

Spring 4-27-2022

Use of intramodal odor cues in shelter recognition by an amblypygid

Nathan Bostelman
nbostel@bgsu.edu

Follow this and additional works at: <https://scholarworks.bgsu.edu/honorsprojects>



Part of the [Behavioral Neurobiology Commons](#), [Behavior and Ethology Commons](#), [Entomology Commons](#), and the [Integrative Biology Commons](#)

[How does access to this work benefit you? Let us know!](#)

Repository Citation

Bostelman, Nathan, "Use of intramodal odor cues in shelter recognition by an amblypygid" (2022). *Honors Projects*. 814.

<https://scholarworks.bgsu.edu/honorsprojects/814>

This work is brought to you for free and open access by the Honors College at ScholarWorks@BGSU. It has been accepted for inclusion in Honors Projects by an authorized administrator of ScholarWorks@BGSU.

Use of intramodal odor cues in shelter recognition by an amblypygid (*Phrynos marginemaculatus*)

Nathan M. Bostelman

Bowling Green State University Department of Biological Sciences

Faculty Mentors: Dr. Verner Bingman, Dr. Daniel Wiegmann

Graduate Mentor: Patrck Casto, MS

27 April 2022

ABSTRACT

Amblypygids are nocturnal arthropods which live in cluttered habitats and possess the ability to navigate home after displacements of up to ten meters. Homing amblypygids rely on olfactory and tactile information gathered by their antenniform legs to navigate successfully. Given that odor signals encountered in nature are complex and dynamic, navigation via olfaction presents unique challenges related to signal uncertainty. To understand how amblypygids perceive complex odors and what they learn from them, individuals of the subtropical amblypygid *P. marginemaculatus* were trained to associate a blend of two odors with a shelter and tested on three treatments: the reinforced blend (AB+ v. CD-) and each of its elements presented in isolation (A v. CD-) (B vs. CD-). The animals spent more time near entrances to shelters cued with both the learned blend and its individual elements than the shelter cued with the unconditioned stimulus. There was no effect of treatment, as behavior between the three treatments did not vary. Therefore, elemental information of the odor blends was preserved by amblypygids and used to facilitate shelter recognition, but there is no evidence that paired odor stimuli form configural representations to aid in navigation.

INTRODUCTION

Many arthropods are well-known for their impressive navigational behavior. Ground-burrowing wasps perform stereotyped learning flights above entrances to their nests to learn about nearby visual landmarks, and they use these memories to home successfully (Collett et al., 2016).

Honeybees (*A. mellifera*) use polarized light to obtain compass information during foraging (Evangelista et al., 2014). Monarch butterflies (*D. plexippus*) are sensitive to geomagnetic cues and use a magnetic compass to guide their transcontinental migrations (Guerra et al., 2014).

Particularly well-studied is the desert ant *cataglyphis fortis*, which use wind-direction and skylight cues to navigate in expansive, featureless salt pans (Müller & Wehner, 2007). Notably, these

animals all possess relatively simple nervous systems, enticing for neuroscientists who wish to study the neural bases of navigation strategies used by arthropods. Animal navigation also applies to computer science: studying the neural architecture, cognitive processes, and sensory systems supporting animal navigation strategies provide biological models for navigating robots (Cheng, 2012). Less studied are the seemingly modest yet cognitively impressive navigation strategies employed by whip spiders, discussed below.

Field Studies of Amblypygid Navigation

The present study investigates the remarkable navigation of amblypygids, an understudied order of arachnids known colloquially as “whip spiders.” These animals are unique among arachnids because they possess only six ambulatory legs; their front two legs have been evolutionarily modified into sensory organs which gather mechanosensory and chemosensory information from the environment. Multiple neotropical amblypygids demonstrate sophisticated navigational abilities and utilize unique homing methods. For instance, *Phrynus*, *Paraphrynus*, and *Heterophrynus* species are highly faithful to specific refuges and reliably home after nightly foraging and mating excursions (Hebets 2002). Experimental displacements of individuals from *Phrynus pseudoparvalus* reveal that these animals can successfully home after displacements of more than six meters; many individuals took indirect paths home and rested at stopover sites when transits took more than one night to complete (Hebets et al., 2014).

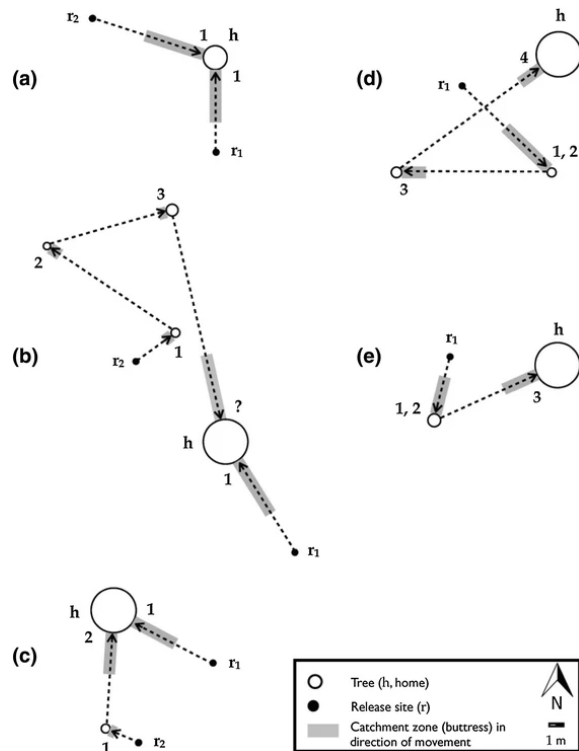


Figure 1: from Hebets et al., 2014. Homing routes of *P. pseudovarvalis* displaced more than six meters from their refuge. The animals were tracked with radio telemetry, allowing researchers to record subjects' positions each morning following displacement. Animals often took indirect routes home, occasionally travelling linear distances of over thirty meters over the course of several nights before finally returning.

