Electrophysiology of peg sensilla mechanoreceptors on the scorpion pecten.

A biology cornerstone manuscript by:

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Summary

Scorpions possess unique chemoreceptive and mechanoreceptive organs known as pectines. The sensory processing capability of the organ arises from abundant microscopic structures called peg sensilla, located in dense patches along the teeth of each pecten. Past studies have shown that these sensilla are sensitive to different types of chemical stimulation; however, the mechanoreceptive function is mostly unexplored. A previously developed method involved delivering chemical stimulants onto the peg sensilla via a glass micropipette; using a modified version of this technique, we set out to record accurate and consistent instances of the mechanoreceptor firing. We made electrophysiological recordings from small patches of peg sensilla of the scorpion *Paruroctonus utahensis* while lowering a micropipette onto them and eliciting a mechanoreceptive response. These recordings allowed for the classification and analysis of electrical activity, measured as action potentials, for the differentiation of chemosensory and mechanosensory responses. Comparisons with previous studies, in which a putative mechanoreceptor was detected while recording from the peg base, suggests the success of this method in reliably stimulating the mechanoreceptor. Our records also show what appears to be an inhibitory effect of the mechanoreceptor on the chemoreceptive units in the pegs. We believe this new technique could be useful in further exploring the mechanoreceptive properties of the scorpion pectines.

Introduction

An elaborate and enigmatic structure unique to scorpions is the pecten. Pectines are paired, ground-facing, chemosensory and tactile sensory organs that scorpions use for substrate detection, mating, and perhaps locating the home burrow (Gaffin & Zhao 2014). They are sexually dimorphic appendages with a comb-like structure varying in the number of teeth (Kladt et al. 2007). Each pecten consists of dense patches of chemosensory peg sensilla on the teeth and ultrasensitive macro- and microsetae called pectinal hair sensilla on the spine capable of detecting air vibrations (Gaffin & Brownell 1997; Kladt et al. 2007). Of particular interest are the peg sensilla. However, the microscopic size and localized density of the pegs, have limited the available techniques and instruments to quantify their individual physiological properties.

Early studies on the function of peg sensilla relied primarily on behavioral observations and ultrastructure analysis (Abushama 1964; Carthy 1966, 1968). Advances in electron microscopy made it possible to map the innervation patterns of the pectines, use neuroanatomy to discern the role of those neurons, and visualize the structural organization of the peg sensilla. But technical constraints limited approaches to understand the pegs' physiological functions. In 1964, Hoffmann performed the first electrophysiological study on the peg sensilla using whole nerve endings from the base of the pectines. The author showed that they were responsive to mechanical deflection of the peg tip; however, the pegs were unresponsive to the variety of chemical stimuli applied (Hoffmann 1964). Gaffin and Brownell (1997) adapted this study to show that the pegs do respond to chemical odorants, when presented as aerosols across the pectinal surface, as well as to mechanical deflection. These experiments developed the basis for electrophysiological data collection, but had important

shortcomings that illustrate the need for consistency and precision in future pecten research.

More recent work by Knowlton and Gaffin (2009, 2010, 2011) on the development of the mineral oil flood technique established an improved method for chemical stimulant delivery. However, there is currently no established method for stimulating mechanical responses in the pectines, and little is known about the mechanoreceptor. The ability to stimulate the mechanoreceptors in localized pegs will significantly broaden the experimental potential for testing new hypotheses. In this study, we implemented electrophysiological techniques for extracellular recording from individual peg sensilla of the desert sand scorpion *Paruroctonus utahensis* and tested the peg's responsiveness to mechanical stimuli from deflections. We argue that this method will allow for accurate and consistent stimulation of the mechanoreceptors.

Methods

Animal care.—The subjects of this study were two large male desert grassland scorpions (*P. utahensis*). We maintained the animals in 3.8 L (15 cm diameter) glass jars containing desert sand of 3.0-5.0 cm depth. Every two weeks, we fed each animal two crickets, and twice a week we moistened the sand with 5 mL potable water to hydrate them. We stored them at constant temperature of 21°C, with relatively stable humidity conditions. We set a 14:10 light-dark cycle (2300-1300h light, 1300-2300h dark).

