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Ogre-Faced, Net-Casting Spiders Use Auditory Cues to Detect Airborne Prey

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Summary

Prey-capture behavior among spiders varies greatly from passive entrapment in webs to running down prey items on foot. Somewhere in the middle are the ogre-faced, net-casting spiders [1] (Deinopidae: Deinopis) that actively capture prey while being suspended within a frame web [2–5]. Using a net held between their front four legs, these spiders lunge downward to ensnare prey from off the ground beneath them. This "forward strike" is sensorially mediated by a massive pair of hypersensitive, night-vision eyes [5–7]. Deinopids can also intercept flying insects with a "backward strike," a ballistically rapid, overhead back-twist, that seems not to rely on visual cues [4, 5, 8]. Past reports have hypothesized a role of acoustic detection in backward strike behavior [4, 5, 8]. Here, we report that the net-casting spider, *Deinopis spinosa*, can detect auditory stimuli from at least 2 m from the sound source, at or above 60 dB SPL, and that this acoustic sensitivity is sufficient to trigger backward strike behavior. We present neurophysiological recordings in response

to acoustic stimulation, both from sound-sensitive areas in the brain and isolated forelegs, which demonstrate a broad range of auditory sensitivity (100–10,000 Hz). Moreover, we conducted behavioral assays of acoustic stimulation that confirm acoustic triggering of backward net-casting by frequencies in harmony with flight tones of known prey. However, acoustic stimulation using higher frequency sounds did not elicit predatory responses in *D. spinosa*.We hypothesize higher frequencies are emitted by avian predators and that detecting these auditory cues may aid in antipredator behavior.

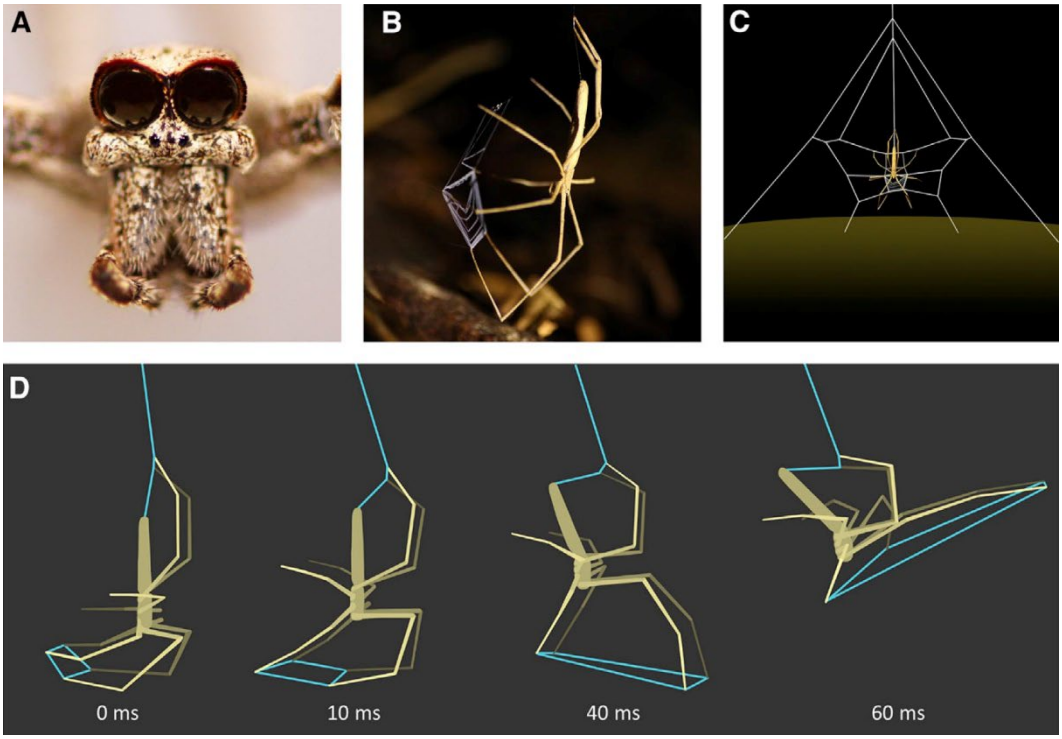

Figure 1. The Backward Strike Behavior of *Deinopis* Spiders. (A) Photograph depicting the massive eyes of *Deinopis* spiders, used in detecting prey items walking beneath their web at night. When visually occluded, spiders remain able to capture flying insects, though unable to capture prey off the ground. (B) Photograph of a *Deinopis* spider in foraging posture in its natural habitat. When hunting, spiders grasp a rectangular capture-net between their front four legs while looking down, face forward, at the substrate below. (C) Diagram of a typical frame web and net-casting spider when in foraging posture. The spider is suspended in mid-air while grasping the frame web (with its back pairs of legs) and the capture-net (with its front pairs of legs). (D) A time series of a backward strike, illustrated by overlaying still frames from a high-speed video recording (2,000 fps). This behavior is used to capture flying insects and has been previously hypothesized to be elicited via acoustic cues emitted by the flapping of insect wings. See Video S1 for high-speed video recording of a backward strike.

Methods

Experimental Model and Subject Details

All experiments were performed with *Deinopis spinosa* spiders (Araneae: Deinopidae). Juvenile male, juvenile female, and mature female spiders were used. Mature males were not used in this study as male *Deinopis* spiders no longer make nets following upon maturation and thus do not exhibit hunting behavior. All spiders used in this study were from the same population in Gainesville, Florida. Spiders used in the laboratory auditory experiments and neurophysiological recordings were collected from our field site and then brought back to our laboratory in the Department of Neurobiology and Behavior at Cornell University (Ithaca, New York). Spiders were individually housed in plastic cylindrical enclosures under a 12 h:12 h light:dark cycle, 60 ± 10% relative humidity, and at 24°C. Spiders were fed one cricket (*Acheta domesticus*) a week and allowed water ad libitum.

Method Details

Auditory neurophysiology from brain and isolated legs

We used previously established techniques for our neurophysiological recordings of isolated legs and intact brains [9–12]. All recordings were conducted on a vibration-isolating air table (Micro-G, Technical Manufacturing Corporation, Woburn, Massachusetts, USA) fitted with a custom-built wire-mesh Faraday cage and acoustic grid foam. For brain recordings, spiders were cold anesthetized and held in place using a specifically designed 3D-printed holder and Kerr dental sticky wax (58°C melting point; Syborn Kerr, Emeryville, California, USA) placed on the air table. Extracellular brain recordings were made using a 4MΩ glass-insulated tungsten electrode (Micro-Probes, Gaithersburg, Maryland, USA) passed through a small hole in the cuticle and directed into the vicinity of the arcuate body [9, 12]. This part of the brain is thought to be one of the main cites of multisensory integration in spiders [11]. Recording location was based on external morphological features readily identified under a stereomicroscope (Wild M3Z Leica Microsystems GmbH, Wetzlar, Germany; maximum magnification of 800×), with electrode placement guided by stereotactic micromanipulators (MM-3, Narishige International USA, East Meadow, New York, USA). Once in place, the electrode was advanced using a digital hydraulic microdrive (Model 607W, David Kopf Instruments, Tujunga, California, USA). A second sharp tungsten electrode was inserted into the abdomen to serve as a ground.

For isolated leg recordings, front legs were dissected off at the trochanter-femur joint and held in place between two 2 mm–wide wooden dowels. The proximal end of the leg, where the dissection took place, was inserted into a well with Ringer's solution, which was held in place by Kerr dental sticky wax. Extracellular recordings were made using a sharpened tungsten wire as an electrode, which was etched by being placed in a potassium hydroxide solution while passing a current through the wire. The tungsten electrode was then inserted into the metatarsal leg segment. A second tungsten electrode was inserted into the well of Ringer's solution to serve as a ground.

