Are mechanoreceptors involved in the neural circuitry of scorpion peg sensilla?

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Summary

Scorpion pectines are paired, ventral appendages that extend from their eleventh body segment. Each pecten resembles a comb with a jointed spine connecting numerous teeth. The ventral surface of each tooth contains a dense patch of truncated hairs, called peg sensilla. Morphological and electrophysiological studies have concluded that numerous chemoreceptive neurons are present in each peg while only one mechanoreceptor is present. Synaptic interactions between chemosensitive cells have been identified via cross-correlation analysis; however, the mechanoreceptor has received very little attention, and it is unknown if it is part of the peg circuitry. Our research focused on the general characteristics and roles of the mechanoreceptors in peg sensilla of the desert grassland scorpion (*Paruroctonus utahensis).* Extracellular electrophysiological recordings were obtained from the bases of individual pegs during mechanical stimulation. These recordings were then segregated into individual cellular firings using wave-form analysis, which showed the existence of four different waveforms (three chemoreceptors and one mechanoreceptor). Cross-correlation analysis did not reveal signs of synaptic interaction between the mechanoreceptor and two of the chemoreceptors while a third was inconclusive. Taken together with previous morphological studies it appears that the chemoreceptors and mechanoreceptors form synaptically isolated neural populations in scorpion peg sensilla.

Introduction

We are interested in understanding synaptic interactions between neurons within simple neural networks. Our hope is to eventually learn how animals acquire and process information from their surroundings. Much of our current understanding of sensory biology in animals stems from studies of mandibulate arthropods (e.g. insects, crustaceans). In particular, moth and crayfish antennal preparations have generated volumes of data on neural response patterns to chemostimulants. However, in these animals synaptic interaction does not occur until the central nervous system (CNS; Kaissling 1987, Derby and Atema 1988). Due to the inherent complexity and difficulty of recording electrophysiologically from the CNS, a simpler system would be useful to the study of synaptic interactions between primary sensory neurons. Unique ground-directed appendages on scorpions, called pectines, may offer special advantages for studies of information processing in accessible neural networks. Our focus is to determine if any interactions occur between chemo- and mechanoreceptor units in pectines.

Pectines are paired, ventral appendages that extend from the eleventh body segment of all scorpions. Each pecten resembles a comb with a jointed spine connecting numerous teeth, the number depending upon sex and species (Swoveland 1978). The ventral surface of each tooth contains a dense patch of hundreds of truncated setae, called peg sensilla. Individual "pegs" are roughly 1-2 μm in diameter and 2-5 μm in length. Each peg is a double-walled shaft of cuticle, flattened at the end where a pore allows the internal sensilla access to the external environment (Ivanov and Balashov 1979). Morphological and electrophysiological studies have concluded that numerous chemoreceptors are present in each peg, while only one mechanoreceptor is present

(Foelix and Müller-Vorholt 1983; Gaffin and Brownell 1997b, Melville 1999). Anatomical studies have also suggested that chemical synapses exist between peg neurons (Foelix and Müller-Vorholt 1983; Foelix 1985). Cross-correlation studies of peg neural activity corroborate these findings (Gaffin and Brownell 1997a).

To date, most physiological research has focused on the properties of chemosensitive cells in the peg sensilla while the mechanosensitive cells have received only cursory attention (Hoffman 1964; Gaffin and Brownell 1997b; Gaffin 2001). Behaviorally, the mechanoreceptor may be important in spermatophore exchange (Alexander 1957) and discrimination of surface texture (Boyden 1978). It is also feasible that the mechanoreceptor interacts synaptically with chemosensory cells, perhaps marking the time when contact is made with the ground.

Our research examined the basic characteristics of mechanoreceptors in scorpion peg sensilla. Several extracellular electrophysiological recordings were made from the bases of pegs while gentle tapping induced the mechanoreceptor cell. The multiunit recordings were parsed into identifiable cell firings, which were then cross-correlated for possible interactions between the mechanoreceptor and chemoreceptors in individual pegs.

Methods

Animals

Male and female *Paruroctonus utahensis* collected from sand substrate in Winkler County Park near Kermit, Texas, were the subjects of this study. Only animals judged to be sexually mature were used (Stahnke 1970). Animals were kept individually in 3.8 L glass jars containing sand from the scorpions' natural habitat. Each animal was fed one cricket every week and lightly watered twice a week. The scorpions were maintained at the University of Oklahoma laboratory facilities at constant temperature (22^{o}C), relative humidity (55-65%), and light / dark cycle (2000-0730 hours dark, 0730-2000 hours light).

