Euscorpius — Occasional Publications in Scorpiology. 2004, No. 17

Scorpion peg sensilla: are they the same or are they different?

Douglas D. Gaffin[1] and Mark E. Walvoord[1]

[1]Department of Zoology, University of Oklahoma, Norman, OK 73019-0235, USA

Summary

Thousands of peg sensilla adorn the ground-facing surfaces of the elaborate chemosensory organs of scorpions called pectines. From external appearances, these sensilla appear identical, however, it is not known if they are functionally the same. The answer to this question will influence our thinking on the organization and function of the pecten chemosensory system. Identical sensilla would suggest a parallel sampling scheme, lending support to an "information enhancement" hypothesis. Conversely, functionally distinct sensilla would support a "segmentation" hypothesis similar to the decomposition of sensory elements in the well-studied mammalian visual processing system. We are using a newly developed chemical delivery approach to test peg response patterns to consistent, repeatable stimulation. We report our findings based on electrophysiological recordings of stimulated peg sensilla of desert scorpions *Paruroctonus utahensis* (Vaejovidae). We also report on other relevant characteristics, including the nature and time course of a typical pecten "sniff" and the density of peg sensilla relative to substrate particle size from the animals' natural sand habitat.

Introduction

Scorpion pectines are featherlike, mid-ventral appendages that form the largest sensory input to the scorpion brain. The pectines are important in guiding males to prospective mates (Gaffin & Brownell, 1992, 2001) and perhaps in spermatophore exchange (Alexander, 1957, 1959) and in relocating stung prey (Krapf, 1986; Skutelsky, 1995). Each pecten is composed of a flexible spine and a series of movable teeth (Fig. 1 A-C; Cloudsley-Thompson, 1955). On the ground-facing surface of each tooth are dense patches of peg-shaped sensilla (Carthy, 1966, 1968; Ivanov & Balashov, 1979; Foelix & Müller-Vorholt, 1983). The peg sensilla are the main chemosensitive elements of the pectines, and previous studies have established their responsiveness to a range of organic molecules (Gaffin & Brownell, 1997a, b). Externally, the pegs appear identical, with their pores directed at the same angle relative to the ground (Gaffin, 2002).

Here we ask the question: are the pegs functionally the same or different? The answer to this question will affect how we think about this organ and its functional organization. In one case, we might find a distinct partitioning of the real world geometrically, where individual pegs contain distinct elements that respond differently to specific chemicals. We term this the *Information Segmentation Hypothesis* (Fig 2A). An example of this type of model is the cochlea in the inner ear, where receptors tuned to different frequencies distribute in an ordered manner along the length of the organ.

A second possibility is that the pegs are essentially repeated units, each possessing the same types of chemosensitive neurons (Fig 2B). This type of arrangement might be useful as a parallel sampling system, amplifying the amount of information received. We term this the *Information Enhancement Hypothesis*. Mammalian taste buds appear to be such a model. Each taste bud contains units that respond to various tastants, and these buds distribute across the tongue.

Initial accounts of peg chemosensitivity have suggested a segregated pattern of responsiveness (Gaffin & Brownell, 1997a). Pegs were stimulated by blowing volatile organic molecules from a syringe tip about a centimeter away as diagramed in Figure 1D. The response pattern for a given peg was consistent within a given stimulant series and orientation, whereas the response patterns for other pegs appeared different (Gaffin & Brownell, 1997a). Still, these results need to be approached with caution since the blowing of stimulant from a distance may have delivered an inconsistent stimulus concentration to different peg tips.

