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RESEARCH ARTICLE

Multisensory integration supports configural learning of a home refuge in the whip spider *Phrynos marginemaculatus*

Kaylyn A. S. Flanigan^{1,2}, Daniel D. Wiegmann^{1,2}, Eileen A. Hebets³ and Verner P. Bingman^{2,4,*}

ABSTRACT

Whip spiders (Amblypygi) reside in structurally complex habitats and are nocturnally active yet display notable navigational abilities. From the theory that uncertainty in sensory inputs should promote multisensory representations to guide behavior, we hypothesized that their navigation is supported by a multisensory and perhaps configural representation of navigational inputs, an ability documented in a few insects and never reported in arachnids. We trained *Phrynos marginemaculatus* to recognize a home shelter characterized by both discriminative olfactory and tactile stimuli. In tests, subjects readily discriminated between shelters based on the paired stimuli. However, subjects failed to recognize the shelter in tests with either of the component stimuli alone. This result is consistent with the hypothesis that the terminal phase of their navigational behavior, shelter recognition, can be supported by the integration of multisensory stimuli as an enduring, configural representation. We hypothesize that multisensory learning occurs in the whip spiders' extraordinarily large mushroom bodies, which may functionally resemble the hippocampus of vertebrates.

KEY WORDS: Amblypygi, Spatial cognition, Navigation, Multimodal, Olfactory, Tactile

INTRODUCTION

Many arthropods are superb navigators (Menzel et al., 2005; Wehner, 2003; Cheng et al., 2009; Layne et al., 2003), and species that inhabit structurally uncluttered environments (e.g. deserts) typically rely on sensory inputs that are organized in parallel as independent, sometimes weighted inputs (Collett et al., 2013; Müller and Wehner, 2007; Wehner et al., 2016). In environments where sensory inputs are associated with uncertainty, such as in structurally cluttered environments (e.g. dense forests), behavior guided by multisensory inputs may be particularly advantageous (Munoz and Blumstein, 2012). Multisensory inputs, when used, can be processed independently (Wehner et al., 2016) or bound together and integrated in the form of a configural representation (Pearce, 2002).

During navigation, the simultaneous or sequential perception of environmental cues can interact functionally. In the Central Australian desert ant, *Melophorus bagoti*, for example, foragers

use celestial compass cues such as polarized light, the sun's position, and spectral and intensity gradients. If these cues are in conflict, they appear to be averaged in a weighted fashion (Wystrach et al., 2014). The functional integration of environmental cues perceived through multiple sensory modalities (multimodal cues) is also common, and scientists predict that the simultaneous use of multisensory cues should increase robustness and overall accuracy of insect navigation (Buehlmann et al., 2020a). Examples of functional multisensory interactions include the integration of celestial and wind inputs to support the directional movements of dung beetles (Dacke et al., 2019), or the priming effects of one sensory cue for another as observed in many arthropods. In host-seeking mosquitos, for example, sensing carbon dioxide (Gillies, 1980) initiates a preliminary phase of host-seeking navigation and increases attraction to visual objects (Bidlingmayer and Hem, 1980; van Breugel et al., 2015). Following visual attraction, thermal cues confirm a mosquito host (van Breugel et al., 2015). Hawk moths (Raguso and Willis, 2002), bumblebees (Reinhard et al., 2004) and fruit flies (Chow and Frye, 2008; van Breugel and Dickinson, 2014) also show similar odor-gated attraction to visual cues. More complex multisensory interactions are seen in desert ants *Cataglyphis fortis*, whose navigation relies on visual, olfactory and wind direction cues. Wind direction, for example, is important in predicting an ant's initial outward path while visual cues initiate learning walks (Vega Vermehren et al., 2020). The structure of those learning walks, however, is again influenced by wind direction (Vega Vermehren et al., 2020). A recent review of multisensory interactions in insect navigation discusses numerous additional examples (Buehlmann et al., 2020b).

