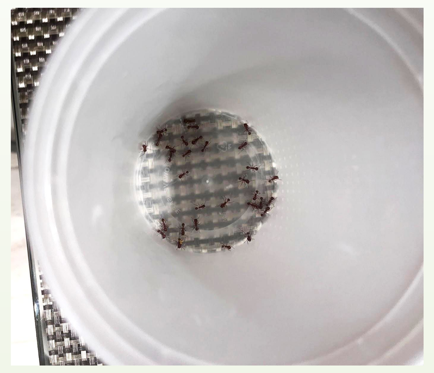
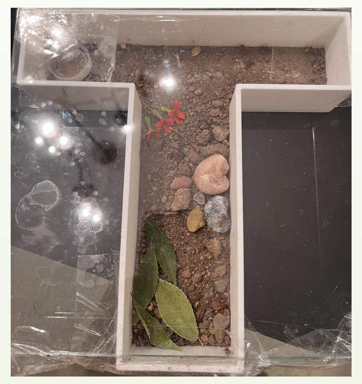
Image taken by AntsWeb [3]

Investigating spatial memory formation in harvester ants through operant conditioning.



separated trained ants from the ant farm
*photo was taken by the student



- Pilot study of T-maze
- Photo taken by student

Past research

My previous project investigated which sensory cues - smell, sight, or sound - harvester ants rely on most to form memories for finding food to survive. I discovered that olfactory senses play the strongest role in ants' memory formation and development. Here, I further investigate whether harvester ants can form spatial maps to navigate during foraging.

Background

- Ants have a unique ability different from mammals in that they can navigate a much bigger despite having smaller scaled view.
- Primates have brain region called hippocampus that forms spatial memory [1].
- Collett and his team studied ability of some insects to navigate [2]. However, it is not known to date whether *Pogonomyrmex barbatus* have spatial memory.

Abstract

I investigated whether harvester ants form spatial maps and cues they use to forage. Using a T-maze with natural stimuli, I show that ants did not strongly remember reward locations. Instead, they relied heavily on local stimuli cues. In particular, when stimuli were moved, ants interacted with them more. Further when the stimuli were moved, ants frequently navigated based on their initial experience. For example, I initially placed the food in line with stimuli A to B and had ants explore the location of food. When I swapped the location of A and B, most ants continued to move from A to B, ending up in the location with no food. Taking together, my data suggest that ants navigate based on local cues and provide insights into ants evolution of memory formation.

Hypothesis

1. Harvester ants do have spatial memory; they can construct spatial map of their surrounding environment.
2. Harvester ants will memorize their surrounding space by overall direction.
3. Regardless of changes in local stimuli, ants will interact with the stimuli in the same manner.

Methods

DATA COLLECTION

- 200 Pogonomyrmex barbatus ants (red harvester ants) were obtained.
- Ants were housed in AntsCanada ant farm and given free access to fresh food (celery, lettuce, and carrots) and water every two days.

EXPERIMENTAL PROCEDURES

- T-maze (from Amazon) had three sets of stimuli in the main arm of the maze.
- Memory test (Phase 1) had fake leaves on the bottom left, sea rocks on the middle right, and a blue LEGO piece on the top left].
- Stimuli swap test (Phase 2) had fake leaves on the bottom right, sea rocks on the middle left, and a blue LEGO on the top right.
- Training phase: 15 untrained ants were placed in a T-maze with a tangerine reward on the left arm for three hours to learn its layout.
- Intermediate phase: Ants were removed for a 30-minute break in a plastic container
- Testing phase: ants are individually placed in Phase 1 position (no reward) to assess memory recall then Phase 2 to see if ants relied on visual cues or memory.
- This process was repeated across 13 trials with new ants each time.
- The independent variable was positive conditioning to go left with certain stimuli. The dependent variables were directional choice and interaction with stimuli. I recorded the direction each ant chose and the number of different stimuli it interacted with in both phases. Untrained ants were used as a control.

