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I, Elizabeth C Kozak , hereby submit this original work as part of the requirements for the degree of Master of Science in Biological Sciences.

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Cognitive cross-modal integration in a wolf spider, Schizocosa ocreata (Hentz) (Lycosidae)

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Cognitive cross-modal integration in a wolf spider, *Schizocosa ocreata* (Hentz) (Lycosidae)

A thesis submitted to
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Abstract

Across animal species, males convey important information to potential mates through signals in multiple sensory modalities. In order to choose the best possible mate, female receivers must accurately perceive and assess male multimodal signals, especially when those signals occur simultaneously with those of other males. Cross-modal integration, i.e., cognitive binding of information transmitted in more than one sensory signal mode, is therefore important in animal communication, especially in complex, noisy environments in which many signals overlap. However, it is currently unknown how perception of multiple, disparate male signals plays a role in female mate choice decisions, especially for invertebrates. Males of the brush-legged wolf spider *Schizocosa ocreata* (Hentz) use multimodal communication (visual and vibratory signals) in courtship. Because female *S. ocreata* may be courted by multiple males at the same time, they must evaluate co-occurring male signals originating from separate locations. Moreover, due to environmental complexity, individual components of male signals may be occluded, altering detection of sensory modes by females. While experiments with live spiders and video playback have shown that female *Schizocosa ocreata* wolf spiders display receptivity to males courting in either isolated signal mode and show increased receptivity for multimodal courtship, it is unknown whether this is the case when females are presented with a choice between multimodal vs. isolated unimodal male courtship signals, and how these preferences are affected by disparity between signals. I first used digital multimodal playback to present females with a choice between 1) isolated unimodal (visual or vibratory), 2) multimodal vs. vibratory, and 3) multimodal vs. visual male courtship signals. I next used digital multimodal playback to investigate the effect of spatial and temporal disparity of visual and vibratory components of male courtship signals on female mate choice, and presented females with male courtship signals consisting of components that varied in spatial location or temporal synchrony. When presented with a choice between either isolated unimodal male courtship signal (visual or vibratory), there was no significant difference in the average number of receptive displays directed to either male signal. When presented with a choice between a multimodal male courtship signal and a vibratory male courtship signal, females directed, on average, significantly more receptive displays to the multimodal signal. However, when presented with a choice between multimodal and visual-only male courtship signals, there was no significant difference in receptivity directed by females to either signal, in contrast with the prediction generated from previous research. Females responded to spatially disparate signal components separated by ≥ 90° as though they were separate sources, but responded to slightly disparate signals separated by ≤ 45° as though they originated from a single source. Responses were seen as evidence for cross-modal integration. Temporal disparity (synchrony) in signal modes also affected female receptivity. Females responded more to male signals when visual and vibratory modes were in synchrony than either out-of-synch or interleaved/alternated. These findings are consistent with those seen in both humans and other vertebrates, and provide insight into how animals overcome communication challenges inherent in a complex environment.

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Introduction

In human communication, cognitive integration of information transmitted in more than one sensory mode (e.g., acoustic and visual cues), known as cross-modal integration, is essential for accurate perception of complex signals such as speech (Bee & Micheyl 2008). Integration of these multimodal signals in humans is thought to contribute to language acquisition (Aboitiz & Garcia 1997; Giraud et al. 2001), learning and memory formation (Freides 1974), and to reading ability (Rose et al. 1999), in addition to serving as a compensatory sensory mechanism in both the blind and the deaf (Kujala et al. 2000). Cross-modal integration is especially important when perceptual interference arises as the result of multiple signals occurring simultaneously, known as "the cocktail party problem" (Bee & Micheyl 2008; McDermott 2009). Although well-studied in humans, cross-modal integration and cognitive processing have only recently garnered attention in animal communication research (Shettleworth 2001). Research on cross-modal integration in animals has concentrated primarily on neurophysiology of receiver sensory capacity (Stern-Tomlinson 1981; Benedek et al. 2004; Narayan et al. 2007; Schmidt, & Römer 2011; Nagarah et al. 2011) and signal production (Fuster et al. 2000). Behavior studies have concentrated largely on vertebrate recognition of and preference for conspecific signals, but not in the context of mate choice (Martin-Malivel & Fagot 2001; Narins et al. 2005; Lombardo et al. 2008; Proops et al. 2009; Velez & Bee 2010; Lampe & Andre 2012; Bee 2012; Taylor 2014).

Across many animal species, males convey information on species identity, health and parental care ability to potential mates through signals in a variety of sensory modalities, including acoustic, visual, chemical, and vibratory modes (Candolin 2003; Michaelidis 2006; Murai & Backwell 2006). In order to choose the best possible mate, female receivers must be able to accurately perceive and assess male multimodal signals, especially when those signals occur simultaneously (Candolin 2003; Bee & Micheyl 2008; McDermott 2009; Richardson & Lengagne 2010; Taylor 2014). However, it is currently unknown how perception of multiple, disparate male signals plays a role in female mate choice decisions (Miller & Bee 2012; Ronald et al. 2012), especially for invertebrates, whose comparatively simple nervous systems are assumed to be less capable of such a cognitive process. However, mounting evidence of plasticity in invertebrate behavior (Bushman 1999; Hopper 2003), as well as higher-level cognitive processes, e.g., learning, decision-making and risk-balancing behaviors (Jackson et al. 2001; Wullschleger & Nentwig 2002; Li et al. 2003), suggest otherwise. As such, invertebrate models are providing insights to mechanisms of cognitive processes in simpler nervous/neural systems (Hochner et al. 2003; Giurfa 2003; Jackson & Li 2004; Hochner et al. 2006; Nagarah et al. 2011).

Among invertebrate models, the well-studied wolf spider *Schizocosa ocreata* is an excellent organism for the study of cross-modal sensory integration, particularly in the context of mate choice. They perceive the world via multiple sensory inputs, e.g., eight eyes and myriad vibration sensors on eight legs, and communicate in multiple modes (Uetz 2000). Males produce courtship signals in both visual (active tapping, raising and extending the first pair of legs) and vibratory (production of substratum-borne vibration by stridulation and percussion) modes (Stratton & Uetz 1981, 1983; Scheffer et al. 1996; Uetz 2000; Gibson & Uetz 2008). Males have demonstrated plasticity in signaling based on the substrate and the amount of available light (Taylor et al. 2005, 2006; Gordon & Uetz 2011), indicating they may be compensating for attenuated signal transduction in the complex environment in which they live (Cady 1984; Scheffer et al. 1996; Uetz et al. 2013). Additionally, they exhibit eavesdropping and signal matching behavior (Clark et al. 2012), demonstrating a level of behavioral complexity and cognitive processing similar to that seen in some vertebrate animals (Peake et al. 2005; Phelps et al. 2007). In the field, a female likely encounters several males throughout the breeding season (Cady 1984), and multiple males may court a single female simultaneously (Clark et al. 2012), hence the synchrony of signal components in different modes, either spatial and/or temporal, may affect female perception of male signals. In order to assess potential mates in such complex environments, females must be able to determine from which direction male signals arise, and discriminate between them, regardless of modality. Depending on the ability of their nervous system to integrate these signals, any disparity in signal modality or synchrony may affect female evaluation of males, their ultimate choice of mate, and thus fitness.

This M.S. thesis research seeks to answer the question of whether evidence for cognitive cross-modal integration (rather than in the context of neuro-sensory processing) can be found in the behavior of female *Schizocosa ocreata*, and if so, how that might affect females' choice of mate. The hypotheses to be tested are: 1) female *S. ocreata* preferences for male courtship signals depend on the modality in which females perceive male signals; 2) female *S. ocreata* cognitively integrate male courtship signals differently depending on the degree of congruence (spatial or temporal) between signals; and 3) this integration affects female *S. ocreata* mate choice decisions. *Schizocosa ocreata* makes an excellent model organism for asking these questions, because females in the field are potentially courted by multiple males signaling in multiple sensory modalities in a physically complex environment, similar to the conditions under which cross-modal integration has been found in vertebrates.

While experiments with live spiders as well as video playback have shown that female *S. ocreata* display receptivity to males courting in either isolated signal mode and show increased receptivity for multimodal courtship (Uetz & Roberts 2002; Taylor et al. 2006; Gibson & Uetz

2008; Uetz et al. 2009), it is unknown whether this is the case when females are presented with a choice between multimodal vs. isolated unimodal male courtship signals. Consequently, before testing for cognitive cross-modal integration in *Schizocosa ocreata* and female evaluation of disparate male courtship signals, it was essential to establish a digital multimodal video/vibration playback apparatus and a set of research protocols for testing multi- vs. unimodal choice (Chapter 1). This new multimodal playback choice apparatus was then used to test the hypotheses above and examine cross-modal integration of both spatially and temporally disparate courtship signals from visual and vibratory modes (Chapter 2). This research will shed light on how animals overcome the challenges inherent in communicating in complex environments, and in a larger context, whether certain complex cognitive processes may be possible even in comparatively simple neuro-sensory systems.

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Chapter 1

Male courtship signal modality and female mate choice in the wolf spider, *Schizocosa ocreata*

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Abstract

Across animal species, males convey important information to potential mates through signals in multiple sensory modalities. In order to choose the best possible mate, female receivers must be able to accurately perceive and assess male multimodal signals, especially when those signals occur simultaneously with those of other males. However, it is currently unknown how perception of multiple, disparate male signals plays a role in female mate choice decisions, especially for invertebrates. While experiments with live spiders and video playback have shown that female *Schizocosa ocreata* wolf spiders display receptivity to males courting in either isolated visual or vibratory signal modes and show increased receptivity for multimodal courtship, it is unknown whether this is the case when females are presented with a choice between multimodal vs. isolated unimodal male courtship signals. We used digital multimodal playback to present females with a choice between 1) isolated unimodal (visual or vibratory), 2) multimodal vs. vibratory, and 3) multimodal vs. visual male courtship signals. When presented with a choice between isolated unimodal male courtship signals (visual or vibratory), there was no significant difference in the average number of orientations, approaches or receptive displays directed to either male signal. When presented with a choice between a multimodal male courtship signal and a vibratory male courtship signal, females directed, on average, significantly more orient, approach and receptive behaviors to the multimodal signal. However, when presented with a choice between multimodal and visual-only male courtship signals, while there were significantly more orients and approaches to the multimodal signal, there was no significant difference in receptivity directed by females to either signal. This contrast with predictions generated from previous research illustrates the importance of testing preference behavior using

a choice paradigm, as female preferences likely depend on the context (e.g. environmental context, social context) in which they are presented with male signals.

Introduction

Animal communication, especially in the context of courtship displays, often utilizes multiple sensory modalities (acoustic, visual, chemical, vibratory). In some cases, multimodal signals may contain different information within each sensory mode (multiple messages, Partan & Marler 1999, 2005). Alternatively, the information contained within these multiple sensory signals may be redundant (Møller & Pomiankowski 1993), with each mode acting as a backup signal to the other, as both encode the same information (Johnstone 1996; Hebets & Papaj 2005). This may be the result of selection for signals that enhance detection and/or perception in complex sensory environments (Candolin 2003; Partan & Marler 2005), or for signals that enforce honesty from the signaler (Hebets and Papaj 2005). Studies in the past decade have focused on categorizing the function and form of multimodal signals across animal taxa (anurans: Taylor et al. 2007; bowerbirds: Doucet & Montgomerie 2003; swordtails: Hankison & Morris 2003; spiders: Scheffer et al. 1996; Hebets & Uetz 1999; Elias et al. 2005; Uetz et al. 2009), demonstrating support for the above classifications of multimodal signals.

