Probing the Pathology of Anorexia Nervosa Using the Activity-Based Anorexia Model

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Introduction

Anorexia nervosa (AN) is an eating and psychiatric disorder that is characterized by significant weight loss, prolonged food refusal, irrational fears of weight gain, and distorted self-images.

Limit calorie intake despite feeling hungry

Uncontrollable refusal to eat

4% of Females Worldwide Affected

Current Treatments

Behavioral Therapy

Antidepressants

A pathology-based treatment for AN does not currently exist. Researchers have been working to find an effective treatment for AN.

Source: Weiselberg et al., 2011; van Eeden et al., 2021; UK Addiction Treatment Centers

ABA Model – Overview

Animal models of AN have the potential to expedite research to find a treatment. The activity-based anorexia (ABA) model replicates some of the hallmark features of AN, such as limited food intake and significantly reduced bodyweight.

ABA Model

Mice habituated to a single-wheel setup for 5 days with free access to food and water

On the first food-restriction day ("Day 0"), all food is removed for 24 hours

On each subsequent day, the mice are fed for 2 hours, during which food intake is measured

Our previous experiments found that mice consume less food on each subsequent day of ABA. This lack of food intake, which is especially prevalent on Day 2, results in significant weight loss of ABA mice

Traditional Animal Models

1

Stress Models

Subject the animal to cold swimming, tail pinching, or direct brain stimulation to induce stress and alter consumption behavior.

2

Diet Restriction

Limit food consumption to less than 50% of daily ad libitum food intake – mimics AN consumption but is involuntary.

3

Genetic Models

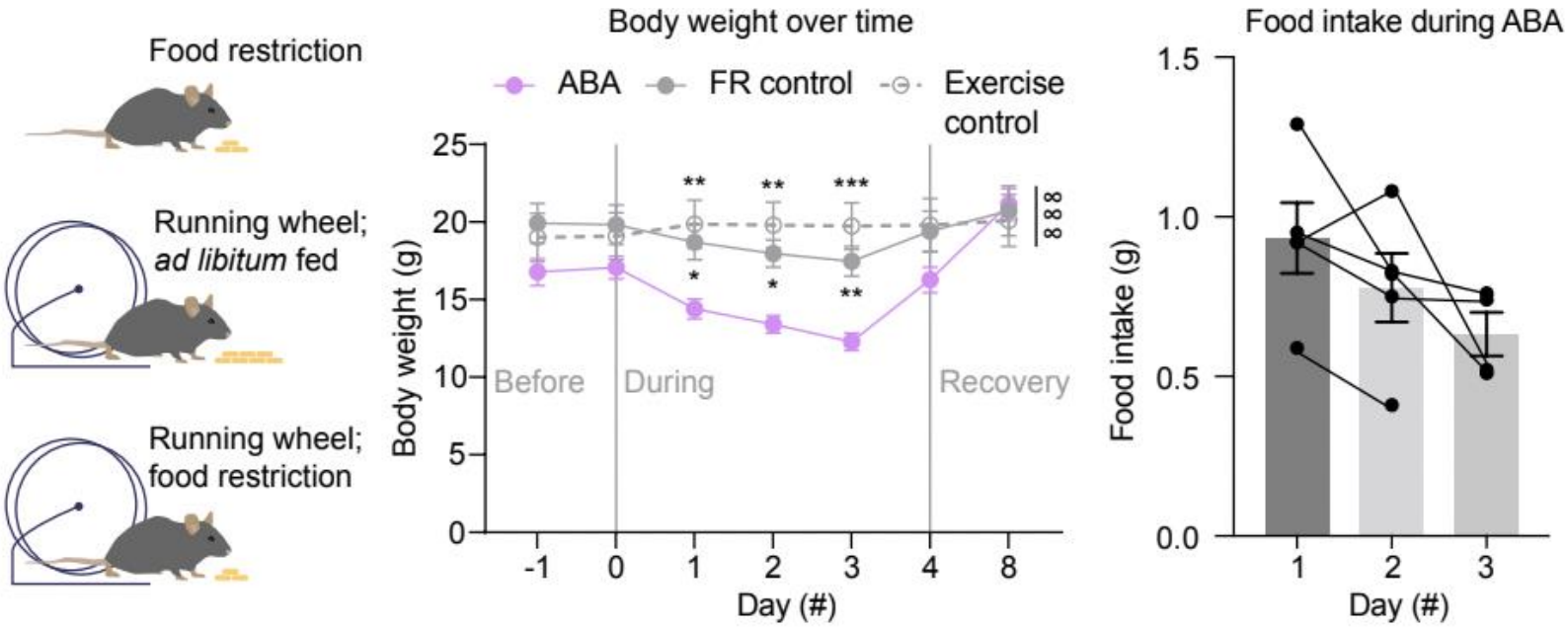
Mutate specific genes which regulate food intake and energy balance – creates various phenotypes related to eating disorders