Electrophysiology.—We tested each animal individually using the same technique. Immediately prior to experimentation, we immobilized the animal by placing it in a 100 mL glass beaker in a freezer for two minutes, or until it was unresponsive to gentle puffs of air. We then prepared the animals for extracellular recordings. We placed the animal on a glass microscope slide (76x26 mm) ventral side up and completely secured its appendages to the slide with modeling clay. We cut a clear cover glass (18x18 mm) in half and placed it over the ventral side of the mesosoma. We then placed a piece of double-sided tape on top of the cover glass and carefully pulled out the right pecten with fine forceps and placed it atop the tape.

We used a micropipette puller (Sutter Instrument Co. Model P-87) to pull a glass capillary tube to create two glass micropipette electrodes with approximately 10 μm tip diameters and filled these micropipettes with a 0.01 M KCl solution. We inserted a sharpened silver wire, which functioned as an indifferent electrode, 2-3 mm into the soft tissue between two segments of the animal's tail, securing it with additional clay.

Next, we transferred the prepared animal to an adjustable platform under the microscope and secured the micropipette to a Leitz mechanical micromanipulator
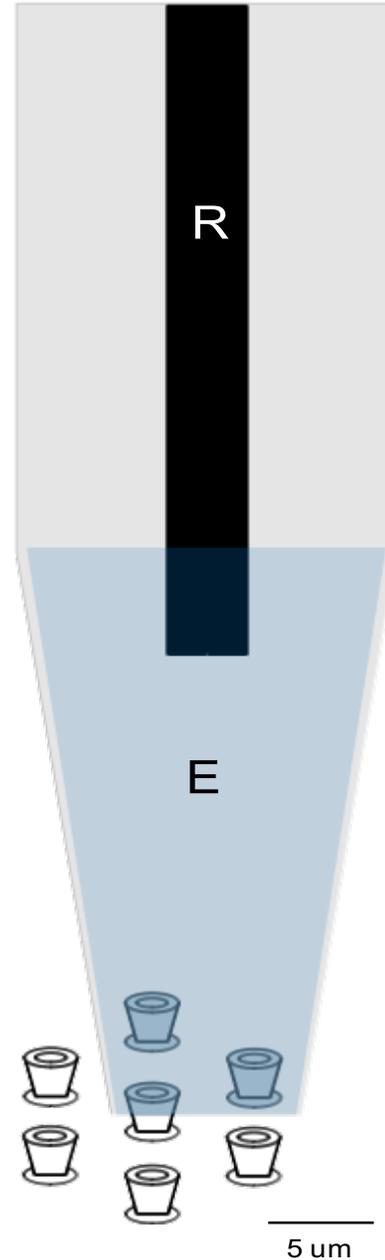

Fig. 1: Illustration of the experimental setup of the micropipette on a peg. [R = recording electrode, E = electrolyte]

near the animal. Under a microscope (Olympus BX5OWI), we located a field of peg sensilla. We identified individual pegs and maneuvered the pipette over a single sensillum or a patch of two to three sensilla (Fig. 1); we used the micromanipulator to position the pipette and stimulate the mechanoreceptor. We repeated

this step, testing 2-3 pegs on 4 different teeth for a total of 10 peg sensilla on each animal.

We recorded the signal using a Micro1401-3 analog to digital converter and Spike2 laboratory software (version 8.03a x64, both from Cambridge Electronic Design Limited). To improve the signal-to-noise ratio, we amplified the response 10,000 times using an amplifier (World Precision Instruments, Inc. DAM80) and band-passed the signal between 300 and 3000 Hz. We analyzed the action potentials of the mechanosensory neurons using the Spike2 software and correlated these spikes to the movement of the pipette. Using the software, we classified different types of spikes in the records and then from those classifications we identified the waveforms of the putative mechanoreceptors.

Results

We examined our records for sections where we had clearly defined spikes, ones that did not appear to be electrical noise. Once these sections were identified, they were exported and processed, and the most promising were analyzed and used to create the figures. We used Spike2's wavemark toolbox to categorize the spikes based on their waveform characteristics. We used the upper amplitude threshold in the wavemark toolbox to identify spikes because many of the tonic spikes did not have prominent leading down-spikes; the lower threshold would have missed spikes or caught a lot of extraneous noise.