The brush-legged wolf spider *Schizocosa ocreata* (Hentz) (Lycosidae) is a well-studied model for questions of multimodal communication. Males court females using multimodal courtship displays, which consist of visual signals (tapping, raising and extending the first pair of legs; tufts of bristles on the forelegs), accompanied by vibratory signals (substratum-borne vibration produced by stridulation and percussion) (Stratton & Uetz 1981, 1983, 1986;

McClintock & Uetz 1996; Uetz 2000). These vibratory and visual components appear to be redundant, as females display receptivity to males courting in either mode when isolated and display enhanced receptivity to multimodal male signals (Scheffer et al. 1996; Hebets & Uetz 1999; Uetz 2000; Gibson & Uetz 2008; Uetz et al. 2009), which lends support for the backup signal hypothesis for the function of these multimodal male signals (Uetz et al. 2009). When signal components were isolated, it was found that females prefer larger tufts over smaller tufts in visual signals (McClintock & Uetz 1996; Uetz & Norton 2007), and prefer higher peak amplitudes and peak frequencies in the vibratory signal modality (Gibson & Uetz 2008).

However, some questions remain about the design of studies that utilize both video and vibratory playback. Previous work that has attempted to present females with multimodal male signals has either paired pre-recorded male visual signals with vibratory signals from live males (Hebets 2008) or has paired video with (unsynchronized) vibratory playback (Uetz & Roberts 2002). Moreover, this research was conducted without the benefit of current technologies (i.e., laser Doppler vibrometry) for calibration of vibratory playback. Additionally, these studies presented females with male signals using a no-choice paradigm, which raises the question whether female preferences for male signals would change in a different context (choice). Many studies of the role of multimodal courtship displays measure female preference, but not female mate choice (Wagner 1998; Hebets & Papaj 2005; Dougherty & Shukar 2015). For example, female mate preferences may differ or change depending on the context in which females perceive male courtship signals (Wagner 1998; Bateson & Healy 2005; Dougherty & Shukar 2015). Here we use a new method for digital multimodal playback to investigate whether female preferences for isolated and multimodal courtship signals vary depending on the manner in which signals are presented, i.e. in choice experiments.

Materials and Methods

Study species The Brush-legged wolf spider, *Schizocosa ocreata*, is a sexually dimorphic species found in deciduous leaf-litter habitat throughout the eastern United States (Dondale & Redner 1978; Stratton 2005). Immature *S. ocreata* spiders were collected in the field from the Cincinnati Nature Center Rowe Woods, Clermont County (39°7’31.15” N; 84°15’4.29” W) in the fall of 2011 and reared in simulated springtime conditions until maturity. Laboratory conditions were maintained at 23-25°C and relative humidity of 65-75%, and a 13:11 hour light:dark cycle to simulate late spring, when spiders mature. Spiders were maintained in the laboratory in individual cylindrical plastic deli containers (9cm diam. x 5cm ht.) with lids that visually isolated spiders. Spiders were fed twice each week with 3-5 small crickets (*Acheta domesticus*), and water was provided *ad libitum*. Female *S. ocreata* were tested approximately three weeks after reaching maturity, when they are at peak receptivity (Norton & Uetz 2005; Uetz & Norton 2007).

Experimental apparatus Video playback has been demonstrated as an effective method for presenting *Schizocosa ocreata* spiders with visual displays, since wolf spiders and jumping spiders perceive and react to video images as though they are real (Clark & Uetz 1990, 1993; McClintock & Uetz 1996; Uetz & Roberts 2002; Uetz & Clark 2013). Several methods have been employed to present spiders with vibratory signals (live spiders: Hebets & Uetz 1999; Gibson & Uetz 2008, Uetz et al. 2009; playback methods Uetz & Roberts 2002) each successfully meeting the needs for which it was designed. However, digital multimodal playback, especially in a choice paradigm, requires a method for vibratory playback that is appropriately scalable to video playback, small in size (i.e., two devices would need to fit in a 20 cm-diameter arena and provide a directional vibratory signal), and able to reliably transmit the same vibratory signal for multiple trials.

Piezoelectric actuators, or disc benders, contain a piezoelectric crystal between a copper and a porcelain disc that vibrates when voltage is applied across it—in this case, the voltage resulting from an audio signal being played through the crystal—fit all three above criteria. Male vibratory signals were transmitted via piezoelectric disc benders (APC International, Ltd. #20-1205) affixed flush with the poster board substrate of the trial arena using clear adhesive tape, and placed in the center-front of each iPod Classic® (Fig. 1.1). We used a 12mm diam. circular disc bender, as it was 0.23mm thick, and could therefore be placed in front of a video iPod®—to effectively pair its vibratory signal with the iPod's® video signal—and easily laid beneath a piece of paper, through which vibratory signals could be transmitted. Copy paper was placed over the entire area of the arena, on top of the disc benders but under the polycarbonate arena wall, such that spiders could perceive vibration from disc benders via the copy paper throughout the arena. Vibration signals from pre-recorded male *S. ocreata* courtship signals were delivered to the disc benders from an iPod® classic via an amplifier (Pyle model PTA2). Disc bender output was calibrated using a Laser Doppler Vibrometer (LDV, Polytech model PDV-100) and Raven (Cornell laboratory of Ornithology, version 1.3 Build 23) software to closely match the playback amplitude and frequency to original recordings from live male *S. ocreata* courtship, and to ensure that vibratory signals from each disc bender propagated throughout the area of the arena. Disc bender output was also measured over distance across the copy paper surface and matched to natural levels (Uetz et al. 2013), so that spiders would be able to perceive signal direction from attenuation patterns.

Male visual courtship signals were presented using two iPod Classics® inserted into slots cut into the poster board at 90° to each other such that the bottom of screens were flush with the arena substrate, and male video exemplars would be within females' line-of-sight. Video male exemplars represented the population mean for body size, leg tuft size, and courtship vigor as in many previous studies (McClintock & Uetz 1996; Uetz & Roberts 2002; Uetz & Norton 2007; Roberts et al. 2007; Roberts & Uetz 2008; Uetz et al. 2011; Clark et al. 2012), and their vibratory signals were synchronized when both signal modalities were presented together. Vibratory signals accompanying each exemplar were previously recorded on the video soundtrack (16bit; 48kHz) by a PCB Piezotronics ICP® accelerometer (PCB-352C23) via an amplifying signal conditioner (PCB –480). To minimize background noise, recordings were made in a sound-attenuating room. When presenting females with only male vibratory signals, the iPod® matching the disc bender displayed a blank leaf-litter background (which matched the background of the male video exemplar), as a control for the presence of the iPod itself as a possible visual stimulus to females.

Trials were conducted in a 20 cm-diameter, clear plastic polycarbonate, circular arena placed upon a 0.092m^2 (1ft^2) piece of poster board that rested on four 1 8cm-high granite "feet", all of which was situated in an anechoic chamber, effectively isolating the arena—and therefore female spiders--from extraneous environmental vibrations.

Experimental trials Females (N=81) were presented with one of three experimental treatments in which they had a choice between isolated unimodal signals (visual alone vs. vibratory alone, n=17), between a multimodal (visual + vibratory) and visual-alone signal (n=38), or between a multimodal and a vibratory -alone signal (n=26). Signal origin (left or right iPod$^{®}$) was varied at random between females to control for any side biases. All trials were conducted with females that were between 15-25 days mature, when females are at peak receptivity (Uetz & Norton 2007). Female hunger was controlled by feeding all females one 10-day old cricket 12-24 hours before trials were conducted. Each female was placed in the center of the experimental arena under a translucent plastic vial and allowed to acclimate for 1-2 minutes; during this time there was no playback of visual or vibratory signals. Trials commenced with the start of playback and the careful removal of the vial so as not to disturb the female; trials lasted 10 minutes and were video recorded and later scored for female detection (orientation, approach) and receptive (settle, tandem leg extend, slow turn/pivot) behaviors.

Statistical analyses All statistical analyses were performed using JMP ver. 10 (SAS Institute, Cary, NC, USA). Three major response variables (mean number of orientations, mean number of approaches, mean comprehensive receptivity score) representing spider behavior toward each iPod$^{®}$ screen in choice tests were analyzed using matched-pairs analysis. The comprehensive receptivity score was computed as a sum of the total number of receptive behaviors (tandem leg extend, slow turn/pivot, settle) the female exhibited toward each screen.

Results

When analyzing female responses to multimodal vs. visual-only male signals, three females were eliminated from analysis due to lack of any behavior or movement during the trial period. When females were presented with a choice between isolated vibratory and visual signals, there were no significant differences between any female behaviors directed to either unimodal signal (Table 1.1, Fig. 1.2). Matched-pairs analyses yielded significant differences in mean number of orient, approach, and receptivity behaviors for treatments presenting multimodal male courtship signals against either unimodal male courtship signal (Table 1.1). Females oriented to and approached multimodal male courtship signals significantly more often than they did unimodal male courtship signals, and were significantly more receptive to multimodal signals than to isolated vibratory -only signals (Figs. 1.3, 1.4). However, there was no significant difference in receptivity to isolated visual signals compared to multimodal signals once outliers were removed from analysis.

Discussion

This study set out to investigate the effect of unimodal vs. multimodal courtship signals of male *Schizocosa ocreata* wolf spiders on female mate choice using a new method for synchronized digital multimodal playback. Previous work in this species had tested female preferences for male courtship signals without presenting females with a choice between those signals, leaving unknown if or how female preferences would change when presented with options to choose from when selecting a mate. Results indicate that female *Schizocosa ocreata* preferences for male courtship signal modality may be dependent on the context in which they are perceived, and confirm the utility of this new method for presenting spiders with digital multimodal playback. When presented with a choice between male courtship signals, females displayed no preference for either individual signal mode, but significantly preferred multimodal courtship signals over isolated vibratory male signals, and tended to prefer multimodal signals over isolated visual signals. Females also detected multimodal signals more quickly than isolated signals, indicating that multimodal courtship signals may help in compensating for any loss of signal due to environmental complexity (Taylor et al. 2009; Gordon & Uetz 2011; Uetz et al.

2013). Finally, because we have found further evidence of equivalence in female *S. ocreata* preferences for individual modalities of male courtship signals (visual, vibratory), our study also lends additional support for the backup signal hypothesis for the function of these redundant signals (Johnstone 1996; Candolin 2003; Hebets & Papaj 2005; Uetz et al. 2009).

These results demonstrate the importance of testing for female preferences under different contexts, e.g, when females are offered a choice vs. no-choice paradigm (Wagner 1998; Dougherty & Shukar 2015). Wagner (1998) defined female mate choice as "differential mating by females as a result of the interaction between environmental conditions, mating preferences, and sampling strategies", which means it is possible that female responses may be different in a choice paradigm that more closely mimics conditions in the field, than when females are not given a choice of stimuli to respond to. For example, female satin bowerbirds *(P. violaceus*) change which male display trait they prefer depending on the age of the female and the stage of the mate choice process the female is in (Coleman et al. 2004). However, if female bowerbirds in that study had only been tested once and were all the same age, variation in preference for male traits might not have been clear, and preference for only a single trait would likely have been found. In this study, females displayed more receptivity to multimodal signals over isolated vibratory signals. However, when presented with a choice between multimodal signals and visual signals, this strong preference relaxed, perhaps because a visual signal was present in both choices. Because females' preference for multimodal male signals varied depending on the signal modality it was paired with (visual or vibratory), this might indicate a possible hierarchy of preference, with multimodal signals as most preferred, followed in order by visual signals and vibratory signals. These results differ slightly from those of earlier, preference-based studies, which found equivalency or redundancy of the visual and vibratory modes in multimodal signals when females make mating decisions (Gibson & Uetz 2008; Uetz et al. 2009; Gordon & Uetz 2011). These results thus demonstrate the importance of choice paradigms when investigating female preferences for male sexual characters (Dougherty & Shukar 2015).