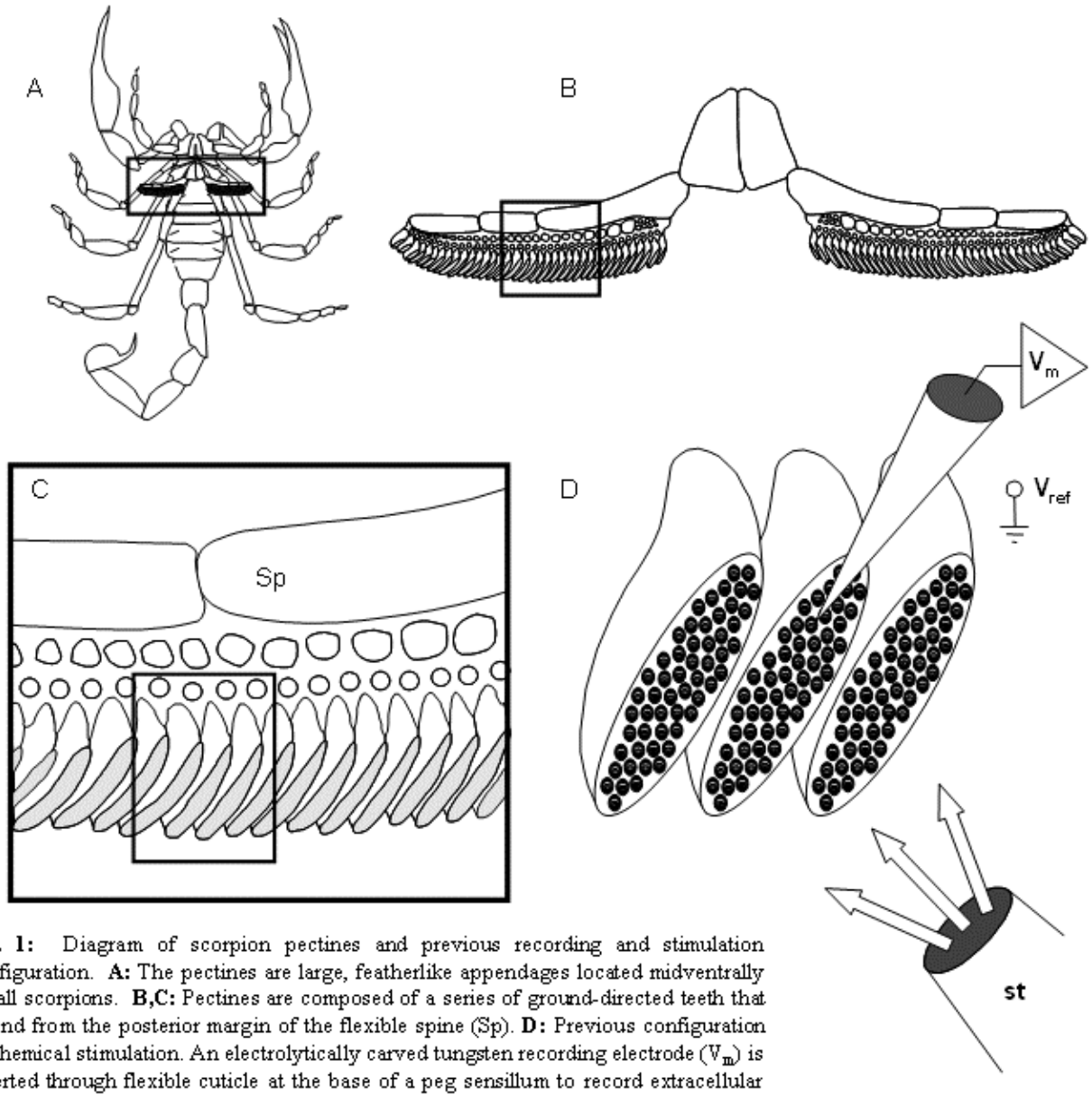

Fig. 1: Diagram of scorpion pectines and previous recording and stimulation configuration. **A:** The pectines are large, featherlike appendages located midventrally on all scorpions. **B,C:** Pectines are composed of a series of ground-directed teeth that extend from the posterior margin of the flexible spine (Sp). **D:** Previous configuration of chemical stimulation. An electrolytically carved tungsten recording electrode (V_m) is inserted through flexible cuticle at the base of a peg sensillum to record extracellular potentials from sensory neurons. Chemical stimuli (st) are blown across peg fields from a distance of about 1 cm (drawing not to scale). A reference electrode (V_{ref}) is placed in contact with hemolymph at some distance from the recording electrode.

While previous studies were important in establishing the chemosensitive nature of peg sensilla, they do not address the functional organization of chemosensitive elements among the peg population. In this study, we investigate this question by using a new method of peg stimulation that depends on stimulant diffusion from a pipette tip within a few microns of the peg pore rather than forceful delivery of stimulant from a distance (Gaffin & Hines, 2003). This method delivers a more controllable and consistent stimulant dose to the pore and allows for better comparison of responses between pegs.

Here, we first describe and characterize this new method of chemical delivery. Next, we use this method to test the sensitivity of adjacent peg sensilla to the identical chemostimulants. In addition, we use high-speed videography to characterize the time course of a typical pectinal brushing ("sniff") of the substrate. Finally, we calculate the density of pegs relative to a sand particle from the scorpions' environment. We use these data to argue that the pegs appear to be functionally redundant, supporting the Information Enhancement Hypothesis.

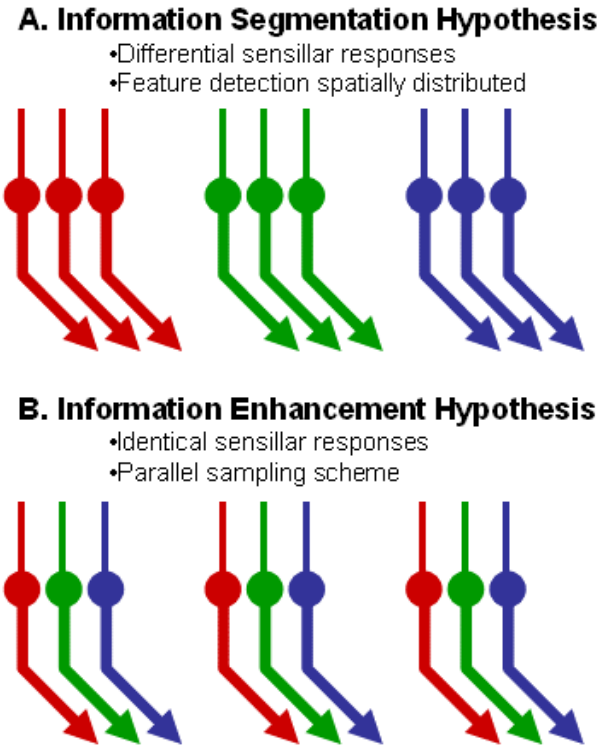

Fig. 2: Alterative hypotheses on the functional neural organization of peg sensilla. **A:** Information Segmentation Hypothesis. In this arrangement, chemical sensitivity is parsed to distinct peg sensilla. **B:** Information Enhancement Hypothesis. In this arrangement, each peg sensillum contains a similar complement of chemically sensitive neurons.