Multisensory interactions may also include functional binding of multimodal cues, or configural representations (Pearce, 2002). A good example of configural learning in invertebrates can be found in *Lymnaea* (Swinton et al., 2019; Kagan and Lukowiak, 2019). The ant *Lasius niger* can learn the conditional relationship between an odor and color cue to locate food rewards (De Agrò et al., 2020) and bumblebees exhibit cross-modal recognition, i.e. they can integrate sensory information in a way that requires modality independent internal representations (Solvi et al., 2020). In the context of navigation and as noted above, a multisensory representation should reduce spatial uncertainty, and if such a representation were configural, it could reduce uncertainty even further as error-prone input from one cue would have little control over behavior when not processed with the companion, configural input(s). An example of a multisensory-configural representation supporting a component of navigation can be seen in the binding of visual and odor inputs to guide locating a nest entrance in desert ants (Steck et al., 2011). Additionally, in wood ants *Formica rufa*, foragers appear to bind cues together, but only under certain circumstances. After learning to navigate to a feeder based on combined olfactory, visual and airflow cues, ants failed to subsequently approach the learnt feeder

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when either olfactory or visual cues were removed, suggesting a configural representation of multisensory cues (Buehlmann et al., 2020a). When the visual cue was placed in a different location, however, the ants successfully navigated to the feeder after the removal of individual sensory components. Ultimately, while providing further evidence of configural representation, or cue binding, this last study also suggests a potential role for flexibility or plasticity in cue binding during navigation (Buehlmann et al., 2020a).

Beyond insects, spiders also exhibit multisensory control of learning. The jumping spider *Habronattus dosseus*, for example, is more likely to learn to avoid a color associated with a heated substrate in the presence of a vibratory stimulus (VanderSal and Hebets, 2007). Multisensory, attention-priming effects have also been documented in other jumping spider species during foraging (Clark et al., 2000; Cross and Jackson, 2009). Additionally, the importance of multisensory signaling in wolf spider communication is well documented, with often-complex interactions reported between multisensory (e.g. visual and vibratory) elements displayed during courtship (reviewed in Hebets, 2005; Hebets and McGinley, 2019).

Similar to the previously mentioned insect groups, many arachnids are also central place foragers, navigating back to a home refuge or burrow after a period of hunting. But in contrast to their insect relatives, multisensory control of navigation in arachnids appears rare. The wolf spider *Arctosa perita* uses the sun and polarized light to home as does the agelenid spider *Agelena labyrinthica* (reviewed in Görner and Claas, 1985; Ortega-Escobar, 2020). Use of single-cue, visual substrate structure for homing has been studied in *Lycosa tarantula* (Ortega-Escobar, 2006, 2011), and single modality landmark orientation has been studied in the Namib desert spider *Leucorchestris arenicola* (Nørgaard et al., 2008). Two recent reviews summarize the current knowledge about the capacity of and mechanisms underlying arachnid navigation, including research on non-spider arachnids (e.g. scorpions and amblypygids; Ortega-Escobar, 2020; Gaffin and Curry, 2020). Based on the existing literature on arachnid navigation, there is little evidence supporting the idea that arachnids can form configural representations of different sensory modalities to guide any component of navigation.

Non-spider, nocturnally active arachnids of the order Amblypygi (commonly called ‘whip spiders’) are excellent navigators, successfully returning to their home refuge after a night of hunting and even after experimental displacement of 10 m or more (Beck and Görke, 1974; Hebets et al., 2014a,b; Bingman et al., 2017). They possess a number of sensory capabilities, many of which are associated with receptors on their specialized antenniform legs (Weygoldt, 2000; Wiegmann et al., 2016; Bingman et al., 2017) and a brain organization, specifically the mushroom bodies, capable of multisensory integration (Sinakevitch et al., 2020). The array of sensory capabilities displayed by whip spiders could be exploited in the learning of multisensory and possibly configural representations that could control navigation; a capability to the best of our knowledge not yet documented in arachnids. In the Florida Keys, the whip spider *Phrynus marginemaculatus* resides in dense scrub habitat cluttered with limestone and plant litter. Wiegmann et al. (2016) proposed that, in such an environment, multisensory control of whip spider navigation would be advantageous, and any eventual multisensory control could be processed as a potentially error-resilient, configural representation. We therefore hypothesized that *P. marginemaculatus* can learn an integrated multisensory and perhaps configural representation of a shelter location, which, if supported, would provide the first evidence that arachnids can learn

a multisensory and configural memory representation that can guide what can be considered the terminal navigational phase of a night’s hunting.

