

Electrophysiological investigation of the sensitivity of scorpion basitarsal compound slit sensilla to vibratory stimuli.

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Summary

Previous research has shown that sand scorpions can locate their prey by detecting and orienting to the seismic disturbances produced by the motion of their prey. It has been suggested that the basitarsal compound slit sensilla (BCSS), mechanoreceptive organs located on the tarsal segments of each of the scorpion's eight legs, are important in the sensing of vibrations produced by prey. However, very little information exists concerning the physiological response properties of the basitarsal slit sensilla. I investigated the neural response of the BCSS to vibrational stimulation of the tarsal joint. Detection of sensory response was accomplished by placing microelectrodes on the BCSS of leg R4 of 3 *Paruroctonus utahensis*. An electrical stimulator connected to a small speaker was used to produce vibratory stimuli that varied in both amplitude (in steps of 0.01, 0.05, 0.10, 0.50, 1.00, 5.00, 10.00, and 15.00 V) and frequency (increments of 200, 400, 600, 800, & 1000 Hz). Recordings from slit sensilla contained large amplitude, biphasic action potentials, which fired at a baseline spike frequency of approximately 5 Hz. From autocorrelation analysis, each of the BCSS slits examined contained only one active cell. The frequency of action potentials in BCSS was correlated with increases in stimulus amplitude. A maximum response of 30 Hz was obtained in response to the 15.00 V stimulation. The response of BCSS did not vary with changes in stimulus frequency. Based on this information, we hypothesize that that scorpions use BCSS to detect prey based on stimulus amplitude, while stimulus frequency is not discriminated by BCSS.

Introduction

Desert sand scorpions are nocturnal, sit-and-wait predators that use vibratory stimuli conducted through the ground to detect and locate their prey (Brownell 1977, 1984; Brownell & Farley 1979a, 1979b, 1979c). Small disturbances in sand elicit an abrupt turn by these scorpions toward the source of the nearby disturbance. Additionally, these animals are cannibalistic and it has been proposed that male scorpions use seismic information during pre-courtship activities to inhibit normal female aggressive behavior (Gaffin & Brownell 1992).

It has been suggested that the basitarsal compound slit sensilla (BCSS), groups of cuticular grooves located on

the distal basitarsal segment near the tarsal joint of all eight legs, are potentially important in the transduction of vibratory stimuli (Brownell 1977, Brownell & Farley 1979a, Root 1990). A mechanical disturbance on the surface of sand produces vibrations that can be decomposed into two distinct waveforms: compressional and Rayleigh waves. Of these, the slower Raleigh surface waves are purported to carry the information transduced by BCSS in prey detection (Brownell 1977, Brownell & Farley 1979a). However, other than some coarse electrophysiological readings from leg nerves, the BCSS have not been investigated functionally.

Slit sense organs are unique to arachnids. In spiders, where they have been much more widely studied than in scorpions, they are widely distributed across the cuticle of the cephalothorax, abdomen, and appendages. These

organs serve as mechanoreceptors and relay proprioceptive information as well as information about gravity and sound (Juusola 1995). Barth and Wadepuhl (1975) described the location of slit sensilla on the legs of scorpions and found that both isolated slits and grouped slits are located throughout the lateral leg surfaces. The BCSS appear as grooves in the cuticle of the distal basitarsal segment of each of the scorpion's eight legs. Each BCSS has approximately six slit sensilla which vary in length and orientation. Foelix and Schabronath (1983) studied the structure of BCSS on tarsi of *Androctonus australis* in detail. They found that each slit was supplied with two neurons innervating different membranes in the structure.

My goal was to determine the specific qualities of stimuli detected by the BCSS. In this set of experiments, we investigated the electrophysiological responses of BCSS to vibratory stimuli that vary in both frequency and amplitude characteristics. Ultimately, we

are interested in determining how the BCSS is tuned to specific components of natural seismic stimuli, and how scorpions use this information to judge the direction and distance to a disturbance.