Electrophysiology

The scorpions were immobilized ventral side up on a rigid glass microscope slide using modeling clay. The pectines were elevated onto a glass microscope cover slip and secured with a piece of double-sided tape. A reference electrode (.250mm silver tef wire with insulation peeled off) was inserted between two metasomal segments until contact with the hemolymph was observed. All recordings were made on an air-cushioned table to minimize vibrations from the ground; pegs were visualized using epi-illumination and magnified 500-1000x with a compound microscope equipped with long working distance objectives (Olympus BX-50WI). Extracellular recordings were made using electrolytically sharpened tungsten wire inserted through the flexible cuticle at the base of the peg with the use of a micromanipulator (fig. 1A). Pegs subjected to electrode penetration were allowed five minutes to recover before mechanical stimulation began. AC-coupled, electrical signals were amplified 1000 fold over a bandwidth of 1-5 kHz and displayed on an oscilloscope. These signals were recorded on audio magnetic tape for subsequent playback, digitization, and analysis.

Mechanical Stimulation

Mechanoreceptor units were induced by gentle tapping of the micromanipulator holding the recording electrode. In some recordings, the peg was either slightly deflected or physically coupled to the edge of the table so that the vibration of the table edge relative to the air-cushioned table top induced intermittent firings of the mechanoreceptive cell (see fig. 1B). Recordings were variable in length and stability, but some lasted as long as 90 minutes while others lasted only 3 to 4 minutes.

Mechanical Stimulation

Extracellular recordings were digitized (Cambridge Electronics 1401 Plus) and stored on a hard drive for analysis. A filtering program (DOS-based Spike 2, Cambridge Electronics) was then used to reduce the 60 Hz artifact. A spike analysis program (Windows-based Spike 2, Cambridge Electronics) was used analyze all digitized recordings. Individual cells were discriminated based on their characteristic waveforms and then stored as separate time series. Auto-correlation—activity plots of one cell against itself—was used to verify that a single waveform resulted from one cell. The existence

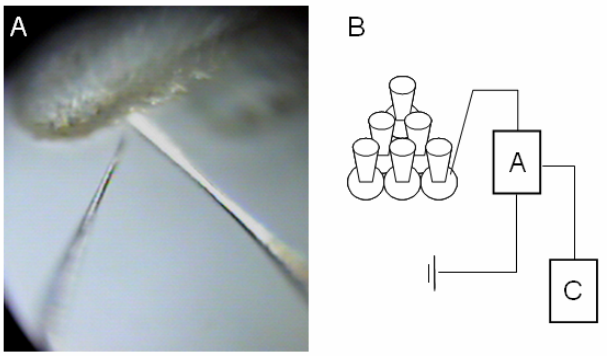

Fig. 1: Extracellular electrode placement. **(A)** Light microscopy view of electrode placement. **(B)** Diagram of electrophysiology apparatus. Tungsten electrode is inserted at the base of the peg while a reference electrode is inserted between two metasomal segments. The signal was processed through an amplifier (A) and analyzed at a computer (C).

of a refractory period would result in a noticeable decrease of cell activity after the firing of that cell, indicating that a particular waveform was the product of an individual cell. Refinement of waveform classifications was performed until all auto-correlations resulted in the presence of a refractory period.

The time activity of each cell was then cross-correlated with the occurrence of all other cells to reveal activity-dependent interactions (Gerstein and Perkel 1972). The results were then displayed in a histogram format with 50 bins (bin width = 10 ms) before and after the reference spike.

Results

The peg sensilla of *P. utahensis* were easily penetrated with tungsten electrodes, allowing for good signal-to-noise ratios. Some recordings could be maintained for over an hours before the electrode was dislodged. Five spike waveforms were identified by computer analysis, but these were assigned to only four types of neurons -- two of the waveforms were found to originate from the mechanoreceptor. The rapid firing of the mechanoreceptor produced a waveform whose amplitude graded quickly from one wave class to a second. Auto-correlations confirmed that each spike was the result of an individual sensory unit (fig. 2A).

Spike forms A1 and A2 displayed similar triphasic waveforms characterized by relatively small amplitudes (fig. 2A). A1 activity displayed more activity than all other classified waveforms combined. A1 also tended to fire regularly with roughly 15 ms intervals between each firing, which can be seen by examining the peaks on the auto-correlation analysis (fig. 2A). The firing of A2 was considerably less frequent and less regular than A1.

