SENSORY DISCRIMINATION AND REFUGE RECOGNITION IN AMBLYPYGIDS

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Displacement experiments with amblypygids, nocturnal arachnids that inhabit the tropics and subtropics, revealed that they are rather extraordinary navigators and that their abilities are severely impeded when access to olfactory information is experimentally abolished. These results and the fact that amblypygids possess exceptionally large mushroom bodies, brain regions that process olfactory and, perhaps, spatial information led to the hypothesis that olfaction facilitates their navigation behavior. The amblypygid *Phrynus marginemaculatus* wanders nightly and shows strong shelter fidelity in a laboratory arena. Here, individual subjects were trained over a 26-night session in a square arena that contained two shelters to determine the extent to which shelter recognition is dictated by odor. The target shelter was positioned at a specific location in the arena near an acrylic well that contained 15 μl of geraniol. The other shelter was likewise positioned at a particular location in the arena, but near an acrylic well that contained 15 μl of water. The session consisted of nights on which the entrance to the target shelter was open and the entrance to the other shelter was closed, referred to as forced choice trials, and nights on which a subject had access to both shelters, referred to as probe trials. Probe trials involved manipulations of the locations of the shelters and their associated acrylic wells after a subject emerged from the target shelter. The probes consisted of three types of manipulations: control manipulations in which the shelters and associated acrylic wells were
removed and replaced with identical shelters and dishes in their original locations; manipulations in which the positions of the two acrylic wells were swapped; and manipulations in which both shelters and their respective acrylic wells were moved from their original, trained locations. The odor-cued shelter was chosen in more than 90 percent of the control probes, but in the other two probe conditions shelters appear to have been chosen randomly. Thus, shelter choice was not dictated by odor. In addition, subjects did not rely primarily on path integration, a commonly applied navigation strategy in arthropods, as they did not chose the shelter in the original location of the odor-cued shelter in probes where only the positions of the acrylic wells were swapped. Instead, the results suggest that amblypygids, like ants, may use a configuration of cues to relocate and identify a shelter.
This is for the spirit of learning, Bowling Green State University, my lab, my friends, and my family.
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INTRODUCTION

Navigation has been studied in a variety of aquatic and terrestrial arthropods, including lobsters, fiddler crabs, beetles, spiders and desert ants (reviewed by Dyer, 1998; Boles and Lohmann 2003; Cheng, 2012; Perry et al., 2013; Ortega-Escobar and Ruiz, 2014, respectively). The most commonly studied of these animals inhabit largely two-dimensional environments and their navigation strategies share a number of properties (Wiegmann et al. 2016). Fiddler crabs, spiders and desert ants all use path integration, for instance, where an accumulator encodes continually updated distance and directional information (reviewed by Collett and Collett, 2000). The direction these animals travel is frequently derived by use of a time-compensated sun compass and distance is calculated from idiothetic information (Mittelstaedt and Mittelstaedt, 1982; Wehner, 2003). Path integration allows a fiddler crab or a desert ant—most notably, the desert ant Cataglyphis fortis—to wander in a circuitous route in search of food and later return to its nest or burrow on an efficient, near straight-line trajectory (Merkel et al. 2006).

The efficiency and feasibility of path integration almost certainly depends on the species life histories, traits, and habitats under which animals navigate (Cheng et al., 2009; Wiegmann et al., 2016). For instance, the desert ant Melophorous bagoti inhabits the semi-arid, moderately cluttered Central Australian desert and relies less on path integration to relocate its nest than C. fortis, which inhabits the relatively featureless salt pans of Tunisia (Kohler and Wehner, 2005; reviewed by Cheng et al., 2009). In particular, M. bagoti uses path integration only partially during its return path, later switching to visual cues, where the majority of the return route of C. fortis is path integrated. But successful navigation by even the path integrator C. fortis may involve local cues, like odor, to pinpoint the entrance of its nest (Steck et al., 2009, 2011).
Indeed, *C. fortis* can be trained to utilize a variety of cues—odor, vibration or magnetic landmarks—to locate its nest (Steck, 2009; Buelmann et al., 2015a, b). Perhaps even more extraordinary is the fact that relocation of the nest entrance by *C. fortis* can be thwarted by experimental manipulations of the odor landscape around the nest (Steck et al., 2011). The latter result suggests that the relocation of a nest entrance may involve the construction and use of some sort of olfactory map, the demands for which may explain the relative size of certain brain regions in arthropods—specifically, the mushroom bodies—and other animals (Jacobs, 2012).