Most model organisms in arthropod navigation inhabit comparatively simple terrains, making the homing behavior of amblypygids notable for their ability to navigate through their highly cluttered and heterogenous habitats. Structurally complex environments render useless certain ubiquitous arthropod navigation strategies. For example, the desert ant *cataglyphis* is among the most well-studied arthropod navigators, and these animals live on expansive Saharan salt pans. In such open environments, desert ants employ a commonly used navigation strategy called path integration. During path integration, an animal tracks directional and distance information during its outbound journey to take a straight-line route home, a behavior known as dead reckoning. Path integration is quite common among animals navigating through open environments, used by desert ants (Wehner, 2003), fiddler crabs (Layne et al., 2003), and wolf spiders (Collett & Collett, 2000). The relative complexity of a cluttered, uneven rainforest floor reduces the efficacy of path integration, placing selective pressure on animals to develop alternate navigation strategies (Kohler & Wehner, 2005; Wiegmann et al., 2016). Indeed, Hebets et al. (2014) confirms that amblypygids do not rely

on path integration; they did not conduct their own outward journey and, thus, did not gather the necessary distance and direction information to enable path integration. Yet, these animals still found their way home, often via indirect paths.

Given their non-reliance on path integration, researchers hypothesized that amblypygids rely on sensory information to navigate. Many nocturnal arthropods are visually-oriented navigators (Warrant & Dacke, 2016), but early studies of amblypygid navigation reported that an individual with experimentally ablated olfactory tissue was unable to home (Beck & Gorke, 1974). To assess the relative importance of visual and olfactory information, researchers performed sensory manipulations on wild amblypygids before experimental displacement; individuals deprived of vision (by painting over their eyes with nail polish) successfully homed at similar rates to control animals (no sensory manipulations). Ablation of olfactory receptors on the antenniform leg, however, resulted in a near-complete loss of navigational ability, indicating that amblypygids rely primarily on olfactory information gathered by the antenniform legs to home successfully (Bingman et al., 2017). Amblypygids' reliance on olfactory information, but not visual cues or path integration, is somewhat unique among arthropods, and it is not clear how these arachnids use their sense of smell to home.

Neuroscientific Studies of Amblypygids

Perhaps unsurprisingly, whip spiders possess distinctive neuroanatomical features that potentially support sensory and cognitive traits underlying their navigational behavior. For instance, olfactory receptors on the antenniform legs send information to the central nervous system where they feed into a relatively large number primary olfactory glomeruli, suggesting that whip spiders possess a keen sense of smell (Sinakevitch et al., 2020). The central nervous systems of these animals also contain extraordinarily large mushroom bodies, which are higher-order arthropod brain regions

implicated in sensory processing, learning, and memory (Strausfeld et al., 1998). Notably, mushroom bodies are thought to be loosely analogous to the mammalian hippocampus, a brain region which supports spatial cognition in animals like birds and rats (Strausfeld et al., 2009). Indeed, the exceptionally large mushroom bodies found in amblypygids are thought to support these animals' spatial abilities by storing a cognitive "odor map" to aid in navigation, enabling amblypygids to understand the spatial orientation of odor landmarks (Wiegmann et al., 2019). Mushroom bodies also act as multisensory processors, a site where information from multiple sensory modalities can be integrated to produce a behavioral response (Sinakevitch et al., 2020; Flanigan et al., 2021). This suggests that large amblypygid mushroom bodies may have evolved to cope with signal uncertainty encountered during excursions through the cluttered environments.

Behavioral Experiments on Amblypygids

Training whip spiders in the lab allows researchers to investigate the sensory and cognitive abilities of whip spiders in a controlled setting. While work in the field demonstrates that amblypygids rely first and foremost on olfactory information to navigate in a natural setting, these animals can recognize a shelter through multiple sensory modalities. Captive amblypygids have been trained to localize a shelter using not only olfactory cues (Wiegmann et al., 2019), but tactile (Santer & Hebets, 2009) and visual (Flanigan et al., 2021) cues as well. The fact that whip spiders can learn through multiple sensory modalities is significant on its own, but an even more intriguing is how these animals integrate cues across senses. If amblypygid mushroom bodies are multisensory processors, it is reasonable to hypothesize that these animals attend to multiple cues simultaneously to obtain positional information. This potentially gives rise to configural learning, a supposedly advanced cognitive trait observed in other animals with simple nervous systems, like freshwater snails (Swinton et al., 2019) and lobsters (Livermore et al., 1997). In configural learning, animals exhibit a learned behavioral response to a configural representation of two or more cues.

For instance, an animal trained to a paired multimodal stimulus (AB+) exhibits configural learning if it responds to the paired stimulus, but not either of its components alone. A phenomenon called neural integration is thought to underly multimodal configural learning, in which multisensory inputs are bound together to create a configural representation (Pearce, 2002). Some theorize that multisensory configural learning evolves to cope with signal uncertainty in cluttered and structurally complex environments (Munoz & Blumstein, 2012). Amblypygids inhabit complex environments and learn about cues through multiple sensory modalities, leading researchers to hypothesize multimodal configural learning in whip spiders. Indeed, whip spiders integrate tactile and olfactory cues to create configural representations that guide shelter recognition. Animals trained to a paired odor-tactile stimulus recognized their shelter when both stimuli were presented simultaneously, but not in isolation (Flanigan et al., 2021).