Methods

Animals

The subjects of these studies were adult male, desert grassland scorpions, *Paruroctonus utahensis*. Animals were collected using portable fluorescent lamps at night from Monahans Sand Hills State Park, approximately 5 miles east of Monahans, Texas. In the lab, animals were maintained individually atop natural sand substrate in glass pickle jars (3.8L) within a room that maintained constant temperature (22°C) humidity (RH 55-65%) and photoperiod (dark 9 hours 50 minutes, light 14 hours 10 minutes). Animals were maintained on

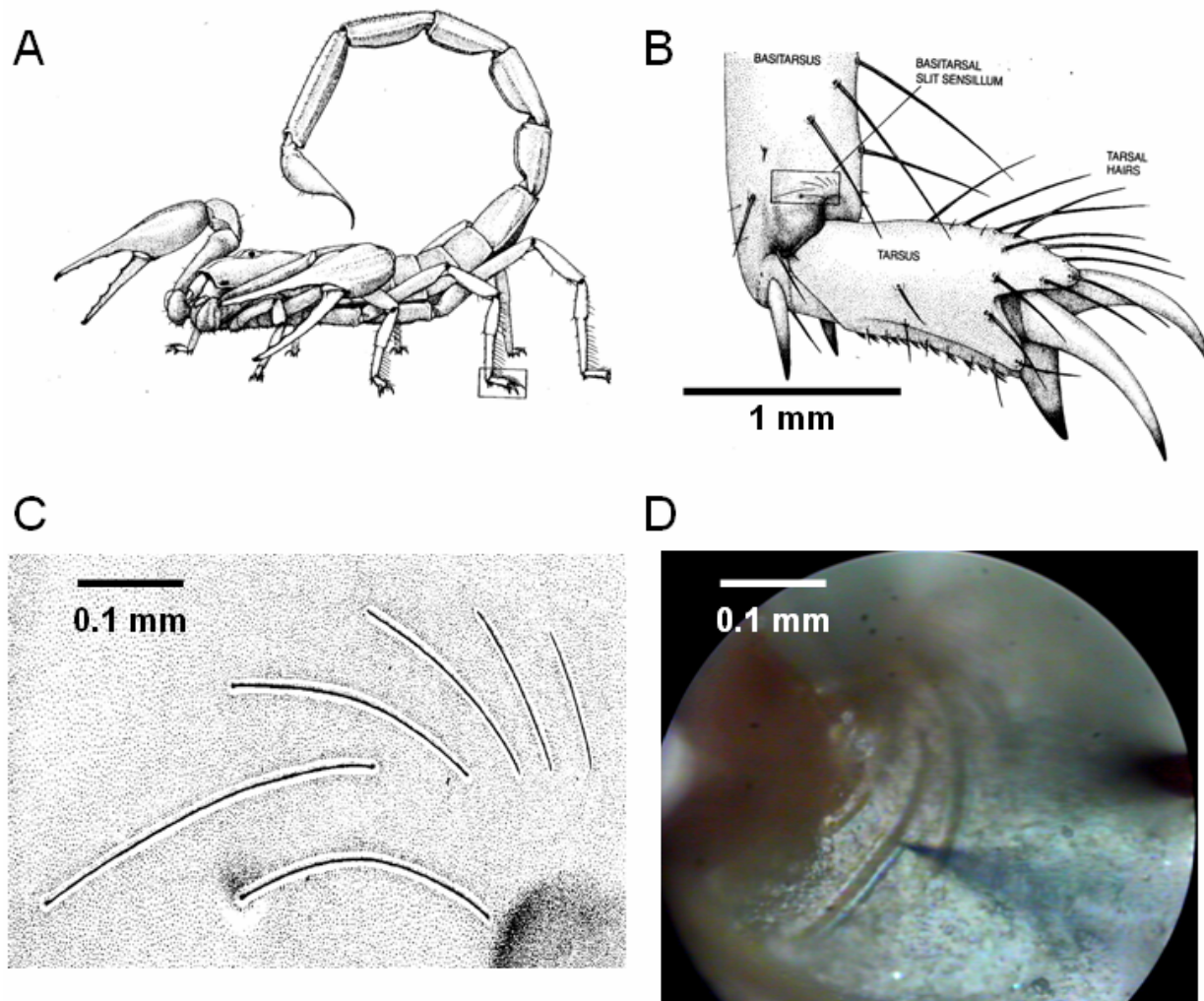


Fig. 1: Location of basitarsal compound slit sensilla. **A-C** These drawings show in increasing detail the location of the BCSS on the scorpion leg (adapted from Brownell 1984). **D** This photograph shows the actual slit sensilla as well as the placement of a tungsten electrode in the middle of one slit sensillum.

a diet of one to two small live crickets (*Acheta domestica*) per week and misted with water twice a week.

