will finish up and have plans to submit my homeostatic manuscript. I will be annotating more data for my maze model to be more robust across the visually diverse 5XFAD mice (coat colors include black, albino, agouti, and tan). I will analyze the 5XFAD mice and compare them to their non-carrier littermates. Since 5XFAD mice are known to have cognitive deficits, this will serve as a validation of the tool. Unless the project scope changes, I should be then writing up these results as a paper to show the usefulness of this tool in sensitive measurement of cognitive decline and spatial learning in a naturalistic environment. I will also be attending the TAGC meeting to present my homeostatic project work, and will hopefully be attending CVPR for the feature representation paper in June.

Highlights of Results:

Homeostatic behaviors of BTBR, C57BL/6J, Lepr Hom, and Lepr Het mice
(This is the abstract I submitted to TAGC)

Understanding nuances in feeding and drinking behaviors is crucial when assessing disorders such as obesity, diabetes, and metabolic syndrome. Obtaining a reliable and efficient method to assess these behaviors in mice can provide powerful insight into the effects of these disorders. Technological advances in the computer vision field have enabled studying mouse behavior with a higher degree of sensitivity. Our group has applied these techniques to study mice in a social context, which allows for more naturalistic behavior in group-housed animals with the potential to produce more relevant data. Using machine vision over 46 long-term, continuous four-day experiments with three mice in each arena, I capture feeding and drinking behaviors across the classic inbred strain C57BL/6J, the autism spectrum disorder model BTBR *T+ Itpr3*^{tf}/J (BTBR), heterozygous B6.BKS(D)-*Lepr*^{db}/J (Lepr) control mice, and homozygous Lepr diabetic mice.

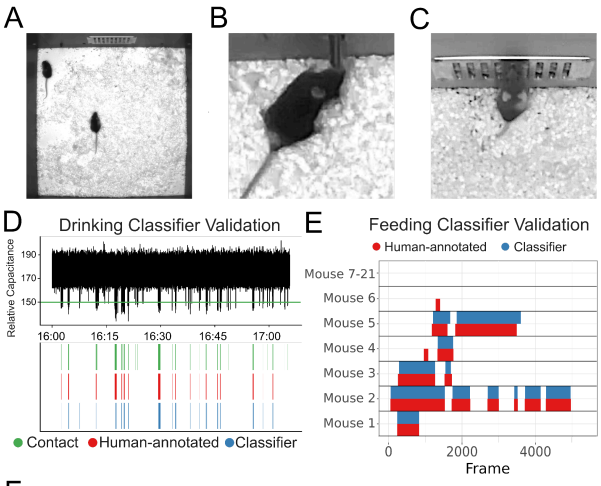
We trained machine learning (ML) classifiers for feeding and drinking through supervised learning. To validate the drinking classifier, I ran a Lickometer experiment, which is a mechanical solution to measuring drinking behavior, that logs contact events with the water spout. I manually scored each contact bout as a drinking bout or a non-drinking contact bout. We validated the feeding classifier through a classical ML approach of densely annotating videos. We used these datasets as ground truth to measure precision, recall, and F1 scores.

Because BTBR mice are used as a model of autism spectrum disorder, we compared social feeding behavior where mice eat at overlapping times from the same food hopper. Interestingly, we find the C57BL/6J mice participating in more social feeding bouts with longer durations

compared to BTBR mice, aligning with the BTBR strain's known antisocial tendencies, which have been previously characterized.

We also compared heterozygous and homozygous Lepr mice in terms of their feeding, drinking, and general activity. Activity levels are typically used as a measure of circadian rhythm. However, while homozygous Lepr mice have circadian rhythms that are extremely arrhythmic when looking at activity, they preserve diurnal feeding and drinking rhythms. This highlights the usefulness of measuring other homeostatic behaviors to glean circadian insights of less active mouse strains. These novel ML tools enable a new generation of assays that can use group-housed mice to produce more naturalistic data.

Here I provide some examples of results from the homeostatic manuscript that I will be presenting during the TAC meeting.



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Behavior	Precision	Recall	F1	Type
Drinking	0.8545	0.8952	0.8744	ML
Drinking	N/A	0.7282	N/A	Lickometer
Feeding	0.8571	1	0.9231	ML

Figure 1. Validation of Drinking and Feeding Machine Learning classifiers. **A).** Entire arena shot. **B).** Drinking screenshot. **C).** Feeding screenshot. **D).** Lickometer validation. One hour-long analysis of four mice during their active period comparing Lickometer sensor data and Machine Learning (ML) Drinking classifier. (Top) Raw sensor data from the Lickometer setup. (Bottom) ML classifier-called bouts and Lickometer-called bouts over the hour. **E).** Feeding ground truth bout plot F. Table of performance metrics. Comparison of the classifier-predicted bouts and the ground truth human-annotated bouts for each mouse in the ground truth dataset. **F).** Performance metrics of drinking and feeding classifiers. Drinking metrics are based on agreement between the Lickometer ground truth and the ML classifier predicted on the same overnight experiment. Feeding metrics are based on annotated ground truth randomly and semi-randomly selected videos.