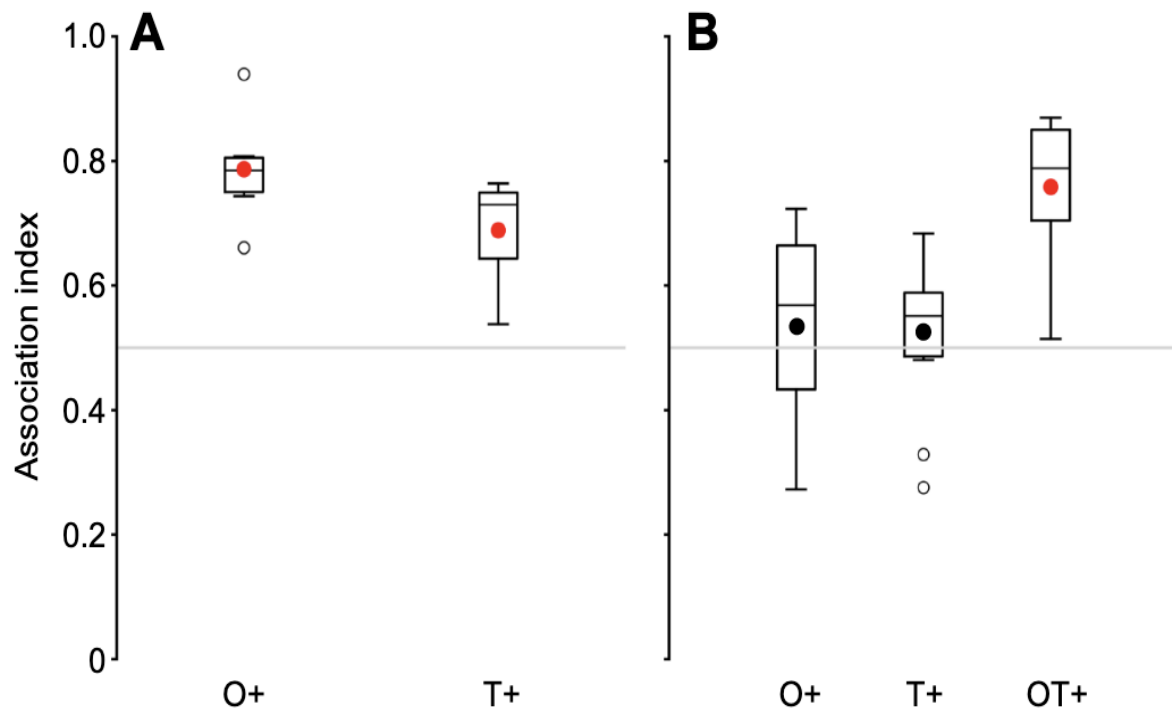


Figure 2: from Flanigan et al., 2021. Amblypygids can recognize a shelter using odor and tactile information. When trained to a paired stimulus (OT+), subjects did not recognize a shelter with only one of the cues presented in isolation and only responded when both cues were presented simultaneously.

The Present Study

As demonstrated, much progress has been made in determining how amblypygids perceive and learn about both unimodal and multimodal sensory information in a homing context. However, less attention has been paid to complex signals within a single sensory modality. This is especially relevant to amblypygids because they rely on olfactory information to home, and odors encountered in nature are complex, dynamic, and often ephemeral. Natural odors are rarely pure substances; instead, they are typically composed of multiple distinct chemical entities which humans perceive as novel and unique sensations (Purves et al., 2001). However, not all animals perceive odor blends as unique, novel, and distinctive from their elements. Many animals, including arthropods like the spiny lobster *P. argus*, can detect and learn about the individual elements composing an odor blend (Livermore et al., 1997). So-called *elemental learning* may be an important part of a homing amblypygid's cognitive toolkit, as it would preserve meaningful olfactory information even when odors unexpectedly mix. Elemental learning would also safeguard the integrity of olfactory cues emanating from beacons or landmarks, which may compose an amblypygid's "odor map." Moreover, odor blends may give rise to configural representations that facilitate more robust shelter recognition, similar to behavior observed in Flanigan et al. (2021).

The current study investigates how amblypygids perceive and learn about complex odors by training the animals to associate a complex odor with a shelter. Then, their shelter recognition

behavior can be studied by presenting both the complex odor and its elements during testing. This experiment asks three questions:

- 1) Can amblypygids use complex odor blends to recognize a shelter?
- 2) Do amblypygids use the elemental information within a blend to recognize a shelter?
(elemental learning)
- 3) If amblypygids can detect and learn about the elements of an odor blend, do these sensory cues give rise to configural representations that aid in navigation?

METHODS

Hypotheses

This experiment tests three hypotheses. First, I hypothesize that amblypygids use complex odor blends to recognize a shelter. Support for this hypothesis demands that animals exhibit a preference to their odor blends they are trained to over the C-.

H_0 : AB+ = CD-

H_a : AB+ > CD-

Second, I hypothesize that elements of odor blends will support shelter recognition. Support for this hypothesis requires that subjects prefer elements over the C- at above chance rates.

H_0 : A = CD-, B = CD-

H_a : A > CD-, B > CD-

Third, I hypothesize that amblypygids will use configural representations of odor blends to enable more robust shelter recognition. Data that supports this hypothesis should indicate that amblypygids demonstrate a stronger preference to the paired stimulus they were trained to than the elements of the reinforced stimulus presented in isolation.