MATERIALS AND METHODS

Subjects

Twenty-one *Phrynus marginemaculatus* (C. L. Koch 1840), collected in Florida (Key Deer National Wildlife Refuge, Big Pine Key, Monroe County, FL, USA), served as the experimental subjects. The sex of the subjects was not recorded. We trained and tested six naive *P. marginemaculatus* in an olfactory-only shelter-discrimination task. In a separate experiment, we trained and tested three naive animals and three subjects from the olfactory-only task in a tactile-only shelter-discrimination task. The three subjects tested on both tasks experienced the tactile-only task second, which was separated by at least 4 weeks from the olfactory-only discrimination task to minimize any cue interference. (The performance of the three subjects tested on the tactile-only discrimination task after being trained on the olfactory task was indistinguishable from that of the three subjects tested solely on the tactile-only task.) In the last experiment, we trained 12 naive animals on the critical, multisensory shelter-discrimination task.

Single-cue shelter discrimination

Earlier studies with experimental designs that differed from ours demonstrated that *P. marginemaculatus* can locate a shelter using olfactory or tactile cues (Wiegmann et al., 2019; Santer and Hebets, 2009). Here, we trained and tested subjects on olfactory-only ($N=6$) and tactile-only ($N=6$) shelter discrimination tasks under conditions similar to those used in the primary multisensory discrimination experiment to better compare performance of single-cue and multi-cue guided behavior in the experiments of the current study.

Olfactory-only discrimination training occurred over four consecutive nights, and the measurements and details of the experimental apparatuses employed can be found in Fig. 1. A subject was placed in a pre-exposure shelter made of opaque PVC pipe, which contained 10 μ l of geraniol ($C_{10}H_{18}O$, Sigma-Aldrich, product number 163333; three subjects) or 1-hexanol ($C_6H_{14}O$, Sigma-Aldrich, product number 471402; three subjects) 2 h before lights-out occurred. *Phrynus marginemaculatus* can use either odor to locate a shelter (Wiegmann et al., 2019). Pre-exposure served to facilitate the association of an odor with the safety of the shelter. After lights-out, the subject was transferred to a training arena containing two shelters identical in size to the pre-exposure shelter. One shelter, which was accessible, contained the pre-exposure odor (CS+). The second shelter, whose entrance was blocked by mesh, contained the other, unconditioned odor (CS-). After 5 min, we allowed subjects to leave the pre-exposure shelter, which was then removed from the arena. (If, after 5 min, the subject failed to exit, we gently coerced it out with a paintbrush.) Subjects were allowed to freely move in the training arena until morning. The next morning (lights-on), a subject was enclosed in the CS+ shelter, where they were typically found, and held there until testing. If the subject was not in the CS+ shelter, it was gently coerced inside.

We conducted tests 2–4 h after lights-on (Fig. 1). Each end of the test arena (for dimensions, see Fig. 1) had an entrance to a shelter, one of which contained 10 μ l of the CS+ odor (side determined randomly) and one that contained 10 μ l of the CS- odor. A mesh door rendered both shelters inaccessible. The arena was illuminated by two 13 W Phillips Mini Twister (120 V, 60 Hz) light bulbs to motivate subjects to escape the light and locate a shelter. Test trials lasted 10 min. We recorded the time a subject spent in goal areas