Relocation of a goal by arthropods that inhabit extremely complex environments, environments such as a rainforest dense with plants, other organisms, and varying topography, would seem to preclude the use of path integration as a primary navigational strategy (Wiegmann et al., 2016). Indeed, many diurnal ants that inhabit rainforests use pheromones and manicured trails as navigational guides (Jackson et al., 2004; Collett and Collett, 2007). Navigation by nocturnal arthropods in such environments is further challenged by low light levels and obstructed celestial cues, which may impede use of a light-based compass (Kelber et al., 2006; Somanathan et al., 2008; but see Warrant and Dacke, 2010; el Jundi et al., 2015).

Navigation by amblypygids, nocturnal arachnids that inhabit the tropics and subtropics, appears to be guided, like diurnal arthropods that inhabit rainforests, by olfactory cues, although vision may be partially involved (Beck and Görke, 1974; Hebets et al., 2014a, b; 2016; Bingman et al., 2017). These animals emerge from refuges—typically, crevices of tree buttresses—at night to forage for invertebrate prey (Weygoldt, 2000; Chapin and Hebets, 2016). Unlike true spiders, amblypygids walk on six legs. Their anterior pair of legs, called antenniform legs, are elongated sensory structures, highly articulated and covered with mechanosensory, chemosensory and, possibly, humidity-sensing sensilla (Foelix, 1975; Beck et al., 1977; Santer
and Hebets, 2011). Multiporous sensilla, located on the distal tips of these legs, serve an olfactory function and, when experimentally disabled, severely impair the ability of amblypygids to navigate (Hebets and Chapman, 2000; Bingman et al., in press). Interestingly, the multi-lobed mushroom body of amblypygids is, in comparison to insects, exceptionally large and elaborately folded, larger, relatively, than those of any other studied arthropod (Strausfeld et al., 1998). Thus, both anatomical and behavior data suggest that navigation by amblypygids likely relies on olfactory information (Wiegmann et al., 2016).

The amblypygid *Phrynus marginemaculatus* reliably wanders from a shelter in the laboratory and exhibits shelter fidelity to an odor-cued shelter after two nights in an arena (Graving et al., in press). In this study, we trained *P. marginemaculatus* to a refuge that was cued by an odor, with intermittent manipulations of the location of the odor-cued shelter, to explore how an odor may guide the relocation and the recognition of a shelter. The results suggest that odor alone is insufficient for shelter recognition and that perhaps, like ants, amblypygids learn an odor landscape to pinpoint the location of a shelter.
MATERIALS AND METHODS

Subjects

Eight *Phrynus marginemaculatus* were used, collected \((n = 7)\) from the Florida Keys (USFWS Permit Number FFO4RFKD-2015-06) or \((n = 1)\) bred from wild-caught individuals. Individuals were housed separately in plastic deli cups \((\text{diameter x height: } 17.1 \text{ cm x } 10.8 \text{ cm})\) that had a soil substrate to retain moisture and a piece of cardboard egg carton for shelter. The amblypygids were fed crickets two or three times weekly and had continual access to water. The room in which subjects were housed was lit by overhead broad-spectrum fluorescent lights \((400 – 750 \text{ nm})\) that were set to a 12:12 h light:dark cycle \((19:00-07:00 \text{ laboratory dusk-dawn})\). The room temperature ranged from 21-26 °C and humidity ranged from 20-60%.