Acknowledgements

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Table 1.1: Matched-pairs analysis of mean Orient, Approach, and Comprehensive Receptivity behaviors exhibited by females with a choice between Multimodal and vibratory-only (Vis/Vib v Vib), Multimodal and visual-only (Vis/Vib v Vis), or vibratory-only and visual-only (Vis v Vib) male courtship signals. P-values in bold are significant at alpha level 0.05.

Treatment	Response	t-ratio	DF	p-value
Vis/Vib v Vib	Orient	-3.21996	22	**0.0039**
	Approach	-1.89929	22	**0.0354**
	Receptivity	-2.62681	22	**0.0154**
Vis/Vib v Vis	Orient	-3.36844	34	**0.0019**
	Approach	-3.2432	33	**0.0027**
	Receptivity	1.103569	32	0.278
Vib v Vis	Orient	0	16	1
	Approach	-0.33282	16	0.7436
	Receptivity	-0.43295	16	0.6708

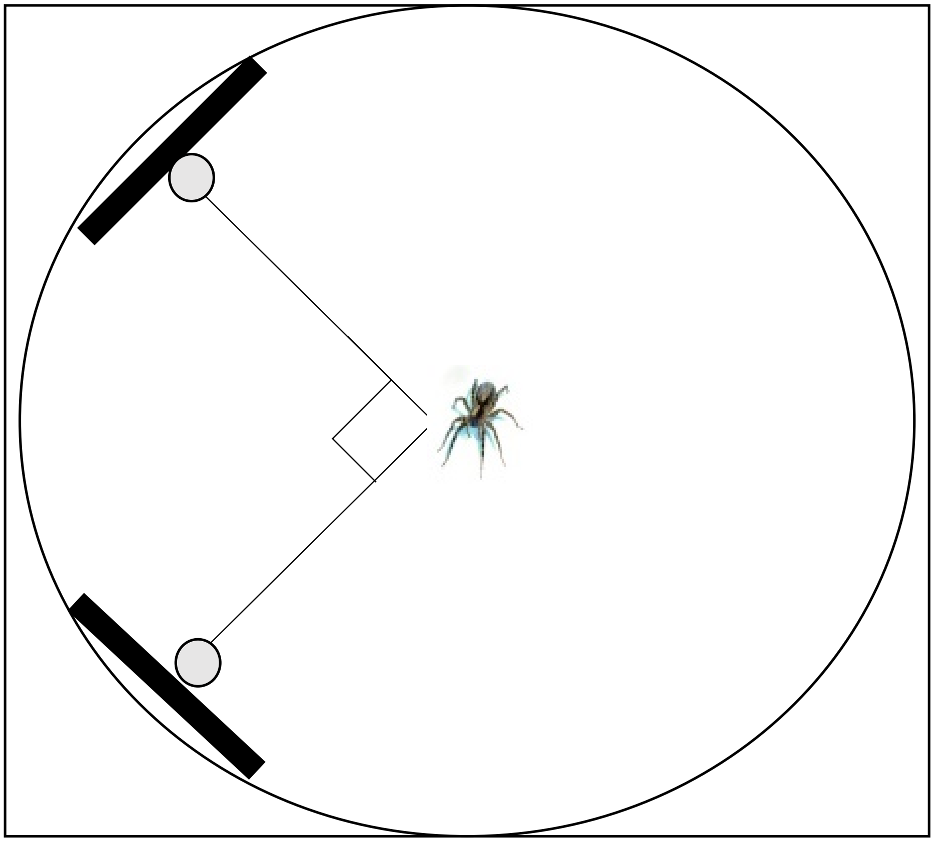


Figure 1.1: Experimental arena for female choice trials. Black rectangles signify iPod Classics®, grey circles represent disc benders.

Figure 1.2: Mean number of female behavioral responses (± S.E.) to unimodal visual and to unimodal vibratory male courtship signals: a) orientations; b) approaches; c) receptivity displays.

Figure 1.2a:

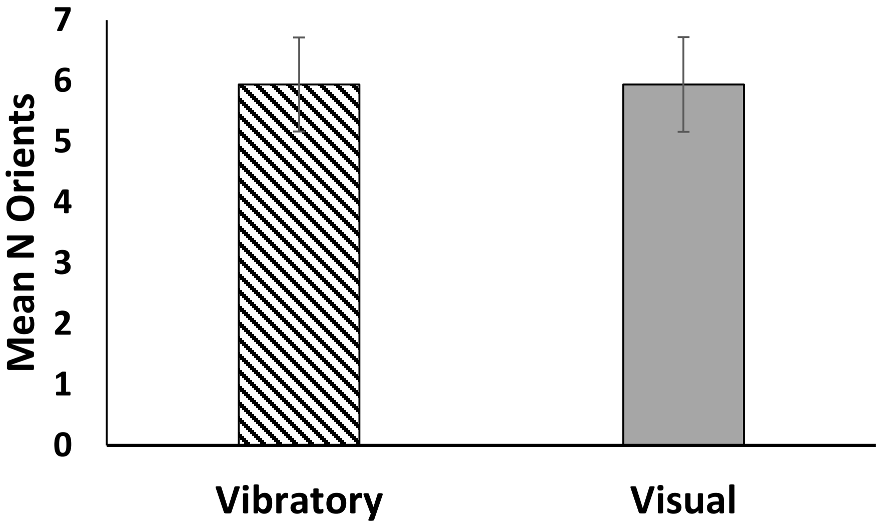
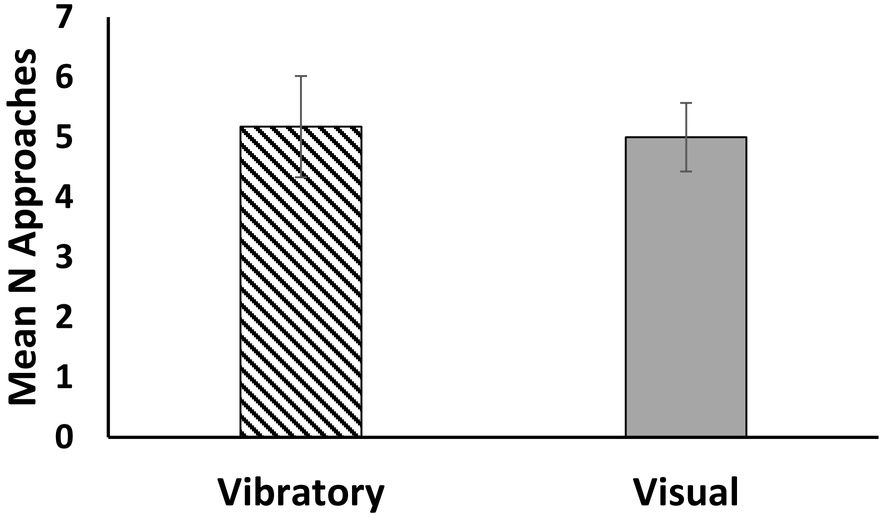


Figure 1.2b:



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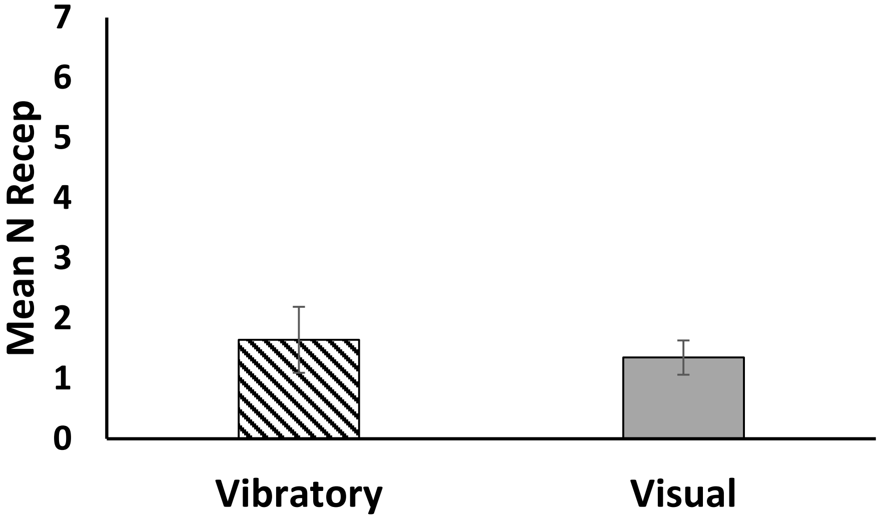
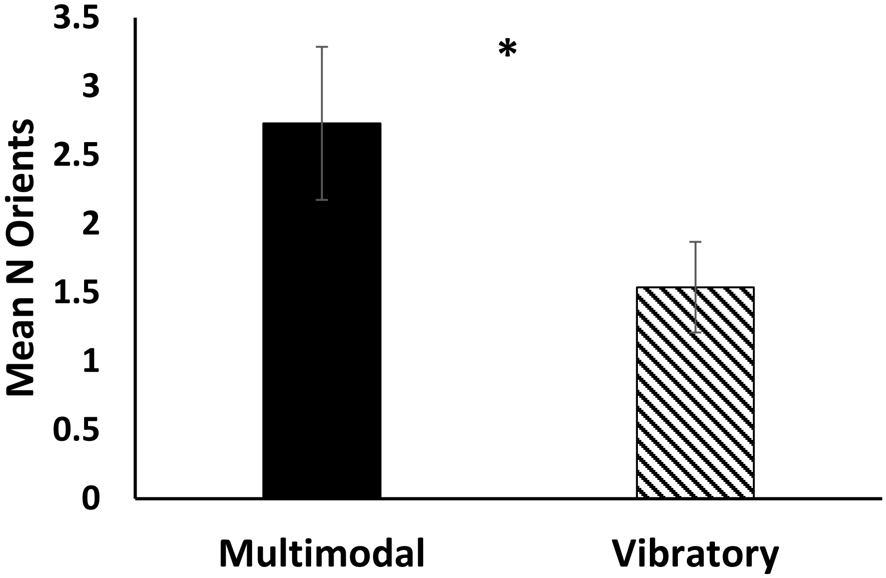


Figure 1.3: Mean number of female behavioral responses (± S.E.) directed to multimodal and to unimodal vibratory male courtship signals: a) orientations; b) approaches; c) receptivity displays.