Electrical activity from both types of recording was amplified by an extracellular headstage (Model 1800 A-M Systems, Sequim, Washington, USA) and a differential AC microelectrode amplifier (amplified 10,000×; bandpass filtered 100 Hz–5,000 Hz with a 60 Hz notch; Model 1800 A-M Systems, Sequim, Washington, USA). This analog signal was then converted into a digital signal (NI PCI-MIO-16E-1, National Instruments, National Instruments, Austin, Texas, USA) and recorded on a PC (Windows 7; 64-bit; Microsoft Corporation, Redmond, Washington, USA) using the data acquisition software Spike Hound [30] at 20,000 samples per second. Recordings often yielded one or two clearly distinguishable spiking units and the spike sorting program Wave_clus [15] was used to isolate responses of individual neural units by grouping candidate spike waveforms based on amplitude and coefficients of a wavelet decomposition.

Acoustic stimuli were generated using custom-written MATLAB programs and were played via a studio monitor speaker (Mackie HR824) driven by a stereo amplifier (Nikko NA-790). The speaker was located 2 m away from the animal. A calibrated ¼-inch microphone (model 4135, amplifier model 5935 Brüel & Kjær) was oriented toward the sound source and placed within 5 cm of the animal such that the distance between the source and the microphone was equal to the distance between the source and the animal. Microphone signals were converted from analog to digital and recorded in the same manner as the electrophysiological recordings.

To gather results similar to those depicted in Figures 2A and 2B, we acoustically stimulated spiders with 500 ms duration pure tones, repeated 16 times in a pseudorandom order at 80 dB SPL. Stimulus presentations varied in the step size of frequency interval and frequency ranges tested (Isolated leg recordings: $N = 2$ [100 Hz–2,500 Hz; in 100 Hz steps], $N = 1$ [2,000 Hz–10,000 Hz; in 500 Hz steps]; Brain recordings: $N = 4$ [100 Hz–1,000 Hz; in 100 Hz steps], $N = 5$ [100 Hz–2,500 Hz; in 100 Hz steps], $N = 3$ [2,000 Hz–13,000 Hz; in 500 Hz steps]). Response curves (Figures 2C, 2J, and S3) were created using a frequency with durations of 500 ms and 1 sec between each presentation; different amplitude values were presented in a pseudorandomized order. To create the isolate leg response curve depicted in Figure 2J, the full combination of frequencies (100 Hz–5,000 Hz, in 100 Hz steps) and amplitudes (~55–85 dB SPL, 10 dB SPL intervals) were tested, with each combination presented 16 times. To create the brain response curve in Figure 2C, a similar combination of frequencies (150 Hz–5,050Hz, in 100 Hz steps) and amplitudes (~50–90 dB SPL, 5 dB SPL intervals) were tested, with each combination presented 16 times.

We have adapted our methodology from previously published works [9, 10, 13, 14]. Importantly, Shamble et al. [10] utilized the same protocols, equipment, and acoustic stimuli used in the current study and tested whether neural activity detected could be due to equipment vibrations caused by the acoustic stimuli. Using a laser Doppler vibrometer, they found that while vibrations were detected at 94 dB SPL and above, vibrations in the equipment were not detected at 89 dB SPL. As such, our acoustic stimuli amplitudes were set, a priori, below 90 dB SPL to ensure that equipment vibrations were not affecting the neural responses here reported.

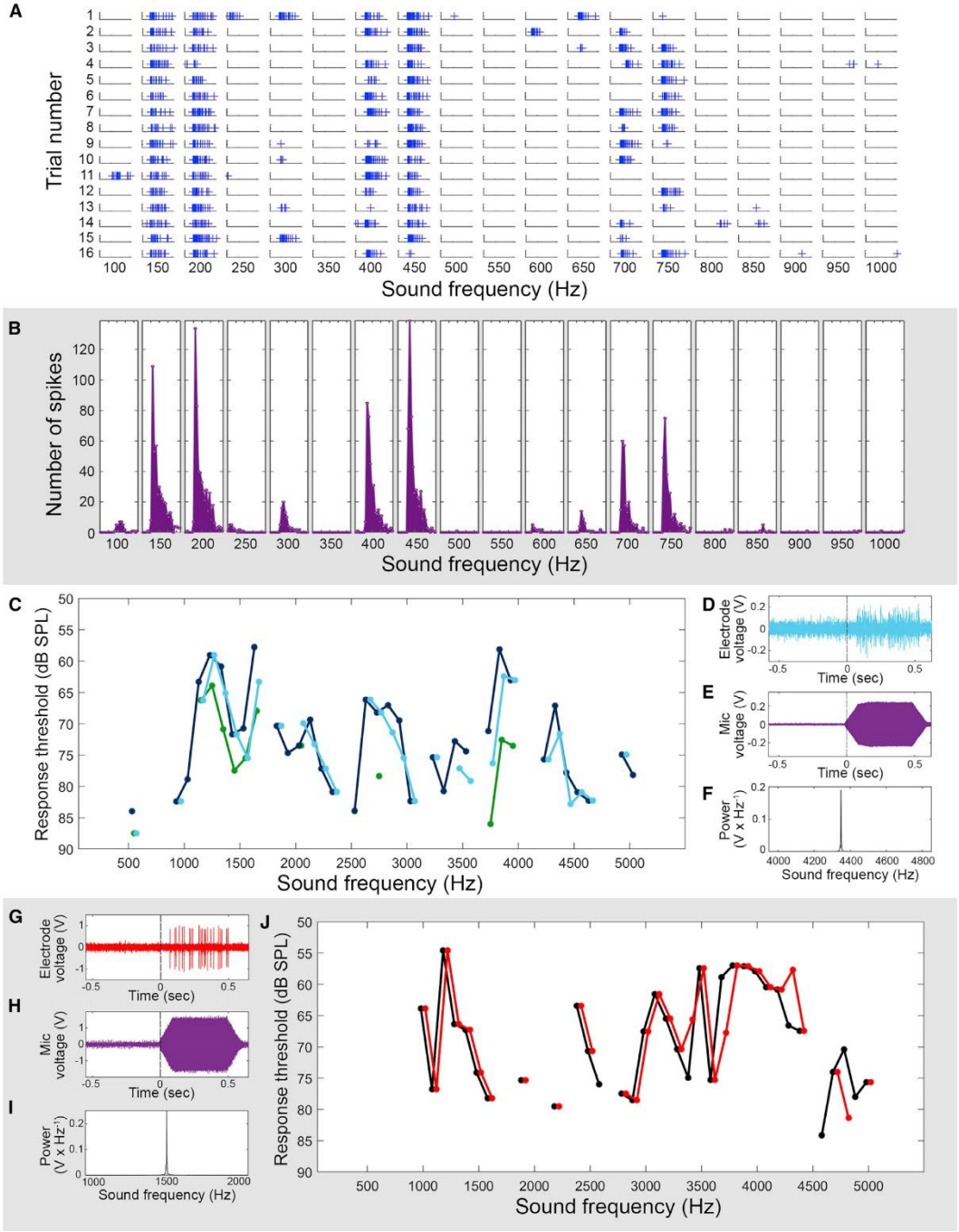

Figure 2. Extracellular, Microelectrode, Neural Recordings from the Brain and Isolated Legs of *Deinopis spinosa* Yield Evidence for Responsiveness to Auditory Stimulation. (A) Raster plots depicting neural spikes associated with detection of separate tonal frequencies. Plots are derived from a single brain recording of *D. spinosa*, where the spider was stimulated by pure tone frequencies from 100 to 1,000 Hz in 50 Hz steps. Each tone