Electrophysiology

In preparation for electrophysiological investigation, an animal was first placed into a freezer (0°C) for approximately five minutes for anesthesia. Following this, the scorpion was mounted on a microscope slide using strips of modeling clay to secure the body, tail, pedipalps and proximal leg segments. Double-sided tape was used to secure the tibia and basitarsus of leg R4, being careful to leave the BCSS exposed and the tarsus free of restraint and extended over the edge of the slide. A piece of silver wire was inserted into the hemolymph, in between two cuticular segments on the dorsal side of the scorpion to provide an electrical ground. The slide, with scorpion fixed on top, was then placed under a microscope, equipped with long working distance objectives (Olympus model BX50WI), inside a Faraday cage atop a vibration free table (TMC MICRO-g).

Recording electrodes were fabricated by electrolytically carving tungsten wire to a sharp point (tip diameter < 1µm). A micromanipulator (Leitz) was used to maneuver the recording electrode until it rested on the midpoint of a single slit of the BCSS (Fig 1); action potentials were usually detected coincident with initial slit contact. The signal from the electrode was bandpass filtered between 300 and 3 kHz, amplified 1000-10000 times, displayed on an oscilloscope, and audibilized using a pair of speakers. The signal was also relayed to a video tape recorder (Panasonic 420C) where it was recorded onto magnetic tape for storage and subsequent analysis. All recordings in this study were obtained from slit sensilla on the BCSS of the right fourth leg.

Stimulation

Stimulation was applied to the preparation using an electrical stimulator (Grass S48) connected to a small speaker (Realistic 2") placed approximately 30 cm from the recorded BCSS. Eight different amplitude stimuli were chosen with the frequency held constant at 1000 Hz. The amplitudes were 0.01 V, 0.05 V, 0.10 V, 0.50 V, 1.00 V, 5.00 V, 10.00 V, and 15.00 V. Then the frequency was manipulated while the amplitude was held constant at 5 V. The frequency steps were 200 Hz, 400 Hz, 600 Hz, 800 Hz, and 1000 Hz. For each sensillum recorded, four randomized series of stimuli (1 s duration) were presented at ten to fifteen second intervals.

Data Analysis

For data analysis, VCR tapes containing recorded signals were played back and digitized at 20 kHz sampling frequency with the stimulus and the

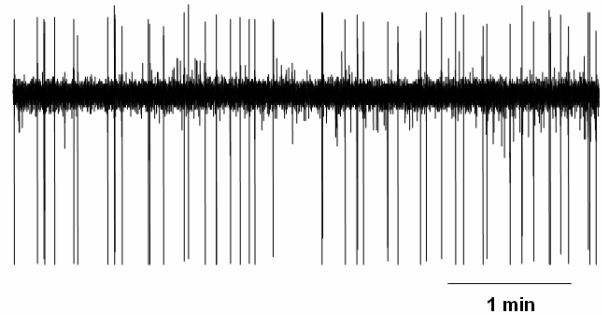


Fig. 2: Baseline recording from scorpion BCSS. This figure shows approximately five minutes of continuous unfiltered data recorded from one slit sensillum. Each of the vertical lines represents an action potential, with the thick line in the middle representing background noise.

response written to different channels. Data was then passed through a digital high-pass filter and further processed to isolate the action potentials from the background noise. Finally, the files were imported as wavemark data to a data analysis program (Spike 2, Cambridge Electronics). The number of spikes per second was counted for each stimulus, then the four trials for each stimulus increment were averaged together. All of the spikes within a record were superimposed, and tested with an autocorrelogram.

Results

We found that electrode placement is critical to obtaining high-fidelity sensory recordings from the BCSS. In early recordings we learned that inserting the electrode into the slit sensillum may disrupt the function of the organ. Instead of recording true action potentials, the movement of the electrode was recorded, not the activity of the cells. With additional experience, the electrode could be placed gently on an individual slit in the BCSS, coming into contact with the sensillum but not penetrating it (Fig 1D). This technique was highly reproducible and yielded high quality records with good signal to noise ratio (Fig 2).