Figure 1.3a:



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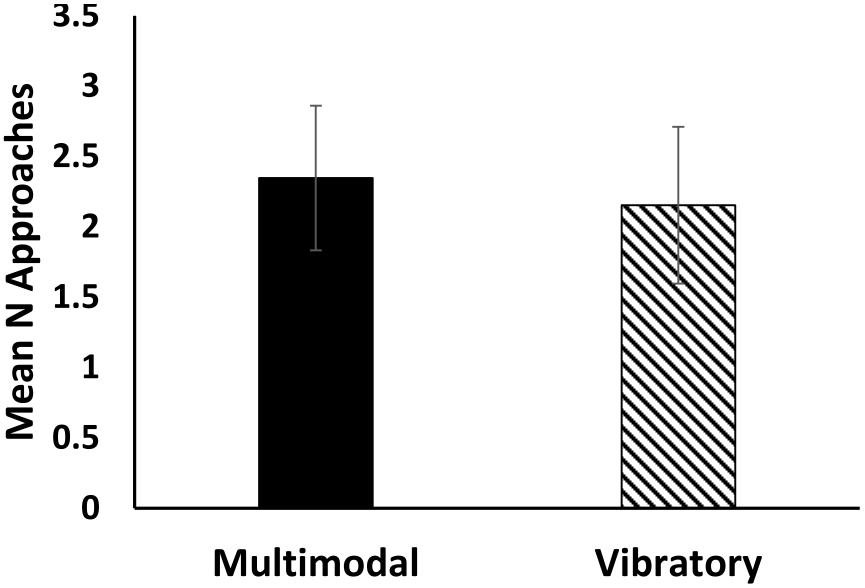

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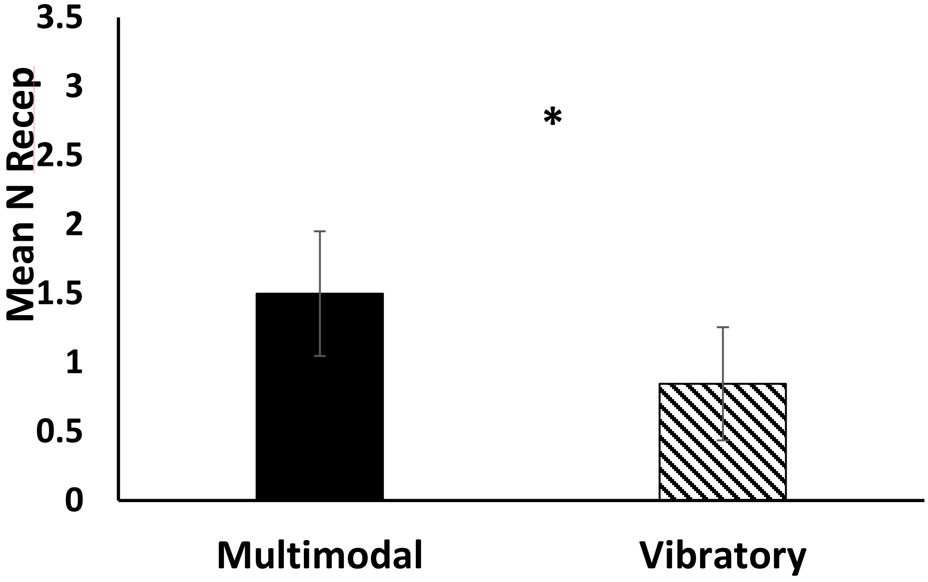


Figure 1.4: Mean number of female behavioral responses (± S.E.) directed to multimodal and to unimodal visual male courtship signals: a) orientations; b) approaches; c) receptivity displays.

Figure 1.4a:

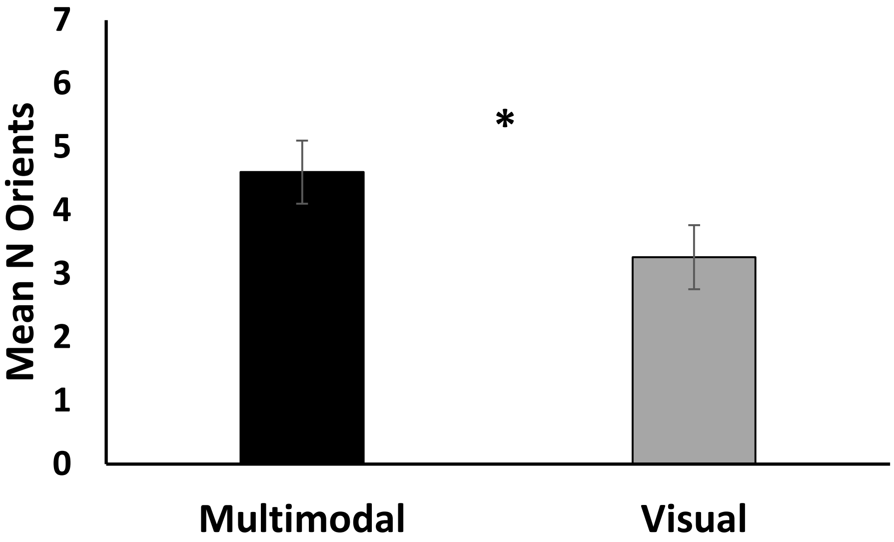
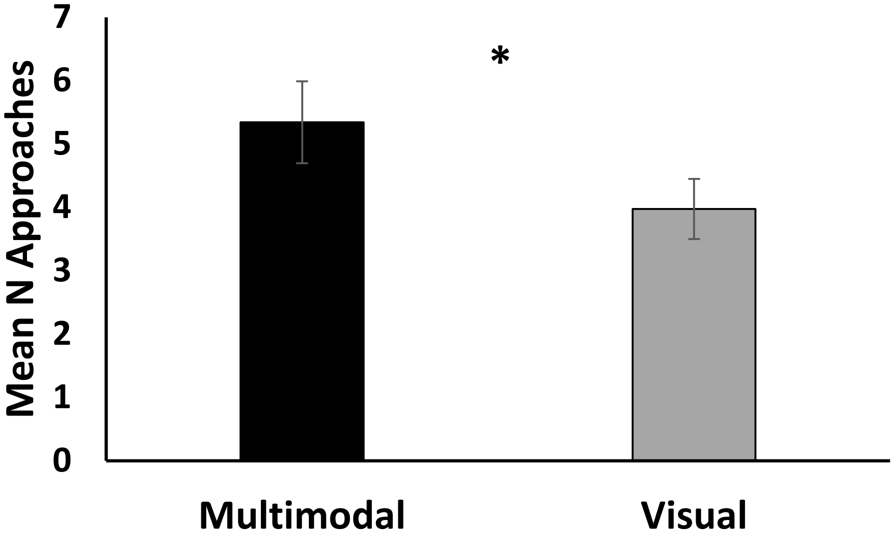


Figure 1.4b:



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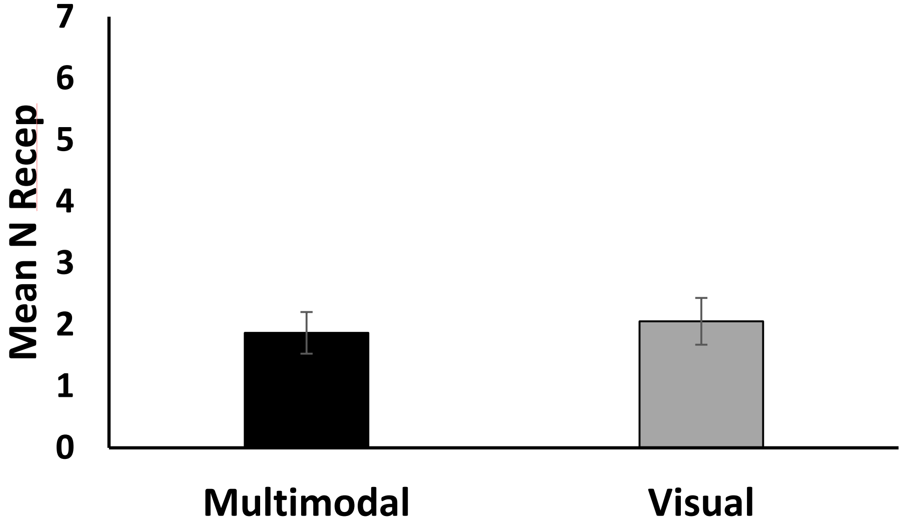

Chapter 2

Cognitive cross-modal integration of multimodal courtship signals in a wolf spider

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Abstract

Cross-modal integration, i.e., cognitive binding of information transmitted in more than one sensory signal mode, is important in animal communication, especially in complex, noisy environments in which many signals overlap. Males of the brush-legged wolf spider *Schizocosa ocreata* (Hentz) use multimodal communication (visual and vibratory signals) in courtship. Because females may be courted by multiple males at the same time, they must evaluate co-occurring male signals originating from separate locations. Moreover, due to environmental complexity, individual components of male signals may be occluded, altering detection of sensory modes by females. We used digital multimodal playback to investigate the effect of spatial and temporal disparity of visual and vibratory components of male courtship signals on female mate choice. Females were presented with male courtship signals with components that varied in spatial location or temporal synchrony. Females responded to spatially disparate signal components separated by ≥ 90° as though they were separate sources, but responded to slightly disparate signals separated by ≤ 45° as though they originated from a single source. Responses were seen as evidence for cross-modal integration. Temporal disparity (synchrony) in signal modes also affected female receptivity. Females responded more to male signals when visual and vibratory modes were in synchrony than either out-of-synch or interleaved/alternated. These findings are consistent with those seen in both humans and other vertebrates, and provide insight into how animals overcome communication challenges inherent in a complex environment.

Introduction

In human communication, cognitive binding of information transmitted in more than one sensory mode (e.g., acoustic and visual cues), known as cross-modal integration, is important in perception and/or localization of complex signals (Bee & Micheyl 2008; Miller & Bee 2012;

Ghazanfar 2013). The innate nature of cross-modal binding of auditory and visual signals in human speech is often illustrated by lip-reading in noisy environments (Sumby & Pollack 1954), the "McGurk effect" (McGurk & MacDondald 1976) created by combined visual and auditory input, and the "ventriloquism effect" (Hauser 1996), in which co-occurring signals slightly offset in space or time are perceived to be a single, synchronous multimodal signal originating from a single location. This cognitive process is less well-known in animals, despite the fact that the ability to accurately perceive multimodal signals may have high fitness consequences. For example, in courtship and mating, receivers need to be able to perceive multimodal signals and integrate the information they contain in order to localize the sender and respond appropriately (Miller & Bee 2011; Taylor et al. 2011). This is especially important when a signal from one individual occurs simultaneously with signals of others (Bee & Micheyl 2008; McDermott 2009; Taylor et al. 2011). Although well-studied in humans, cross-modal integration and cognitive processing have only recently garnered attention in animal communication research (Shettleworth 2001; Narins et al. 2005; Taylor et al. 2011), with a focus on neurophysiology of receiver sensory capacity (Fuster et al. 2000; Narayan et al. 2007; Schmidt & Römer 2011) and signal production (Lombardo et al. 2008; Vélez & Bee 2010; Bee 2012), but almost exclusively in vertebrates (but see VanderSal & Hebets 2009).

Across many species, males convey information on mate quality through a variety of sensory modalities, i.e. acoustic, visual, chemical, and vibratory (Candolin 2003; Michaelidis et al. 2006; Murai & Backwell 2006). In order to choose the best possible mate, females must be able to accurately perceive and assess male signals in different modalities, and determine their location (Candolin 2003; Michaelidis et al. 2006; Murai & Backwell 2006; Bee & Micheyl 2008; McDermott 2009; Richardson & Lengagne 2010). However, it is currently unknown how perception of multiple, disparate male signals plays a role in signal localization and female mate choice decisions (Miller & Bee 2012; Ronald et al. 2012). This is especially true for invertebrate animals, for which cross-modal integration is largely unstudied.

Although cross-modal integration in animals has recently been studied in a few vertebrate models (Martin-Malivel & Fagot 2001; Narins et al. 2005; Hoke et al. 2007; Lombardo et al. 2008; Proops et al. 2009; Lampe & Andre 2012), invertebrates have been considered too neurologically simple to possess more complex cognitive mechanisms other than simple responses to stimuli. There is, however, mounting evidence of flexibility in invertebrate behavior (Bushman 1999; Hopper 2003), as well as the possibility of higher cognitive processes, e.g., risk-balancing behavior (Jackson et al. 2001; Wullschleger & Nentwig 2002; Li et al. 2003). As such, invertebrate models are providing insights to mechanisms of cognitive processes in so-called simple nervous/neural systems (Giurfa 2003; Hochner et al. 2003; Jackson & Li 2004; Hochner et al. 2006; Nagarah et al. 2011).