We categorized the extracted wavemarks into what seemed to be distinct response patterns (based on the overdrawn wavemarks, Fig. 2), which were then averaged. This was used to create Fig. 3, an overlaying of the wavemarks, from which we identified the largest negative initial amplitude and subsequent positive amplitude spike as the putative mechanoreceptor (Kladt et al. 2007). We believe that the raw sample above displays the tonic response of the peg sensilla, which is a result of a chemoresponse to the potassium chloride electrolyte solution, followed by a section where the mechanoreceptor has been triggered, followed by a section where the mechanoreceptor has adapted and gone silent, returning to just the tonic response. The putative mechanoreceptor response is most apparent in the large downspikes that the chemoresponse lacks.

We observed several instances of a possible interaction between the mechanoreceptors and chemoreceptors. While the mechanoreceptor was active, for about the first 0.3 seconds, or about half its total period of activity, the chemoreceptors seemed not to be active. This pattern can be observed in Fig. 3, with the chemoreceptor resting spikes at the left and the right of the record not appearing in the middle when the putative mechanoreceptor is firing (i.e. when the spikes with the large negative amplitude are occurring). A closer view of this can be seen in Fig. 4, a
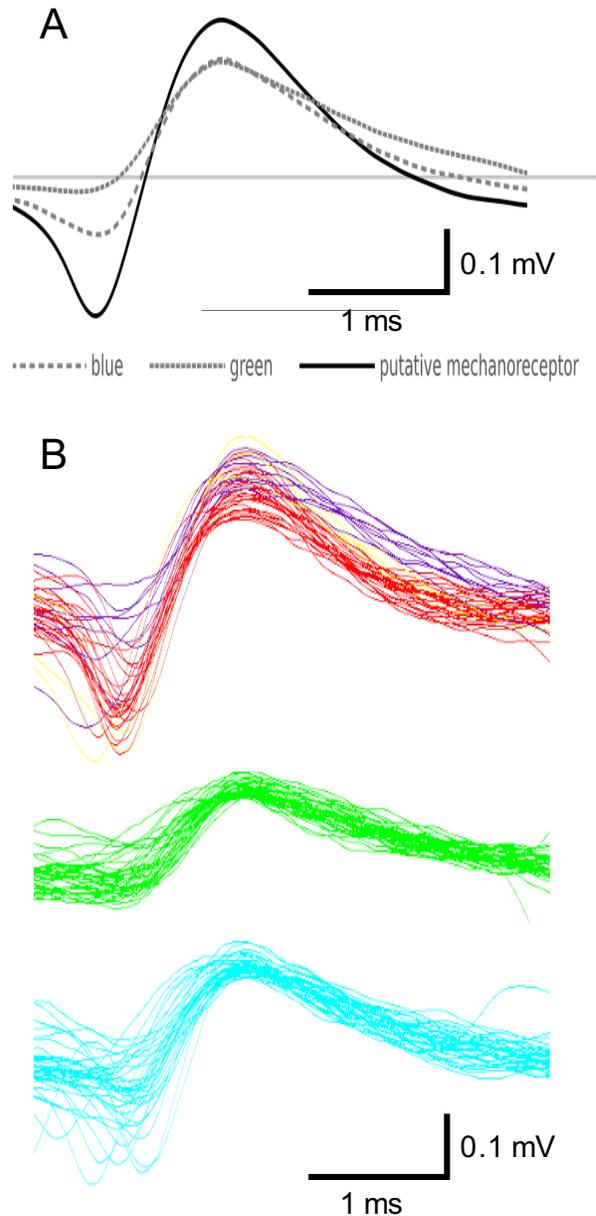

Fig. 2: Spike shapes. **A:** A comparison of the average waveforms of the distinct spikes from our Spike2 wavemarks. **B:** The overdrawing of the waves averaged in A; the putative mechanoreceptor is in red at top; chemoreceptors a shown below in green and blue.