Methods

Animals

Mature *Paruroctonus utahensis* (Williams, 1968) (Scorpiones: Vaejovidae) obtained from sandy regions near Kermit, Texas, were the subjects of these experiments. We housed animals individually in 3.8 L clear glass jars containing 250 ml of sand collected from the scorpions' natural habitat. The jars were kept in a room with consistent temperature (22° C). The room lighting alternated between periods of light (0730-2000 h) and dark (2000-0730 h). Each week, we fed each scorpion one cricket and misted each with 10 ml of deionized water.

Electrophysiology and chemical stimulation

Scorpions used for electrophysiology were immobilized ventral side up in modeling clay on a glass microscope slide. An indifferent silver electrode was inserted between metasomal segments until contact was made with hemolymph. The pectines were then attached to a glass cover slip using double-sided adhesive tape; the individual "teeth" of the pectines were straightened and aligned to facilitate recording. The prepared scorpion was then fastened to the microscope stage and pegs were located using a high-powered (500-1000x) compound microscope (Olympus BX-50WI) equipped with epi-illumination and long working distance objectives.

Extracellular recordings were obtained by inserting an electrolytically sharpened tungsten electrode (tip diameter about 1 μm) into the cuticle at the base of the desired peg (Fig. 3). Electrodes were maneuvered into place using a Leitz mechanical micromanipulator. After insertion, the peg was allowed several minutes to recover to a consistent baseline activity. Electrical signals detected by the electrode were amplified 1000 to 10,000 times over a bandwidth of 1-3 kHz, displayed on an oscilloscope, and relayed through digitizing hardware (1401-plus, CED, Cambridge, England) at 20 kHz sampling rate to a computer for storage and analysis. Acquired records were further filtered with a digital high pass filter and analyzed using a spike recognition and analysis program (Spike 2, CED). Spiking events were traced using 100 samples spread evenly across the spike wave (100 points at 20 kHz sampling frequency gives 5 ms between point). Events with peak amplitude above background noise were isolated from the record and categorized to discrete classes using a spike recognition algorithm in the Spike 2 program.

The chemical delivery device consisted of a glass pipette with a tip pulled to a diameter of about 5 μm. The pipette was formed from a glass capillary tube (World Precision Instruments 1.00 mm OD, 0.58 mm ID, 152 mm length, with filament) pulled in a glass micropipette puller (Sutter Instrument Co. Model P-87).

Stimulant pipettes were backfilled by immersing the pipette tip into a vial containing the pure substance to a depth of about 1 cm for 2 hours. This method allowed enough chemical to backfill into the pipette tip to allow several hours of experimentation. We used pure 1-hexanol as the stimulant chemical in this study.

The stimulant pipette was attached to a glass electrode holder and affixed to the head of an electronically controlled micromanipulator (Burleigh step driver PZ-100). The electronic head was mounted to a mechanical micromanipulator for movement of the pipette to within 100 microns of a recorded peg. The left-to-right and up-and-down positioning of the pipette tip was also adjusted using the fine controls of the mechanical micro-manipulator. The electronic micromanipulator produced precise in-and-out movements, which allowed us to control the distance of the pipette tip from the tip of the recorded peg. The distance of travel of the pipette tip was monitored by reading the output from the electronic manipulator. This information was sampled at 100 hz on a second channel of the digitizer and stored on a separate channel in the Spike 2 program.

Once precisely positioned within microns of the peg tip, our experiments consisted of using the electronic manipulator to move the pipette tip close to a recorded peg, away, close, etc. while monitoring the neural response and the position of the pipette as shown in Figure 3.

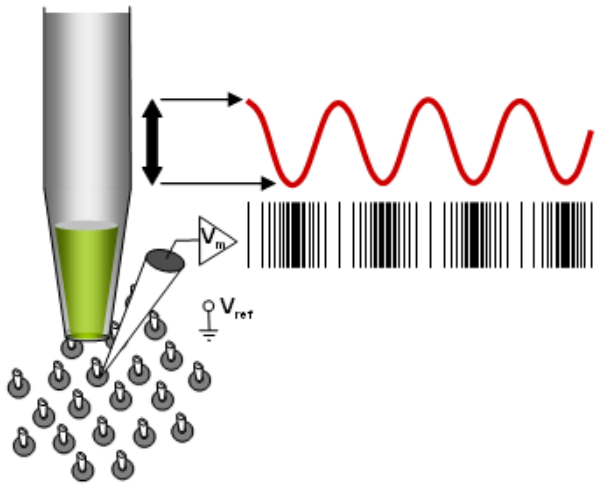

Fig. 3: Recording and stimulation configuration of new static odorant method. Tungsten microelectrode is inserted through flexible base of individual peg sensillum. A glass pipette with tip diameter of about 5 microns is backfilled with a pure substance and maneuvered via an electronically controlled manipulator to within microns of the peg tip. The travel of the stimulant pipette tip is monitored relative to distance from the peg tip while the neural response is recorded.