Among invertebrate models, the well-studied wolf spider *Schizocosa ocreata* is an excellent organism for the study of sensory integration. They detect environmental stimuli via multiple sensory inputs (e.g., eight eyes and myriad vibration sensors on eight legs), and communicate in multiple sensory modes (Uetz 2000; Taylor et al. 2006; Uetz et al. 2009). Males produce courtship signals in both visual (active tapping, raising and extending the first pair of legs – see Uetz 2000; Delaney et al. 2007 for details) and vibratory (production of substratum-borne vibration by stridulation and percussion – see Stratton & Uetz 1981, 1983; Scheffer et al. 1996; Gibson & Uetz 2008 for details) modes. These signals may be redundant, as female *S. ocreata* display receptivity to males courting in either isolated signal mode (Scheffer et al. 1996; Gibson & Uetz 2008; Uetz et al. 2009). Males have demonstrated plasticity in signaling based on the substrate and the amount of available light (Taylor et al. 2005, 2006; Gordon & Uetz 2011), indicating they may be compensating for attenuated signal transduction in the complex environment in which they live (Uetz et al. 2013). Additionally, they exhibit eavesdropping and signal matching behavior (Clark et al. 2012), demonstrating a level of behavioral complexity and cognitive processing similar to that seen in some vertebrate animals (Peake et al. 2005; Phelps et al. 2007).

Female *S. ocreata* likely encounter several males throughout the breeding season (Cady 1984), and may be courted simultaneously by multiple males (Clark et al. 2012; Uetz, pers. obs). Because the complex leaf litter environment may obscure or degrade visual and vibratory signals (Uetz et al. 2013), females may receive signals from multiple males in different sensory modes from different locations. Consequently, we investigated how female *S. ocreata* integrate spatially and temporally disparate male signals in multiple sensory modes (visual and vibratory), and how that affects mate choice decisions.

Methods

Study species

Immature *S. ocreata* spiders were collected in the field from the Cincinnati Nature Center Rowe Woods, Clermont County (39°7'31.15" N; 84°15'4.29" W) in the fall of 2012. Spiders were reared in the laboratory in individual cylindrical plastic deli containers (9cm diam. x 5cm ht.) with lids. Spiders were fed twice each week with 3-5 small crickets (*Acheta domesticus*), and water was provided *ad libitum*. Laboratory conditions were maintained at 23-25^{0}C and relative humidity of 65-75%, and a 13:11 hour light:dark cycle. Females (N=185 in all) were tested approximately three weeks after reaching maturity, i.e., during peak receptivity (Uetz & Norton 2007).

Ethical Note

To our knowledge, no animal welfare laws or regulations in the USA or the State of Ohio govern the use of invertebrates such as spiders in research. Wherever possible, we adhered to the “Guidelines for the treatment of animals in behavioural research and teaching” (Animal Behaviour 85 (2013) 287–295) of the Animal Behavior Society. At the end of this study, spiders were either transferred to another researcher in the lab for further study, or ultimately humanely euthanized with CO_2 and freezing.

Experimental apparatus

Trials were conducted in a 20 cm-diameter, clear plastic polycarbonate, circular arena placed upon a black granite base (30.48cm x30.48cm x 3.81cm). Sorbothane® (Isolate it! #0510131-30-4-PSA) rubber bumpers underneath the granite served to effectively isolate the base from extraneous environmental vibration. Piezoelectric disc benders (APC International, Ltd. #20-1205) were affixed flush with the granite using adhesive tape, and Reynolds Wrap® parchment paper was placed over the entire area of the arena, on top of the disc benders but under the polycarbonate arena. Vibration signals were delivered to the disc benders from an iPod® touch via a pre-amp (FiiO #EO6) and amplifier (Pyle model PTA2). Disc bender output was calibrated using a Laser Doppler Vibrometer (LDV, Polytech model PDV-100) and Raven bioacoustics software (Cornell laboratory of Ornithology, version 1.3 Build 23) to closely match the playback amplitude and frequency to original recordings from live male *S. ocreata* courtship. In addition, disc bender output was measured to assure that directional signal attenuation over distance across the parchment paper surface matched natural levels (Uetz et al. 2013). A single iPod Touch® was placed at one end the arena such that the bottom of the screen was flush with the top of the granite base, in a notch cut into the granite. Disc benders were placed at different angles in a 360° array around the inside circumference of the arena, creating a range of potential angles (measured from the position of females at the center of the arena at the start of a trial) for vibration source separation from the iPod® (Fig. 1).

Experimental trials

All trials were conducted when females were between 15-25 days mature, when females are at peak receptivity (Uetz & Norton 2007). Female hunger was controlled by feeding all females one 10-day old cricket 12-24 hours before trials were conducted. Each female was placed in the center of the experimental arena under a translucent plastic vial and allowed to acclimate for 1-2 minutes; during this time there was no playback of visual or vibratory signals. Trials commenced with the start of playback and the careful removal of the vial so as not to disturb the female; all trials lasted 10 minutes and were video recorded from two perspectives: a) directly in front of, and b) directly above the arena (facing and aerial shots, respectively) using high definition digital camcorders (Sony #HDR-XR260V).

Digital video recordings of trials were scored for female signal detection (orientation latency, number of approaches) toward each stimulus location. Female receptivity toward a stimulus is indicated by specific display behaviors (slow pivot, tandem leg extend, settle) that would typically precede acquiescence to copulation (Stratton & Uetz 1981, 1983; Scheffer et al. 1996), and was scored as the sum of displays as in previous studies of this species (Uetz & Norton 2007; Uetz et al. 2009).

Spatial disparity: Experimental treatments

Females (N = 107) were presented with experimental treatments in a repeated measures design over the course of 4 days (1 trial/treatment/day); only those females that were tested in all four treatments were later included in analysis. Order of presentation of treatments was varied across the four groups, to which females were randomly assigned, in order to control for any effect the order of treatment presentation may have had. Treatments consisted of 4 disc bender positions relative to the iPod Touch®. Degree of separation between the iPod and the disc bender was measured in terms of the angle between them, rather than the linear distance between signals, because of the nearly 360° range of visual and vibration senses of lycosid spiders (DeVoe 1972; Rovner 1993), and conditions female *S. ocreata* likely experience in the field (Cady 1984; Uetz et al. 2013). All angles were measured from the center of the arena as above. Disc benders were placed at 0°, 45°, 90°, and 180° relative to the iPod Touch®; in the 45° and 90° treatments disc benders were placed on both sides of the arena, which allowed for presentation from either side of the arena and therefore controlled for any side bias (Fig. 2.1). In all treatments vibratory playback was synchronized with spider behavior in video playback.

Temporal disparity: Experimental treatments

These experiments were conducted in the same apparatus as spatial disparity experiments (above, Fig 2.1). Females (N = 78) were presented with each of three temporal disparity treatments in a repeated measures design over three consecutive days (1 trial/treatment/day); additionally females were sorted into one of three treatment order presentation groups, in which order of treatments females were presented with was varied, to control for both priming and habituation effects. Temporal disparity treatments consisted of an in synchrony (IS) stimulus, in which both visual and vibratory male signals were completely synchronous; an out of synchrony

(OS) stimulus, in which male vibratory signals were delayed by 1.2s; and an interleaved/alternating (IL) stimulus, in which male vibratory and visual signals were alternated in time such that there was no overlap between signals (i.e., with the vibratory signal commencing only after the visual signal completed, and vice versa as in Fig. 2.2).

Statistical analysis

A series of one-way ANOVA analyses (with repeated measures accounting for variation among individuals) were first performed on the three major response variables (orient latency, approach, comprehensive receptivity score) to test for any priming or habituation effects. As none were found, all data were pooled over time periods and the analysis was collapsed around treatment as the main effect, with the same main response variables.

Repeated measures ANOVA and subsequent matched-pairs analyses were run on the spatial disparity data. These analyses were followed by a series of one-way repeated-measures ANOVA with Tukey HSD post-hoc testing on responses to individual signal modes (visual- and vibratory-only signals) across treatments. The temporal disparity data set was subject only to repeated measures ANOVA with Tukey HSD post-hoc testing. An alpha level of $P < 0.05$ was held as the standard for statistical significance.

Results

Spatial Disparity Experiments

One-way ANOVA analyses (with repeated measures accounting for variation among individuals) showed no evidence of behavioral priming or habituation effects; i.e., neither order of treatment presentation nor day of trial were significant predictors of any response: Order of Treatment (latency to orient $F_{3,105} = 0.0404$; $p = 0.989$; number of approaches $F_{3,102} = 1.067$; p

$= 0.367$; comprehensive receptivity score $F_{3,109} = 0.076$; $p = 0.973$); Day of Trial (latency to orient: $F_{3,424} = 1.048$; $p = 0.371$; number of approaches $F_{3,424} = 0.429$; $p = 0.732$; comprehensive receptivity score $F_{3,424} = 0.539$; $p = 0.656$). As a consequence, data were pooled over time periods and the analysis was collapsed around treatment as the main effect, with orient, approach, and a comprehensive receptivity score as the main response variables.

One-way ANOVA analyses (with repeated measures as above) showed a significant effect of treatment on all response variables (latency to orient $F_{3,451} = 39.782$; $p < 0.0001$; number of approaches $F_{3,451} = 16.141$; $p < 0.0001$; comprehensive receptivity score $F_{3,451} = 28.574$; $p < 0.0001$) (Table 2.1). Subsequent one-way analyses compared responses to individual signal modes across treatments (multimodal, visual-only or vibratory-only) (Table 2.2, Fig. 2.3). Results showed no significant difference in latency to orient to the visual signal across treatments, but latency to orient to vibratory signals did vary significantly, with females orienting most slowly to vibratory signals separated from visual signals by 45° (Table 2.2, Fig. 2.3). Matched-pairs analysis showed there was no significant difference in the total number of approaches to either signal when separated by 180° (Table 2.1), otherwise females approached the visual signal significantly more often. When approach responses to individual signal modes were compared across treatments, approaches to either signal mode varied significantly (Table 2.2). Females tended to approach multimodal signals most often and least often to vibratory signals separated from visual signals by 45° (Fig. 2.4). There was a reduction in approaches to the visual signal when separated by ≥90° but an increase in approaches to the vibratory signal, with no significant differences seen between the 90° and 180° treatments for either visual or vibratory signal responses (Fig. 2.4).

Females were significantly more receptive to the visual signal in all treatments (Table 2.2), although this disparity decreased with increasing spatial separation of signal modes (Fig. 2.5). When receptivity to individual signal modes was compared across treatments, females were least receptive to vibratory signals separated by only 45° from visual signals (Fig. 2.5). Mean comprehensive receptivity score was highest for the multimodal signal, and not significantly different from the mean score for visual signals in the 45° treatment, but was significantly different from all other signals (Fig. 2.5). Females tended to exhibit increasing receptivity to vibratory signals as they became more spatially disparate from visual signals, and there was no significant difference between the 90° and 180° treatments in the mean level of receptivity directed to visual signals (Fig. 2.5).