Traditional models are not as effective because the food intake for animals is controlled, which is not representative of AN in humans

Source: Lo Sauro et al., 2008; Avraham et al., 2001; Johansen et al., 2003

ABA Model – Previous Findings

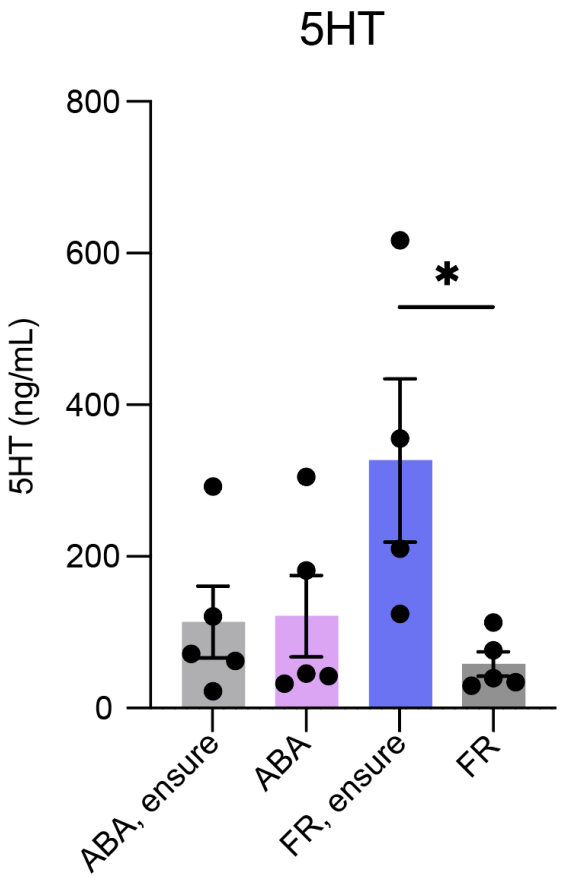
Our previous experiments discovered that mice consume less food on each subsequent day of ABA. This lack of food intake, which is especially prevalent on Day 2, results in significant weight loss of ABA mice. Through in vivo neural recordings, we also observed an attenuated dopamine response to post-ingestive stimuli in ABA mice.



Source: Betley Lab

ABA Model – Previous Findings

One critical peripheral experiment we performed was collecting and analyzing blood plasma after these ABA experiments, through which we found that ABA mice exhibited lower levels of peripheral serotonin relative to non-ABA mice.



Source: Betley Lab

Study Rationale

Because of these findings, we decided to probe possible treatments for AN using ABA. The current literature suggests a variety of potential pathogeneses for AN. These studies, along with our own findings, shed light on possible therapeutic targets for AN.

Hypotheses for Pathogenesis of AN in Current Literature

1. **Gut Microbiota Disruptions**
2. **Irregular Metabolite Profiles**
3. **Combination of Social & Environmental Factors**

+

Our Previous Findings in ABA Experiments

Our New Hypothesis

We hypothesized that restoring peripheral serotonin may rescue the eating habits and bodyweights of ABA animals. As such, we performed two separate experiments aimed to increase peripheral serotonin levels of ABA mice.

Source: Fan et al., 2023; Westwater et al, 2022

Study Overview

We performed two separate experiments aimed to increase peripheral serotonin levels of ABA mice.

1

Mice

Before the experiments, the mice were subjected to a 2–3 habituation period and exposed to experimental conditions, including handling, i.p. injections, and experimental chambers.

2

Habituation Stage

The mice were randomly selected to 1 of 3 experimental groups: treatment group (6 mice), control group (4 mice), and food-restriction group (4 mice). Treatment and control group mice were provided with ad libitum access to food, water, and a running wheel for five days. Food-restriction group mice were provided with ad libitum access to food and water but no running wheel. Bodyweight and total food intake were assessed daily.