ANALYSIS

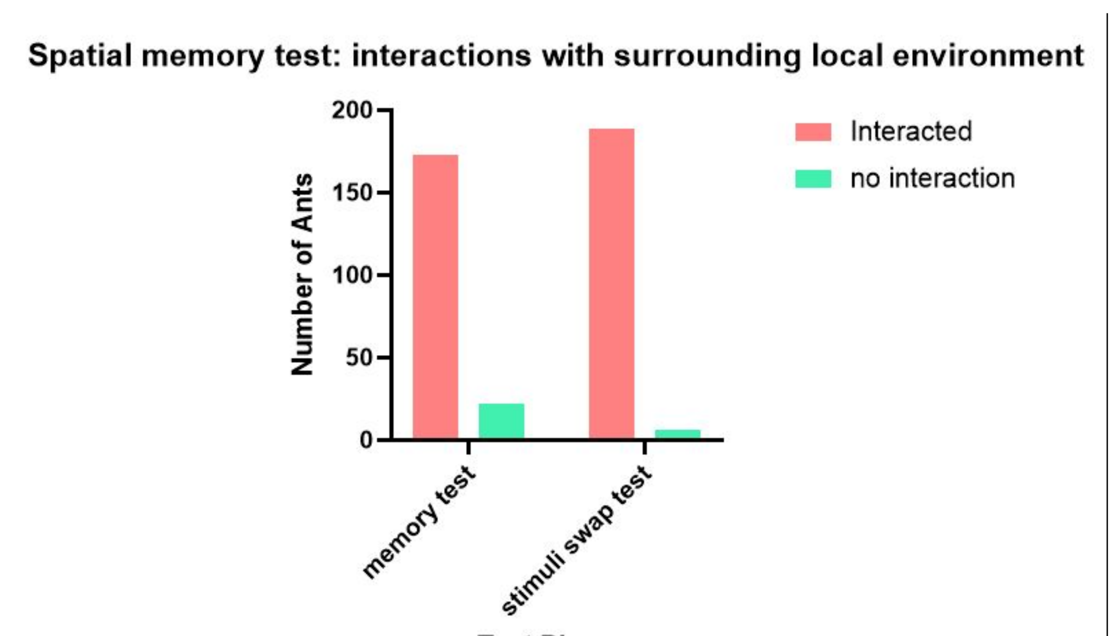
- I used one-sided, paired-samples t-test, comparing Phase #1 and Phase #2 to test whether spatial memory is constructed based on local stimuli or the overall direction.
- I used the one-sided, one-sample t-test, which compared against the population mean of 0.5 to test if harvester ants have spatial memory.
- I used the Chi-square test of independence, examining whether the pattern of interactive behaviors of ants change during different phases of experiments when local surroundings alter.

Results

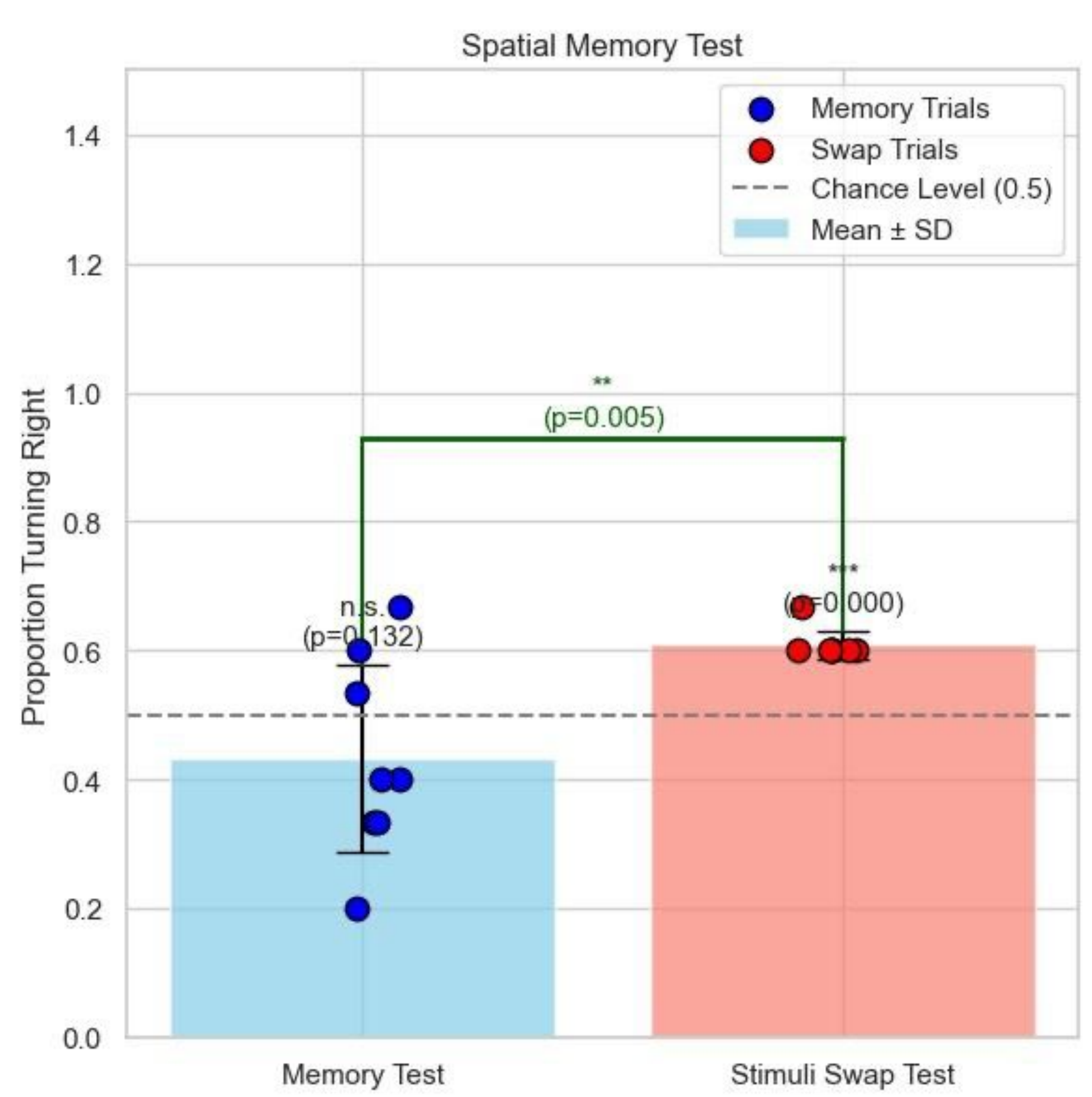
Table 1: Directional Decision Making in the harvester ants