In our studies we found only evidence of a single neuron in our recording of individual slits of BCSS. Four subjects were used in this study, and data was taken and presented from three subjects. Superimposed action potentials had almost identical waveforms and produced a very clear image of the waveform (Fig 3 A). The average waveform was biphasic, about 3 ms in duration, and had a characteristic notch in the initial negative-going phase of the wave (Fig 3 B). Autocorrelogram results showed that 0.0045-s before and after each spike there was no activity. This time corresponds to the refractory period of an action potential, or the time required for a neuron to recover. With this we conclude

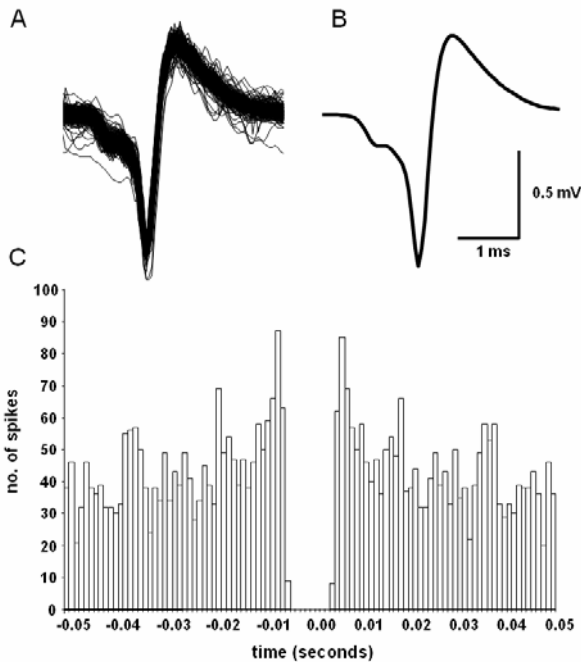


Fig. 3: Evidence that each slit sensillum possesses one cell type. **A** Superimposed action potentials occurring within a five-minute time frame. **B** The average waveform of all of the action potentials shown in A. **C** Autocorrelogram of spikes occurring in A.

that all of the action potentials recorded for a particular slit sensillum originated from a single active neuron.

We found that changes in the amplitude of a stimulus cause a differential response in the BCSS we investigated. As the stimuli increased in amplitude, there were more action potentials per second (Fig 4). The amplitudes were chosen on a logarithmic scale in order to incorporate the entire range of the stimulator. With no stimuli at all, the cell fired about 5.5 action potentials per second. As stimulus amplitude increased logarithmically, the frequency of action potentials increased linearly (Fig 5). The greatest response recorded was 30 action potentials per second.

There was no correlation between stimulus frequency and cell response. Again, the firing rate of the cell with no applied stimulus was approximately 5.5 Hz. With an applied stimulus of any frequency and amplitude of 5 V, the response was around 16 Hz, very close to the response in the experiments with varying amplitude. When the frequency was increased from 200 to 1000 Hz, no change was recorded (Fig 6).

Discussion

An important methodological finding of this study was that recording from the BCSS requires placement of the electrode on, and not penetrating, the organ.

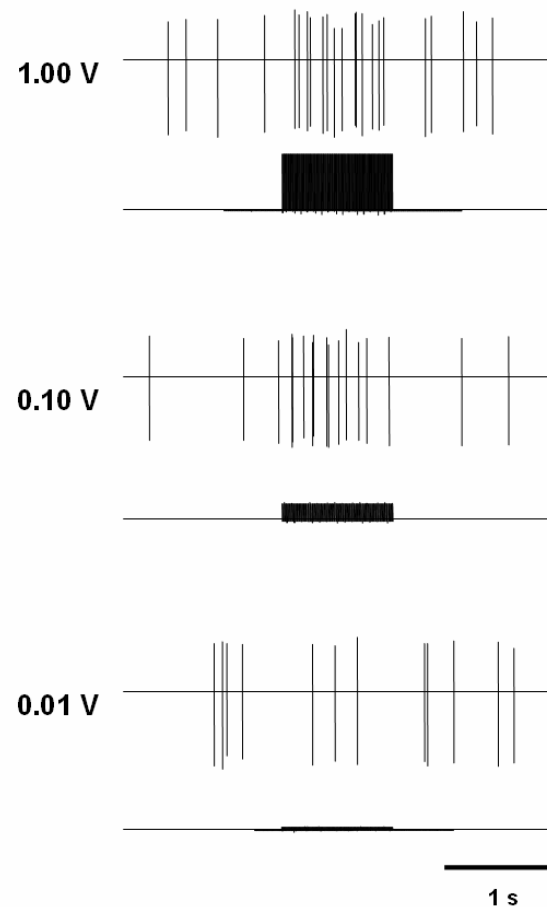


Fig. 4: Differential response to stimuli of three different amplitudes. Each stimulus is shown as the solid bar at the bottom of each graph, and the response shown above as action potentials after the background noise was removed.