3

ABA Model

- On “Day 0”, all food was removed from the home cages and the mice were food deprived for 24 hours.
- On the successive 2 days, the mice were given ad libitum food access for 2 hours in their respective home cages. They were also provided with unlimited access to a running wheel.
- On each of the experimental days, bodyweight of the mice were recorded before the 2-hour feeding period. At the end of the feeding period, the total food consumption was measured.

4

Peripheral Serotonin-Related Experiment

Mice either subjected to a selective serotonin reuptake inhibitor (SSRI) injection or a tryptophan-enriched diet.

5

Blood Plasma Collection & Analysis

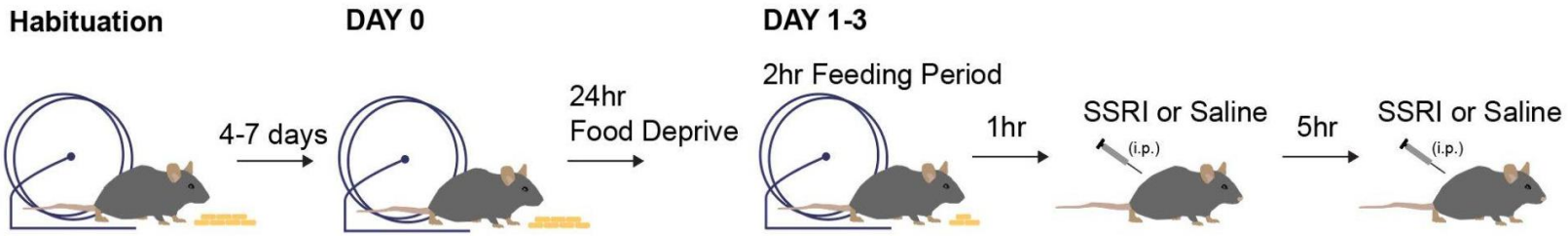
Perfusion was performed and blood plasma was collected and analyzed.

Source: Betley Lab

4

Experiment 1 – SSRI Injections

In the first experiment, we injected the ABA mice with Selective Serotonin Reuptake Inhibitors (SSRIs). Our aim was to understand how the food intake and bodyweight of ABA mice would be impacted by increasing peripheral serotonin levels.



Methodology

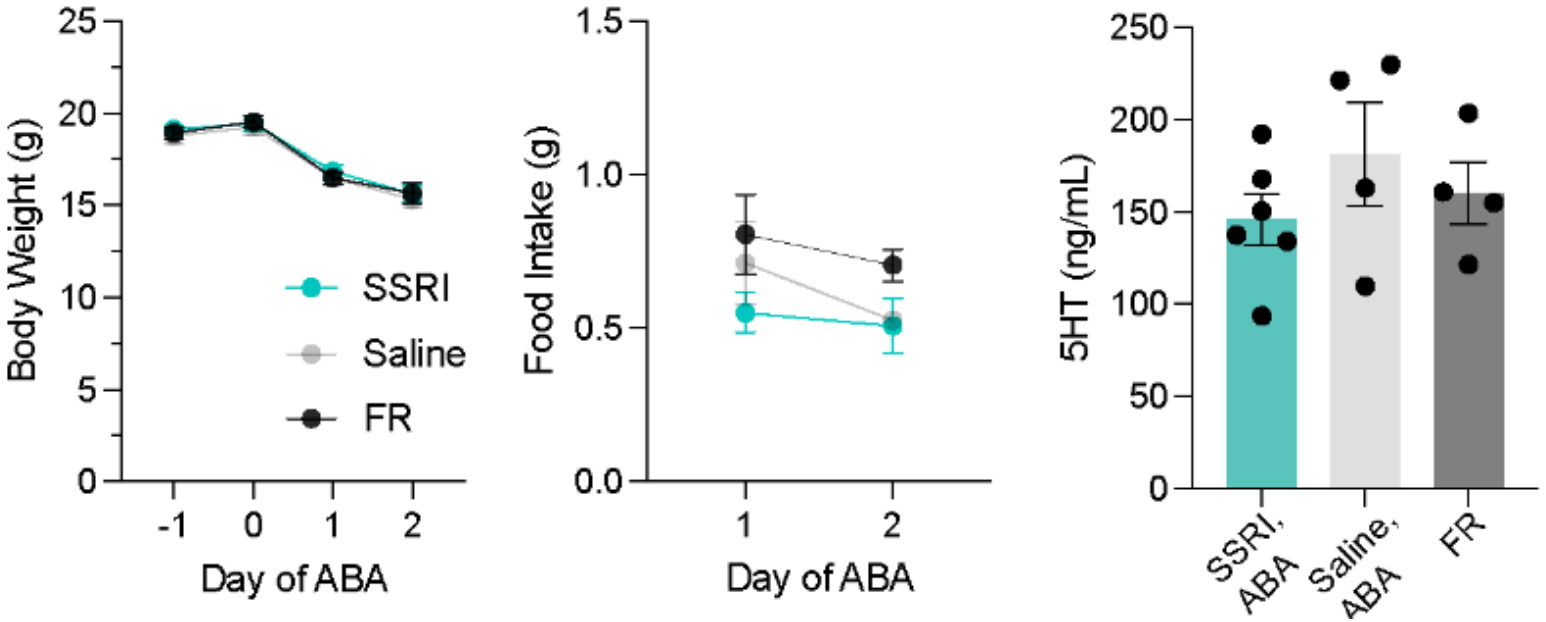
- In the feeding period of the SSRI injection experiment, a SSRI (either Citalopram Hybromide or Fluoxetine) was injected into the 6 mice in the treatment group and saline was injected into the 4 mice in the control group.
- Injections were performed 1 hour before the feeding period, during which the mice were given ad libitum food access for 2 hours.
- We then recorded total food consumption and bodyweight.