Temporal Disparity Experiments

As in the previous experiment, repeated measures ANOVA showed no clear evidence of behavioral priming or habituation effects overall, as order of treatment presentation and day of trial were not significant predictors of female responses: Order of Treatment (latency to orient $F_{2,73} = 0.096$; $p = 0.909$; number of approaches $F_{2,73} = 317$; $p = 0.729$; comprehensive receptivity score $F_{2,73} = 0.343$; $p = 0.711$); Day of Trial (latency to orient: $F_{2,219} = 2.070$; $p = 0.129$; number of approaches $F_{2,219} = 1.214$; $p = 0.299$; comprehensive receptivity score $F_{2,219} = 0.669$; $p = 0.513$). As above, data were pooled across time periods and the analysis was collapsed around treatment as the main effect.

Latency of orientation to stimuli did not vary significantly with temporal disparity treatment (ANOVA: $F_{2, 219}=0427$, $p=0.669$). Likewise, female approaches to the stimuli did not vary significantly with treatment (ANOVA: $F_{2,219}=2.546$, $p=0.0807$) (Fig. 2.6). While some females were receptive to all three stimulus treatments, frequency of receptivity was not independent of temporal synchrony (Friedman's χ^2=6.25, df=2, p=0.0439). Female receptivity score (measured as sum of receptivity displays) varied significantly with treatment (ANOVA: $F_{2, 219}$=3.556, p=0.0302). Females displayed significantly higher levels of receptivity (Fig. 2.7) to the IS stimulus over both the OS and IL stimuli (Tukey's post-hoc tests, $\alpha < 0.05$).

Discussion

Results of these studies strongly suggest that female *S. ocreata* demonstrate cross-modal integration of spatially and temporally disparate visual and vibratory components of multimodal signals. It has previously been demonstrated in this species that while females are receptive to either courtship signal when unimodal (visual alone or vibratory alone), they exhibit greater levels of receptivity (enhancement) to multimodal signals (Uetz et al. 2009). Here, there was no significant difference in the mean level of receptivity directed to the visual signal in the 45° treatment and to either signal in the 0°/multimodal treatment, strongly indicating that females perceived the 45° visual signal as being multimodal. If this signal was not perceived as multimodal, there likely would have been reduced receptivity to the visual signal, and/or more behaviors would have been directed to the vibratory signal in that treatment. The standard test of a hypothesis of cross-modal binding, suggested by the "ventriloquism effect", is based on the prediction that disparate signals will be bound to the visual signal as the stronger stimulus (Alais & Burr 2004; Pages & Groh 2013), and that response behaviors will be directed to the origin of the visual signal. Here female *S. ocreata* directed the majority of their responses in the 45° treatment to the visual signal, and responded to that signal as though it were multimodal. Females thus behaved in a manner indicating cross-modal binding of spatially separate signals, as suggested by the ventriloquism effect and previous tests for cross-modal integration (Narins et al. 2005).

In contrast, female *S. ocreata* appeared to recognize signals separated by ≥90° as arising from distinct individuals. Females oriented to and approached both signals, indicating signal disparity did not affect detection or recognition of signals. They approached the visual or vibratory signal with similar frequency and there were no significant differences in the level of receptivity directed to either signal. Compared to the multimodal signal, females displayed reduced receptivity to spatially disparate signals in a pattern similar to that seen with isolated unimodal (visual alone or vibratory alone) male courtship signals (Uetz et al. 2009). This suggests that females perceive spatially separate signals as coming from different sources.

With respect to temporal synchrony of signal modes, female responses are more difficult to interpret, as both signals originated from the same location. In this case, any differences in orientation or approach responses to individual signal modes would be lost. However, there is some indication that a temporal equivalent of the ventriloquism illusion might be in effect, even though there were no significant differences in female orientation and approach behaviors across treatments. It is clear that temporal binding affects the way females perceive male courtship signals, as females were significantly more receptive to signals with temporally synchronous components (IS) than to those with alternating (IL) signals. However, females showed no differences between the IS and OS treatments, suggesting that temporal binding was in effect for the OS treatment. However, in this case it is uncertain whether the overlap of visual and vibration signals might be perceived as a slightly longer multimodal signal (perhaps with an "echo") or as an atypical or even novel signal. Future experiments might include comparing treatments with overlapped signals with the visual component leading vs. one with the vibration component leading to fully parse out female perception of temporally disparate signals.

Signalers and receivers must both contend with environmental complexity, and it is possible that this may have influenced the evolution of cross-modal integration. Environmental complexity presents a challenge to animals attempting to communicate, as signal components may be occluded or altered, and thus the perception and/or interpretation of signals may be affected. A male whose signals reach the female without occlusion or alteration by the environment, or interference from another individual, would definitely have an advantage over males whose signals do. On the other hand, it is essential that a female be able to discriminate among multiple males, and in order to choose the best possible mate, must correctly attribute signals to the appropriate male.

To our knowledge, this is the first study to demonstrate cognitive binding of multimodal signals in an invertebrate, although evidence is mounting that spiders and other invertebrates possess more cognitive ability than given credit for. Previous studies have shown behavioral plasticity in this species (Taylor et al. 2006), as well as both learning and risk-balancing decision-making in other spider species (Jackson et al. 2001; Jackson et al. 2003; Skow et al. 2006). Taken together, results strongly indicate that spiders are capable of more complex perceptual and cognitive processes than had previously been thought.

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Table 2.1: Repeated measures matched-pairs analysis ANOVAs of spatial disparity data (N = 107).

Response		F-ratio	p-value
Orient Latency	Within pairs	44.6291	**<0.0001**
	Among pairs	40.8102	**<0.0001**
N Approaches	Within pairs	62.2697	**<0.0001**
	Among pairs	16.3633	**<0.0001**
Receptivity Score	Within pairs	38.9199	**<0.0001**
	Among pairs	29.0161	**<0.0001**

Table 2.2: One-way ANOVAs for individual signal modes (visual- or vibratory-only) across treatments in spatial disparity experiments (N =107).

Response	Signal	DF	F-ratio	p-value
Orient Latency	Visual	3, 422	0.8225	0.482
	Vibratory	3, 423	48.1664	**<0.0001**
N Approach	Visual	3, 422	5.50528	**0.0022**
	Vibratory	3, 422	39.9006	**<0.0001**
Receptivity Score	Visual	3, 422	9.3825	**<0.0001**
	Vibratory	3, 422	75.1745	**<0.0001**

FIGURE LEGENDS

Figure 2.1: Experimental arena for both spatial and temporal disparity trials. Small circles represent disc benders, black rectangle represents the iPod Touch®.

Figure 2.2: Diagram of temporal disparity treatments. Small black rectangles represent visual signals, below are oscillograms of the vibratory signals, placed according to the time of vibratory signal onset.

Figure 2.3: Matched-pairs analysis of mean latency (sec) to orient to spatially varied visual and vibratory signals (N = 107). Vertical error bars indicate one SEM. Letters over bars indicate significance across treatments by visual- or vibratory-only Tukey HSD post-hoc test of one-way repeated measures ANOVA ($\alpha=0.05$). All pairs were significantly different ($p<0.0001$).

Figure2. 4: Matched-pairs analysis of mean number of approaches females made to male courtship signals that varied by spatial disparity (N = 107). Vertical error bars indicate one SEM. Letters over bars indicate significance from Tukey HSD post-hoc test of one-way repeated measures ANOVA. Brackets over bars indicate outcome of matched-pairs analysis.

Figure 2.5: Matched-pairs analysis of mean comprehensive receptivity scores for spatially disparate male courtship signals (N = 107). Vertical error bars indicate one SEM. Letters over bars indicate outcome of Tukey HSD post-hoc testing of one-way ANOVA for visual-only and for vibratory-only data. All pairs within treatments were significantly different ($p<0.0001$).

Figure 2.6: Mean number of approaches females made to temporally disparate or synchronous male courtship signals (N = 78). Vertical error bars indicate one SEM. There were no significant differences.

Figure 2.7: Mean comprehensive receptivity score to multimodal video-vibratory playback for temporal disparity stimulus treatments (N = 78). Vertical error bars indicate one SEM (different letters indicate significance by Tukey's post-hoc test).