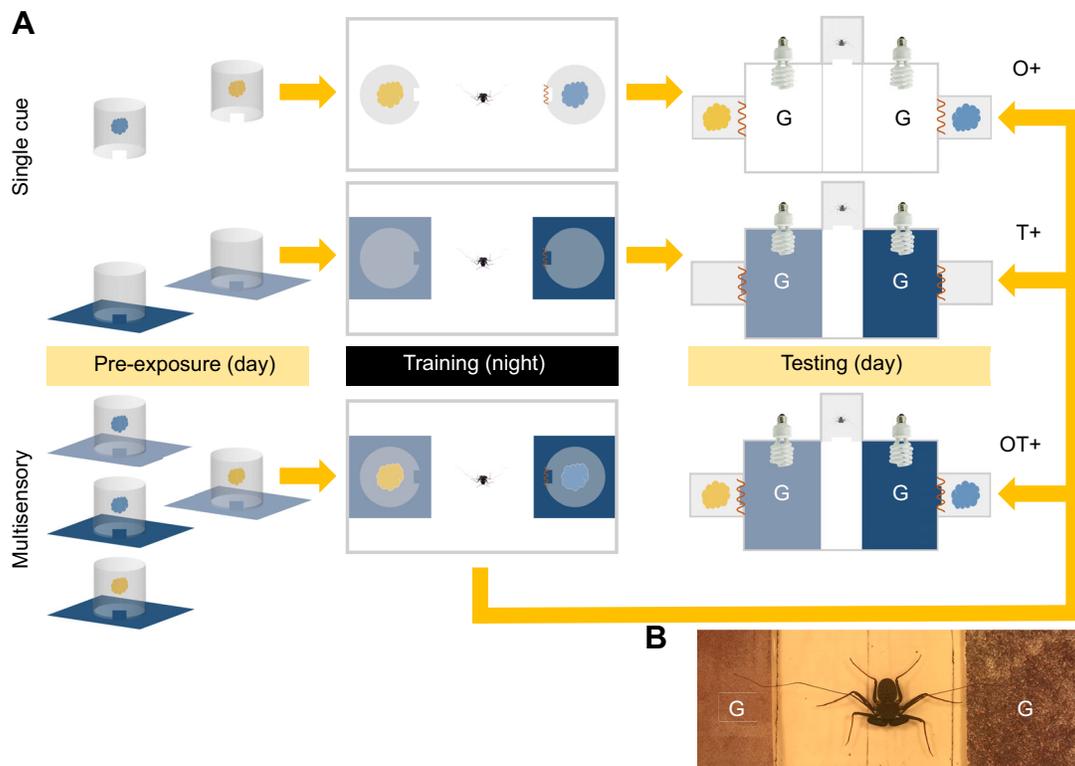


Fig. 1. Experimental procedure. (A) Schematic drawing of training and tests. Subjects were placed in a pre-exposure shelter (diameter, 10.2 cm; height, 12 cm) that contained a CS+ odor (blue and yellow 'clouds') or sandpaper (light and dark blue surfaces) for the single-cue experiment, and a CS+ paired odor and sandpaper (four combinations) for the multisensory experiment. At night, we released subjects into a training arena (length, 1.0 m; width, 0.5 m; height, 30.0 cm) that contained two shelters. One shelter, which was accessible, contained the CS+. The other shelter, which was inaccessible because of a mesh barrier (wavy lines), contained the CS-. (The sandpaper extended beyond the boundaries of the shelters.) The next morning, subjects were tested. The floor of the test arena (length, 29.0 cm; width, 14.0 cm; height, 9.5 cm) was demarcated into two goal areas, G (length, 14.0 cm; width, 10.5 cm). We separated goal areas by a central 'neutral zone' (length, 14.0 cm; width, 7.0 cm). A 'start chamber' (length, 7.0 cm; width, 5.0 cm, height, 2.5 cm) opened into neutral zone. Two shelters at the ends of the arena, neither of which could be entered because of a mesh barrier, contained the CS+ and CS- odors. The CS+ and CS- sandpaper occupied their respective goal areas. The test arena was illuminated by two light sources. O+, olfactory only; T+, tactile only; OT+, paired olfactory and tactile stimuli. See Materials and Methods for more details. (B) Image of a subject in the neutral zone during a test. Note: the antenniform legs could be extended to touch both sandpaper surfaces. The average prosoma width of the test subjects was approximately 0.9 cm and the antenniform legs, when extended, were approximately 9.0 cm.

near shelter entrances. Our dependent measure to assess the strength of the learned association to the CS+ shelter was an 'association index' (θ), which was the time spent near the CS+ shelter divided by the total time spent near both shelters. After testing, we placed individuals in a pre-exposure shelter until lights out, when we again placed them in the training arena. This sequence was repeated 4 times for a total of four tests per individual.

Procedures for the tactile-only discrimination task were the same, except for the conditioned stimuli (Fig. 1). Three animals were trained to coarse sandpaper as the CS+ and three were trained to smooth sandpaper (3M Pro Grade Precision Sandpaper, P60 and P320, respectively). We covered the floor of the pre-exposure shelter with the CS+. In training, we placed sheets of sandpaper (28×23.8 cm) inside and around each shelter. The tactile features covered the goal areas in the test arena.

