Electrophysiological properties of sensory neurons in peg sensilla of *Centruroides vittatus* (Say, 1821) (Scorpiones: Buthidae)

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Summary

Pectines are unique, substrate-directed appendages that are important in mating and food-finding behaviours in scorpions. In this paper, investigations of electrophysiological properties of pectinal peg sensilla in *Centruroides vittatus* (Buthidae), are reported. Twenty minutes of spontaneous electrical activity, from six different peg sensilla, were recorded for each of eight adult animals for a total of 48 pegs and 16 hours of recording time. Distinct spiking events were digitally sorted and subjected to cross-correlation analysis to detect interactions between sensillar neurons. Most records contained three spontaneously active spikes of distinctive waveform, which we labelled A1, A2, and B. Cross-correlation analysis revealed excitatory interactions between spike types A1 and A2, while type B showed no effects on the spiking behaviour of A1 or A2. A fourth cell, a putative mechanoreceptor, was induced in some records in response to light mechanical stimulation. This cell fired in high-frequency bursts with rapid adaptation and recovery, characteristic of other arthropod mechanoreceptors. Taken together with previous studies, it appears that peripheral synaptic interactions may be a common feature of scorpion peg sensilla, although distinct patterns exist in the activity patterns and electrical characteristics of sensory neurons in peg sensilla from different species.

Introduction

The pectines of scorpions are ground-directed appendages that are involved in mating (Alexander, 1959; Polis & Farley, 1979; Gaffin & Brownell, 1992, 2001) and food-finding (Krapf, 1986) behaviours in these animals. These comb-shaped organs extend from the ventral body wall just caudal to the genital opening. Each pectine is composed of a flexible spine and a series of teeth, each supporting dense fields of hundreds to thousands of peg-shaped, chemosensory sensilla (Carthy, 1966, 1968; Swoveland, 1978; Ivanov & Balashov, 1979; Foelix & Müller-Vorholt, 1983; Gaffin & Brownell, 1997a, 2001). There is no comparable structure in the animal kingdom; closest are the chemosensitive malleoli that extend from the hind legs of solpugids, a related group of arachnids (Brownell & Farley, 1974). Elaborate, midventral, ground-directed chemoreceptors have so far only been described in these two groups. Of these, only the pectines have been the focus of behavioural or physiological studies.

Despite the unusual nature of the pectines, little is known of how they transduce and process information. The only detailed study to date describes some of the electrophysiological properties of peg sensilla of the vaejovid scorpion *Paruroctonus mesaensis* Stahnke, 1957 (Gaffin & Brownell, 1997a). Extracellular recordings from the bases of peg sensilla revealed several distinct spike waveforms, which showed various activation patterns when organic stimulants were blown across the peg tip. In addition, a mechanosensitive unit was activated upon gentle deflection of the peg tip. Interestingly, activity interactions were found among some spike classes in the peg recordings. Cross-correlograms of spontaneous activity revealed inhibitory interactions between some units within individual peg sensilla. These interactions appear to play a role in shaping chemosensory response patterns at the level of the receptor, prior to information relay to the central nervous system (Gaffin & Brownell, 1997b).

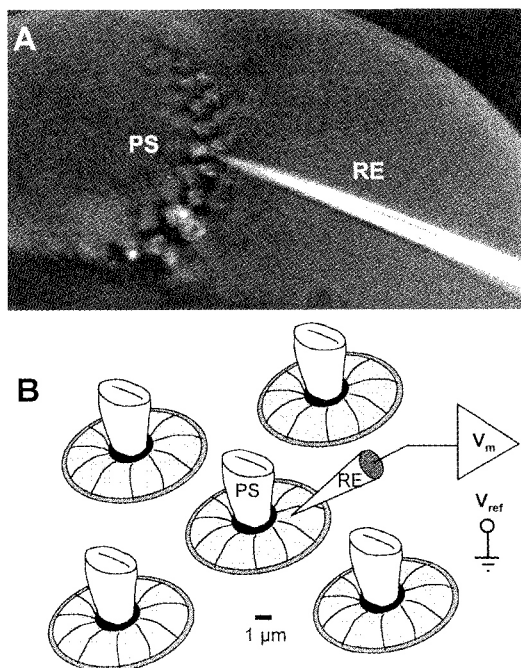Peripheral synaptic contacts appear to be a rare phenomenon in chemo- and mechano-sensory