subsection taken during the firing of the putative mechanoreceptor, which illustrates the absence of any of the resting spikes -- spikes without the large negative initial phase.
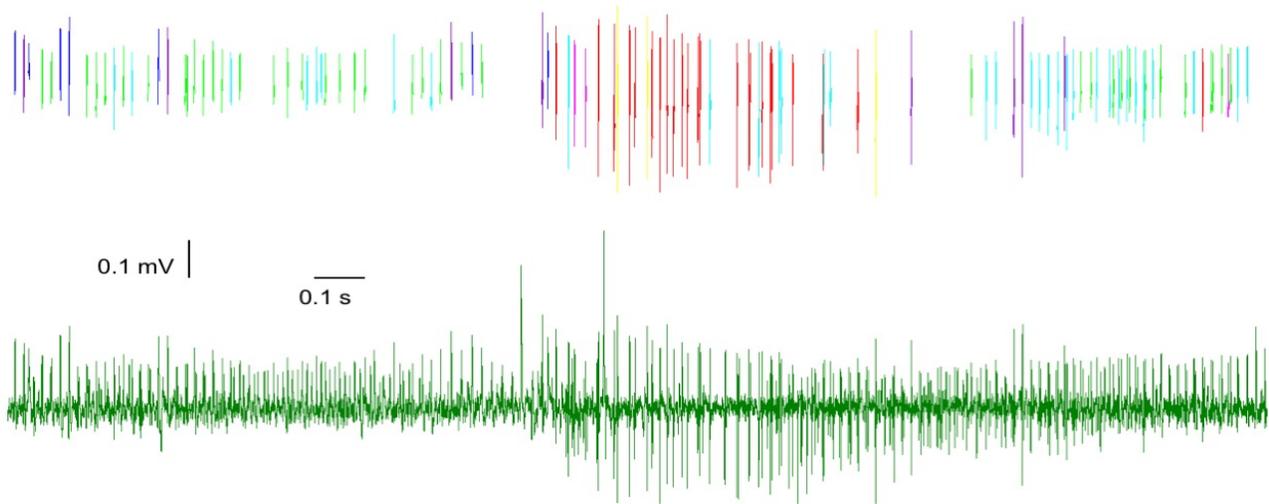
Fig. 3: The raw data with the Spike2 "wavemarks" above, highlighting the distinction between the chemical and the putative mechanoreceptor's signals. The "resting" reactions are depicted on either side, with the putative mechanoreceptors displaying much deeper downward spikes than the resting waveforms.

Discussion

Based on electrophysiological data, the pecten tip-recording technique can be adapted to investigate both the chemo- and mechanoreceptive characteristics of individual peg sensilla (Knowlton & Gaffin 2011). We show proof of concept that deflection of the peg is clearly correlated with activation of the mechanoreceptor and that the technique provides more stability for better recordings. Additionally, the use of the micromanipulator provides the user with an increased level of control and precision compared to previous studies. Using this technique, we identified a possible regulatory association between the chemoreceptors and mechanoreceptor within the peg sensilla.

Following mechanical deflection of the peg, a novel response was recorded and the resulting action potential displayed characteristics consistent with the mechanoreceptor. However, as an example of the tip-recording technique's limitations, Fig. 4 displays a record in which the pipette tip apparently deflected two pegs, causing two mechanoreceptors to fire. We recognized this triphasic waveform by its large-amplitude initial negative phase and subsequent positive phase, and noticed that the mechanoreceptor's negative amplitude degraded throughout the phasic response (Figs. 2, 3). These characteristics are consistent with the mechanoreceptor of the pectinal hair sensilla (PHS), and we think this consistency is due to the multiple innervation patterns of the peg sensillum's mechanoreceptor (Kladt et al. 2007). This similarity suggests that a single, common mechanoreceptor exists within a given peg, and together, the pegs and PHS may be involved in controling movements, positioning, and orientation of the pectines. This is important because the pecten is primarily a chemosensory organ that relies on the movement of each pecten to contact substrate structures intermittently through "taps," each lasting 0.033 s, to detect the chemical composition of heterogeneous substrate (Gaffin & Walvoord 2004). Understanding the interplay between the mechanoreceptors of the PHS and the peg sensilla and the chemoreceptors of the peg sensilla would provide important insight to the evolutionary significance of the pecten's complexity.