was presented 16 separate times, in a pseudorandomized order. Each frequency bin depicts the 500 ms of stimulus presentation. (B) Spike histograms of the recording depicted in (A). Each binned frequency represents the sum of spikes over the 500 ms of stimulus presentation for all 16 trials. See Figures S1 and S2 for additional spike histograms of brain and foreleg recordings. (C) Response curves from a single *D. spinosa* extracellular brain recording. Response threshold (*y* axis) indicates the lowest sound intensity (dB SPL) required to elicit a significant response for each unit over the range of all tested frequencies (50 to 5,050 Hz, in 100 Hz steps). Three separate units are depicted (light blue, dark blue, and green). Brain recordings illustrate neural units with much overlap. As such, a 40 Hz jitter was used to aid in visually representing overlapping responses (–20 Hz dark blue, +20 Hz light blue). Gaps between solid lines indicate frequencies that failed to produce a significant response in any of the recorded units at any sound intensity ≤ 90 dB SPL. It is possible that spiders may respond to these frequencies at higher intensities, yet are untested because of limitations in our experimental facilities. See Figure S3 for an additional response curve of acoustic responses from a separate brain recording. (D) An unprocessed, extracellular brain recording before and during stimulus onset (dashed line). Activity shown in response to acoustic stimulus depicted in (E) and (F), prior to applying spike sorting algorithms. (E) Exemplar acoustic stimulus (3,850 Hz at 58.13 dB SPL) as recorded at microphone, used to construct the response curve shown in (C). Dashed line indicates stimulus onset. (F) Amplitude spectrum of acoustic stimulus from (E). (G) An unprocessed, extracellular leg recording before and during stimulus onset (dashed line). Activity shown in response to acoustic stimulus depicted in (H) and (I), prior to applying spike sorting algorithms. (H) Exemplar acoustic stimulus (1,500 Hz at 74.11 dB SPL) as recorded at microphone, used to construct the response curve shown in (J). Dashed line indicates stimulus onset. (I) Amplitude spectrum of acoustic stimulus from (H). (J) Response curves from a single *D. spinosa* extracellular leg recording. Response threshold (*y* axis) indicates the lowest sound intensity (dB SPL) required to elicit a significant response for each unit over the range of all tested frequencies (100 to 5,000 Hz, in 100 Hz steps). Two separate units are depicted (red and black). Both units share a broad sensitivity to tones ranging from 1,000 to 5,000 Hz, with significant overlap. As such, a 40 Hz jitter (–20 Hz black, +20 Hz red) was used to aid in visually representing overlapping responses. Gaps between solid lines indicate frequencies that failed to produce a significant response in any of the recorded units at any sound intensity ≤ 90 dB SPL, again because of experimental limitations, but it is possible that spiders may respond to these frequencies at higher, untested intensities. See Figure S3 for an additional response curve of acoustic responses from a separate leg recording.

Field behavioral assays

We visually located foraging *Deinopis spinosa* at night in Gainesville, Florida, for use in our field behavior assay. Spiders were used only if they had finished constructing a complete net, as deinopids do not hunt for prey without a net grasped in their front legs (unpublished data). Once a spider was located, we set up stimulation and recording equipment and began a trial. Spiders were used only once.

Stimuli consisted of previously made recordings of pure tones of 150 Hz, 400 Hz, 750 Hz, 2,300 Hz, and 4,400 Hz as well as pulses of white noise. Sound files for stimulation were created using Audacity software (V2.1.2). Each trial consisted of a single playlist that comprised 6 sound files at one sound file per frequency. Each sound file was 5 s long and

contained two tones (500 ms duration tones) of a given frequency, separated by 2 s of silence. Randomly assorted playlists were created such that each playlist began with 10 s of silence, followed by 6 randomly ordered sound files. As such, each spider was exposed to each tone and white noise in a randomized order. The prerecorded playlists were played from a Samsung cellular phone that was linked remotely to a Bluetooth speaker (JBL Flip 3, Los Angeles, California, USA), held at a distance of 1 m to the spider in its web. All auditory trials were video recorded using a GoPro HERO 4 (GoPro, San Mateo, California, USA) camera for later analysis.

Laboratory behavioral assays

Spiders were individually housed in testing arenas 48 h prior to testing. Arenas were made of a plastic cylinder placed atop a square of cardboard with a small vertical stick glued to the middle of the square. Arenas were constructed such that spiders would build webs in the middle of their arena, allowing for the removal of the wall of plastic cylinder that encircled the spider and stick immediately before the trials, without destroying their web structure. As long as these spiders remain relatively undisturbed, they will remain in their webs in hunting posture. Following removal of the plastic cylinder, no obstructions were present between the spider, its web, and the stimulus sound source.

Spiders that had completed the construction of their capture snare/net were chosen for behavioral trials. Prior to acoustic exposure, the plastic cylinder was lifted up and away from the spider and its web. To account for potential disturbances caused by this procedure, we let spiders settle for 5 min following removal of the cylinder. The same media devices and wireless speaker used in field trials were also used in laboratory trials, as were the GoPro HERO 4 cameras and sound file playlists. The speaker sound level and distance between the speaker and the spider were also held constant across field and laboratory assays. Nondisturbing, deep infrared light (920 nm) was used to illuminate testing arenas, as the GoPro cameras used to record laboratory behavior were modified to detect IR light. Using the wireless and Bluetooth capabilities of our equipment within our department building at Cornell University, we were able to conduct trials remotely (i.e., we were able to expose spiders to sounds and record their behavior without having to physically remain in the testing room during trials). Following acoustic stimulation, plastic cylinders were replaced and spiders were later returned to their standard housing enclosure. Spiders were used only once.

Quantification and Statistical Analysis

Auditory Neurophysiology from Brain and Isolated Legs

To create our response curves, we needed to determine whether activity during a tone was significantly different from background activity. Thus, we used a *t* test to compare the number of threshold-based spikes per unit time during the stimulus to the number of threshold-based spikes per unit time that occurred when the stimulus was not present. To insure that this measure of "nonstimulus" background activity was not unfairly biased by the global statistics of the recording, "silent-shuffled-periods" were generated by taking spike times from nonstimulus portions of the recording, shuffling these times, then

sampling them to generate > 100 "nonstimulus background responses" with durations equal to that of the stimulus tone. This process ensured that we had a notion of background neural activity that was specific to a given recording site. For each frequency-amplitude combination, 16 tones were presented. If more than half (9 or greater) resulted in a statistically significant response based on our *t* test, we concluded that the given frequency was detectable at the given amplitude. For example, in the recording shown in Figure 2J, spiders experienced 16 repeats of tones with frequencies from 100 Hz to 5,000 Hz (in 100 Hz steps) for a single amplitude level. The amplitude of the signal was then adjusted and the stimulus was presented again.

Field behavioral assays

Video files were observed to quantify behavioral responses to each sound stimulus (150 Hz, 400 Hz, 750 Hz, 2,300 Hz, and 4,400 Hz and white noise) for each spider. For every trial, each sound stimulus was scored as either a (0) no response or (1) response, depending on whether the focal spider responded to the sound file within 1 sec following presentation. All responses occurred within 0.5 sec following presentation; no responses were seen after 0.5 sec post-sound-presentation. A response was defined as a "backward strike," as described in the main text, in which hunting spiders flip themselves backward, as if an insect had flown past. Here, we defined a response as any instance when a spider lifts its front legs past the midline of its body (Figure 1D).

Laboratory behavioral assays

Laboratory trials were scored identically to field trials.

Results

Auditory Neurophysiology from Brains and Isolated Legs

Previous studies have hypothesized an important role of acoustic sensitivity in the backward strike behavior of net-casting spiders, where spiders might locate prey by detecting acoustic cues produced by the flapping wings of flying insects [4, 5, 8]. To directly investigate the auditory detection abilities of net-casting spiders, we used extracellular recording techniques while simultaneously stimulating focal *Deinopis spinosa* spiders with airborne acoustic stimuli. We recorded neural responses to acoustic stimuli both in higher-order processing centers of the brain [9–12] and in peripheral nerves of isolated legs [13, 14]. As past reports have located acoustic sensors on the legs of other spiders [13, 14], isolated leg recordings were used to help locate putative sensory organs in *D. spinosa*. All recordings took place on a vibration isolated air table surrounded by sound-absorbent foam. Individual, acoustically sensitive units were isolated and identified through spike sorting with Wave_clus software [15].

To investigate the frequency sensitivity of acoustic detection in *D. spinosa*, we recorded neural activity from intact spiders or isolated forelegs while stimulating the preparation with pure tones of the same intensity (80 dB SPL) over a wide range of frequencies (Figures 2A and 2B). We presented 500 ms duration pure tones, repeated 16 times in a pseudorandom order from the loudspeaker at a distance of 2 m from the spider/recording site.

Stimulus presentations varied in the step size of frequency interval and frequency ranges tested (brain recordings: n = 4 [100–1,000 Hz; in 100 Hz steps], n = 5 [100–2,500 Hz; in 100 Hz steps], n = 3 [2,000–13,000 Hz; in 500 Hz steps], n = 1 [100–800 Hz, in 20 Hz steps]; isolated leg recordings: n = 2 [100–2,500 Hz; in 100 Hz steps], n = 1 [2,000–10,000 Hz; in 500 Hz steps]). Responses from both intact *D. spinosa* brains and isolated legs displayed auditory sensitivity over a wide range of frequencies (Figures 2A, 2B, S1, and S2), with some recordings illustrating high specificity (Figure S1B), while others displayed broader sensitivity to tonal frequencies (Figure S1C).