Source: Betley Lab

4

Experiment 1 – SSRI Injections Results

The SSRI injection **did not increase** peripheral serotonin or food intake levels in ABA mice. Rather, the mice in the treatment group were found to have **overall lower levels** of peripheral serotonin compared to the mice in the control and food-restriction groups.

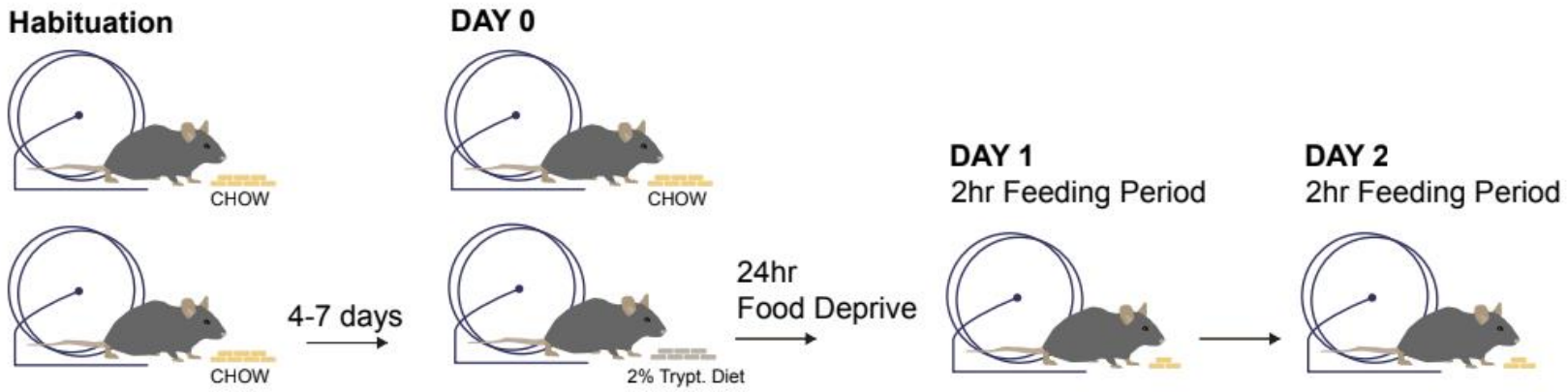


Source: Betley Lab

4

Experiment 2 – Tryptophan-Enriched Diet

In the second experiment, we supplied the ABA mice with a tryptophan-enriched diet. Our aim was to understand how the food intake and bodyweight of ABA mice would be impacted by increasing peripheral serotonin levels.



Methodology

- Tryptophan is the sole precursor of peripheral serotonin.
- In the feeding period of the tryptophan experiment, ad libitum 2% tryptophan-enriched diet access was given to the 6 mice in the treatment group during the 2-hour feeding period of the ABA experiments.
- The 4 mice in the control group were given access to a regular chow diet during the 2-hour feeding period.
- We then recorded total food consumption and bodyweight.