	Phase 1 (no swap)			Phase 2 (stimuli swapped)		
Trial	L	R	Ratio*	L	R	Ratio*
1	10	5	0.33	6	9	0.60
2	10	5	0.33	6	9	0.60
3	9	6	0.40	6	9	0.60
4	7	8	0.53	6	9	0.60
5	9	6	0.40	5	10	0.67
6	9	6	0.40	7	8	0.53
7	7	8	0.53	5	10	0.67
8	8	7	0.46	6	9	0.60
9	9	6	0.60	4	11	0.73
10	10	5	0.33	5	10	0.67
11	8	7	0.46	5	10	0.67
12	8	7	0.46	6	9	0.60
13	9	6	0.40	5	10	0.67

*ratio was calculated by dividing the number of ants taking the right turn by the number of total ants Table created by finalist using Python, 2025



The number of ants interacting or not interacting with at least one stimuli was recorded in memory test and stimuli swap test. Graph created by finalist using Prism, 2025



The proportion of ants turning right was recorded and compared in memory test and stimuli test. For stimuli swap test, the p-value is so small that it is shown as 0 in Prism. Graph created by finalist using Python, 2025.

Results

1. One-samples t-test (one-sided)

• **Null hypothesis 1:** The mean ratio of ants turning right is not less than the random chance of 0.05.
• Alternative hypothesis 1: The mean ratio of ants turning right is less than the random chance of 0.05.

2. Paired-samples t-test (one-sided)

• Null hypothesis 2: The mean ratio of ants turning right in Phase 1 is not less than that in Phase 2.
• **Alternative hypothesis** 3: The mean ratio of ants turning right in Phase 1 is less than that in Phase 2.

3. Chi-square test of independence

• Null hypothesis 3: There are no differences in the number of interactions between Phase 1 and Phase 2.
• **Alternative hypothesis 3:** There are differences in the number of interactions between Phase 1 and Phase 2.

Conclusion

In Phase 1, I expected ants to turn left toward the reward. However, the results were not statistically significant, providing no evidence that ants remembered the reward location. This result did not support my hypothesis and suggests that ants do not form strong spatial memory under these conditions.

My second hypothesis was that harvester ants use overall direction to form spatial memory. Phase 2, when the location of the stimuli was swapped, ants significantly changed their turning behavior. This indicates that ants navigate based on local, neighbouring cues, rather than a fixed internal compass.

My third hypothesis was that regardless of changes in local stimuli, ants will interact with the stimuli in the same manner. However, the Chi-square test results showed a significant difference in how many ants interacted with the stimuli between Phase 1 and Phase 2. This demonstrates that the ants interaction behavior changed when the stimuli's positions were swapped, showing that the ants notice and respond differently to changes in their environment.

Reference

[1] Rolls, Edmund T, and Sylvia Wirth. "Spatial Representations in the Primate Hippocampus, and Their Functions in Memory and Navigation." Progress in Neurobiology, Pergamon, 13 Sept. 2018, www.sciencedirect.com/science/article/abs/pii/S0301008218300625.

[2] Collett, Matthew, et al. "Spatial Memory in Insect Navigation." Current Biology, Cell Press, 9 Sept. 2013, www.sciencedirect.com/science/article/pii/S0960982213008439.

[3] "Specimen: CASENT0006306 Pogonomyrmex Barbatus." Antsweb Version 8.114, California Academy of Science, www.antweb.org/specimen.do?name=casent0006306. Accessed 30 Sept. 2025.