Multisensory shelter discrimination

We trained naive ($N=12$) subjects in a multisensory discrimination task with the same olfactory and tactile cues, in this situation paired, where we balanced the four possible CS+ combinations across subjects. Tests involved either the paired cues or the component stimuli alone (Fig. 1). Subjects were required to meet a recognition criterion $\theta \geq 0.70$ with the paired stimuli on three consecutive tests

before formal tests were initiated. We imposed a criterion on the multisensory shelter discrimination task because subjects underwent test trials that differed from training trials. We wanted to ensure the subjects had learned the discrimination with the paired CS+ stimuli before testing them on trials where only one element of the combined CS+ was present (see below). All 12 subjects rapidly reached criterion (none were excluded from the experiment for failure to reach criterion). Testing involved four blocks of three tests, where each block included an odor-only, tactile-only and a paired-stimuli test. Test order within blocks was determined randomly except for the first test in the first block, which was restricted to a single-cue test to avoid habituation after criterion tests. We conducted tests over 12 days, one test per day. Procedures otherwise followed those described for the single-cue experiment.

Quantitative analysis and statistical procedures

Larger values of the association index ($0 \leq \theta \leq 1$) indicate a stronger conditioned association between the CS+ and access to shelter. For the single-cue experiment, indices from the four tests for each subject were averaged and one-sample *t*-tests were used to compare mean indices with the random expectation of $\theta=0.5$. For the multisensory experiment, the means for each individual within test-trial types were averaged and likewise compared with $\theta=0.5$. In

addition, a repeated measures ANOVA, with Tukey HSD *q post hoc* tests to control for Type I error, was used to compare performance between the single-cue and paired-cue tests. Lastly, we compared performance in single-cue tests of the multisensory experiment with performance in the single-cue experiments with two-sample *t*-tests.

RESULTS

We provide goal-area occupancy times for all subject–test trials in Table S1.

Single-cue shelter discrimination

The mean±s.e.m. association index θ for the odor-only shelter discrimination task was 0.79 ± 0.04 and differed significantly from the chance expectation $\theta=0.5$ (Fig. 2A; $t_5=7.67$, $P<0.0006$). The mean association index for the tactile-only shelter discrimination task was 0.69 ± 0.04 and likewise differed significantly from chance (Fig. 2A; $t_5=5.09$, $P=0.0038$). Hence, in the experimental context of this study, *P. marginemaculatus* learned to recognize an accessible shelter cued solely by an odor or tactile stimulus (see also Higham and Hebets, 2013; Santer and Hebets, 2009; Wiegmann et al., 2019).

Multisensory shelter discrimination

In the multisensory discrimination experiment, subjects required on average 3.33 ± 0.19 nights to reach criterion to move on to formal test trials. The repeated-measures ANOVA revealed that association indices differed across the three test conditions ($F_{2,22}=15.35$, $P<0.0001$). *Post hoc* tests revealed that the mean association index in odor-only (0.53 ± 0.05) and tactile-only (0.53 ± 0.04) tests did not differ ($q_{22}=0.19$, $P=0.9804$) and that performance in paired-stimuli tests (0.76 ± 0.03) was better than in either single-stimulus test (paired versus odor only: $q_{22}=4.70$, $P=0.0003$; paired versus tactile only: $q_{22}=4.89$, $P=0.0002$). Performance in odor-only and tactile-only tests did not differ from chance (Fig. 2B; $t_{11}=0.76$, $P=0.4631$ and $t_{11}=0.72$, $P=0.4876$, respectively). By contrast, performance in the multisensory shelter discrimination was significantly biased toward the CS+ (Fig. 2B; $t_{11}=7.85$, $P<0.0001$).

Discrimination performance in single-cue tests in the multisensory experiment was also poorer than in analogous tests in the single-cue experiments (Fig. 2; olfactory-only tests: $t_{16}=3.63$, $P=0.0022$; tactile-only tests: $t_{16}=2.89$, $P=0.0105$). Performance in paired-stimuli tests, however, was no better than that of subjects trained on either of the element stimuli in the single-cue experiments (olfactory cue: $t_{16}=0.53$, $P=0.6054$; tactile cue: $t_{16}=1.30$, $P=0.2114$). Together, these results indicate that the component

stimulus from either sensory modality could control shelter recognition when stimuli were individually conditioned, but neither stimulus alone could support shelter recognition when we trained subjects to the paired olfactory and tactile stimuli.