Experimental Apparatus

The experiment was conducted in two 1 m x 1 m x 0.34 m \((L \times W \times H)\) arenas, with walls of clear acrylic plastic and a floor of sanded, opaque white acrylic plastic. Petroleum jelly was rubbed on the inside top 7.5 cm of arena walls to ensure that any subject that managed to climb a wall would not escape from the arena. The arena was elevated approximately 12 cm from the floor by four 12 cm diameter PVC pipes, one placed near each corner. The outline of these supports, which were just visible through the floor of the arena, specified four prospective locations for shelters. The arenas were located in the same room in which individuals were housed.

Two shelters, also constructed of 12 cm diameter PVC pipe, were positioned directly over the supports in diagonal corners of the arena. The top of each shelter was fitted with a circular piece of black opaque acrylic plastic, which blocked light in daylight hours, covered by similar piece of white opaque plastic. Each shelter had a 3 cm x 1 cm \((L \times H)\) entrance directed
toward the nearest corner. The interior perimeter of the shelters were covered with the loop part of Velcro® fastener tape and the roof of each shelter was covered with the hooked compliment of the tape to provide surfaces on which subjects could crawl. To provide water for drinking and to retain humidity in a shelter, a piece of cellulose sponge was sewn to a loop piece of Velcro® tape and attached to the ceiling of the shelter. In the corners of the arena near each shelter we placed a 2.5 cm x 2.5 cm x 1 cm (L x W x H) cube of clear acrylic plastic into which a small well was drilled to hold a scented liquid or water. The shelters and supports were positioned 10 cm from the nearest walls of an arena, which situated the shelter entrance approximately 15 cm from the cube that contained the odor cue or water.

**Procedures**

The primary objective of this study was to determine whether subjects that are trained to utilize a shelter that is cued by an odor use the odor to recognize the shelter. A subject was first trained over three nights to a shelter cued by an odor. In particular, a subject was placed several hours before lights-out in a shelter that was then positioned over one of the four supports (Figure 1). An acrylic well that contained 15 μL of geraniol (Sigma-Aldrich, Product Number 163333) in its well was placed in the adjacent corner. The second shelter was placed above the support in the diagonal corner and an acrylic well filled with 15 μL of reverse osmosis water was put in the adjacent corner. The entrance to the latter shelter was covered with a piece of white electrical tape so that in daylight hours a subject was forced to utilize the shelter nearest to the cube filled with geraniol. Nights on which a subject could only take refuge in the shelter near the cube filled with geraniol are referred to as forced choice (F) trials. In all F-trials the locations of the odor-cued and inaccessible shelters were always identical within subjects and two subjects
were used for each of the four configurations (Figure 1). It is around this F that the other trials are composed.

The experiment involved the manipulation of shelters into four configurations after a subject emerged, referred to as control (C), dissociative (D), and transposition (T) probes, in which the entrance of both shelters was open (Figure 1). In C probes the two shelters and their associated acrylic wells were removed and replaced with clean shelters and newly filled acrylic wells in the same configuration as in F trials, one well with geraniol and one with water. In D probes the same procedure was followed, except that the acrylic well with a geraniol-filled well and the acrylic well with the water-filled well were reversed. In T probes the replacement shelters and acrylic wells were positioned diagonally over the supports in the corners of the arena where there were initially no shelters. The shelters and acrylic wells were removed and replaced after a subject emerged and walked to a position in the arena that was approximately equidistant from the old and new shelter locations so as not to disturb a subject as it wandered.

No shelter manipulations were conducted on the first three nights after a subject was placed in the arena, which forced a subject to utilize the shelter nearest to the acrylic well filled with geraniol. The sequence of manipulations that followed was grouped into four blocks of three probes, where each block contained one of each of the probes (C, D, T). Probes were separated by an F trial and the order of the probes within each block was determined randomly. There were two possible T probe configurations and one of each occurred in the first and second blocks and, likewise, in third and fourth blocks (Figure 1). For example, a potential sequence is F, F, F, (C, F, D, F, T₁), F, (T₂, F, C, F, D), F, (T₂, F, D, F, C), F, (D, F, T₁, F, C), where parentheses delimit blocks of probes and the subscripts on T indicate the two possible configurations for T. The tops of shelters were lifted in daylight hours to determine the shelter
that a subject entered on nights of probes. The complete sequence of trials consequently lasted 26 days, after which a subject was returned to its original container and fed. No food was provided in the arena, where eight animals were tested for a period of approximately twenty six days each.