Penetration of the organ disrupts its function and does not provide useful recordings. When the electrode was correctly placed resting on the sensillum, we obtained high fidelity recordings with a low signal to noise ratio. This finding will be useful in future studies of the BCSS.

In the sensilla we studied, only one cell was spontaneously active. Both the superimposed action potentials and the autocorrelogram results support this conclusion. Since we restricted our investigations to individual slit sensilla within BCSS on the right fourth leg of male scorpions, we are unable to extrapolate this result to other sensilla on the same leg or other legs.

As the amplitude of the stimuli increased, the frequency of response increased. This increase showed a positive linear relationship when plotted on a logarithmic scale. One functional interpretation of this result may be that the scorpion is very sensitive to small changes in amplitude, corresponding to the small amplitude stimuli produced by normal scorpion prey. It also suggests that the scorpion is not very sensitive to, or

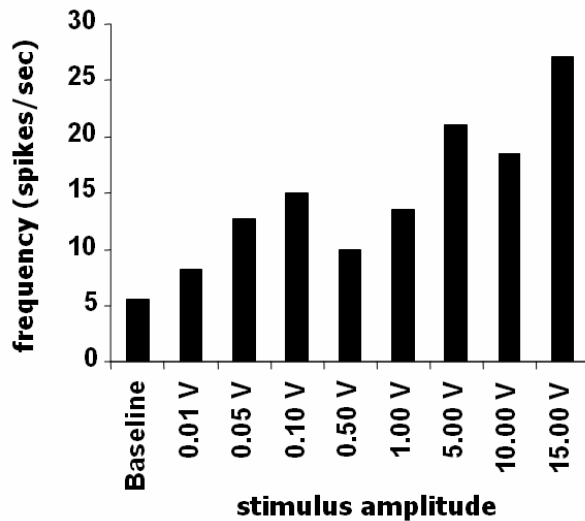


Fig. 5: Neurophysiological response of BCSS to increasing amplitude of stimuli.

able to differentiate between, much larger stimuli. In relation to behavior, it may be that large amplitude stimuli do not need to provide information about the exact size of the stimuli, since anything that would produce stimuli of this magnitude would represent potential danger and thus only need to provide the scorpion information to retreat.

Within the parameters of our experiments, changes in the frequency of stimuli had no effect on the neural response of BCSS. This suggests that these organs may only provide information on size and location of objects around the scorpion, and that the frequency components of Rayleigh waves may not be used in prey detection. Of course, this hypothesis is based on a limited sample size and stimulation set. It is possible that the other slit

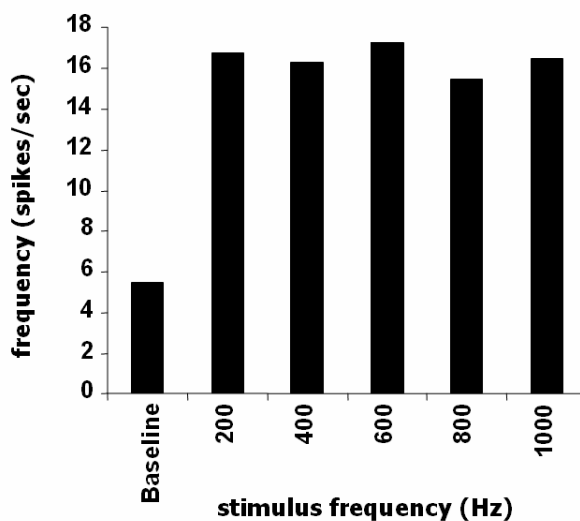


Fig. 6: Neurophysiological response of BCSS to increasing frequency of stimuli.

sensilla on the same basitarsal segment, or slit sensilla located on other legs, may detect changes in frequency. Both of these are valid questions for further research in this area.

Acknowledgments

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