



Centella asiatica and exercise together improve spatial memory learning in C57BL6 male mice to a greater extent than either intervention separately



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Background

Centella asiatica



Mouse running wheel



- In the United States it is estimated that two out of three individuals will experience some form of cognitive impairment by age 70.¹
- Recently, multimodal treatments, targeting multiple physiological systems, have shown promising results in reversing cognitive decline.²
- Age-related changes in inflammation, oxidative stress and mobility have also been reported to accompany cognitive impairment in rodents and humans.^{5,6,7}
- Here we present a model to study salutogenesis, or health restoration, in aging.
- This model is designed to test the effect of multiple complex interventions on multiple physiological systems.
- Our group has previously demonstrated that a water extract of the botanical Centella Asiatica (CAW) can improve learning and memory in aged mice.³
- Exercise has also been shown to improve memory in aged mice.⁴
- For this study, we explored the effects of CAW and exercise, alone and in combination, on cognition as well as on mobility, oxidative stress and inflammation to gain insights into the whole organism health restoration effects of the interventions.
- Data from these endpoints can be integrated into a bioinformatic model to look at the relationship between outcomes in response to these treatments, as well as the effect on multi-omic domains.

Project Objective

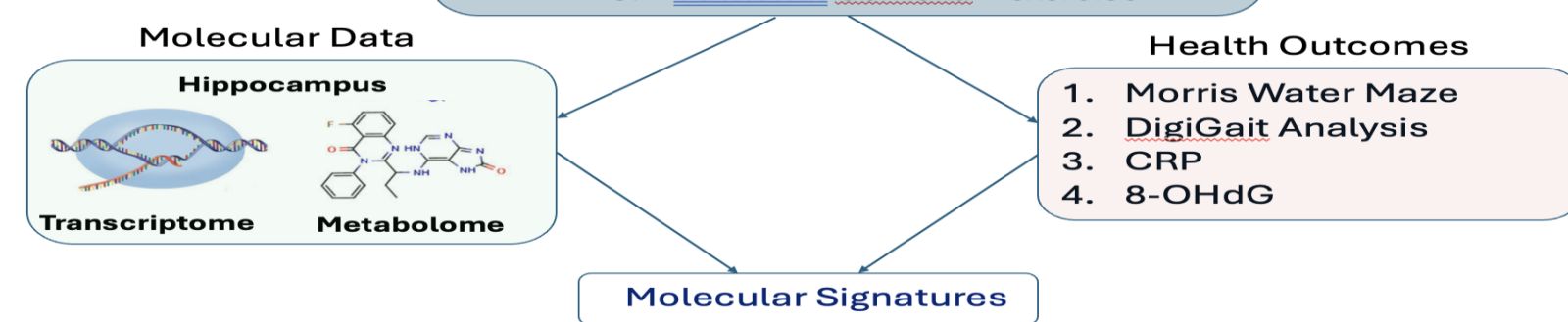
Evaluate the effects of CAW and exercise on cognition, inflammation, oxidative stress and mobility, as well transcriptomic and metabolomic changes in the hippocampus of aged mice.

Methods

- 4 and 18 month old C57BL6 mice were obtained from the NIA aged rodent colony and divided into the following treatment groups.
- The plant material to create the CAW powder was obtained from Oregon's Wild Harvest (OWH), then prepared by reflux extraction with water for 90 minutes, in the ratio 1g herb:12.5mL water, then followed by biofiltration and lyophilization.
- CAW powder was dissolved in the drinking water of the mice at 10g/L.
- Exercise was provided by constant voluntary access to a running wheel in their cage.⁸
- Treatment lasted for 35 days. At the conclusion of treatment behavioral testing was initiated.