*Predictions and Statistical Analyses*

The primary variable of interest was the proportion (θ) of nights that a subject utilized the odor-cued shelter for each of the probe types. The probe types were chosen so that this proportion, when compared between probes and to chance expectations, would reveal clues as to how a shelter is recognized. The proportion was expected to be highest on C probes because the odor cue (and any uncontrolled cues) was not manipulated and subjects exhibit high levels of site fidelity in such conditions (Graving et al., *in press*). The D probes dissociated the trained location of the shelter and the odor cue and a low proportion under such conditions would implicate shelter relocation and recognition by path integration or some an uncontrolled cue(s). Transposition probes involved a shift of the odor and location of the odor-cued shelter and subjects were expected to choose more or less randomly between the shelters, unless the odor was used in the recognition of a shelter. The influence of probe conditions on shelter choice was compared in a repeated measures analysis of variance (rANOVA) followed by post-hoc Fisher Least Significant Difference (LSD) *t* tests to discern which proportions in percent form differed after testing the eight amblypygids (Jones and Kenward, 2015). The twelve probe trials per animal were used in the calculations, not the forced choice. In the rANOVA, the SSw, or sums of squares within subjects, was used to account for the multiple tests. In addition, one-sample *t* tests were used to compare the realized proportion of choices to the odor-cued shelter within probe types to the random expectation of θ = 0.5 (Glover and Mitchell, 2016).
RESULTS

Results of the repeated measures analysis of variance revealed a significant effect of probe type on shelter choice in probes (Table 1; Figure 3). Post-hoc t tests revealed that subjects were more likely to choose the odor-cued shelter in C probes as compared to either D probes ($t_{14} = 3.69, P = 0.0024$) or T probes ($t_{14} = 3.45, P = 0.0039$). This result was expected, as in C probes—unlike D or T probes—the odor cue was not manipulated and path integration, the odor cue and any uncontrolled cue(s) could be used by a subject to relocate or recognize the shelter. In addition, subjects chose the odor-cued shelter in C probes significantly more often than the random expectation of $\theta = 0.5$, which indicates that subjects were site faithful under these conditions ($t_7 = 8.88, P < 0.0001$).

The repeated measures analysis of variance indicated that the odor-cued shelter was chosen equally frequently in D and T probes ($t_{14} = 0.25, P = 0.8090$). However, the probability that subjects chose the odor-cued shelter did not differ from the chance expectation of $\theta = 0.5$ under either of these conditions (D: $t_7 = -0.68, P = 0.5165$; T: $t_7 = -0.31, P = 0.7627$; Fig. 3). Thus, in D and T probes the conditioned odor was not sufficient to dictate shelter choices. These results, taken together, suggest that shelter choice could be reliably predicted only when path integration was feasible and subjects had access to the entire configuration of cues available in the forced choice trials.
DISCUSSION

Interpretation of Probes

Amblypygids are unique animals with unique sensory capabilities attributed in part to their antenniform legs. As previously mentioned, due to the densely foliaged, dark, three dimensional environment they navigate in every night, they have developed appropriate tools to home back to their refuge (Hebets, et al., 2013). In captivity, when given enough space, they emerge nightly and travel hundreds of meters before returning home (Graving, 2015). In the current study, training and control probe trials, where positions of the shelters are not moved, simulate in part what occurs in the field. The results from control probe trials demonstrated that the training protocol used resulted in the experimental subjects displaying a high degree of shelter fidelity (Figure 2).

The intent of the current study was specifically to determine the relative importance of olfactory, path integration or the possible integration of those cues in enabling the refuge fidelity displayed on control probe trials. Toward this end, two types of manipulative probe trials were carried out. On dissociative probe trials, when the positon of the odor-cued and non-odor-cued shelters were switched, shelter choice dropped to chance. What this result suggests is that neither path integration nor the odor cue alone was used to recognize the home shelter and indirectly supports the hypothesis of some kind of sensory integration of the two cues as essential for home recognition.