Behavior

After entering the test arena from the start chamber, subjects would typically move toward and come into contact with one of the two far walls with the shelter entrances (both entrances were closed by mesh during the test trials). Subjects would then periodically move from one side of the test arena to the other, perhaps motivated by the inability to escape the light because the shelter entrances were closed by the mesh. Across each subject's four OT+ versus OT– test trials, the mean number of side crossings in the arena/trial was 2.69 ± 0.27 .

DISCUSSION

The results of this study support our hypothesis that whip spiders can use multisensory integration to guide the terminal phase of navigation following a night's journey. Further, the multisensory integration supports the learning of a configural representation of a shelter; when subjects were trained to recognize a refuge characterized by both olfactory and tactile stimuli, neither component stimulus by itself supported shelter recognition. As such, whip spiders are among a growing list of invertebrates shown to be capable of configural learning (e.g. the snail, *Lymnaea*; Swinton et al., 2019; Kagan and Lukowiak, 2019). This exclusion of behavioral control by either component stimulus of a trained compound stimulus is a defining feature of configural learning as described in vertebrates (Pearce, 2002). In the context of arthropod navigation, configural learning has been observed to control aspects of navigation in two species of ants (Steck et al., 2011; Buehlmann et al., 2020a), but to the best of our knowledge it has not been previously reported to control arachnid navigation. Following on from the signal uncertainty (Munoz and Blumstein, 2012) likely associated with the structural complexity and nocturnal habits of whip spiders, it is perhaps not surprising that they are capable of integrated, multisensory learning to support navigation (see Wiegmann et al., 2016).

Our experiment was designed such that, during the single-cue tests of the subjects trained to the compound olfactory and tactile stimulus, the test arena was polarized with a trained (familiar) CS+ element on one side and a trained (familiar) CS– element on the other (Fig. 1). Future experiments could build on this design by contrasting an element of a compound CS+ with no competing

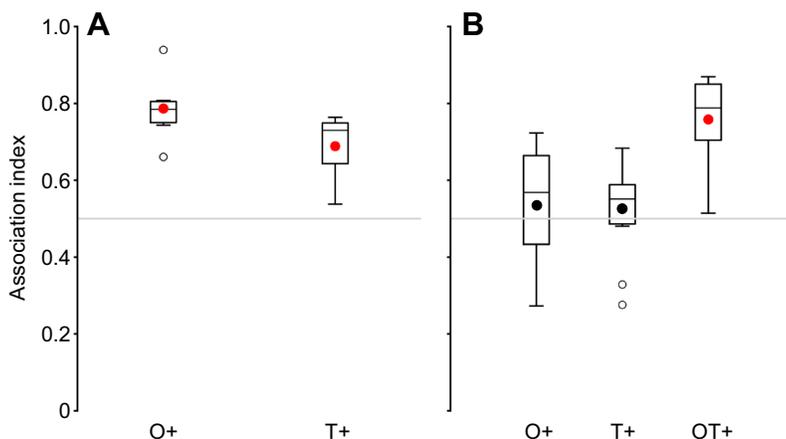


Fig. 2. Box plots of association index (θ) in shelter discrimination experiments. (A) Single-cue ($n=6$ for each) and (B) multisensory cue ($n=12$) trials. Filled circles are group means and open circles are outliers. Lines within the standard error boxes are medians. O+, olfactory-only tests; T+, tactile-only tests; OT+, tests with paired olfactory and tactile stimuli. Association index means near one indicate a preference for CS+. Red association index means differ significantly from the chance expectation (gray horizontal line) of $\theta=0.5$.

stimulus or an unfamiliar stimulus of the same modality. The alternative testing regimes could reveal whether the indifference to the trained CS+ element without the companion CS+ element of the compound stimulus observed in the current study (Fig. 2B) would generalize to conditions when no familiar CS− element would be present.

We also expected that discrimination (correct choice performance) would be better when a shelter was associated with multiple cues compared with a single stimulus. This was not supported, as the performance of subjects trained and tested on paired stimuli was no better than that of subjects trained on either of the element stimuli by itself. However, the advantage of configural, multisensory learning has been theorized to manifest in settings where there is uncertainty in the information carried by stimuli. In retrospect, therefore, this result is perhaps not surprising as the controlled experimental conditions generated no environmental uncertainty with regard to shelter identity and, hence, no advantage to multisensory learning with regard to shelter recognition.