Fig. 1: Electrode placement in peg sensillum of *C. vittatus.* **A** A microelectrode is shown under a light microscope (500×) as it enters a field of peg sensilla. **B** The insertion of an electrode though flexible cuticle at the peg base. RE = recording electrode, PS = peg sensillum.

organs of insects and crustaceans (Moulins & Noirot, 1972; White *et al.*, 1990). In arachnids, however, such synapses may be more the rule than the exception. Synaptic contacts are seen between sensory neurons in the tarsi of the modified first legs of whip spiders (Amblypygi) and harvestmen (Opiliones) (Foelix, 1975, 1985). Morphological accounts of peg sensilla on the pectines of the buthid scorpion, *Androctonus australis* (L., 1758) indicated putative synaptic contacts between axons of primary afferents, in a region just proximal to the sensory cell body layer, just 50 μm below the pegs themselves (Foelix & Müller-Vorholt, 1983).

It is not known how widespread synaptic interactions are in peg sensilla of different scorpion species and whether different patterns of interaction exist. In this paper, I describe some electrophysiological properties of peg sensillar neurons on the pectines of *Centruroides vittatus* (Say, 1821), representing the family Buthidae. In addition, we subject identified units to cross-correlation analysis to determine if activity interactions

exist between sensory neurons in peg sensilla of this species.

Methods

Animals: Eight adult *Centruroides vittatus* were collected from leaf litter in post-oak woodlands near Lake Thunderbird (Cleveland County, Oklahoma). Animals were housed singly in clear glass pickle jars (4150 ml) on natural soil substrates in a room with a light cycle of 15 h light : 9 h dark, temperature of 22°C, and 65% relative humidity. Animals were maintained with weekly feedings of small gray crickets (Fluker's Cricket Farm, Baton Rouge, LA) and misted with approximately 12 ml of water twice weekly. At the end of each recording session the animal was returned to its home container.

Animal preparation: Following anesthetization by cooling (-5°C for 5 min), each animal was secured, ventral side up, to a glass microscope slide using modelling clay. A bridge consisting of a glass cover slip supported by modeling clay was erected to span the ventral body surface just posterior to the genital aperture. The pectines were lifted and secured on the cover slip with a piece of double-sided tape aligned with the anterior edge of the cover slip. The pectine teeth were then arranged and pressed into the tape using fine forceps or a fine-bristled brush. The indifferent electrode (an 8 cm long, presharpened length of tungsten wire with attached lead) was inserted by hand between cuticular plates on the animal's ventral mesosoma. The free end of the electrode (with lead exposed) was secured with clay to keep the electrode in place.

Electrophysiological recording: Recording electrodes consisted of tungsten wires electrolytically etched to a tip diameter of approximately 1 μm. The electrode tips were bent downward approximately 45 degrees to improve the angle of approach to the peg base. Peg sensilla were visualized under epi-illumination and magnified 500–1000× with a compound microscope equipped with long working distance objectives (Olympus BX-50WI). Electrodes were manoeuvred and inserted through the flexible cuticle at the peg base (Fig. 1) using a micromanipulator (Leitz). Penetration of the recording electrode was accompanied by changes in audio frequencies of the amplified signal, indicating electrical contact with hemolymph. Spiking events were typically detected simultaneously with penetration, but electrode penetration was continued

to maximize spike resolution against background noise. Electrical signals between a bandwidth of 300 Hz and 1 kHz were amplified 10,000 times (DAM 50, WPI) and displayed on an oscilloscope (Tektronix Dual Storage Scope). Recordings were stored on 120-minute VHS tape using a VHS videocassette recorder (Panasonic Omnivision model 420 L) for subsequent playback and analysis.