This impression is supported by the results of the transposition probe trials, where the shelters were manipulated in such way that path integration was rendered useless and home shelter identification could only be mediated by scent. Again, shelter choice dropped to chance
indicating that scent alone was insufficient to support home shelter recognition even when use of path integration would have brought the subjects to a location without any shelter. This again implies that it is the configuration or integration of the two identifiable sources of spatial information that enabled the shelter fidelity observed on control probes. The design of the study allowed for the experimental manipulation of odor, and what is clear from the results is that odor alone was insufficient to support refuge fidelity on the critical probe trials. The animals may have been using something in conjunction with odor, considering the success rates of the trials. However, no direct proof is offered that path integration was used and the possibility that some unspecified cue such as variation in the earth’s magnetic field or detection of some stable source of mechanical energy provided the additional spatial information cannot be excluded. There is no reason to think the additional spatial information was limited to one source. However, path integration should be considered the most important of alternative cues because in an amblypygid’s typical lifespan, they are not displaced by humans and tend to remain faithful to a home location while venturing out hundreds of meters foraging and performing other activities nightly (Graving, 2015). Given that a field study displaced the amblypygids about 10 meters, the organisms also could have been using different strategies not only because of the distance but because of the displacement depriving the amblypygids of an outward journey to calculate a vector (Wiegmann, et al., 2016). As the olfaction-deprived organisms had a significantly decreased ability to return, this phenomenon could be called ‘check and random choice if wrong,’ with attention given to two key points. The first is that in this study the animals were left intact and not displaced, but also couldn’t return ‘home’ when something disrupted the configuration to which they were trained. This disruption of the changing of forced choice trials
to probes (and vice versa after a probe) may have caused a conflict with what was learned and the new setup.

These results are very similar to a study by Steck et al. (2010) on the use of olfactory cues by *Cataglyphis*, where ants were given an array of scents in a specific composition to use while they were out foraging to identify the location of a feeder filled with crumbs. During tests, when the array was the same one the subjects were used to before, their search patterns focused on their goal with searches occurring over a smaller area. However, when the scent array was swapped for one with odors in a different location, the search pattern increased in area searched and turn angles widened. The scent sequences were not the only cue—they also paid attention to the direction, left or right, that it was in (Steck, et al., 2010). By removing an antennae from an ant, it didn’t stop them from homing but kept them from orienting in their scent array due to being deprived of the ability to sense the gradient of odors that each antennae would differentially receive (Steck, et al., 2010). Similar to how amblypygids failed to return to their refuge in the field when deprived of antenniform leg information, an ant with one antennae, who focused on the composition of the scents but not the scents themselves, also had trouble finding the feeder. This, combined with the ‘conflict’ between space, shelter, and scent when moved in the probes, may explain these variations in scent choice success.

*Individual Effects and Sociality*

One striking aspect of the study was that there was relatively little inter-subject variation in behavioral performance, shown by the comparison of the means in the rANOVA (Table 1). The implications of this is that the data is likely to generalize to amblypygids as a whole in homing-based situations. There also were no detectable difference in the behavior of the two
sexes, although due to the limited sample size one should remain open to the possibility of sex differences. Finally, amblypygids are semi-social arachnids (Santer and Hebets, 2011), they routinely engage in searching for mates, encountering rivals, evading predators, and other inter- and intra-species interactions. It would be interesting to investigate how social context might influence spatial behavior and navigation.

As previously defined, path integration is when speeds and turning angles of the path leaving the nest or other place are translated into a global vector that is updated continuously during the outward journey, using the direction of the vector at the end of the journey to take the straightest path possible back (Merkle, et al., 2006). As the amblypygids are not choosing the space of the then unscented shelter, this combined with the disruption shows the animals are not path integrating alone in this situation. However, another way of interpreting the configuration of cues is that the amblypygid may use the geraniol scent cue to verify that the shelter is theirs when they arrive to the vicinity of it, and may be confused when there is no scent, and choose randomly otherwise. In reverse, the animal may wander into the vicinity of the ‘forced’ or blocked-off shelter as it was during training, but find it scent cued like the ‘home’ they were trained to, but not in the correct area, also causing a conflict that cannot be resolved, also resulting in a random choice.