Videography of a pecten "sniff"

We filmed individual *Paruroctonus utahensis* during the day in a dark room by placing them into a clear, rectangular, Plexiglas container elevated above an infrared (IR) spotlight (Ultrak, model UL-IR-50-FL 12 V, 50 W). This box was 37 cm long, 5.5 cm wide, and 7 cm tall to give enough space for scorpion movement. An IR-sensitive camera (Panasonic CCTV camera model WV-BP314) was focused on the center of the Plexiglas floor through the side of this box. We placed a female scorpion into this box, covered the box with black paper to reduce glare, turned off room lights, and began recording onto a videotape in a time-lapse video recorder (Panasonic model AG-RT600P) set to 8-hour mode. This allowed us to capture sixty frames/second. Video recording took place for approximately 2 hours at a time, before stopping the tape to check for footage of the scorpion traversing in front of the camera.

We reviewed all taped trials, and the single pecten lowering sequence with the clearest view was used in this paper. This trial took place on 20 May 2004 from 1025–1152 hours at 22°C and approximately 78% relative humidity.

Calculation of peg density vs. sand particle size

We directly counted and calculated the density of peg sensilla on tooth 17 of the right pecten of a female *P. utahensis*. We did this by snapping an overlapping series of digital photos using a digital camera (Flexcam Teaching NTSC Rev. 3.0, Videolabs Inc., USA) connected to a frame grabber (Snappy Video Snapshot, Play Inc.) for computer manipulation and analysis. Photos were taken directly from one of the eyepieces on the light microscope used for electrophysiology under high power. We used a similar approach to determine the area of a "typical" grain of sand retrieved from the scorpions' native habitat. Peg density was calculated by dividing the number of pegs on a tooth by the surface area of the peg field in square microns.

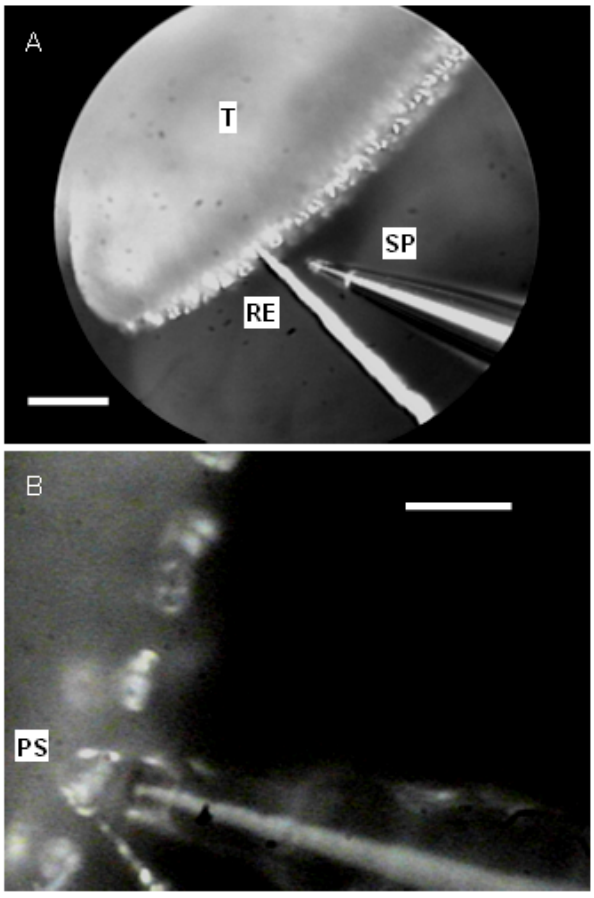

Fig. 4: Stimulus pipette relative to peg and recording electrode. **A:** A pectinal tooth (T) is shown with recording electrode (RE) in place as the stimulus pipette (SP) approaches the recorded peg. **B:** Close up view of tungsten microelectrode inserted in base of peg sensillum (PS) and tip of stimulant pipette manuevered within microns of the peg tip. Scale bars: A: 10 microns; B: 5 microns.

Results

Our extracellular recordings were stable for tens of minutes to hours with little reduction in signal-to-noise ratio. With practice, the recording electrode and stimulus pipette could be configured to avoid crossing each other. We found that the response of peg neurons was highly sensitive to the distance between the pipette and peg tips. Because of this, it was important to move the stimulus pipette as close to the peg tip as possible without making contact. Contact of the tip to the peg usually resulted in flooding of chemical across the peg fields, causing intense firing of peg neurons and several minutes of sensory adaptation. Figure 4 shows the relationship of the recording electrode and stimulus pipette in a typical recording configuration . Figure 5 shows an experimental control recording. Moving an empty pipette tip near to the peg tip had no effect on the spiking activity of the peg neurons.