If H_a : $A > CD-$, $B > CD-$ is accepted (animals learn elements), then...

H_0 : $AB+ = A$, $AB+ = B$

H_a : $AB+ > A$, $AB+ > B$

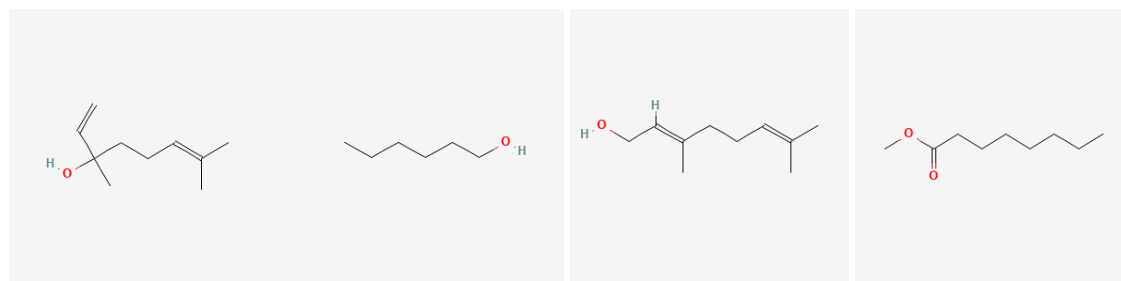
Animals

This study uses amblypygids from the species *Phrynos marginemaculatus*, a small, subtropical Caribbean amblypygid frequently used in the lab. While less is known about the behavioral ecology and navigational abilities of *P. marginemaculatus* compared to its larger neotropical relatives, unpublished data demonstrates these animals home after displacements of two meters (Casto, forthcoming). Moreover, *P. marginemaculatus* behave more readily in a laboratory setting than *P. laevifrons* and *P. pseudoparvalus*, as their microhabitat preferences more closely match the artificial shelters our lab uses as a reward in behavioral assays. All subjects were collected from the Pine Rocklands of the Florida Keys between 2018 and 2020. Animals are housed in plastic deli cups with coconut fiber substrate and a piece of bark that acts as a hide. Plastic mesh lines the wall of each container to enable climbing and assist in molting. All the animals are housed separately and stored in a room with twelve-hour light-dark cycles. The room is consistently maintained at room temperature and at least 70 percent humidity. Each enclosure is misted daily and supplied with food (in the form of a live cricket) every week. Participants were selected randomly from a pool of individuals who were naïve to the odors used in the experiment. Animals were trained and tested

on alternating days, meaning they were given one full day of “rest” after each arena session. Data were collected over seven experimental days (13 days in total).

Odors

Four odors were used to create two blends. Literature indicates that honeybees have trouble discriminating between odors that are similar in chain length and the position and type of their functional groups (Laska et al., 1999). As a precaution, odors were assigned to their blends based on their non-similarity to the other element. Geraniol and methyl octanoate were paired because they share no major functional groups, and linalool and hexanol were paired despite their similar hydroxyl group because they differ in chain length. The paired stimulus “geraniol + methyl octanoate” is abbreviated here as GM, and “linalool + hexanol” as LH.



Linalool

Hexanol

Geraniol

Methyl Octanoate

Arena

Subjects were trained and tested in a 29cm x 14cm arena with opaque acrylic walls and a clear acrylic floor. Arenas sat on top of white paper with faint markings outlining boundaries between the neutral area and the goal areas in front of entrances to a 7cm x 5cm shelter, which were placed on the short, lateral sides of the arena. On one long wall of the arena, a 7cm x 5cm start box sits behind the entrance to the arena. Two lightbulbs were centrally placed over each shelter entrance and pointed toward the center of the arena. Amblypygids exhibit negative phototaxis, so they find

the opportunity to escape from a bright light and into a dark shelter rewarding. Thus, the bright light motivates the animals to recognize a dark shelter they can escape to, facilitating learning. Each shelter and start box contained a damp sponge and a removable lid to keep the interiors of these shelters dark.

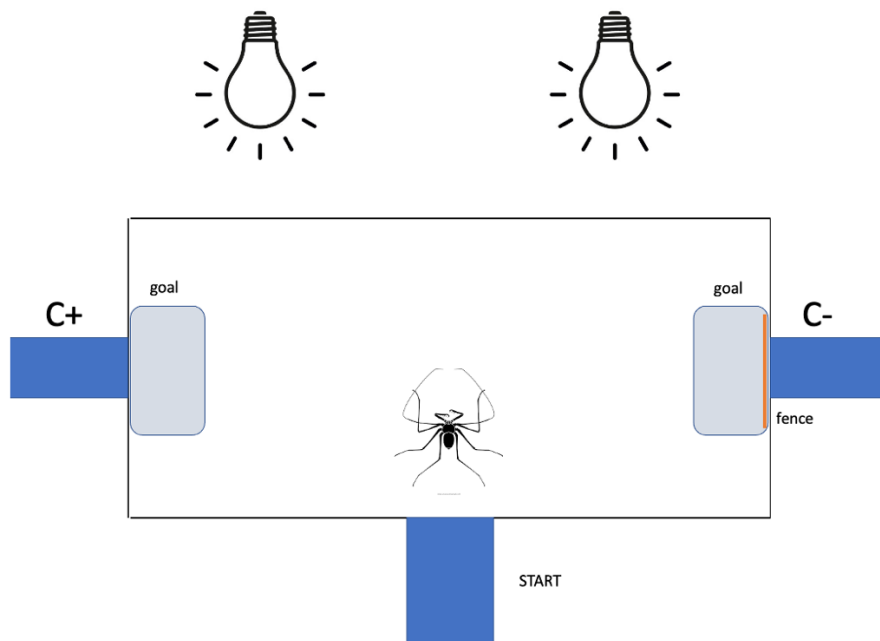


Figure 3: Diagram of training arena.