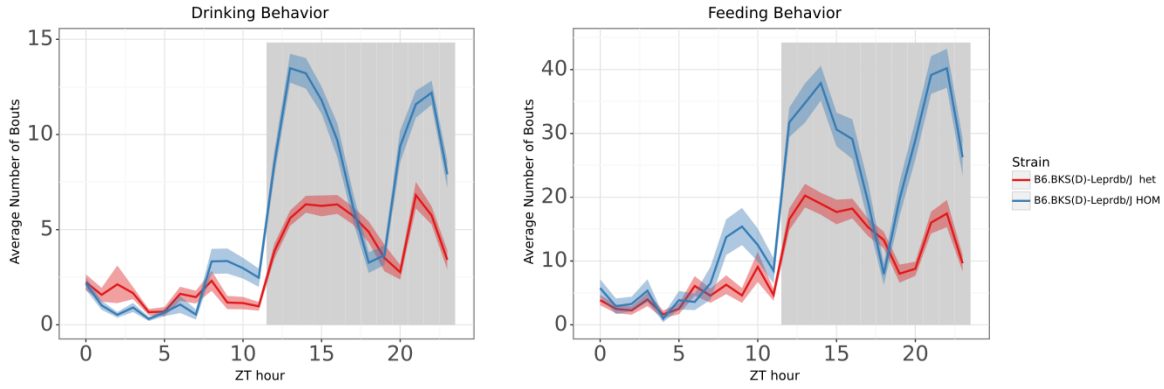

Figure 2. Next, I show the drinking and feeding behaviors of Lepr hom and het mice. The hom (diabetic, obese) mice show elevated levels of drinking and feeding, which is consistent with their known phenotypes to be polydipsic and polyphagic. I will be showing more analysis of these mice during the meeting.

Maze assay to measure spatial learning and cognitive flexibility

In the last meeting I showed my validated maze assay to extract tracking data and learning metrics from the experimental paradigm. Since then, I have run reversal learning experiments of young adult C57BL/6J mice. I have quantified several levels of learning to show. The first is the discrimination between the left and right nose pokes. I show that the mice can learn to differentiate between the two. I have preliminary results below:

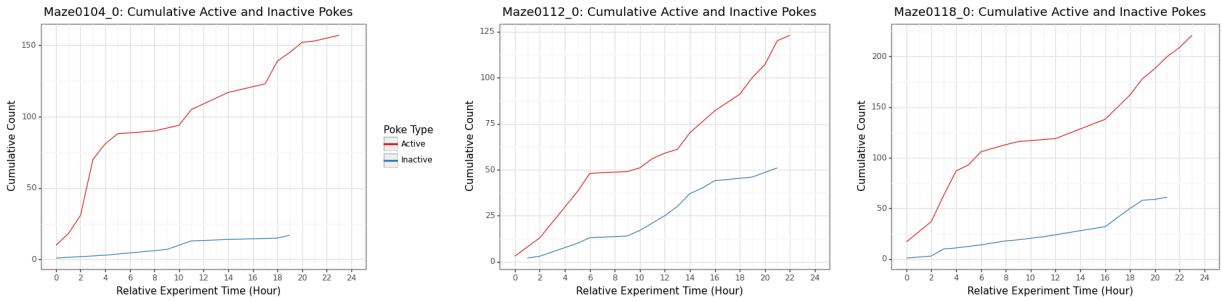

Figure 3. Three examples of mice discriminating between active and inactive poke for a sugar pellet reward. The mice learn to differentiate between the active and inactive pokes.

They also can learn to take perfect paths to the reward at a higher rate than another equivalently located node in the maze. They all learn to find their way to the sugar pellet reward.

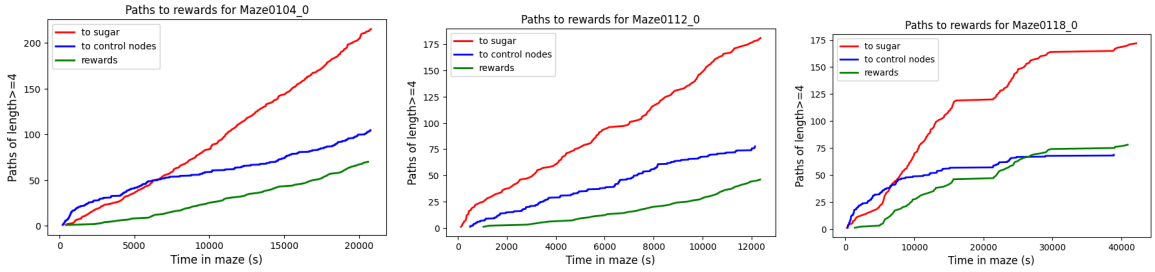

Figure 4. Three examples of mice discriminating between the path to the actual reward (to sugar) vs. to the control node. They learn to discriminate and will take deliberate perfect paths to the reward.

Finally, I show that when I move the location of the reward, they can learn the new location and go to that new location preferentially compared to the old location.

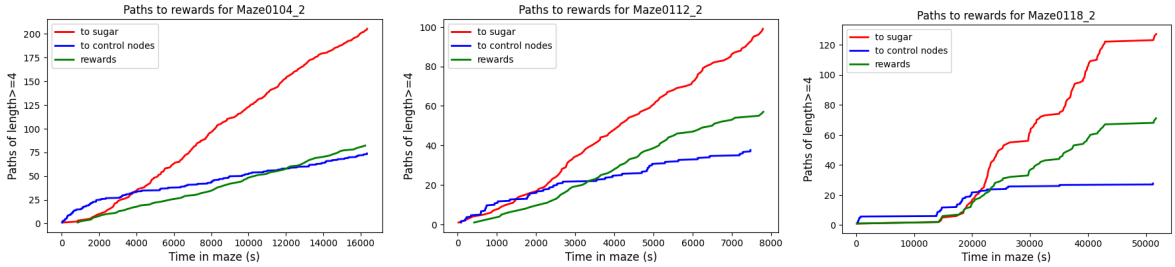

Figure 5. Three examples of mice discriminating to the new location of the reward (previous control location) vs. to the old location. They learn to discriminate to the correct new path of the reward.