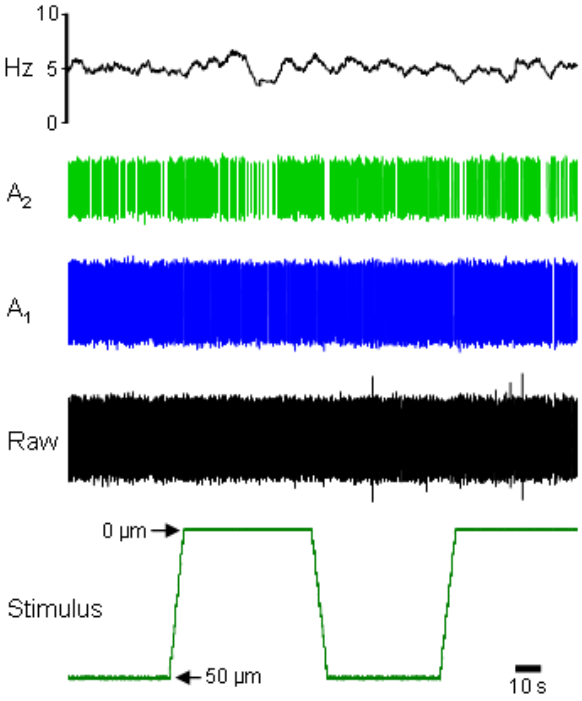

Fig. 5: No stimulus control. The bottom trace shows the travel of an empty pipette tip from 50 microns to within 1 micron of the peg tip and back. The "Raw" trace shows all electrical activity recorded from the peg sensillum while A_1 and A_2 show the isolated activity of two identifiable cells. The top graph depicts the spiking activity of the peg in Hz for the duration of the test.

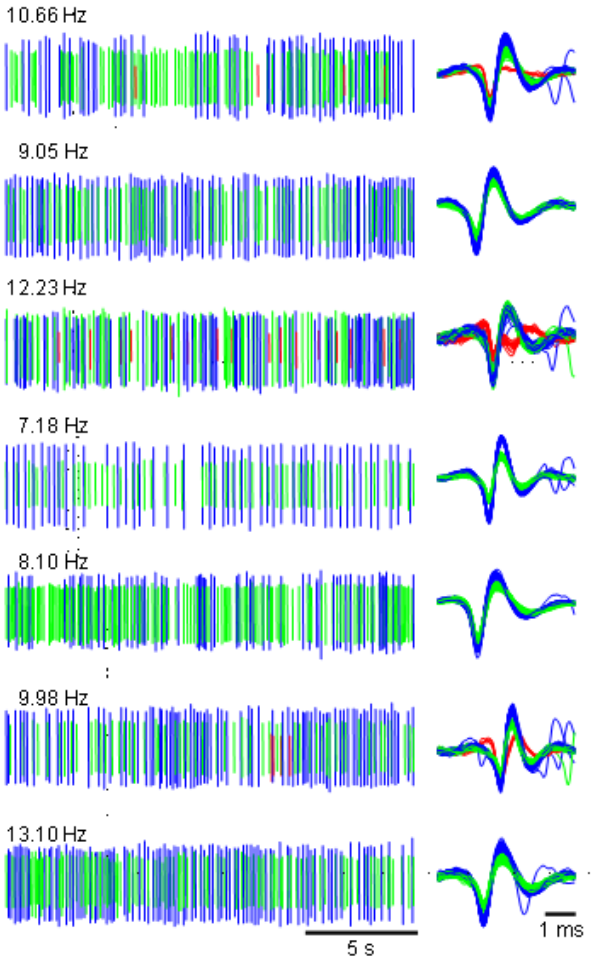

Fig. 6: Baseline series. Shown are no-stimulus recordings of spontaneous activity from seven adjacent peg sensilla. The traces at right show 15 seconds of spiking categorized by spike sorting algorithm. At right are the superimposed waveforms from each record. Spiking frequencies varied from 7.18 to 13.10 Hz in these records.

Spontaneous spiking activity

We recorded spontaneous spiking activity from seven adjacent peg sensilla on a single pectinal tooth (Fig. 6). The baseline spiking frequencies of these samples ranged from 7.18 Hz to 13.10 Hz. All seven records contained two large biphasic waveforms, which appear similar to the A1 and A2 waveforms reported for *Smerigerus mesaensis* (Gaffin & Brownell, 1997a). A third, smaller, triphasic waveform (colored red in Fig. 6) was detected in three records. The patterns of spiking activity appeared similar in each of the records.

Chemical Stimulation

To test for similarity in chemical response, we recorded from six consecutive pegs from tooth 19 of a female *P. utahensis*. Several successive advances and retractions of pure 1-hexanol were made for each recorded peg. In each case, the pipette tip was moved immediately adjacent to the peg, then retracted 20 microns, and returned to the peg tip. The advance and retraction rate was 4 microns per second; one complete cycle took 10 seconds. Shown in Figure 7 are samples of seven such advances and retractions. The spiking patterns of each peg were similar, showing a sharp increase in spiking with approach of the stimulus pipette. Spiking frequencies, smoothed by 3-second bins, are displayed above the spikes in each of the peg records. Peak frequencies ranged from 20 to 40 Hz across the records.