Spike form C was much less frequent than spikes A1 and A2. It was also characterized by a triphasic waveform with noticeably higher positive phase amplitude. No discernable pattern was apparent upon examination of the auto-correlograms. A fourth spike form similar to spike form B seen by Gaffin and Brownell (1997b) was also seen, but was rare in our recordings. This spike was distinctly smaller in amplitude and had a slight deflection in its positive going phase.

The mechanoreceptor (labeled "M") was triphasic with a large-amplitude initial negative phase and subsequent positive phase (fig. 2A). Mechanoreceptor analysis demonstrated that the cell's negative amplitude degrades over a series of close firings, presenting difficulties in classification. M spike firings were clearly correlated with mechanical displacement of the peg tip.

The activity of spike types A1, A2, and C were cross-correlated against the firings of the mechanoreceptor

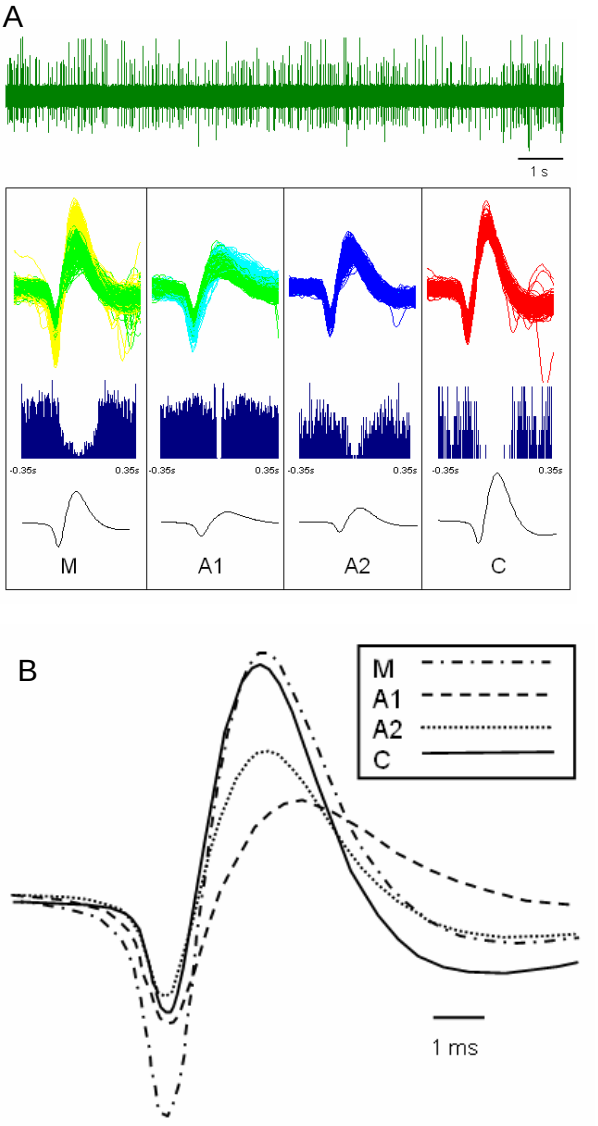

Fig. 2: Spike classification and auto-correlations. **(A)** Baseline recording and spike classifications. Forty-five minute baseline recordings were segregated into different spike classifications. Auto-correlations indicate the degree of certainty that each identifiable spike originates from only one neuron. Auto-correlations are then followed by the average waveform as computed by Spike2. Four spike forms, labeled M, A1, A2,

(fig. 3). The cross-correlograms show the activity patterns of A1 and A2 to be level and consistent both before and after the firing of M. This suggests no apparent interactions between the mechanoreceptor and neurons A1 and A2. The cross-correlogram of spike type C against the mechanoreceptor is irregular and inconclusive due to the infrequent occurrence of type C.