In the present study, we have identified a possible regulatory association between the chemo- and mechanoreceptors of the peg sensilla. For electrical conduction, the pipette contained a 0.01 M KCl electrolyte solution, which produced a tonic response of chemoreceptive peg neurons (Knowlton & Gaffin 2010). The resulting action potentials were also triphasic, but they had a much smaller initial negative phase and subsequent positive phase than observed in the mechanoresponse (Fig. 3). As illustrated in Fig. 2: the small-spike chemoresponses fire at a steady rate when the electrolyte solution has made contact with the peg, but once the micromanipulator is used to deflect the peg a high frequency, large-spike mechanoresponse is activated. At this point in the record, we observed a surprising result – activation of the mechanoreceptor caused the chemoreceptor to go silent (Fig. 2). Then, after approximately 0.3 seconds, the chemoresponse resumed.
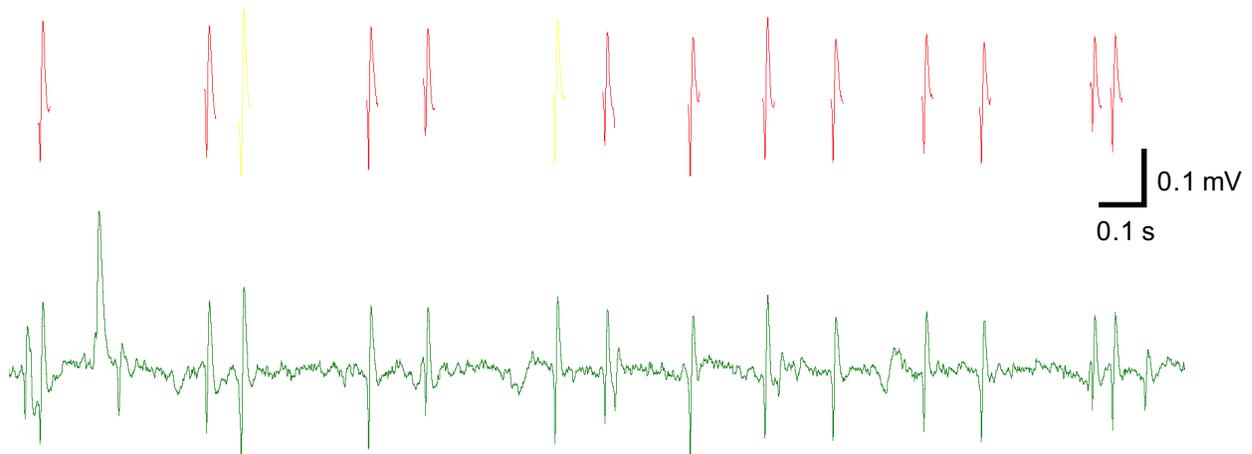
Fig. 4: A section of the wave depicting the double spikes we observed, likely caused by the tip of the pipette covering and stimulating more than one peg at once, as well as depicting the absence of the "resting" spikes during this section of the putative mechanoreceptor firing.

It is difficult to discern the exact duration of this inhibition due to spike-classification errors, but it's clear that the chemoreceptor is at least temporarily suppressed. Although there is no study to date that has investigated such a relationship in scorpions, this phenomenon was observed clearly for a second time in a different record. While there are sources of error that might account for this result, the evidence is sufficient to suggest a relation worthy of further investigation.

This study showed that the tip-recording technique (Knowlton & Gaffin 2010), adapted to exclude the mineral oil medium, is a useful tool for future research on scorpion pectines. We successfully stimulated the putative mechanoreceptor and obtained a triphasic waveform with a large negative initial amplitude and positive subsequent amplitude that was strikingly similar to the mechanoreceptor waveform observed in the pectinal hair sensilla (Kladt et al. 2007). Last, we identified a possible novel regulatory association in which stimulation of the peg's mechanoreceptor temporarily inhibits the activity of the chemoreceptors. Future research on pectines should seek to adapt the tip-recording technique, perhaps by engineering the micromanipulator to include an additional pipette for simultaneous recording from multiple pegs. However, we think the focus should be on adapting the technique to investigate the characteristics of the putative mechanoreceptor, such as directional-sensitivity, velocity-sensitivity, and pressure-sensitivity, to name a few. In addition, our surprising result suggests a possible interplay between the chemo- and mechanoreceptors of individual peg sensilla. Further research is needed to confirm this finding; but if the relationship persists, this result would provide important insight to the evolutionary complexity of the pectinal sense organs.

Acknowledgments

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