Stable recordings were obtained from peg sensilla located centrally in the peg fields on six pectine teeth: the distal-, proximal-, and centre-most teeth on both right and left pectines. Twenty minutes of spontaneous activity were recorded at each location. A total of 48 pegs were recorded for a total recording time of 16 hours. All recordings were conducted between the hours of 20.00 and 02.00, the normal period of surface activity of these animals.

Data analysis: Taped recordings were played back through digitizing hardware (1401-plus, CED, Cambridge, UK) at 20 kHz sampling rate and run through a digital high-pass filter. Spiking events with peak amplitude above background noise level were detected and isolated from the record and categorized to separate classes using a spike recognition algorithm (Spike 2, CED). Generalized waveforms were obtained by using the waveform average utility within the Spike 2 software by sampling 100 points at 20 kHz, yielding 5-ms windows. Cross- and auto-correlation analyses consisted of centering 1s time windows (divided into 100×10-ms bins) around referent spikes to view activity-dependent interactions between particular spike classes (see Gaffin & Brownell, 1997b; Eggermont, 1990 for details).

Results

Extracellular action potentials from the bases of peg sensilla of *C. vittatus* were easily observed above background noise and ranged between 3 and 8 in signal-to-noise ratio. Electrode shape was an important factor in the quality of our recordings. A trade-off existed between the fineness of the point and the rigidity of the electrode for penetrating the peg cuticle. Also, the angle of approach of the electrode relative to the sensillum was an important consideration, with an angle that departed about 20 degrees from vertical producing the best recordings.

The average composite spiking frequency of our baseline recordings ranged from 2–14 Hz. In these recordings, we identified three different
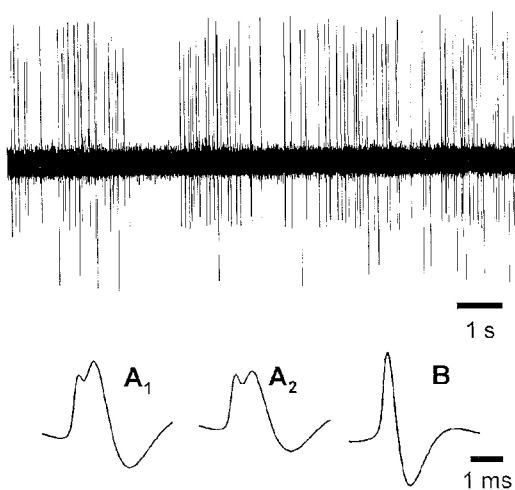

Fig. 2: Recordings of spontaneous activity of peg sensilla of *Centruroides vittatus* reveal three spike classes. This ten-second record of baseline activity shows spikes of two distinct amplitudes. The expanded waveforms of these spikes resolve into three classes, with the two smaller-amplitude units possessing characteristic double peaks in their waveforms.

units based on the consistency of their spike waveforms and firing patterns (Fig. 2). Two of these spikes, whose waveforms were very similar, possessed characteristic double peaks and have been labelled A1 and A2. These spikes were typically the most active units in our peg recordings, firing tonically at about 5 Hz. These cells had waveforms of approximately 5 ms duration, the longest of any in our records for this species.

Spike classes A1 and A2 often overlapped so closely that they were difficult to distinguish and were therefore grouped into one class. Auto-correlation analysis of these units, however, indicated that more than one cell was firing (data not shown). In some recordings, the waveforms were distinctive enough to resolve into two spike classes. In these cases, cross-correlation analysis revealed that one of the units typically excited the other (described below). It is by this criterion that we distinguish A1 from A2, with A1 classified as the unit that excites A2.