Figure 2.1

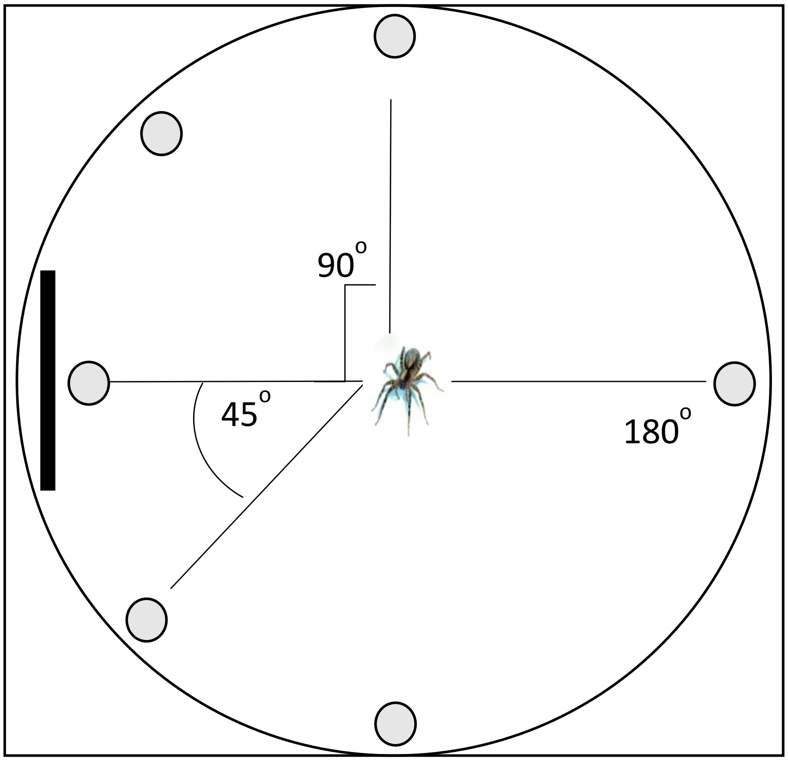


Figure 2.2

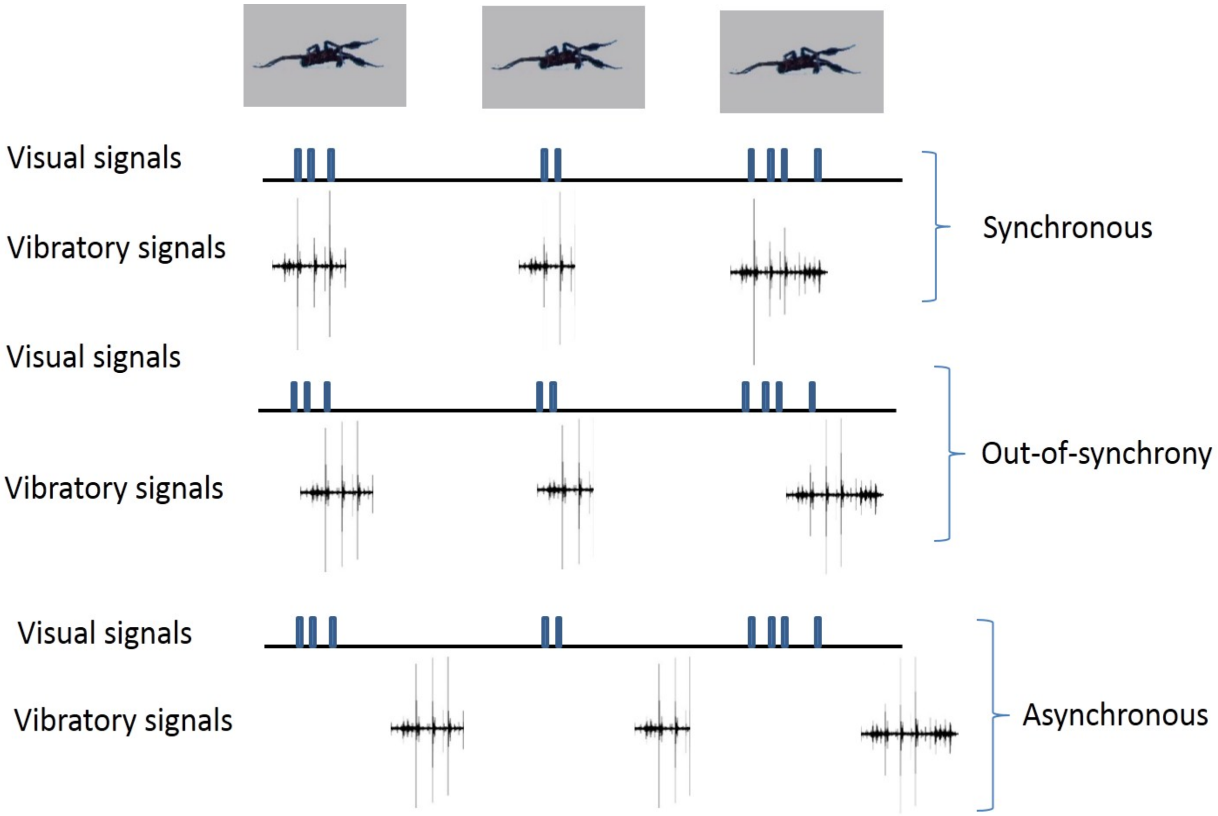

Visual signals
Vibratory signals
Synchronous
Visual signals
Vibratory signals
Out-of-synchrony
Visual signals
Vibratory signals
Asynchronous

Figure 2.3

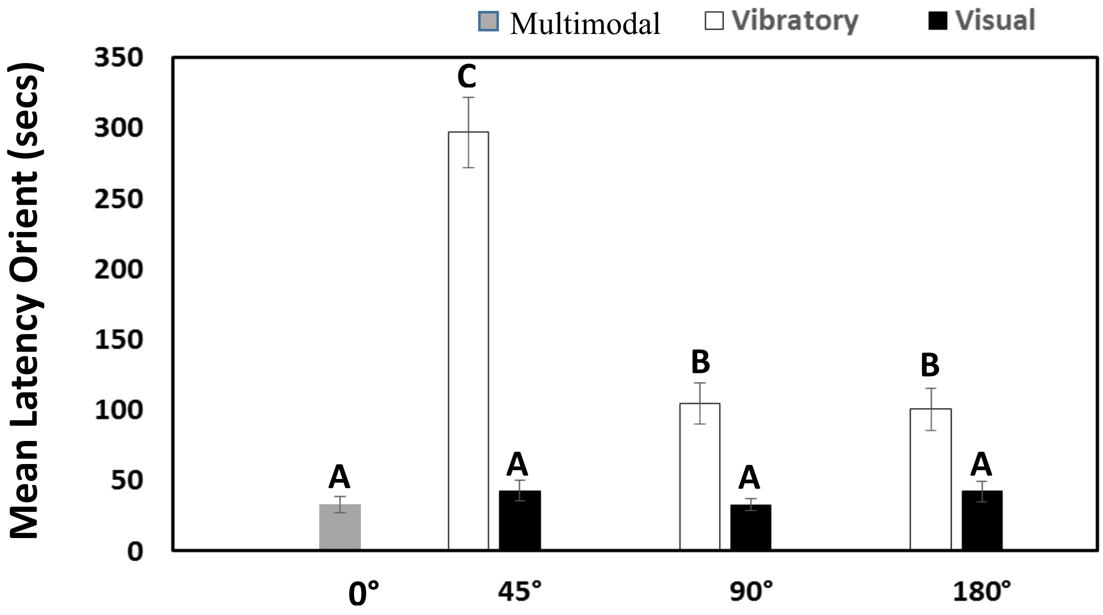

Multimodal
Vibratory
Visual
Mean Latency Orient (secs)
350
300
250
200
150
100
50
0
C
B
B
A
A
A
A
0°
45°
90°
180°

Figure 2.4

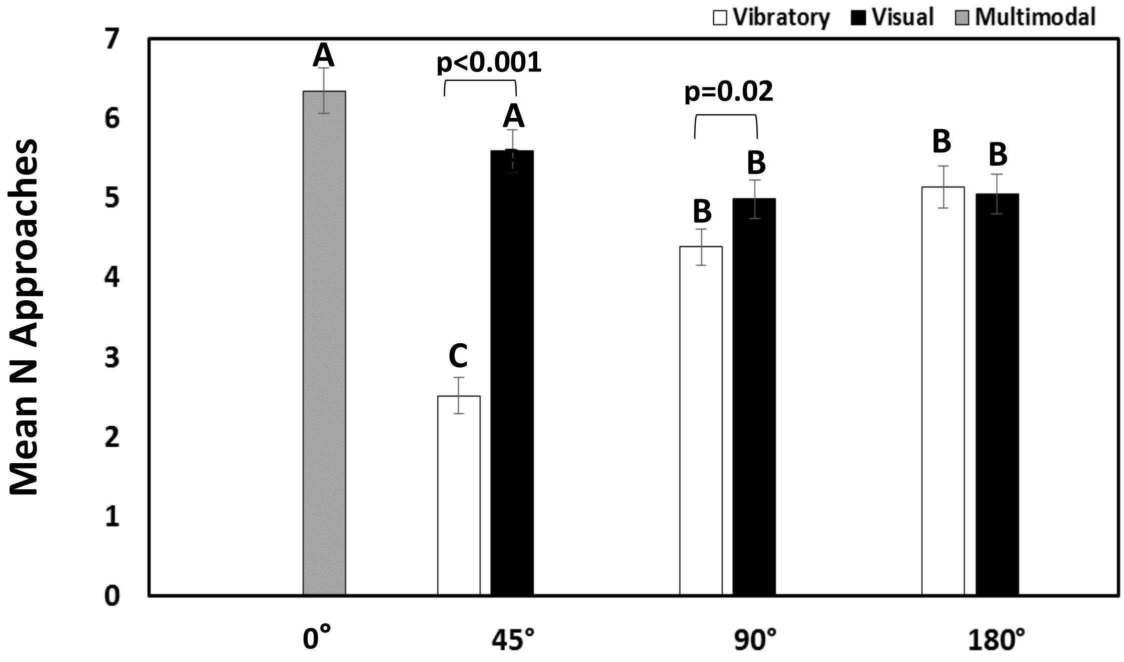
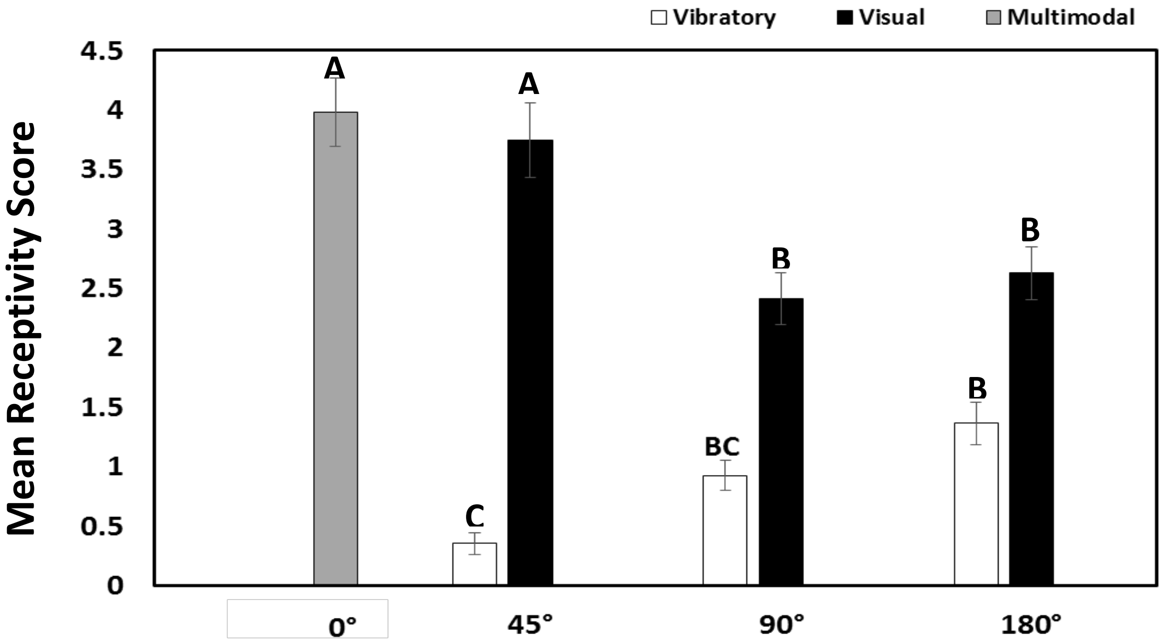

□ Vibratory ■ Visual ▨ Multimodal
Mean N Approaches
7
6
5
4
3
2
1
0
0°
45°
90°
180°
A
p<0.001
A
C
p=0.02
B
B
B
B

Figure 2.5



☐ Vibratory
■ Visual
▨ Multimodal
Mean Receptivity Score
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
A
A
C
B
BC
B
B
0°
45°
90°
180°