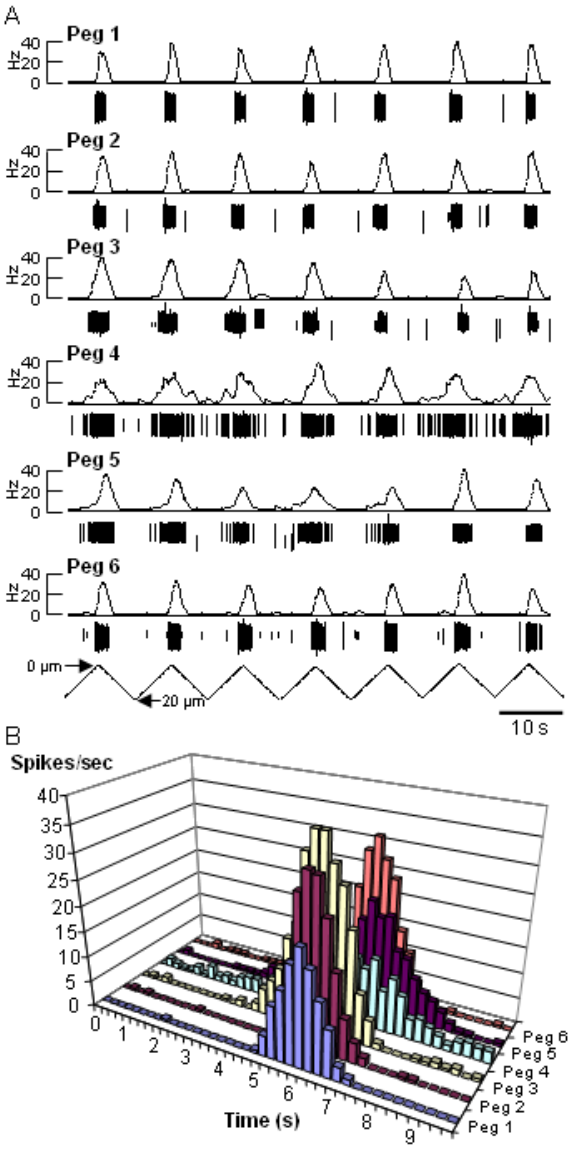

Fig. 7: Hexanol stimulation of six adjacent peg sensilla. **A:** Neural responses of six pegs to seven successive movements of the stimulus pipette from 20 µm to 0 µm (adjacent to the peg tip) and back (pipette travel shown below peg 6 record). For each peg, the bottom trace represents the isolated spiking activity and the top trace the spiking frequency in Hz. **B:** Spiking frequency averaged across the seven stimulus repetitions for each of the six pegs.

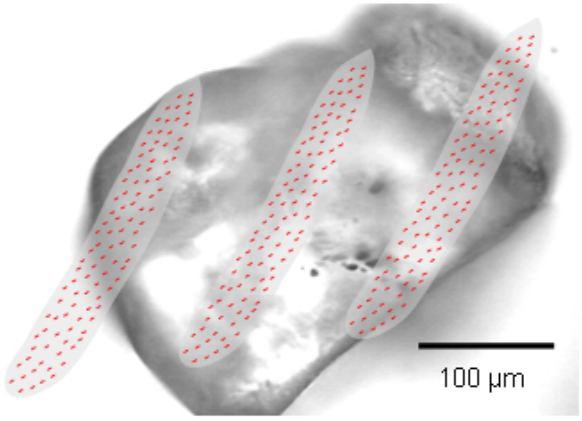

Fig. 8: Peg sensilla density relative to a sand grain. The positions of peg sensilla on a pecten tooth of a female *P. utahensis* was determined using overlapping photos of the peg field taken from high power light microscopy. Here we show this field repeated three times at the appropriate spacing to represent three successive pecten teeth. The fields are superimposed atop a photo of a grain of sand from the scorpion's native habitat.

Peg density vs. sand grain size

The peg density on tooth 17 of a female *P. utahensis* was directly calculated from a series of overlapping photos. We counted 105 pegs on this tooth across a peg field area of approximately 8400 µm^2. This gives a peg density of approximately 0.0125 pegs per µm^2 or 12,500 pegs per mm^2. We also measured the area of a "typical" sand particle from the dune habitat where these animals live. We measured the surface area of this particle directly from the photo as 33,440 µm^2. Put another way, 418 pegs would fit on this sand grain (33,440 µm^2 * 0.0125 pegs/µm^2 = 418 pegs). Figure 8 shows representative peg fields from three pecten teeth superimposed atop the sand grain.

Kinematics of a pecten "sniff"

The pecten sniff consisted of a ~0.20-second lowering sequence followed by a ~0.07 second retraction of the pecten upon contact with the substrate (Fig. 9). Contact with the substrate, in this case, lasted at most 0.033 seconds. During other sequences of this and other trials,