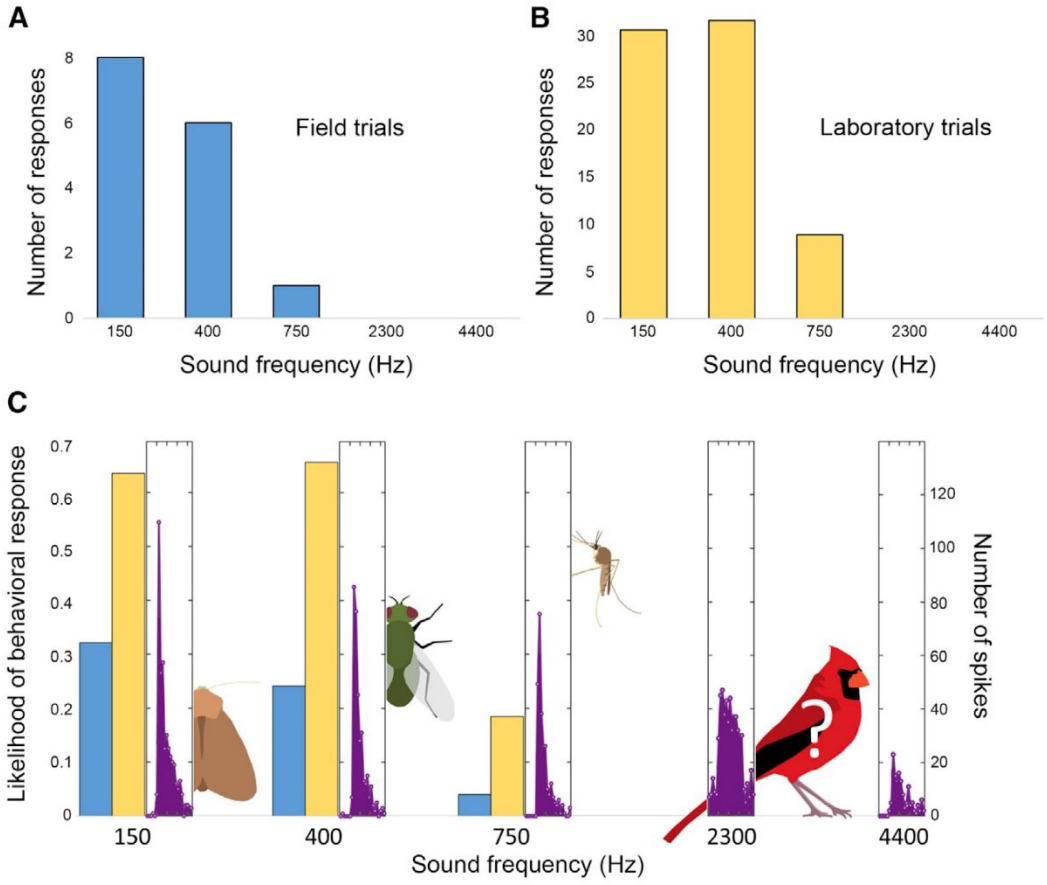

Figure 3. Field and Laboratory-Based Behavioral Assays of Acoustic Stimulation Illustrate Auditory Detection Used in Foraging Context. (A) Behavioral trials were conducted in the natural habitat of *D. spinosa* (Gainesville, Florida), where foraging spiders were exposed to pure tones of different frequencies (n = 25). Spiders made backward strikes at lower frequency tones (Video S1) but did not respond to higher frequency tones in a foraging context. (B) Laboratory trials were conducted in an acoustically controlled environment to determine behavioral responses to different frequencies (n = 51). As with field trials, spiders in laboratory trials responded to lower frequency tones with backward strikes (Video S1) but did not respond to higher frequencies in a foraging context. (C) Behavioral responses depicted from both field trials (blue) and laboratory trials (yellow) alongside

neural response histograms to each frequency (purple). Acoustic frequencies eliciting behavioral and neural responses overlap with wing beat frequencies of known prey species (moths, mosquitoes, and other diptera). We speculate that song birds, often emitting calls higher than 1 kHz in frequency, may prey upon exposed, but cryptically camouflaged, spiders during the day.

We next investigated whether net-casting spiders displayed higher sensitivities (i.e., tuning) to specific frequencies. We created response curves using previously established acoustic stimulation protocols and neurophysiological recording techniques [10, 16], and explored response thresholds (dB SPL) of neural activity across a range of frequency and amplitude pairings. Response curves were separately created from brain recordings ($n = 4$) and isolated leg recordings ($n = 3$). Response thresholds varied from ~55 to ~90 dB SPL in both types of recording. As with previous recordings, we uncovered acoustic sensitivity to a surprisingly broad range of tonal frequencies (100–5,000 Hz; Figures 2C–2J and S3).

Our extracellular methods demonstrated that an auditory sensory organ is located on the legs of *D. spinosa*—presentation of acoustic stimuli produced neural responses in isolated leg recordings. Informed by past studies of acoustic detection in cob-web spiders [13, 14], we investigated whether the metatarsal organ (MTO), a group of slit sensilla sensitive to exoskeleton strain, could be a putative auditory organ. To do so, we acoustically stimulated the isolated legs with lab-generated sinusoids ranging from 1,000 to 3,000 Hz while recording neural activity before, during, and after dampening movements around the joint, used to decrease vibrational responsiveness detected by the MTO. In various ways, auditory sensitivity was reduced when movement at the leg was hindered by mechanical loading (Figure 4). Dampened isolated legs produced fewer overall significant responses, depicted in the loss of significant responses between 1,000 and 1,400 Hz (Figure 4E). In addition, response thresholds were typically higher in dampened legs (Figure 4E) when compared to recordings occurring prior to (Figure 4A) and following (Figure 4I) dampening. Lastly, among responses of similar amplitude and frequency, spike rate decreased in the dampened joint treatment (Figures 4F–4H) when compared to unmanipulated recordings (Figures 4B–4D and 4J–4L). Restricting leg-joint movement, and thus MTO efficacy, decreased auditory sensitivity, supporting previous claims of MTO involvement in auditory sensation in spiders [13, 14].