Figure 2.6

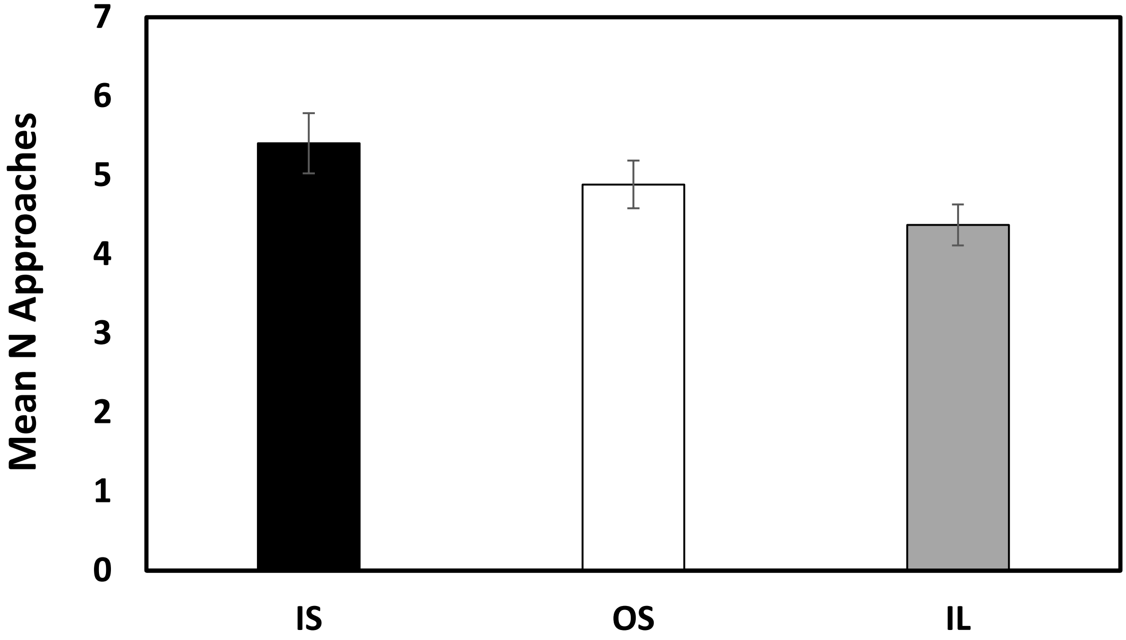
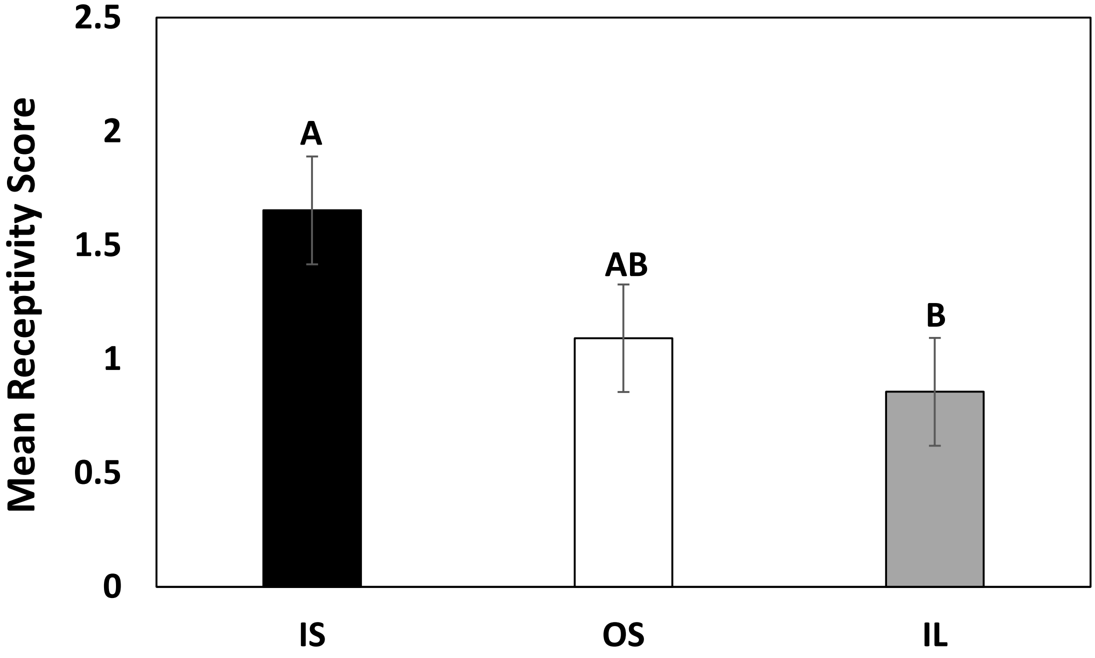


Figure 2.7



Summary and Conclusions

The main objective of this thesis research was to determine whether cognitive cross-modal integration could be found in the behavior of female *Schizocosa ocreata* wolf spiders, and if so how that might affect females' choice of mate. Cross-modal integration on a cognitive level is essential to communication and other cognitive processes across taxa (Garcia 1997; Giraud et al. 2001; Lewald & Guski 2003; Bee & Micheyl 2008), yet is understudied in invertebrates (and in the context of mate choice versus mate preference for both vertebrate and invertebrate species). Given the conditions under which cross-modal integration has been found in humans and vertebrate animal species, *Schizocosa ocreata* wolf spiders are an ideal organism in which to test for this cognitive process in an invertebrate, as females are courted by males in multiple sensory (visual and seismic/vibratory) signal modalities (Stratton & Uetz 1981, 1983; Scheffer et al. 1996; Uetz 2000; Gibson & Uetz 2008), live in an environment that is both physically and socially complex (Cady 1984; Scheffer et al. 1996; Clark et al. 2012; Uetz et al 2013), and exhibit female mate choice based on male characters and behaviors (Uetz & Norton 2007; Delaney et al. 2007; Gibson & Uetz 2008; Uetz et al. 2009).

In previous tests with vertebrate animals, cross-modal integration responses to multimodal signals that were disparate in either space or in time was seen as evidence of cognitive perception of those signals (for example, see Narins et al. 2005; Lombardo et al. 2008; Taylor et al. 2014). Because of this, testing for cross-modal integration in *S. ocreata* needed to involve a clear perception by females of a choice between male signals. It was therefore necessary to establish a baseline for female mate choice behavior when signals were separate in space. While female perception and receptivity to visual and/or vibratory components of male courtship signals is well-established (Uetz & Roberts 2002; Taylor et al. 2006; Gibson & Uetz

2008; Uetz et al. 2009), whether responses remain the same in a choice context using digital playback is unknown. Additionally, a new method for presenting digital multimodal signals to spiders was required in order to present two (or potentially more) multimodal signals simultaneously. Chapter 1 of this thesis addressed these methodological issues and tested the hypothesis that *S. ocreata* female preferences for male courtship signals depend on the modality in which females perceive male signals. This was done by establishing both a new apparatus for presenting digital multimodal signals (visual and vibratory) and a new baseline for female response behaviors in a choice paradigm. Consequently, piezoelectric disc benders were paired with iPod$^{©}$ devices to present females with a choice between male *S. ocreata* courtship signals in differing sensory modalities. Previous work had found that females display receptivity to either unimodal male courtship signal (visual OR vibratory) but exhibit enhanced receptivity to multimodal male courtship signals (visual AND vibratory) (Uetz & Roberts 2002; Taylor et al. 2006, Gibson & Uetz 2008; Uetz et al. 2009). Therefore, it was predicted that these responses would hold within a choice paradigm: i.e., there would be no significant difference in receptivity to isolated unimodal (visual OR vibratory) signals, but females would be significantly more receptive to multimodal signals over either unimodal signal. The spiders in this study behaved as predicted when presented with a choice between isolated unimodal male courtship signals from either mode, as there was no significant difference in the average number of receptive displays directed to either the visual or the vibratory male signal. When presented with a choice between a multimodal male courtship signal and a vibratory male courtship signal, females directed, on average, significantly more receptive displays to the multimodal signal, again as predicted. However, when presented with a choice between multimodal and visual-only male courtship signals, there was no significant difference in receptivity directed by females to either signal, in contrast with the prediction generated from previous research. This underscores the importance of testing preference behavior using a choice paradigm, as female preferences likely depend on the context (e.g. environmental context, social context) in which they are presented with male signals (Wagner 1998; Johnson & Basolo 2002; Murphy 2012). Additionally, results indicate there may be some degree of difference or equivalency between multimodal and visual-only male *S. ocreata* courtship signals, which raises questions of both comparative evaluation and multimodal communication in mate choice (Partan & Marler 1999, 2005; Bateson & Healy 2005).

The responses of females in choice tests (Chapter 1) provided a baseline to use when making predictions about female responses to disparate male courtship signals, an experimental requirement when testing for cognitive cross-modal integration (Chapter 2). The emerging standard when testing for this cognitive process is to present individuals with multimodal signals in which the individual component modalities are disparate in space or in time to varying degrees (Narins et al. 2005, Lombardo et al. 2008, Taylor et al. 2011, for e.g.). Integration of the signal is considered to have occurred if the individual responds to disparate signals as though they are congruent, known as 'the ventriloquism effect' (Lewald & Guski 2003). When presented with a choice between male courtship signals that differed in signal modality and were separated in space by ≥ 90°, females were observed to approach both signals, but to approach and display receptivity significantly more often to multimodal signals (nearly two times as often compared to unimodal signals), followed by visual signals and vibratory signals. Thus, when females were presented with spatially or temporally disparate multimodal male courtship signals (Chapter 2), it was predicted that females would approach and direct receptivity to all signals, but would only display enhanced receptivity to signals perceived as congruent/multimodal (thus demonstrating cognitive cross-modal integration), and this would be seen in signals that had less than 90° spatial separation between them. Concurrently, it was predicted that female receptivity would depend more on female perception of male courtship signals, and hence on each females' integration of the male courtship signals she encounters.

Using a modified version of the apparatus designed for Chapter 1, female *S. ocreata* were presented with courtship signals of male *S. ocreata* that varied in the degree of either spatial (0°, 45°, 90°, and 180°) or temporal (synchronous, out of synchrony, and alternating/asynchronous) congruence between signal modes, in a repeated measures design. Female responses were largely as predicted, and there was no significant difference in the mean level of receptivity directed to the visual signal in the 45° treatment and to either signal in the 0°/multimodal treatment, strongly indicating that females perceived the 45° visual signal as being multimodal. If this signal was not perceived as multimodal, there likely would have been reduced receptivity to the visual signal, and/or more behaviors would have been directed to the vibratory signal in that treatment. This is a demonstration of the "ventriloquism effect" in an invertebrate, as females directed their behaviors to the perceived origin of the signal, which the ventriloquism effect predicts is the visual stimulus location (Alais & Burr 2004; Pages & Groh 2013). Underscoring this conclusion is the contrast in behavior seen as more spatial disparity was introduced between male signals: females oriented to and approached both signals when separated by ≥90°, and there were no significant differences in the level of receptivity directed to either signal. Compared to the multimodal signal (0°/multimodal and 45°/visual), females displayed reduced receptivity to spatially disparate signals in a pattern similar to that seen with isolated unimodal (visual alone or vibratory alone) male courtship signals (Uetz et al. 2009).

This suggests that females perceive signals separated spatially by at least 90° as coming from different sources.

With respect to temporal synchrony of signal modes, female responses are more difficult to interpret, as both signals originated from the same location, meaning any differences in response to individual signal modes is lost. However, there is some indication that a temporal equivalent of the ventriloquism effect was at play, even though there were no significant differences in female orientation and approach behaviors across treatments, as females were significantly more receptive to signals with temporally synchronous components (IS) than to those with alternating (IL) signals. However, females showed no differences between the synchronous (IS) and out of synchrony (OS) treatments, suggesting that temporal binding was in effect for the OS treatment. Future experiments in which signals overlap more closely in time may be necessary to fully parse out female perception of temporally disparate signals.

Taken together, the results of experiments from chapters 1 and 2 develop a clear answer to the question behind this thesis, i.e., that cognitive cross-modal integration of male courtship signals is evident in *S. ocreata*. This demonstrates cognitive cross-modal integration of male courtship signals by female *S. ocreata*, and strongly suggests that the manner in which male signals are integrated by females affects their ultimate choice of mate. This means that if a female in the field is courted by multiple males simultaneously, there are advantages to being a male that the female can see and sense his vibratory signal, or alternatively a male that a female can only see, but is close enough to another courting male to "appropriate" his vibratory signal. This also allows both the signaling male and the receiving female to compensate for any occlusion or loss of signal due to the physical complexity of the environment.

In a larger context, this is the first time, to this author's knowledge, cognitive cross-modal integration of multimodal signals has been tested for in an invertebrate. The unspoken assumption has historically been that the comparatively simple neural systems of invertebrates were unable to carry out the processing required for integration of sensory signals (with the possible exception of the molluscan class Cephalopoda). However, limiting the approach of questions of cognitive processing to a narrow neurophysiological view excludes any possibility for adaptive convergence on certain cognitive processes. If the environmental and social conditions for some processes (e.g. communication, mate choice) are similar across taxa, then there may be a way for "simple" neural systems to produce the same cognitive results as those of more "complex" animals. The results of this study exemplify this point - despite having a brain smaller than the size of a pinhead, *S. ocreata* nevertheless demonstrate cognitive cross-modal integration, possibly as the result of adaptations to overcome or compensate for the challenges of perceiving multimodal signals in complex environments.

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