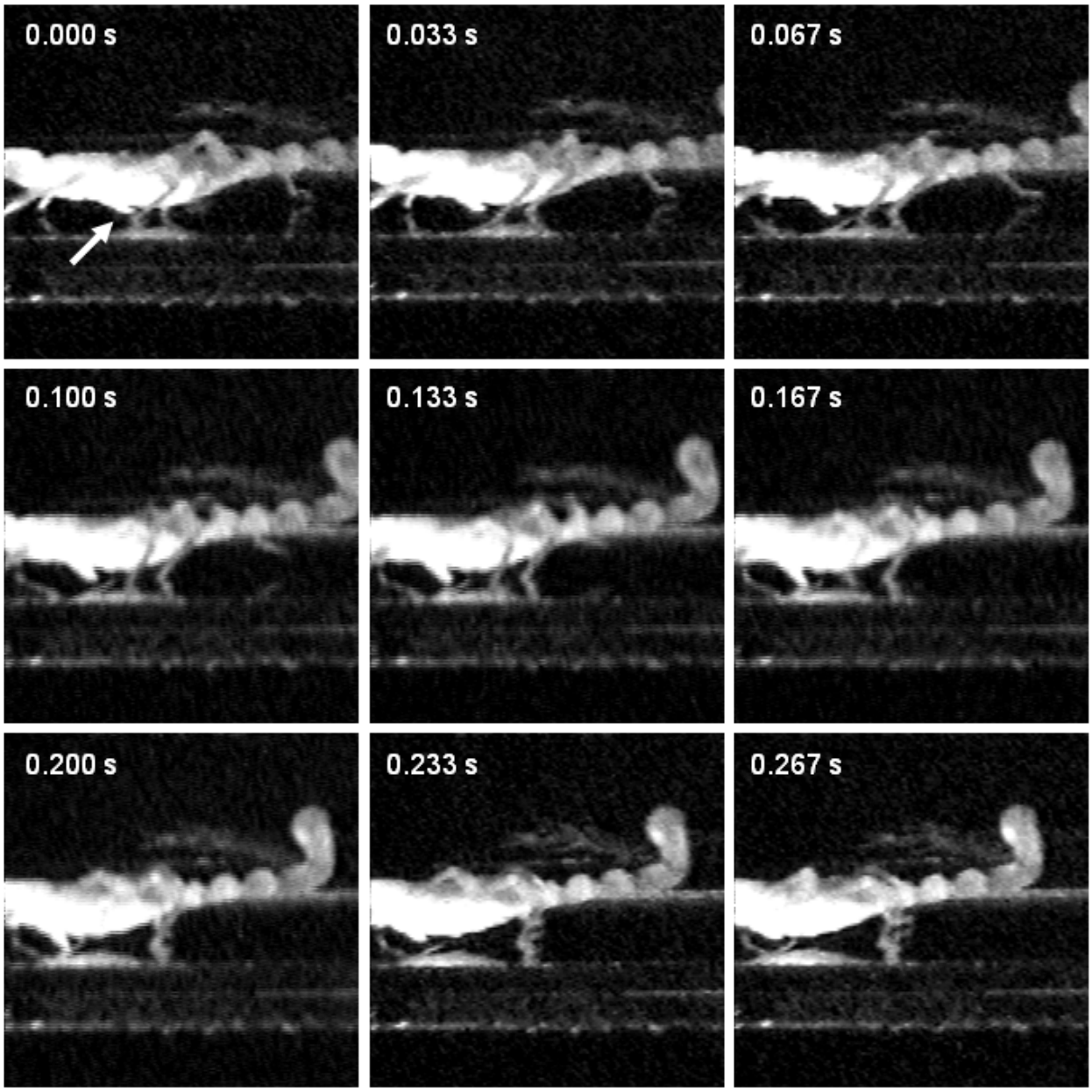

Fig. 9: These pictures represent a time course of a pecten "sniff" in a captive, female *P. utahensis*. Video was recorded at sixty frames/second under infrared (IR) light with an IR-sensitive camera. This time course represents snapshots of the video taken every other frame. The pecten is indicated by the white arrow in the first frame.

scorpions displayed another behavior in which they would lower and drag their pectines for a longer period just above or on the substrate before retracting them.

Discussion

Both the activity patterns and types of units present in recordings from peg sensilla are similar between individual pegs during both spontaneous recordings and under consistent stimulation. The new method of presenting a stimulus by moving a static source near a peg tip gives much more consistent response patterns between pegs than during forceful propulsion of the stimulant across the peg field from a distance (Gaffin & Brownell, 1997a). The small departure in stimulant response for some pegs (such as peg 4 in Fig. 7) is more likely a result of the pipette tip not being as close to the peg pore as in the other recordings; we found that distance from pipette to tip greatly influences the peg response and even a micron can have a significant effect.

The data from videography allowed us to observe pecten lowering in a homogenous environment and to

determine a time course for one pecten "sniff." We also observed that pectines appear to make contact with the substrate. To fully analyze pecten sniffs or dragging behavior, we need to record more pecten sniffs from multiple animals, classify how many events were "touches" and how many were drags, and record an average time course of these drags. We hypothesize that this dragging behavior may be a response to the smooth, homogeneous surface on which they were traveling. More observations are needed of scorpions active at night in their natural habitat to record the exact sniffing behavior on a normal substrate.

The time course of a single pecten "sniff" is important for approximating the amount of time available for a field of peg sensilla to gather an information sample. On the conservative side, the peg fields are near to the substrate (within microns) for only 0.033 s. We have found that chemical responsiveness of peg neurons to a pure stimulant varies significantly with distance of the stimulant from the peg tip. For example, movement of the pipette tip from 20 µm distant to 1 µm distant resulted in an increase of spiking frequencies from 0 to 40 Hz (see Fig. 7) with most of the change occurring as the tip was within 10 µm.