Shelters were cued using odor wells, made by creating small depressions in 2cm x 2cm x 1cm acrylic bricks. Each well was only cued with one odor throughout the duration of the experiment to prevent cross contamination (the hexanol well would only ever contain hexanol, for instance). Depressions in the odor wells were cued with 10ul of the odorant (paired stimuli were placed in an odor well with two depressions and 20ul of total fluid, while single stimuli had one depression and 10ul of fluid). During experimentation, wells were placed inside of shelters behind a plastic mesh fence to prevent the animals from sampling the odors directly with gustatory receptors in their antenniform legs.

The interior of the arena, the start box, and the unoccupied shelter were sanitized with 70 percent ethyl alcohol after each training and test trial (shelters containing an animal were not sanitized). The alcohol dissolves residual experimental odors as well self-scent odor cues deposited by the animal, which they can use to recognize a shelter (Casto et al., 2019).

Training

Training trials began when an animal was placed in the uncued start box for a five-minute acclimation period. Then, using a paintbrush, the animal was gently pushed into the brightly lit arena, and the entrance to the start box was closed behind them. Each subject was given 15 minutes to explore the arena and attempt to locate an escape refuge. One shelter, cued with the C+ blend, was open and easily accessible. The other shelter, placed on the opposite side of the arena, was cued with the C- and made inaccessible by placing a plastic mesh fence in front of the entrance. Animals that did not enter the open shelter after fifteen minutes were gently pushed into the correct shelter with a paintbrush. Subjects were rewarded for choosing the correct shelter with ten-minute reward periods, during which they sat undisturbed in the dark shelter. This way, the animals hypothetically learn to associate the C+ cue with a dark shelter. Once the animal located and entered the accessible arena, the cued shelter was sealed and transferred to the arena entrance and became the start box for the next trial. Thus, at the beginning of each training and test trial (apart from the first trial of the day) the starting chamber is cued with the C+ stimulus. Therefore, the C+ cued starting chamber was from the vicinity of the arena immediately after the animal exited the shelter to prevent odor contamination in the arena. On the first day of experimentation, this process was repeated six times for a total of seven training trials per animal. The left-right orientation of the C+ and C- shelters for each trial was randomized, determined by a coin flip.

Ten animals were trained to associate a blend of linalool and hexanol with an accessible shelter (LH+) and the blend of geraniol and methyl octanoate with an inaccessible shelter (GM-). Ten more animals were trained to the reciprocal arrangement (GM+, LH-).

Testing

On each subsequent experimental day, animals were tested on each of their group's three treatments. Animals in both groups received three experimental treatments: the C+ paired with the C- (AB+, CD-), component "A" of the C+ blend paired with the C- (A, CD-), and component "B" paired with the C- (B, CD-). Each treatment was encountered once every day, with treatments appearing in randomized order. As with training trials, the left-right orientation of the cued shelters was randomized with a coin flip. Six permutations of the order of the three treatments exist, so one permutation was presented on each day. After test trials one and two, two training trials were carried out to reinforce learning to the C+ (Day 1: 7t; Day 2-7: T, 2t, T, 2t, T).

During the test, both shelter entrances are closed off with plastic mesh. Animals are assigned an association score based on sum of time they spent in both goal areas, which were 7cm by 5cm zones in front of the entrance to each shelter. An individual enters a goal area if more than half of its prosoma is within the goal area's boundaries. Tests lasted fifteen minutes. At the conclusion of the test, the animal was coerced back into the C+ cued starting chamber with a paintbrush and left undisturbed for ten minutes in the dark shelter.

Data Collected

By summing the total time that individuals spent in each goal area, a proportion reflecting the amount of time animals spent in the C+ goal area (or the non C- goal area if an individual element of the C+ was presented) can be calculated. This is determined by dividing the time spent in the C+

goal area by the total time spent in both goal areas. A score of 0.5, for instance, indicates the animal spent equal amounts of time in both goal areas. Each animal was assigned an association score that reflected their behavior over the course of the six testing days.

RESULTS

The mean of all association scores for each treatment were taken to analyze support for the stated hypotheses. All averages are well above 0.5, and one-sample t-tests were performed using these values to test if the exhibited preferences to the C+ cues and their components at above-chance rates. Indeed, animal preferred both the C+ and their components at above chance rates. This result indicates that amblypygids used both the paired stimulus and its components to recognize a shelter (GM: $p < 0.05$, G: $p < 0.05$, M: $p < 0.05$, LH: $p < 0.05$, L: $p < 0.05$, H: $p < 0.05$).