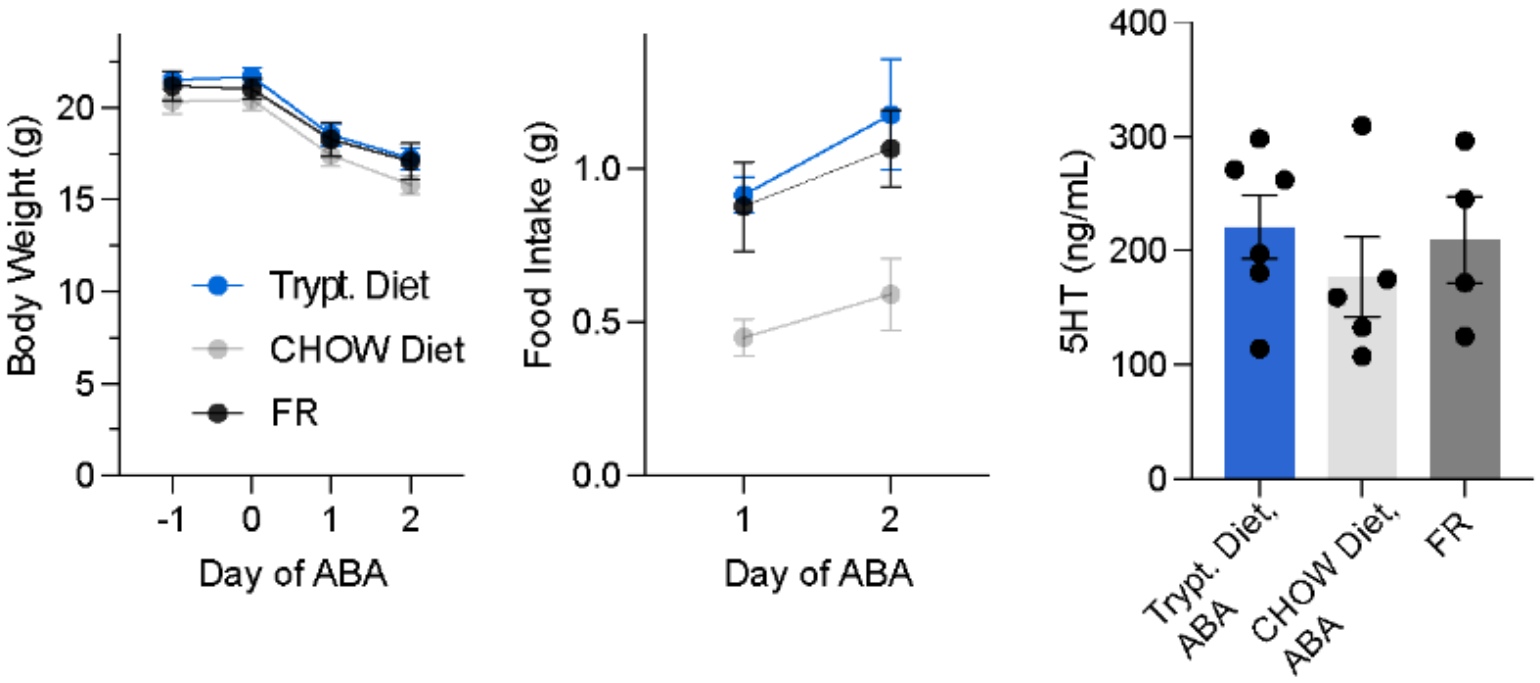
Source: Betley Lab; NIH

4

Experiment 2 – Tryptophan-Enriched Diet Results

The tryptophan-enriched diet **increased** peripheral serotonin and food intake levels in ABA mice. Blood plasma results demonstrated **trending higher** peripheral serotonin and food intake levels in the ABA mice which were supplied with the tryptophan-enriched diet.

The treatment group and food-restriction group mice exhibited similar levels of peripheral serotonin and food intake.



Source: Betley Lab

Study Discussion

These experiments were designed to elucidate the features and characteristics of ABA mice. We predicted that restoring peripheral serotonin via SSRI injection or tryptophan-enriched diet would prevent a decrease in food intake and bodyweight, which are hallmark characteristics of AN.