The peg density for the pecten tooth we measured on a female *P. utahensis* was approximately 0.0125 pegs per μm^2. This calculation is in line with what Brownell (2001) reported for another vaejovid scorpion species, *Smeringurus mesaensis*. Why are there so many pegs? This is an important question, given that the particle size of the animal's habitat is orders of magnitude greater than the inter-peg distances.

The answer to this question may come from a closer examination of the chemical response data. First, we find that the inter-peg distances are approximately 8 µm, which is in line with the proximity of the stimulus pipette tip for which we see significant neural responses. Also, while our frequency graphs of neural activity suggest that individual pegs can track the stimulant within this distance, we obtained those graphs by averaging over 3-second bins. If calculated across a more realistic time window of 0.033 seconds (the time course of a pecten "sniff," calculated above), spiking averages of peg neurons would not be able to resolve stimulant distance with any degree of confidence. Put another way, to achieve the stimulant distance resolution for a single peg sensillum as implied by Figure 7, the animal would need to hold its pectines to the ground for at least three seconds – and the animal simply does not do this.

Taken together, these data support the *Information Enhancement Hypothesis*, with the peg sensilla being functionally repeated units. It appears that the peg fields function in a parallel sampling system, delivering to the brain information sampled from a quick "sniff." In essence, such a system could provide the same resolution of stimulant location as can be obtained from a single sensillum presented with seconds of prolonged chemical stimulation.

Acknowledgments

We thank Drs. J. Bastian and M. Hoefnagels for their valued suggestions, technical advice, use of equipment, and encouragement. We would also like to thank P. McGowan, M. Hines, and G. Blass for assistance during the research project. An Associates Presidential Professorship and the *Life Fund* of the University of Oklahoma Foundation provided funding for this project. The experiments described here comply with the "Principles of animal care," publication No. 86-23, revised 1985 of the National Institute of Health and also with the current laws governing animal care and usage in the United States.

References

ALEXANDER, A. J. 1957. The courtship and mating of the scorpion, *Opisthophthalmus latimanus*. *Proceedings of the Zoological Society, London*, 128: 529–544.

ALEXANDER, A. J. 1959. Courtship and mating in the buthid scorpions. *Proceedings of the Zoological Society, London*, 133: 145–169.

BROWNELL, P. H. 2001 Sensory ecology and orientational behaviors. Pp. 159–183 *In* P. H. Brownell & G. A. Polis (eds.). *Scorpion Biology and Research*. New York, NY: Oxford University Press.

CARTHY, J. D. 1966. Fine structure and function of the sensory pegs on the scorpion pecten. *Experientia,* 22: 89–91.

CARTHY, J. D. 1968. The pectines of scorpions. *Symposium of the Zoological Society, London*, 23: 251–261.

CLOUDSLEY-THOMPSON, J. L. 1955. On the function of the pectines of scorpions. *Annals and Magazine of Natural History*, 8: 556–560.

FOELIX, R. F. & G. MÜLLER-VORHOLT. 1983. The fine structure of scorpion sensory organs. II. Pecten sensilla. *Bulletin of the British Arachnological Society*, 6: 68–74.

GAFFIN, D. D. & P. H. BROWNELL. 1992. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida:Vaejovidae). *Ethology,* 91: 59–69.

GAFFIN, D. D. & P. H. BROWNELL. 2001. Chemosensory behavior and physiology. Pp. 184– 203, *In* P. H. Brownell & G. A. Polis (eds.). *Scorpion Biology and Research*. New York, NY: Oxford University Press.

GAFFIN, D. D. & P. H. BROWNELL. 1997a. Response properties of chemosensory peg sensilla on the pectines of scorpions. *Journal of Comparative Physiology, A,* 181: 291–300.

GAFFIN, D. D. & P. H. BROWNELL. 1997b. Electrophysiological evidence of synaptic interactions within chemosensory sensilla of scorpion pectines. *Journal of Comparative Physiology, A,* 181: 301–307.

GAFFIN, D. D. & M. A. HINES. 2003. A new approach to chemostimulation of scorpion peg sensilla reveals high resolution response properties to near-range chemostimulants. *Abstracts/Annual Meeting of the American Arachnological Society. Denver, Colorado*.

IVANOV, V. P. & Yu. S. BALASHOV. 1979. [The structural and functional organization of the pectine in a scorpion, *Buthus eupeus*, studied by electron microscopy]. *In*, Balashov, Yu. S. (ed) *Fauna i ekologiya* paukoobraznykh [The Fauna and Ecology of Arachnida]. *Trudy Zoologicheskogo Instituta, Leningrad,* 85: 73–87 (in Russian).

KRAPF, D. 1986. Contact chemoreception of prey in hunting scorpions (Arachnida:Scorpiones). *Zoologischer Anzeiger*, 217: 119–129.

SKUTELSKY, O. 1995. Flexibility in foraging tactics of *Buthus occitanus* scorpions as a response to aboveground activity of termites. *Journal of Arachnology*, 23: 46-49.