animal	association scores				association scores		
	LH	L	H		GM	G	M
all	0.73767861	0.66720854	0.62820913		0.66646089	0.67906469	0.64080704
stdev	0.09855392	0.16726284	0.08726461		0.15728627	0.17266509	0.1969618
sem	0.03116548	0.05289315	0.02759549		0.04973829	0.0546015	0.06228479
BPK B.H. 54	0.9159732	0.61110332	0.52638606	NN 10	0.8335962	0.77496147	0.74112203
BPK 86 after	0.70856087	0.59081156	0.67530915	BPK 23 morn	0.71944209	0.59607213	0.79521786
BPK S.C. 12	0.82659813	0.91331991	0.78379044	BPK 45 morn	0.58926545	0.51071194	0.32920617
BPK 48 morn	0.68418598	0.54814599	0.66483479	BPK 67 morn	0.90866455	0.63482589	0.78197084
BPK 38 morn	0.84716222	0.94528458	0.56787567	BPK 91 after	0.58412292	0.71821336	0.54462636
BPK D.C. 44	0.66480601	0.58827919	0.75310424	BPK 83 after	0.78373831	0.77517203	0.71580863
BPK D.C. 31	0.70894965	0.6400575	0.58507431	BPK S.C. 21	0.73776267	0.86442345	0.86295181
BPK B.H. 60	0.68726667	0.52146667	0.58796667	BPK S.C. 01	0.47893333	0.7004	0.63536667
BPK B.H. 61	0.74805	0.83331667	0.5868	BPK S.C. 05	0.41368333	0.89623333	0.71863333
BPK D.C. 36	0.58523333	0.4803	0.55095	BPK B.H. 59	0.6154	0.31963333	0.28316667

Figure 4: Association scores for each animal to each treatment

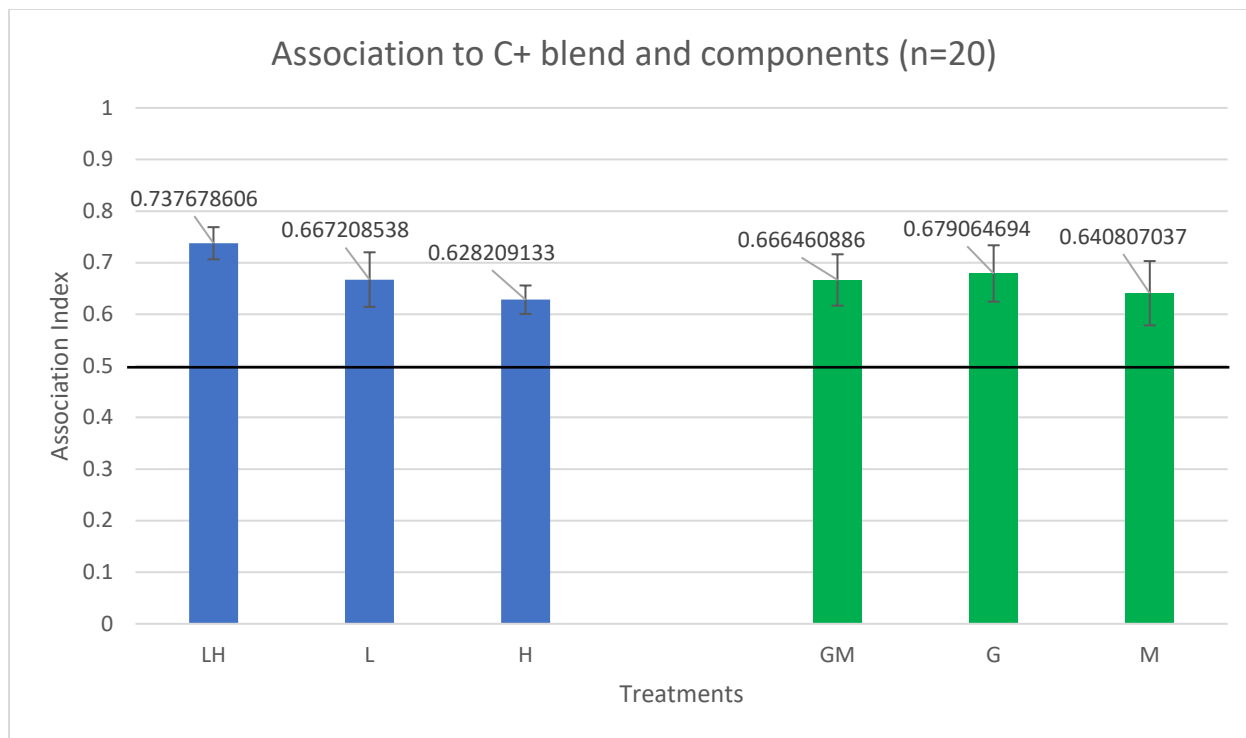


Figure 5: Histogram showing average association scores across all animals to all treatments. One-sample t-tests performed on each treatment show that animals preferred both blends and components at significant rates. (GM: $p < 0.05$, G: $p < 0.05$, M: $p < 0.05$, LH: $p < 0.05$, L: $p < 0.05$, H: $p < 0.05$). A mixed-effects ANOVA shows that none of the treatments differ significantly from each other (LH: $p = 0.11$, GM: $p = 0.24$).

A mixed effects ANOVA was used to investigate whether preferences to these cues differed from each other. For both LH+ and GM+ animals, there was no effect of treatment, indicating that paired stimuli and their elements produced similar shelter recognition behavior (LH: $p = 0.11$, GM: $p = 0.24$).

CONCLUSIONS & FUTURE INQUIRY

Interpreting data using statistical testing permits acceptance or rejection of the null condition for each of the proposed hypotheses. Below, the results of these statistical tests are used to determine whether each of the null hypotheses can be rejected.

Complex Cues

The results indicate that amblypygids can use complex odors to recognize a shelter, rejecting the null hypothesis. Subjects associated with GM+ and LH+ at above-chance rates, suggesting they learned to associate the reinforced odor blend with a dark refuge.

Elemental Learning

Amblypygids associated not only with the reinforced blend, but with the elements of those blends as well. Animals trained to LH+ spent significantly more time near L alone and H alone than the C-, and animals trained to GM+ behaved in a statistically identical manner. Thus, amblypygids can perceive and learn about the elemental information of two-odor blends, and the null hypothesis can be rejected.