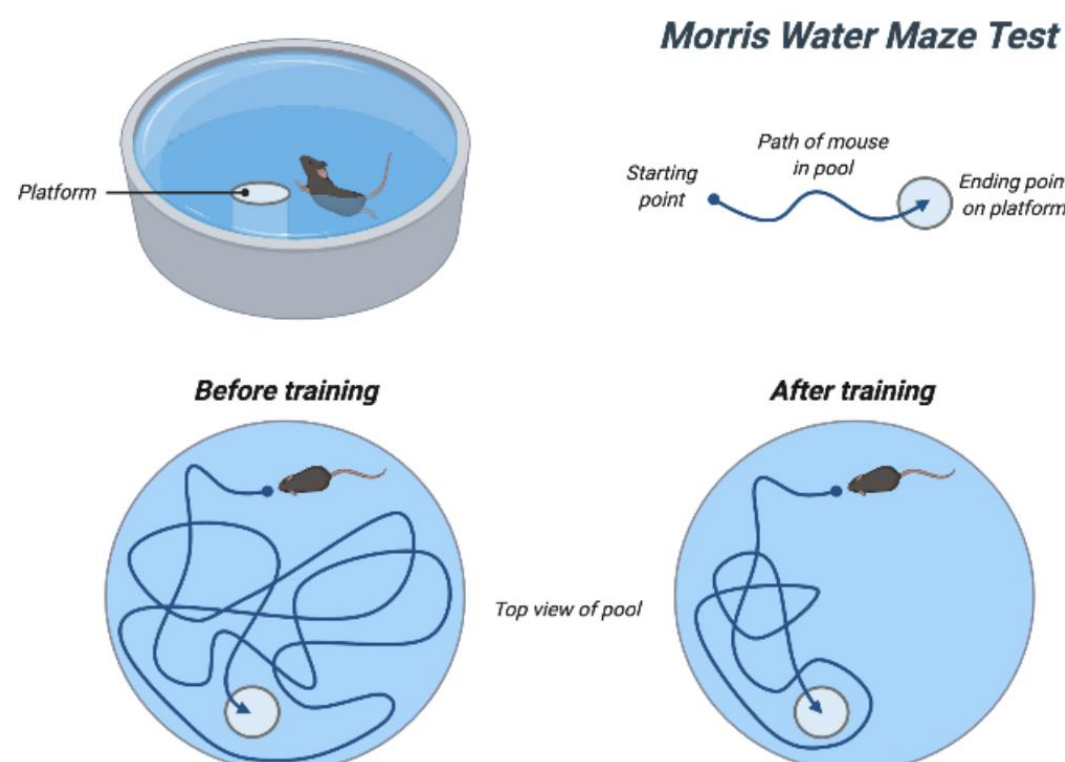
Experimental Design

- 10 males, 10 females in each group
1. 4 month untreated
2. 18 month untreated
3. 18 month Centella
4. 18 month exercise
5. 18 month Centella + exercise



- Behavioral testing:
 - Gait was assessed using the DigiGait treadmill apparatus
 - Spatial learning and memory was evaluated using Morris Water Maze. This test is divided into three phases:
 - The visual platform phase occurs on the first two days of testing and is used to assess swimming ability and vision
 - Next is the hidden platform phase which assesses learning. In this phase the platform is submerged below the water and visual cues on the walls around the tank are used to orient the mice. Three 60 second trials are done twice a day for 5 days (AM and PM) to see how long it takes the mice to find the platform.
 - The probe phase occurs 24h and 72h after the hidden platform phase. In this phase the platform is removed completely, the trial is 60 seconds and the amount of time the mice spend in the quadrant where the platform was is how memory retention is measured
- At the conclusion of behavioral testing, plasma and hippocampal tissue was harvested for analysis.
- ELISA kits were used to measure plasma C-reactive protein (CRP) as an inflammatory marker and Plasma 8-hydroxydeoxyguanosine (8-OHdG) as a marker of oxidative stress.
- Changes in the hippocampal transcriptome and metabolome will be determined by RNA-seq and metabolomic analysis.