*Future Directions*

While this piece of information is but one piece of the puzzle of arthropod navigational strategies, this piece connects to several others. With the knowledge that amblypygids are disrupted by a conflict between scent and other spatial information as well as the change in odor landscape, one can examine aspects of how that information was formed and any interactions.
Currently unexplored questions involve examining different searching strategies in terms of when a shelter is moved after an animal leaves or is presented with a conflicting cue, the situations in which different strategies are used to solve navigational problems, and in a neurological sense, the functions and changes of the amblypygid brain during the phases of training. Because displacing shelters in the field can be difficult, utilizing the fact they are homing to a scent cue can open up a field of inquiry on what cues (and thus their chemical composition) are being used on the exact tree or stone of an amblypygid’s refuge. That in turn can be manipulated, such as isolating a scented compound of their specific location and masking that, for example. Another sample manipulation is removing the use of one antenniform leg and testing the return success. It is worth noting that candidate odor cue(s) that can be used for refuge recognition cue could be deposited by an animal itself, which is a question that could be addressed in a laboratory setting. Answering the question of how scent may guide navigation in amblypygids would necessarily yield more questions about how this unique animal uses its sensitive antenniform legs to venture forth upon its nightly excursions and into becoming the navigational expert that it is.
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APPENDIX A: HOMING AND ORIENTATION

True navigation, as defined by Boles and Lohmann (2003), is the ability of an organism to determine their position relative to a goal after displacement to a novel environment without relying on familiar surroundings, locational cues from the goal, or information gleaned from the trip outwards. Homing, in contrast, is the use of cues to return to a known location, such as Cataglyphis bicolor using visual cues such as the landscape panorama to return to the home nest. This is an allothetic (external) cue to path integration’s idiothetic (internal) cue (Warrant and Dacke, 2010). Many members of the animal kingdom demonstrate navigational abilities, both vertebrates and invertebrates, notable members being pigeons, spiders, dung beetles, fiddler crabs, and desert ants, though here focusing upon arthropods (Wallraff, 2014, Norgaard, et al., 2007, Smolka, et al., 2015, Hong and Layne, 2012, Steck, et al., 2009, Graham and Cheng, 2009, Schultheiss, 2016, Sommer and Wehner, 2005).

Pigeons use a combination of dead reckoning, celestial body interpretation, sensing the geomagnetic field, and recently supported long distance olfactory homing (Wallraff, 2014). The arachnid Leucorchestris arenicola uses local cues after being displaced, but was found that scent from their burrows was not an important sensory cue for return (Norgaard, et al., 2007). Scarabaeus, a genus of dung beetles, use the position of the sun and moon, polarization of light, and skylight gradation as cues to direct themselves when rolling a freshly formed dung ball away from conspecifics (Smolka, et al., 2015). Hong and Layne demonstrated that fiddler crabs use the stable external cue of gravity, along with other sensory cues to navigate extremely accurately along with their allothetic cues (2012). Several species of desert ants use a variety of cues to navigate through their environment. Steck, along with their colleagues, studied how Cataglyphis fortis used bilateral comparison sensing to associate their home nest with scents that could be
distinguished in a mixture in an otherwise rather featureless salt pan desert (2009). Cheng and Graham used *Melophorus bagoti* in their experiment showing the organism used a skyline panorama to return home (2009). The same species of ant is again used by Schultheiss to show that they also use ultraviolet light cues creating a green contrast between the terrestrial panorama and the sky also to navigate (2016). *Cataglyphis fortis* have also been shown using path integration as well, internally tracking their steps and their length and creating a vector that points them back to their nest, essentially leading them to the straightest path back home after taking a circuitous path outward, and in this case using celestial cues to indicate direction (Sommer and Wehner, 2005). Sensory cues are one manner an animal can use to navigate.
APPENDIX B: OLFACITION AND ARACHNIDS