Configural Learning

The subjects associated to each treatment at above-chance rates, but the paired stimuli in the odor blend were no better at facilitating shelter recognition than the elements of that stimulus presented in isolation. Performance to LH paralleled performance to L alone and H alone; similarly, behavior toward GM did not significantly differ from behavior to G alone and M alone. Therefore, the results do not indicate that the animals created configural representations of odors to support shelter recognition. The amblypygid *P. marginemaculatus* appears to be capable of elemental learning, but not configural learning, of the intramodal cues in odor blends.

Future Inquiry

The results of this experiment should be interpreted with caution because it assumes that the observed learning and behavior was primarily motivated by an attraction to the C+ and its elements. However, it is possible that amblypygids in this experiment learned an aversion to the C-,

which was paired with both the reinforced blend and its elements during testing. Because the data do not vary significantly from each other, the results of the experiment could be explained entirely by a learned aversion to the C- blend. One potential solution to this problem is to conduct a follow-up experiment to test the relative control of attractive and aversive during experimentation (treatments: C+ v. C-; C+ v. blank; C- v. blank). Alternatively, elements of the C- could be presented during testing rather than the C- blend itself. In such an experiment, even aversion to one of the components of the C- blend would indicate elemental learning since the animal would be learning to avoid an element of a complex blend it had previously learned an aversion for.

Regardless, the results of this experiment suggest that amblypygids are capable of elemental learning of complex odor blends, but not configural learning. In an ethological context, elemental learning may be useful because odors emanating from potential olfactory beacons would not become compromised after blending with another odor to produce a unique stimulus. Indeed, configural learning of odor blends may not be advantageous to a navigating amblypygid since natural odors are dynamic and ephemeral. In contrast, tactile cues, which were paired with odor cues to demonstrate multisensory configural learning in this species by Flanigan et al. (2021), are relatively stable and static, so forming certain multisensory configural representations may be an effective way to reduce signal uncertainty in complex environments.

The odors used in this experiment were blended in 1:1 ratio to create paired stimuli. An interesting route for future research would involve investigating the olfactory “acuity” of amblypygids.

Neuroscientists have found that the amblypygid central nervous system contains a large number of primary olfactory glomeruli, suggesting an advanced sense of smell in these animals (Sinakevitch et al., 2020). If amblypygids can detect the elements of a 1:1 odor blend, can they detect elements in a 2:1, 5:1 or 10:1 blend? Similarly, the complex odors in this experiment were composed of only two

odors. Can amblypygids detect the elements of more complex blends composed of, say, five odors? These questions are ethologically relevant to amblypygid navigation because the odors they encounter in nature are certainly more complex than simple two-odor blends. Moreover, investigating the olfactory processing and neural circuitry that preserves elemental information of odor blends in an amblypygid's central nervous system. Similar work has been done on the moth *S. littoralis*; calcium imaging suggests that olfactory glomeruli preserve component information of two-odor blends (Carlsson et al., 2007). Investigating the sensory capabilities and underlying neurophysiology enabling elemental learning of odor blends is relevant to answering questions about how an advanced sense of smell evolves.

REFERENCES

- Beck L & Gorke K. (1974). Tagesperiodik, Reviewverhalten und Beutefang der GeiOelspinne *Admetus pumilio* C. L. KOCH im Freiland. - Z. Tierpsychol., 35: 173-186.
- Bingman VP, Graving JM, Hebets EA & Wiegmann DD. (2017). Importance of the antenniform legs, but not vision, for homing by the neotropical whip spider *Paraphrynus laevifrons*. *Journal of Experimental Biology*, 220(5), 885-890. <https://doi.org/10.1242/jeb.149823>.
- Carlsson MA, Chong KY, Daniels W, Hansson BS & Pearce TC. (2007). Component information is preserved in glomerular responses to binary odor mixtures in the moth *Spedoptera littoralis*. *Chemical Senses*, 32(5), 433-443. <https://doi-org.ezproxy.bgsu.edu/10.1093/chemse/bjm009>.
- Casto P, Gosser J, Wiegmann DD, Hebets EA & Bingman VP. (2019). Self-derived chemical cues support home refuge recognition in the whip spider *Phrynus marginemaculatus* (Amblypygi: Phrynidae). *Journal of Arachnology*, 47, 290-292.