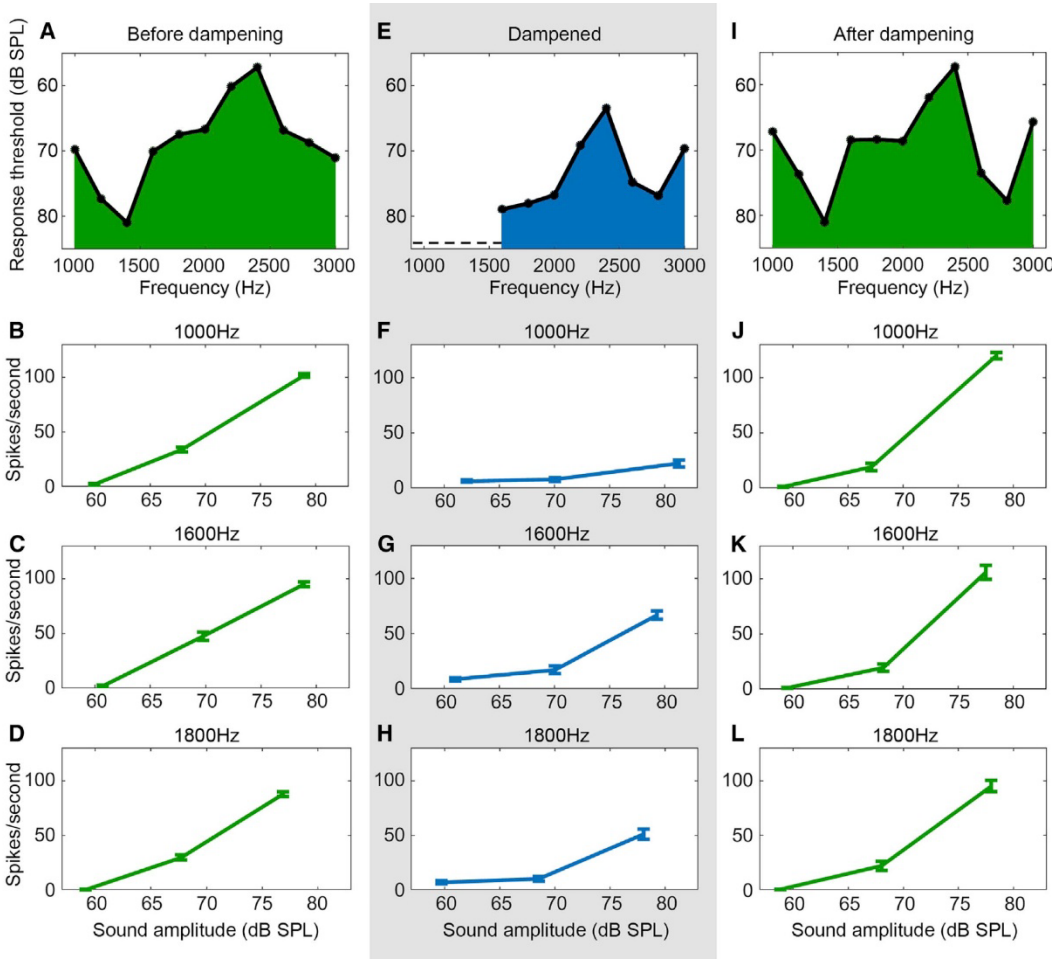

Figure 4. Reversibly Dampening Metatarsal Organ Alters Acoustic Sensitivity in Leg Recordings. Response curves constructed from a single leg recording, prior to (A), during (E), and following (I) our reversible manipulation, decreasing tarsal movement and, thus, dampening metatarsal organ detection (Figure S4). All three curves are constructed from the same acoustic stimulation set, with tones (1,000 to 3,000 Hz, in 200 Hz steps) of three separate intensities, repeated 16 times in pseudorandomized order. Exemplar frequency responses are depicted prior to (B–D), during (F–H), and following (J–L) tarsal dampening. Acoustic sensitivity decreased when tarsal movement was hampered, both in loss of significant neural responses (lack of significant response between 1,000 and 1,400 Hz in E) and in decreased spike counts among significant responses (F–H), when compared to the nonmanipulated recordings. Vertical bars indicate ± one SD.

Field Behavioral Assays

Our extracellular recordings indicated that *D. spinosa* are sensitive to an unexpectedly wide range of tonal frequencies. To uncover which frequencies, if any, are useful in capturing flying prey items, we conducted behavioral assays of acoustic stimulation in the natural habitat of *D. spinosa* (Gainesville, Florida). After an extensive field search, we located and

selected actively hunting *D. spinosa* (i.e., spiders possessing nets; $n = 25$) for use in our assays, which involved presenting spiders with different tonal frequencies and observing behavioral responses. Each spider was presented with 500 ms pure tones of the following frequencies: 150, 400, 750, 2,300, and 4,400 Hz, as well as pulses of white noise. Acoustic stimuli were presented in a randomized order, through a Bluetooth speaker, at 70–80 dB SPL, and from a distance of 1 m from the focal spider.

Of the 25 spiders tested, 13 individuals responded to at least one acoustic stimulus, always responding with a backward strike (Figure 1D), acting as if an insect had flown past (Figures 3A and 3C). Moreover, spiders reacted only to lower frequency tones (150, 400, and 750 Hz), while no backward strikes, or any observable behaviors, were witnessed in response to higher frequencies (2,300 and 4,400 Hz) or pulses of white noise. Forward strikes were never elicited by acoustic stimulation.

Laboratory Behavioral Assays

Following field experiments, behavioral assays were conducted in an acoustically controlled laboratory environment. *Deinopis spinosa* spiders ($n = 51$) were individually housed and maintained under a reversed, 12:12 light-dark cycle. During the night phase, spiders that spun webs were selected for testing. The same speaker, media devices, and sound files used in field trials were also used in laboratory trials. The stimulus sound level and distance between the speaker and the spider were also held constant across field and laboratory assays.

Of the 51 spiders tested, 32 spiders responded to at least one acoustic stimulus (Figure 3B). As with field trials, spiders performed backward strikes only in response to lower frequency tones (150, 400, and 750 Hz), while no observable behaviors were elicited through the presentation of higher frequency tones (2,300 and 4,400 Hz) or pulses of white noise. As in field trials, no forward strikes were elicited via acoustic stimulation.

Discussion

We present the first neuroethological analysis of predatory behavior in the ogre-faced, net-casting spider, *Deinopis spinosa*. These creatures live circadian Jekyll and Hyde lives, avoiding predators by day, through total immobility and camouflage, and stealthily ambushing prey by night. In color, morphology, and behavior, deinopids resemble dry, immobile fronds of their palm plant hosts during daylight hours [8]. However, at nightfall, a flurry of activity transforms them into ambush predators. They build a sparse frame web, shaped like the letter A (Figure 1C), from which they suspend themselves in the air, grasping a relatively small, stretchable net held between their front four legs (Figure 1B). Thus positioned, they wait for insects to pass by. The near approach of prey triggers explosive acts of body movement and net manipulation that underlie an uncanny ability to ambush prey walking beneath (forward strike) or flying above (backward strike; Video S1). Our experiments address the sensory modalities that mediate prey capture behavior and, in particular, interception of aerial prey using auditory cues.

Neurophysiological recordings from the brain and isolated legs of *D. spinosa* display an acute sense of auditory sensitivity over a surprisingly wide range of tonal frequencies from

100 to 10,000 Hz (Figures 2, 3, 4, and S1–S3). Moreover, we uncover multiple neural units that respond to frequencies in the 150–750 Hz range (Figures 2A, 2B, S1, and S3), while our behavioral assays help explain the role of detecting lower frequencies in the context of foraging. Our behavioral assays, conducted both in the field and in the laboratory, illustrate that detecting low-frequency tones in the range of 150–750 Hz is sufficient to trigger a sudden backward strike in *D. spinosa*, where spiders respond as if an insect was flying past, supporting earlier reports that described this behavior as not reliant on vision [4, 5, 8]. As these tonal frequencies overlap with wingbeat frequencies of common deinopid prey, such as moths, mosquitoes, and various other flies (Figure 3C) [17–24], we propose that aerial predation in *D. spinosa* is enabled by detecting acoustic cues emitted by the flapping wings of flying prey.

To our surprise, neurophysiological recordings reveal many neural units sensitive to frequencies between 1,000 and 10,000 Hz (Figures 2C–2J and S1–S3). Neither our field nor laboratory-based behavioral assays suggest such frequencies have adaptive salience in the context of foraging. Since the fastest known wingbeat frequency is ~1,000 Hz, produced by a ceratopogonid midge [20], sound frequencies over 1 kHz in the fundamental flight frequency will not indicate the presence of potential flying prey. The possible benefits, or lack thereof, of high-frequency detection outside of foraging behavior are yet to be tested. We speculate that high-frequency sensitivity is beneficial in a nonforaging context, possibly predator avoidance. The superb diurnal crypsis and mimicry of *D. spinosa*, reinforced by its apparently day-long motionless posture, has almost certainly evolved to avoid day-active, visually guided predators. Small passerine birds possess acute vision [25, 26], emit call frequencies in the kHz range [27], and often forage on or beneath palm plants inhabited by *D. spinosa* (J.A.S., unpublished data). We hypothesize that detection of high-frequency sounds allows net-casting spiders to eavesdrop on foraging birds, providing an early warning to incoming predators. Future work will seek to answer how detecting bird calls might benefit cryptic deinopid spiders, a potential facet of deinopid behavior inferable only because neural recordings uncovered high-frequency responses. When exploring the sensory world of a behaviorally charismatic but relatively rare and understudied animal, the strategy of adopting a neuroethological approach has significant benefit for potential discovery. Having established that these spiders are sensitive and reactive to airborne acoustic stimuli, we next turned to exploring the potential auditory organs of *D. spinosa*.