It is notable that results from prior experiments have suggested that olfactory inputs to the antenniform legs play a large role in guiding navigation in the field (Bingman et al., 2017) and in recognizing a home shelter in the laboratory (Casto et al., 2020), promoting the idea that input from a single sensory modality could enable navigation. However, Hebets et al. (2014a,b) offer evidence of multisensory, vision and olfaction, control of navigation in the field, and the field study of Bingman et al. (2017) relied on deafferenting input from the tips of the antenniform legs, eliminating olfactory but also other sensory inputs, including mechanosensory. Therefore, although there is a suggestion that olfactory inputs play an outsized role in guiding whip spider navigation, there are ample empirical and theoretical considerations supporting the hypothesis that navigation, and home refuge recognition in particular, is guided by integrated, multisensory inputs like that observed in the current study and as originally hypothesized by Wiegmann et al. (2016).

Multisensory, configural learning in the amblypygid *P. marginemaculatus*, as suggested by the current study, raises the question of the underlying neural architecture. We proposed (Wiegmann et al., 2016) that the mushroom bodies, which support learning and memory in insects (Menzel et al., 2006), are the site of multimodal, sensory integration in whip spiders. The mushroom bodies are exceptionally large and elaborately folded in whip spiders (Strausfeld et al., 1998), suggesting their importance in the control of complex behavior and memory-based navigation (Strausfeld et al., 2009). A recent analysis of the amblypygid central nervous system documents a large number of primary olfactory glomeruli (~460) that receive inputs from olfactory neurons of the antenniform leg nerve (Sinakevitch et al., 2020). Olfactory projection neurons then ascend from these primary olfactory glomeruli to terminate on a set of secondary olfactory glomeruli in the mushroom body calyx (Sinakevitch et al., 2020). Notably, the mushroom body calyx also receives input from the secondary visual neuropil, meaning that there is bimodal input into the extraordinarily large amblypygid mushroom bodies (Sinakevitch et al., 2020). Although mechanosensory, or tactile, information appears to be spatially segregated from the olfactory glomeruli in the antenniform neuromere (Strausfeld et al., 1998), this does not preclude the eventual convergence of olfactory and mechanosensory information in the mushroom bodies.

The mushroom bodies of insects play a large role in both navigation and multisensory integration. Honeybees trained to discriminate a reward based on a configural representation of two

odors were unable to carry out the discrimination when the mushroom bodies were inactivated (Devaud et al., 2015). Consistent with this observation are the results from an electrophysiological study revealing that many mushroom body output neurons in the honeybee *Apis mellifera carnica* respond to multimodal inputs, suggesting a role in multisensory integration and perhaps configural learning (Strube-Bloss and Rössler, 2018). Additionally, two new studies that used local anesthetics to inhibit neural activity in the mushroom bodies' vertical lobes (Kamhi et al., 2020) and calyxes (Buehlmann et al., 2020c) of ants demonstrated a direct role of the mushroom bodies in visual navigation. Although both studies focused explicitly on vision-based navigation, they nonetheless lay the path for future studies assessing multisensory integration explicitly.

Finally, Strausfeld et al. (2009) proposed that the mushroom bodies of arthropods are functionally equivalent to the vertebrate hippocampus. Indeed, the hippocampus appears to support configural learning in mammals (Sutherland and Rudy, 1989), although its precise role is still uncertain (Whishaw and Tomie, 1991). If the proposed configural representation of a shelter in whip spiders were a result of multisensory integration in the mushroom bodies, it would provide support for Strausfeld et al.'s (2009) idea of some functional equivalence between the mushroom bodies of arthropods and the vertebrate hippocampus.

Acknowledgements

We thank two anonymous reviewers who were instrumental in improving an earlier draft of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: K.A.S.F., D.D.W., E.A.H., V.P.B.; Methodology: K.A.S.F., D.D.W., E.A.H., V.P.B.; Formal analysis: K.A.S.F., D.D.W., V.P.B.; Investigation: K.A.S.F.; Data curation: D.D.W., V.P.B.; Writing - original draft: K.A.S.F., D.D.W., E.A.H., V.P.B.; Writing - review & editing: K.A.S.F., D.D.W., E.A.H., V.P.B.; Supervision: V.P.B.; Project administration: D.D.W.; Funding acquisition: D.D.W., V.P.B.

Funding

We are grateful for financial support from the National Geographic Society and the National Science Foundation (IOS 1457304).

Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.238444.supplemental>

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