- Cheng K, Narendra A, Sommer S & Wehner R. (2009). Traveling in clutter: Navigation in the central Australian desert ant *Melophorus bagoti*. *Behavioural Processes*, 80(3), 261-268.
<https://doi.org/10.1016/j.beproc.2008.10.015>.
- Cheng K (2012) Arthropod navigation: ants, bees, crabs, spiders finding their way. In: Zentall TR, Wasserman EA (eds) The Oxford handbook of comparative cognition. Oxford University Press, New York, pp 347–365.
- Collett M & Collet TS. (2000). How do insects use path integration for their navigation? *Biological Cybernetics*, 83, 245-259. <https://doi.org/10.1007/s004220000168>.
- Collet TS, Philippides A & Hempel de Ibarra N. (2016). Insect navigation: How do wasps get home? *Current Biology*, 26, R157-R179. <http://dx.doi.org/10.1016/j.cub.2016.01.003>.
- Evangelista C, Kraft P, Dacke M, Labhart T & Srinivasan MV. (2014). Honeybee navigation: Critically examining the role of the polarization compass. *Phil. Trans. R. Soc. B.*, 369.
<http://dx.doi.org/10.1098/rstb.2013.0037>.
- Flanigan KAS, Wiegmann DD, Hebets EA & Bingman VP. (2021). Multisensory integration supports configural learning of a home refuge in the whip spider *Phrynus marginemaculatus*. *Journal of Experimental Biology*, 224. doi:10.1242/jeb.238444
- Flanigan KAS, Wiegman DD, Casto P, Coppola VJ, Flesher NR, Hebets EA & Bingman VP. (2021). Visual control of refuge recognition in the whip spider *Phrynus marginemaculatus*. *Journal of Comparative Physiology A*, 207, 729-737. <https://doi.org/10.1007/s00359-021-01509-y>.
- Guerra PA, Gegear RJ & Reppert SM. (2014). A magnetic compass aids monarch butterfly migration. *Nature Communications*, 5(4164). <https://doi.org/10.1038/ncomms5164>.
- Hebets EA. (2002). Relating the unique sensory system of amblypygids to the ecology and behavior of *Phrynus parvulus* from Costa Rica (Arachnida, Amblypygi). *Can J Zool-Rev Can Zool* 80(2), 286–295.

- Hebets EA, Gering EJ, Bingman VP & Wiegmann DD. (2014). Nocturnal homing by the tropical amblypygid *Phrynus pseudoparvalus* (Class Arachnida, Order Amblypygi). *Animal Cognition*, 17, 1013-1018. DOI: 10.1007/s10071-013-0718-8.
- Kohler M & Wehner R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: How do they interact with path integration vectors? *Neurobiology of Learning and Memory*, 83, 1-12. doi:10.1016/j.nlm.2004.05.011.
- Laska M, Galizia CG, Giurfa M & Menzel R. (1999). Olfactory discrimination ability and odor structure—Activity relationships in honeybees. *Chemical Senses*, 24(4), 429-438. <https://doi-org.ezproxy.bgsu.edu/10.1093/chemse/24.4.429>.
- Layne JE, Barnes WJP & Duncan LMJ. (2003). Mechanisms of homing in the fiddler crab *Uca rapax* 2: Information sources and frame of reference for a path integration system. *Journal of Experimental Biology*, 206(24), 4425-4442. <https://doi.org/10.1242/jeb.00661>.
- Livermore A, Hutson M, Ngo V, Hadjisimos R & Derby CD. (1997). Elemental and configural learning and the perception of odorant mixtures by the spiny lobster *Panulirus argus*. *Physiology & Behavior*, 62(1), 169-174.
- Müller M & Wehner R. (2007). Wind and sky as compass cues in desert ant navigation. *Naturwissenschaften*, 94, 589-594. doi: 10.1007/s00114-007-0232-4.
- Munoz NE & Blumstein DT. (2012). Multisensory perception in uncertain environments. *Behavioral Ecology*, 23(3), 457-462. <https://doi-org.ezproxy.bgsu.edu/10.1093/beheco/arr220>.
- Pearce JM. (2002). Evaluation and development of a connectionist theory of configural learning. *Animal Learning & Behavior*, 30, 73-95. <https://doi.org/10.3758/BF03192911>.
- Purves, D., et al. (2001) Neuroscience. 2nd Edition, Sinauer Associates, Sunderland, MA.
- Santer RD & Hebets EA. (2009). Tactile learning by a whip spider, *Phrynus marginemaculatus* C.L. Koch (Arachnida, Amblypygi). *Journal of Comparative Physiology A*, 195, 393-399. <https://doi.org/10.1007/s00359-009-0417-8>.

- Sinakevitch I, Long SM & Gronenberg W. (2020). The central nervous system of whip spiders (Amblypygi): Large mushroom bodies receive olfactory and visual input. *J. Comp. Neurol.*, 529, 1642-1658. DOI: 10.1002/cne.25045.
- Strausfeld NJ, Hansen L, Li Y, Gomez RS & Ito K. (1998). Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learn. Mem.* 5, 11–37.
- Strausfeld NJ, Sinakevitch I, Brown SM & Farris SM. (2009). Ground plan of the insect mushroom body: Functional and evolutionary implications. *Journal of Comparative Neurology*, 513(3), 265-291. doi: 10.1002/cne.21948.
- Swinton C, Swinton E, Shymansky T, Hughes E, Zhang J, Rothwell C, Kakadia M & Lukowiak K. (2019). Configural learning: A higher form of learning in *Lymnaea*. *Journal of Experimental Biology*, 222. doi:10.1242/jeb.190405.
- Warrant E & Dacke M. (2016). Visual navigation in nocturnal insects. *Physiology (Bethesda)*, 31(3), 182-192. doi: 10.1152/physiol.00046.2015.
- Wehner R. (2003). Desert ant navigation: How miniature brains solve complex tasks. *J Comp Physiol A*, 189, 579-588. doi: 10.1007/s00359-003-0431-1.
- Wiegmann DD, Hebets EA, Gronenberg W, Graving JM & Bingman VP. (2016). Amblypygids: Model organisms for the study of arthropod navigation mechanisms in complex environments? *Frontiers in Behavioral Neuroscience*, 10(47). <http://dx.doi.org/10.3389/fnbeh.2016.00047>.
- Wiegmann DD, Moore CH, Flesher NR, Harper ED, Keto KR, Hebets EA & Bingman VP. (2019). Nocturnal navigation by whip spiders: Antenniform legs mediate near-distance olfactory localization of a shelter. *Animal Behaviour*, 149, 45-54. <https://doi.org/10.1016/j.anbehav.2019.01.005>.