The role of olfaction in navigation is a chemical cue, a cue or signal that can be deposited either by the navigating organism or be present in the environment. Scent is variable in concentration and composition depending on the qualities of the laid chemical, and is mutable, unlike the magnetic field. It is also used in navigation. The worker bumblebee of *Bombus impatiens* walk while out at night instead of flying, laying an odor trail as they progressed towards a feeder, and even though the bees had the same success with the odors removed. The bee had the same success due to other factors such as magnetic sensing, though it showed evidence of organisms depositing hydrocarbon scent cues from their ‘leaking’ tarsal gland to indicate where they have been (Chittka, et al., 1999). For more purposeful scent use, *Cataglyphis fortis* is a species of desert ant that has multiple sensory capabilities. In this study, it uses its skills to follow scent plumes along the rather empty Tunisian salt pans is being studied, the organisms using these odor cues to consistently return to the nest (Buehlmann, et al., 2015). The amblypygid, a member of the arachnid order, navigates in much more complex environments such as the rainforest, the group containing several species found around the equator (Hebbets, et al., 2013). The complex nature of the rainforest environment adds to the challenge of leaving to forage at night due to the nocturnal nature of amblypygids. A rainforest is a three dimensional environment where the organisms could potentially be traversing vertically up trees or on other barriers, adding an intriguing ‘twist’ on the previously two-dimensional studies, with the darkness also making the visual system less advantageous and encouraging olfaction (Wiegmann, 2016).
APPENDIX C: AMBLYPYGIDS

Though they are arachnids, amblypygids do not possess the common body formation of a spider, having not eight walking legs, but six. Instead, they are notable for their front pair of legs, which have are elongated and highly segmented with a femur twenty five to fifty percent longer than a walking leg, with the addition of sensory hair coverage (Weygoldt, 2000). Holding these legs in front of the head and slightly out, they are used to scan the environment in front of their flattened body with their numerous sensillae with different sensory functions. This front pair of legs of the amblypygids, also called whip spiders, are covered in sensory hairs and are used like antennae to sense the environment (Weygoldt, 2000). Their body, like most arachnids, possesses an abdomen and cephalothorax, but unlike the small pedipalps of arachnids such as spiders, amblypygids have exaggerated, lengthened pedipalps covered in a varying amount of spikes depending on the species, used for grabbing and consuming prey (Santer and Hebets, 2011). While the exact specifications of their living habits depend on the species, amblypygids are nocturnal creatures that live in moist equatorial environments, with species coming from places such as Costa Rica, Florida, Kenya, and Mexico. On their cephalothorax, they have eight eyes, two median eyes and sets of three lateral eyes on either side of the median eyes. The cuticle color and patterning also depends on the species, but the species of interest here specifically is *Phrynus marginemaculatus*. This darkly colored species with a yellow spotted posterior ectal angle has twenty seven tibial segments in the antenniform leg, while their distinguished pedipalps possess two spines on their trochanter with two teeth on their basal cheliceral segment, their eyes highlighted by a yellow line near each cluster, distinguishing them from other species (Quintero, 1981). They grow to a length of about eighteen millimeters as adults, though like many arachnids, the females are often larger than the males. *Phrynus marginemaculatus* is native the
area of Florida of the United States and the islands below it such as Jamaica and the Bahamas. They often find shelter below rocks, logs, and other darkened, moist areas, including some human-made materials such as shingles (Quintero, 1981). When they are moved from their home tree it takes them one night to return if the distance is less than seven meters, and two to five nights if it is greater than seven, and it is this ability to leave a refuge and return faithfully that is the inspiration for this inquiry (Santer and Hebets, 2011).