Spiders do not possess insect-like "ears," as no arachnid has been found to have a tympanal membrane. Even so, several spider species have been reported to detect airborne acoustic stimuli, beyond near-field range airflow, using nontympanal hearing organs to accomplish this task. We found that isolated legs respond to acoustic stimuli; therefore, *Deinopis* legs must possess sensory organ(s) capable of auditory sensation. In cob-web (Theridiidae) and fishing spiders (Pisauridae), auditory sensitivity to high-frequency sound [13, 14] (G. Smith, unpublished data; G.M., unpublished data) has been attributed to aggregations of slit sensilla (i.e., lyriform organs) located on the distal portion of a spider's leg, namely the MTO. These sensilla are extremely sensitive to exoskeletal strain and are known for their vibrational detection capabilities [11]. Pioneering work by Charles Walcott and colleagues [13, 14] has illustrated that the MTO can detect minute movements of the tip of the leg (i.e., tarsi) caused by airborne acoustic stimuli. We borrowed methods from these studies to

investigate whether dampening tarsal movement through mechanically loading would decrease responsiveness to high-frequency tones in *D. spinosa*.

Recordings from isolated legs prior to, during, and following our reversible-dampening manipulation provide evidence for a role in high-frequency acoustic detection by the MTO. When tarsal movement was dampened, acoustic sensitivity was significantly decreased across most frequencies tested. When tarsal movement was undampened, acoustic sensitivity recovered to premanipulation thresholds and spike rates. Recent reports of farfield hearing in jumping spiders illustrate the utility of long, thin hairs (e.g., trichobothria) on their forelegs in detecting low-frequency sounds from over 2 m away from a sound source [10]. In *D. spinosa*, we expect low-frequency sounds are similarly detected by the trichobothrial leg hairs. We thus suspect that two distinct sensor types confer auditory sensation to net-casting spiders in the form of high-frequency detection by strain detectors (slit sensilla) and low-frequency detection by hairs (trichobothria).

Deinopids are well known for their hypersensitive, night vision eyes (Figure 1A). Here, we show net-casting spiders also possess an acutely tuned auditory sense, packed into microscale sensory organs that trigger rapid bodily movements. Intriguing questions are now open for further study. Behavioral observations (J.A.S., unpublished data) suggest that the backward strike is not a reflexive, "shot in the dark" act of chance but actively and directionally steered. Thus, the directional sensitivity of the auditory organ(s) is as important a question as its auditory sensitivity. The rapid action of the sound-triggered backward strike raises questions that involve biomechanics of body movements. The apparent stereotypy of the body's twisting motion unfolds within 60 ms, rivaling or exceeding the performance of insect startle responses and prey capture [28, 29]. The question of whether there are "giant" interneurons or electrotonic synapses involved in the neural circuitry of deinopid prey capture, as is known to occur in the ballistically fast escape systems of cockroaches and crayfish [28], naturally arises. The nature of neural processing of the spider's brain has only recently commenced [9, 10] and the deinopid brain is an inviting future target.

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Author Contributions – Conceptualization, J.A.S., G.M., E.A.H., and R.R.H.; Methodology, J.A.S., G.M., E.I.N., E.A.H., and R.R.H.; Software, E.I.N.; Formal Analysis, J.A.S., G.M., and E.I.N.; Investigation, J.A.S. and G.M.; Resources, J.A.S., G.M., E.I.N., E.A.H., and R.R.H.; Data Curation, G.M. and E.I.N.; Writing—Original Draft, J.A.S. and E.A.H.; Writing—Review & Editing, J.A.S., G.M., E.I.N., E.A.H., and R.R.H.; Visualization, J.A.S., G.M., and E.I.N.; Supervision, E.A.H. and R.R.H.; Project Administration, E.A.H. and R.R.H.; Funding Acquisition, E.A.H. and R.R.H.

Declaration of Interests – The authors declare no competing interests.

Key Resources		
Reagent or Resource	Source	Identifier
Experimental Models: Organisms/Strains		
Deinopis spinosa	Gainesville, FL, USA	J. Stafstrom
Software and Algorithms		
MATLAB	MathWorks	https://www.mathworks.com/
Spike Hound	[14]	http://spikehound.sourceforge.net
Custom stimulus-generation code	This paper	Available on request
Custom analysis code	This paper	Available on request
Audacity	https://www.audacityteam.org/	V2.1.2
Windows	Microsoft Corporation, Redmond, WA, USA	Windows 7, 64-bit
Other		
Stimulus-generating loadspeaker	LOUD Technologies, Woodinville, WA, USA	Mackie HR824
Stimulus-generating stereo amplifier	Nikko Audio, Japan	Nikko NA-790
GoPro video camera	GoPro, San Mateo, CA, USA	GoPro HERO 4
Bluetooth Speaker	JBL, Los Angeles, CA, USA	JBL Flip 3
Callibrated microphone	Brüel & Kjær, Denmark	Brüel & Kjær 4135 ¼ in. microphone
Microphone amplifier	Brüel & Kjær, Denmark	Brüel & Kjær 5935 amplifier
Microphone callibration device	Brüel & Kjær, Denmark	Brüel & Kjær 4420 piston phone
Kerr dental wax	Syborn Kerr, Emeryville, CA, USA	58 C melting point dental wax
Extracellular tungsten electrodes	MicroProbes, Gaithersburg, MD, USA	4 MU glass-insulated tungsten electrode
Stereomicroscope	Leica Microsystems GmbH, Wetzlar, Germany	Wild M3Z
Stereotactic micromanipulators	Narishige International USA, East Meadow, NY, USA	MM-3
Digital hydraulic microdrive	David Kopf Instruments, Tujunga, CA, USA	Model 607W
Extracellular headstage amplifier	A-M Systems, Sequim, WA, USA	Model 1800
Differential AC microelectrode amplifier	A-M Systems, Sequim, WA, USA	Model 1800
Analog-digital signal converter	National Instruments, Austin, TX, USA	NI PCI-MIO-16E-1

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Supplemental Information

Ogre-Faced, Net-Casting Spiders Use Auditory Cues to Detect Airborne Prey

Jay A. Stafstrom, Gil Menda, Eyal I. Nitzany, Eileen A. Hebets, and Ronald R. Hoy