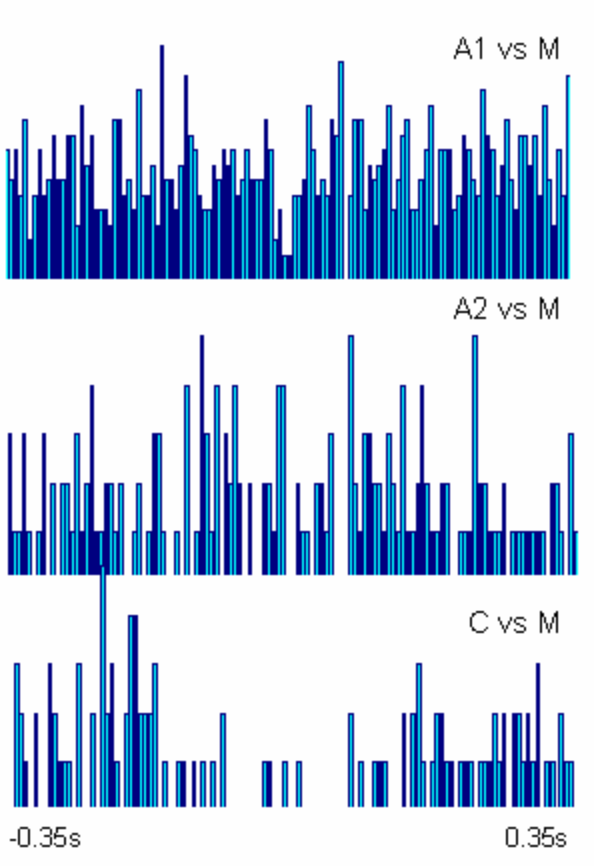

Fig. 3: Cross-correlation with respect to mechanoreceptor. Cross-correlations of all cells with respect to the mechanoreceptor, cell M. In each case, no apparent interactions are present, although the relationship between M and C is inconclusive.

Discussion

Extracellular recording from the bases of peg sensilla were easily obtained and produced long term, high-fidelity recordings. Using the Spike2 software, we were able to clearly resolve four waveforms in our recordings. Spike forms A1 and A2 were remarkably similar to the corresponding A1 and A2 cells in *P. mesaensis*—hence our reasoning for naming these cells. In addition, we describe a class C cell that fired in low frequency in baseline conditions, and that was not reported for *P. mesaensis*. The fourth wave form (type M) was induced during mechanical stimulation of the recording electrode. This putative mechanoreceptor adapted quickly during stimulation and recovered quickly between stimulations. A fifth waveform similar to the B waveform in *P. mesaensis* (Gaffin and Brownell 1997b) was seen, but could not be clearly classified due to its similarity to mechanoreceptor waveforms that gradated into its wave class.

The mechanoreceptor (class M) was extremely difficult to classify due to its tendency to decrease its negative and subsequent positive phase amplitudes. Classification was performed by first looking for "large" and "small" amplitudes separately and isolating these waves to separate traces. Next we selectively combined the waveforms, and ran auto-correlations to determine if they originated from a single source. Also, slight movements of the electrode inside the peg during the some recordings caused the signal-to-noise ratio to change, further complicating classification over an extended period of time. Future investigators should devise a system to minimize electrode displacement inside the peg in order to ease classification.

Cross-correlation analysis revealed no interaction between the mechanoreceptor and other cells under the conditions of this study. A1 and A2 clearly showed no interaction, but cell C was inconclusive due to low firing frequency. These results seem to discount my hypothesis that the mechanoreceptor activates the chemoreceptors, thereby decreasing the stimuli needed to reach threshold when the mechanoreceptor is activated (e.g. when it is in contact with the ground). These results did, however, provide physiological support for a recent morphological analysis which shows the mechanoreceptor cell bodies forming a layer distinct from the cell bodies of the chemoreceptors (Melville 1999). A morphological description of chemoreceptor synapses places them between axons just proximal to the cell bodies (Foelix and Müller-Vorholt 1983). Taken together, if synaptic interactions occur between chemoreceptors and mechanoreceptors, it is unlikely to be of the axo-axonic type reported for the chemoreceptor population.

Future studies of the mechanoreceptor need to focus on greater refinement of its characteristics. Better manipulation equipment is essential to quantify its response to mechanical stimulation. In addition, more firings of cell C are needed to resolve possible interactions between cells. An interesting possibility is that mechanoreceptors from neighboring pegs may be synaptically linked. This could result in lateral inhibition similar to that found in vertebrate retinas and could increase the animal's ability to perceive contours in the substrate (as during spermatophore transfer). Simultaneous recordings of multiple pegs should reveal whether any mechanoreceptor interactions occur among adjacent peg sensilla.

Despite the difficulties associated with extracellular recordings under mechanical stimulation, the scorpion pecten remains an organ with great promise in studying how animals (especially arthropods) perceive the world. The easy accessibility of the peg to extracellular recording allows for non-invasive investigation of peripheral synaptic interactions. We hope to use this system to resolve the role of peg mechano- and

chemoreceptors within individual pegs and how individual pegs function within the pecten as a whole.

Acknowledgments

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