Amblypygids, arachnids that live in a range of habitats from Mexican caves, Floridian forests, to Costa Rican rainforests, are notable for their ability to navigate three dimensional environments (in contrast to the usual two-dimensional models of desert ant navigation) ranging from foraging to predator evasion (Weygoldt, 2000). These actions require an integrated sensory system and neuroplasticity. As previously mentioned, when amblypygids were tracked by telemetry systems and displaced up to 8.7 meters away from their home refuges, it only took anywhere from one to three nights to reach their original refuges, depending on the distance displaced, often using indirect paths back (as they were displaced in this study), showing evidence of a technique other than path integration used by organisms such as the aforementioned desert ants (Hebets, et al., 2013). This was with individuals who were not altered in any way. When their antenniform legs are disabled through the application of nail polish or they are blinded with dental resin covering their eyes, they become less successful at locating their original refuge, more so if they were deprived of olfaction (Hebets et al., 2013). Similar responses have been seen to sensory disruptions, for example the wandering desert spider Leucorchestris arenicola. These desert spiders use local cues to find their burrows, not path integrating like Cataglyphis fortis can (Norgaard, et al., 2007). With the movement of these desert spider burrows, twelve of fifteen spiders failed to find their refuges, indicating no scent
cues were utilized (Noorgaard, et al., 2007). There are still some topics that need researched in relation to amblypygid spatial behavior, such as how these animals, after moving hundreds of meters every night in captivity or navigating a complex landscape in the wild, manage to recognize a burrow, tree crevice, or some other shelter as theirs.
APPENDIX D: AMBLYPYGIDS AND SCENT

In order to see if there is a connection between the sensory cue of scent and refuge recognition and association, the potential for connection must first be tested. Scent associations have succeeded in being formed in the past with desert ants for studying their navigation, and amblypygids could be used as a model organism in tropical areas for navigation, such as *Phrynus marginemaculatus* (Steck, et al., 2009). In the field, the amblypygids show site fidelity that is disrupted when their sensory organs are impaired (Hebets, et al., 2013). This is especially true with antenniform leg tip clips, as they hold a great proportion of chemosensory sensillae (Weygoldt, 2000). This phenomenon contributes to the idea that they are suitable as they navigate in a three dimensional, sensory stimulus-packed, complex environment in challenging conditions. Due to the interest in not only navigational, sensory, and behavioral studies but also cognition in the sense of how sensory input shape behavior and decisions, this experiment tests the associations an amblypygid makes, specifically the effects of scent (in this case geraniol) on shelter choice. The intention of this study is to discover if scent is sufficient to guide shelter recognition and to distinguish how a shelter is chosen a recognized as a refuge. The broader impacts of this work will not only reveal new information about the understudied amblypygid, it will lend new insights into the invertebrate decision making process by examining the connections between space and cue and examining choice when the two are put into conflict. The effects of learning will be quantified as the experiments progress and tracking records are accumulated and analyzed for evidence of path integration vectors versus scent-based homing. This experiment will be the base paradigm for testing other sensory cues and their relation to shelters, including their overlap with sensory integration and multi-modal interpretation of sensory inputs and its effect on decision making.
### APPENDIX E: TABLES

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
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<th>P</th>
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**Table 1.** Results of repeated measures analysis of variance (SS: sum of squares; df: degrees of freedom; MS: mean square).
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control Probe</th>
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<th>Dissociative Probe</th>
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<th>Transposition Probe</th>
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<tr>
<td></td>
<td>% Scent Choice</td>
<td>% Scent Choice</td>
<td>% Scent Choice</td>
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<tr>
<td>41F</td>
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<tr>
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Table 2. Breakdown of individual subject overall scent choice percentages by probe.
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<td>0.0038</td>
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</table>

**Table 3.** Fixed effect test table.
Figure 1. Example shelter configurations for forced choice (F) trials and probes (C, D, T). The red circle indicates the position of the scent-cued shelter. The illustration shows one of four shelter configurations for F trials. The other three correspond to the shelter arrangements depicted in the D and two T probes. The positions of the two shelters were identical in each F trial of a 26-night session and two subjects were tested under each of the four possible F configurations.
Figure 2. Overhead image of arena which shows the location of the two shelters in a forced choice trial and the small acrylic wells placed in the corners next to the shelters.
Figure 3. Proportion (mean ± 2 standard errors) of nights on which the conditioned odor-cued shelter was chosen by subjects in control, dissociative and transposition probes. The horizontal solid black line is the chance expectation $\theta = 0.5$. 