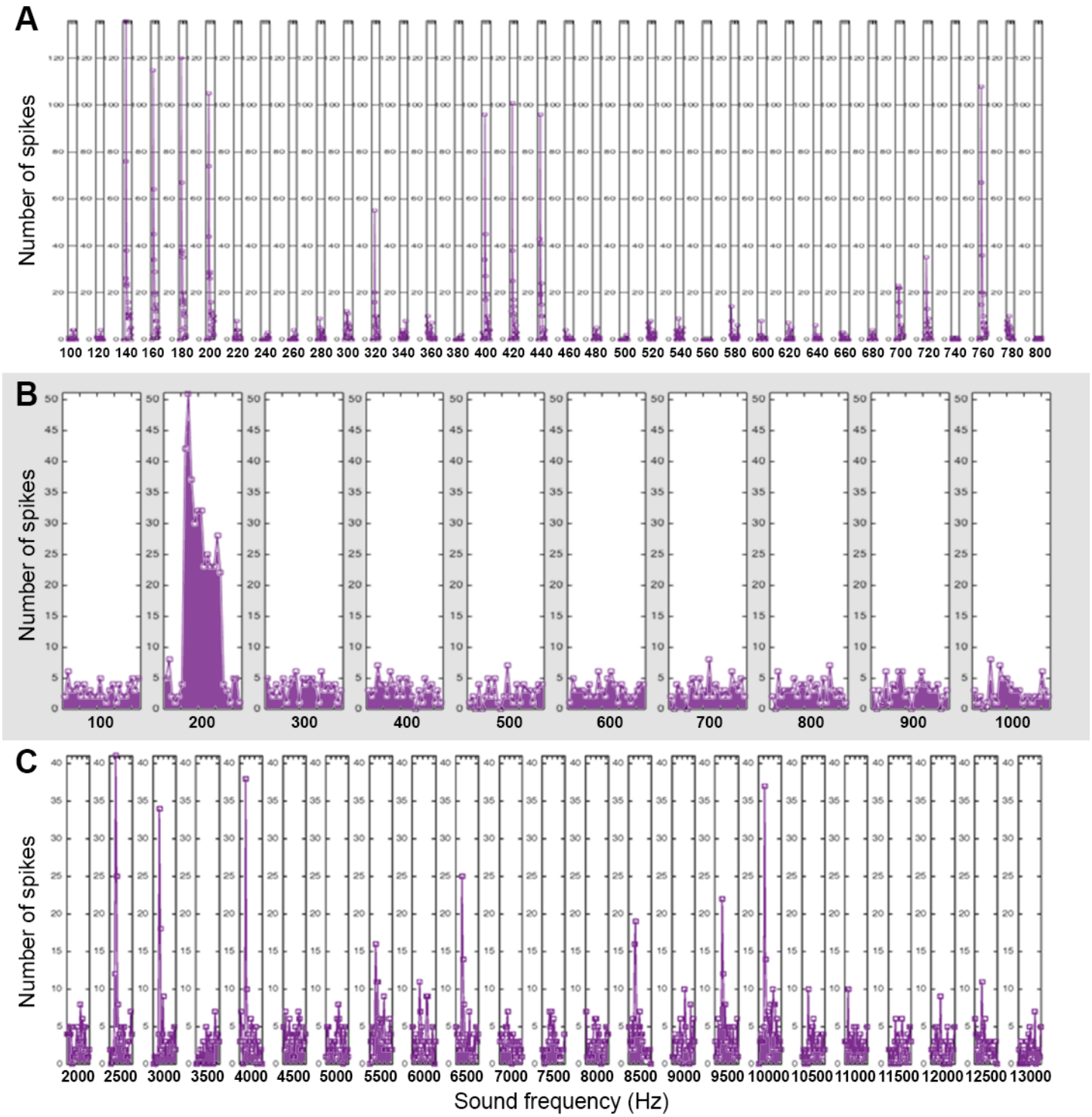

Figure S1. Extracellular, Microelectrode, Neural Recordings from the Brain of *Deinopis spinosa* Yield Evidence for Responsiveness to Auditory Stimulation. Related to Figure 2.

Each panel above (A-C) depicts recordings of brain activity of separate, intact *D. spinosa* spiders, where each intact spider was stimulated by different ranges of pure tone frequencies at 80dB SPL. Panel (A) was stimulated by tones between 100Hz to 800Hz in 20Hz steps, panel (B) was stimulated by tones between 100Hz and 1,000Hz in 100Hz steps, and panel (C) was stimulated by tones between 2,000Hz and 13,000Hz in 500Hz steps. Each tone was presented 16 separate times, in a pseudorandomized order. Each binned frequency represents the sum of spikes over the 500ms of stimulus presentation for all 16 trials.

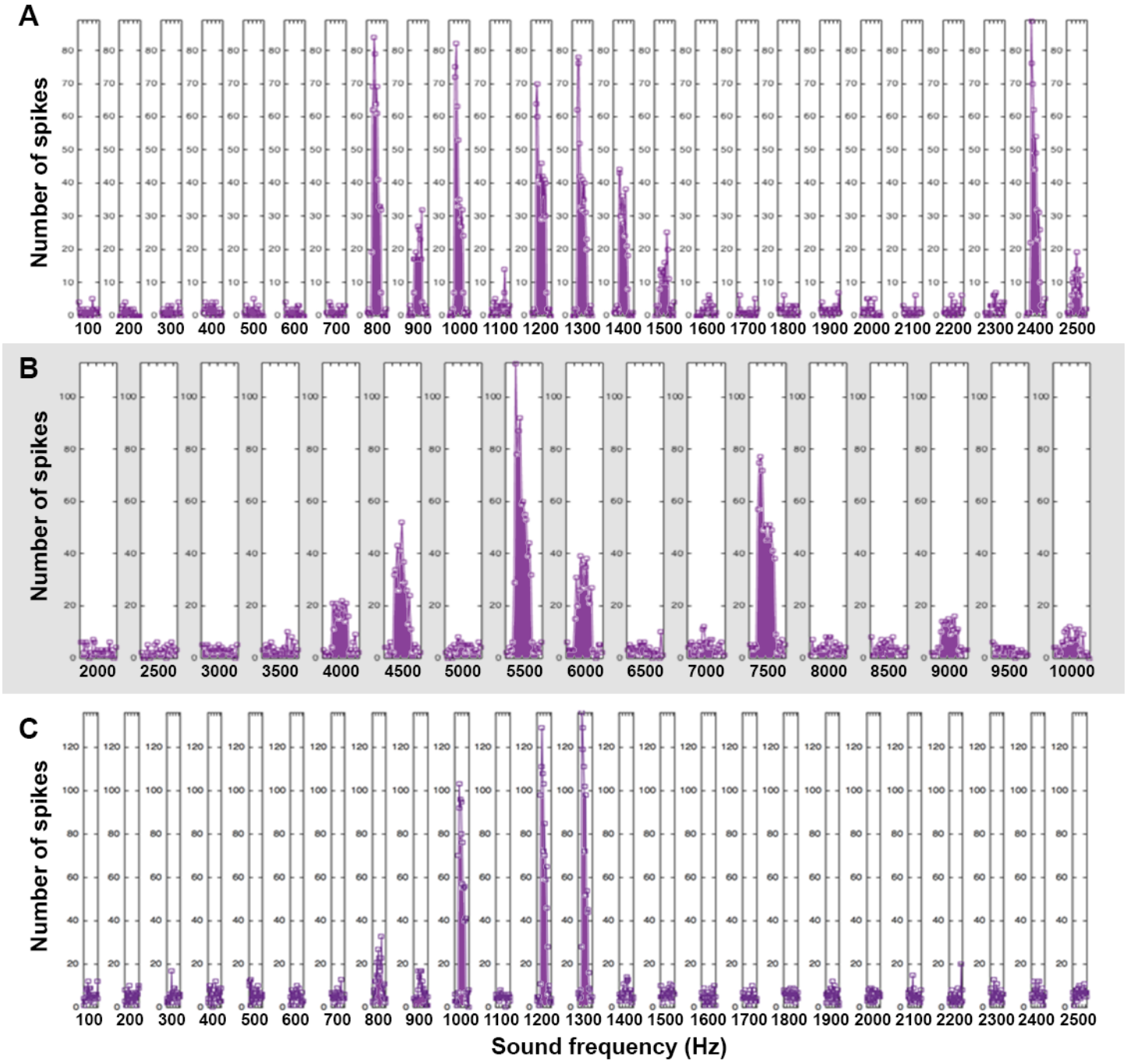

Figure S2. Extracellular, Microelectrode, Neural Recordings from Isolated Forelegs of *Deinopis spinosa* Yield Evidence for Responsiveness to Auditory Stimulation. Related to Figure 2.

Each panel above (A-C) depicts neural recordings from the forelegs of separate *D. spinosa* spiders, where each isolated leg was stimulated by different ranges of pure tone frequencies at 80dB SPL. Panel (A) was stimulated by tones between 100Hz to 2,500Hz in 100Hz steps, panel (B) was stimulated by tones between 2,000Hz and 10,000Hz in 500Hz steps, and panel (C) was stimulated by tones between 100Hz and 2,500Hz in 100Hz steps. Each tone was presented 16 separate times, in a pseudorandomized order. Each binned frequency represents the sum of spikes over the 500ms of stimulus presentation for all 16 trials.