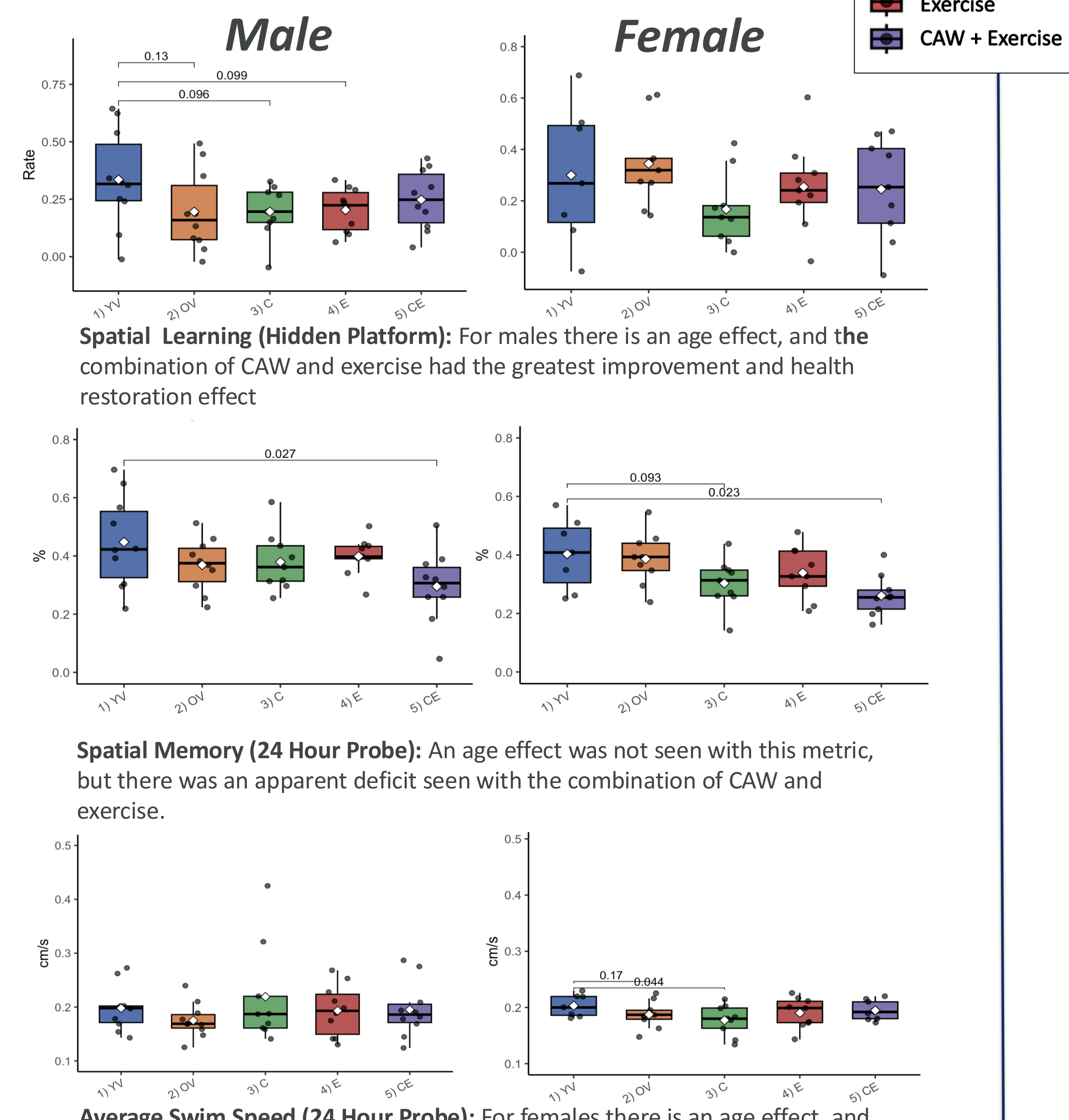
Morris Water Maze Test



Source: <https://www.biorender.com/template/morris-water-maze-test>

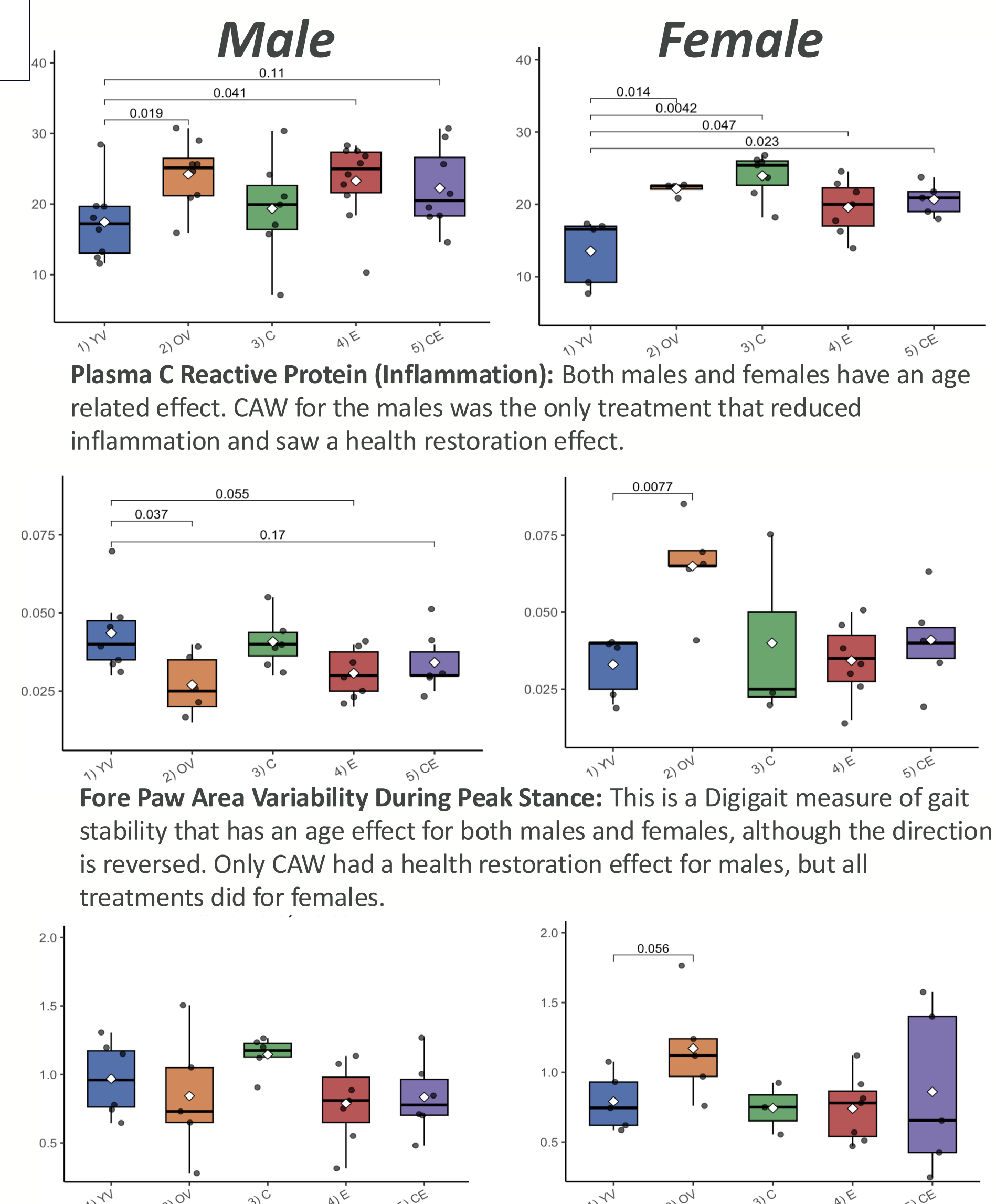
Result Highlights

CAW+Exercise Improves Spatial Learning But Not Spatial Memory



Average Swim Speed (24 Hour Probe): For females there is an age effect, and both treatment groups that included exercise saw the greatest improvement and health restoration effect.

CAW Reduces Inflammation and Improves Gait Stability



Fore Limb Ataxia: This is another Digigait measure of gait stability that only has an age effect for females. All treatments saw improvement and health restoration in this group.

Summary and Future Directions

- Five of the six metrics presented here show an age-related effect that was reversed by at least one of the treatments for at least one of the sexes
- Interestingly spatial memory did not show an age-related effect and also had a deficit with the combination of CAW and exercise, which was the treatment with the best health restoration effect for males for spatial learning.
- Treatments were stopped at the beginning of the testing period, which lasted 10 days, and little is known about how long the effects of CAW last post treatment.
- However, for the males it can be seen there is still a health restoration effect of both inflammation and measures of gait stability
- We are currently performing an additional experiment which will continue treatments until the end of testing for comparison.
- Transcriptomics and metabolomics data are also being collected for correlation to the health restoration effects observed.
- A p-value cutoff of .2 was used for the figures. These are not adjusted for multiple testing.

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Funding: NCCIH K08AT012477 (Chamberlin)