Experiment 1 – SSRI Injection

Limitation: Injection time prior to ABA feeding period

- In our experiments, we used a buffer time of 1 hour based on past published experiments; however, incorrect timing could have contributed to the results we observed.
- In future experiments, we could consider **decreasing the 1-hour period between the injection and feeding period**.

Experiment 2 – Tryptophan-Enriched Diet

Further Confirmation: Taste Preference Test

- To ensure our results were not affected by the preferred taste of the tryptophan diet, we performed a preference test with tryptophan-enriched diet and standard chow.
- We found **no differences in intake between the two types of food**, leading us to believe that the ABA rescue was not a result of food taste.

Impact & Future Steps

- Our study elucidates the pathology of ABA and reveals a potential path of rescue for hallmark ABA characteristics.
- This data contributed to the Betley Lab's holistic understanding of the ABA model and its validity.
- Our team will collaborate with Dr. Kimberly Smith at JHU to compare peripheral serotonin levels in human AN patients.

Ultimately, because the pathology of AN is currently unknown, the hope is for our results to serve as a foundation for AN treatments in the future.

Source: Betley Lab

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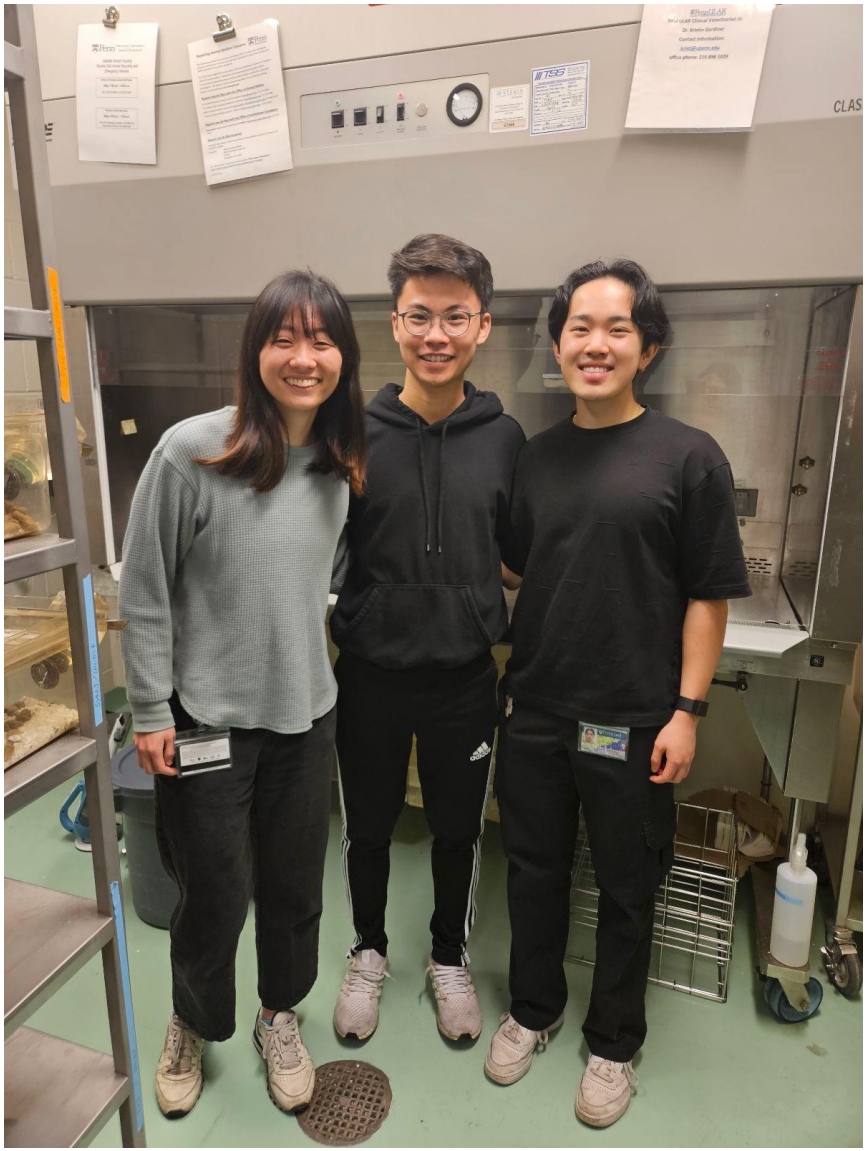

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