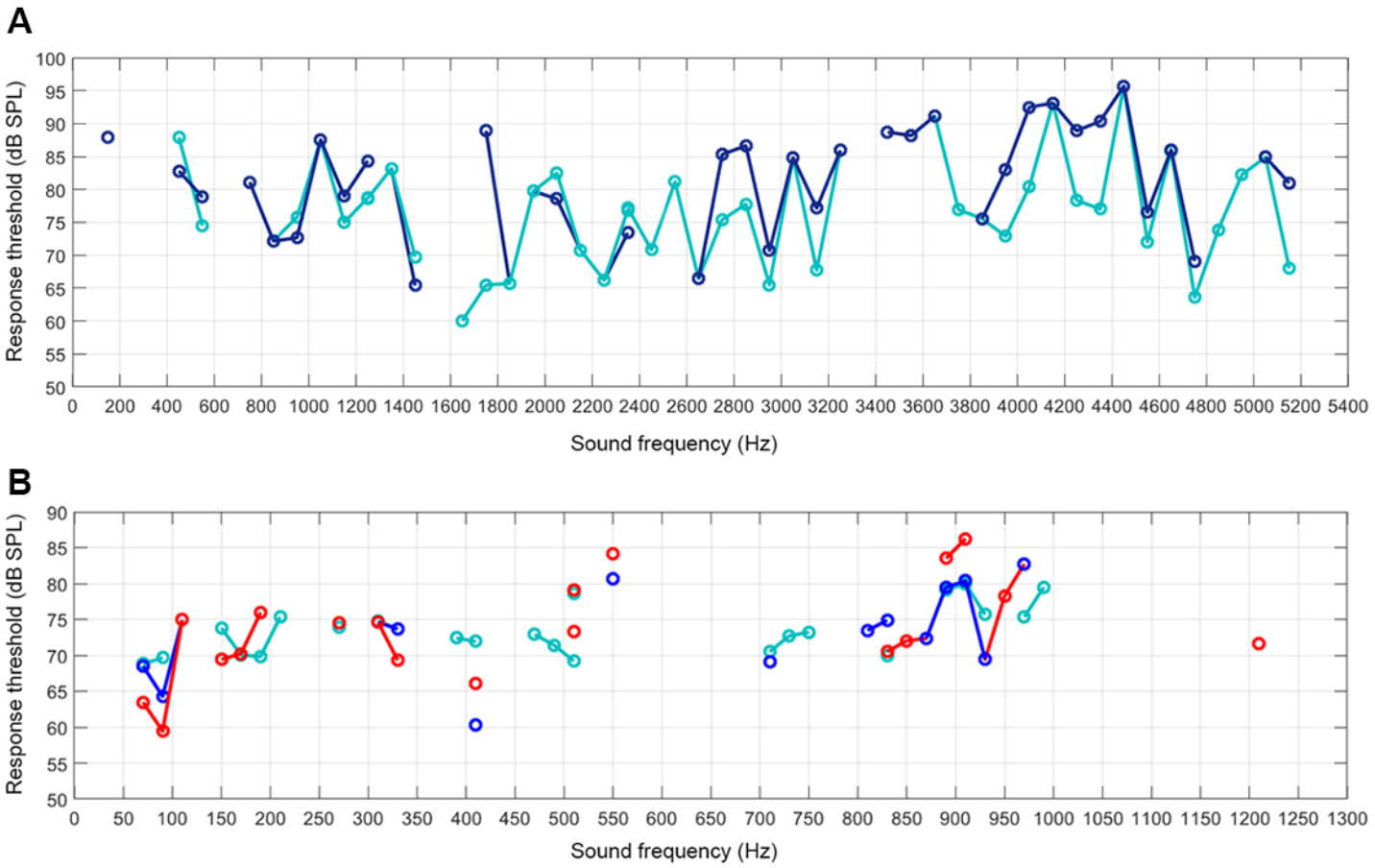

Figure S3. Leg and Brain Recordings Illustrate Broad Auditory Sensitivity in *D. spinosa*. Related to Figure 2.

Response curves created from extracellular recordings of (A) an isolated leg and (B) an intact brain of *D. spinosa*. Response threshold (Y-axis) indicates the lowest sound intensity (dB SPL) required to elicit a significant response for each unit over the range of all tested frequencies. The isolated leg recording (A) yielded results indicating a broad range of acoustic sensitivity across two neural units (light blue, navy blue). The brain recording (B) displays three neural units (light blue, navy blue, and red) similar in acoustic sensitivity. Gaps between solid lines indicate frequencies that failed to produce a significant response in any of the recorded units at any sound intensity ≤ 95/90 dB SPL.

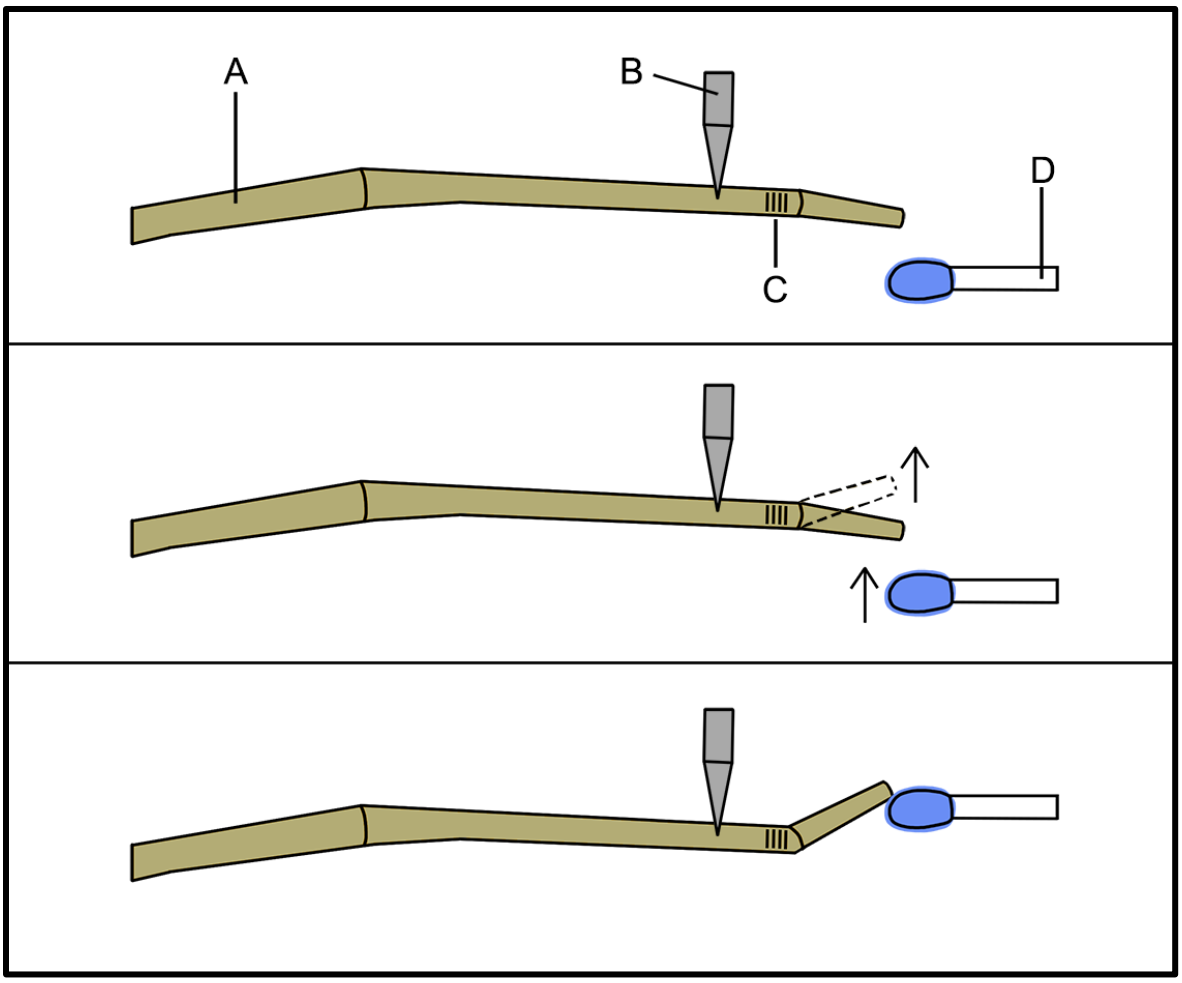

Figure S4. Graphical Representation of Methods Used in Metatarsal Dampening Protocol. Related to Figure 4.

The three panels above illustrate the method used to mechanically load the metatarsal-tarsal joint utilized to construct Figure 4. Joint movement was dampened by carefully contacting the tarsus to a wetted cotton swab, and driving the tarsus upward, depicted across the three above panels. To reverse joint dampening, the cotton swab was carefully lowered down and away from the tarsus. As such, we investigated acoustic sensitivity prior to, during, and following tarsal dampening, which provided evidence suggesting the metatarsal organ plays a role in auditory sensation. (A) an isolate spider leg, (B) recording electrode, (C) location of metatarsal organ, (D) wetted cotton swab.