The third waveform in spontaneous recordings was a large biphasic cell with a smooth waveform, which we labelled B. The amplitude of this cell's waveform was typically 1.5 times that of the A1 and A2 spikes, while its duration was about 2.5 ms, or half that of the A1 and A2 spikes. The frequency of spiking of B cells was
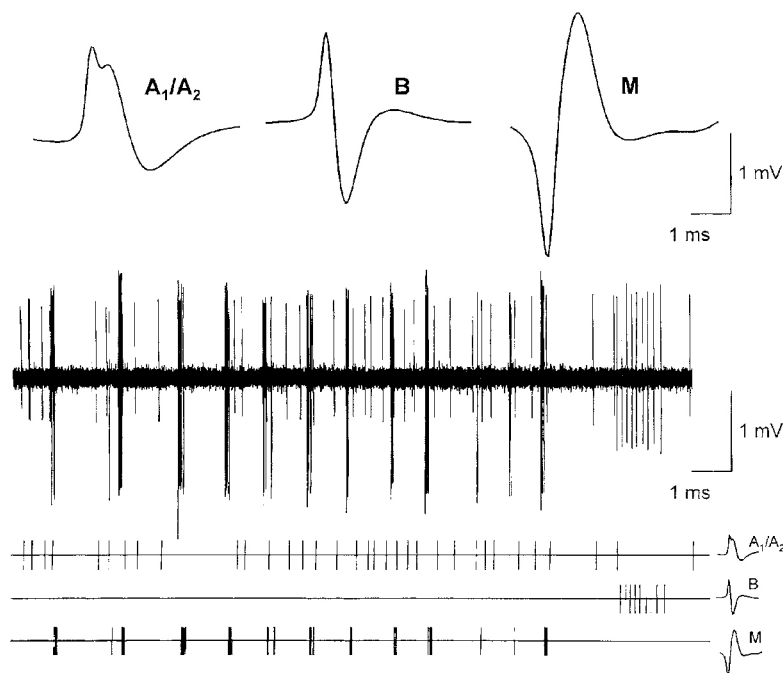
Fig. 3: Inducement of mechanosensory unit in peg recordings. Gentle taps of the recording electrode induced bursts of spikes of large biphasic waveforms labelled M. The lower three traces indicate the decomposition of the composite record into the individual firing patterns of identified units. Type A1 and A2 could not be resolved in this record and are therefore combined as A1/A2. Note the low-frequency burst of spike type B, which characterized this unit's activity in many of our recordings.

much more variable than that of A1 and A2 and its activity often occurred in bursts interspersed with prolonged periods of inactivity (Fig. 3).

In some recordings, gentle tapping on the recording electrode induced a fourth spike to fire (Fig. 3). This putative mechanoreceptor (labelled M) was clearly distinguishable from other units by its waveform and activity characteristics. Its amplitude was typically twice that of the other units in the record and its smoothly varying biphasic waveform had an initial negative phase, which was distinct from the initial positive phase of A1, A2, and B spikes. This cell fired in phasic bursts (up to 200 Hz) with quick adaptation and recovery.

I found indications of interaction between spiking units when the activity of identified waveforms was cross-correlated. An example of cross-correlation analysis applied to a peg recording is shown in Figure 4. In this recording the resolution of A1 and A2 was such that they could be accurately resolved as two separate waveforms. The bottom three histograms in

Figure 4 show the results of correlating each spike's activity against itself (auto-correlation). In this example, the B spike shows a distinct clearing around the reference point, as would be predicted based on the refractory period for the neuron. The histogram profiles for spikes A1 and A2 dip at the reference point yet do not completely fall to zero, indicating errors in the classification of some units. The upper three histograms display the results of pairwise correlation of each of the three units. The activity of A1 showed a distinct increase in the 100 ms prior to firings of spike type A2, indicating that A1 has an excitatory effect on A2. The activities of A1 and A2 showed no alteration when correlated with firings of spike type B.

Discussion

This study presents the first documented electrophysiological recordings of neural activity in peg sensilla of *Centruroides vittatus*. The extracellular signatures of three spontaneously active

units (labelled A1, A2, and B) were clearly detected above background noise. A fourth unit, presumably a mechanoreceptor (labelled M), was induced by gentle tapping on the recording electrode. In records where the waveforms of A1 and A2 units resolved into separate classes, cross-correlation analysis of their activity patterns revealed an excitatory interaction of unit A1 on A2. No activity interaction was seen in cross-correlations of B with either A1 or A2.

Several comparisons can be made between the electrophysiological properties of neurons in peg sensilla of *C. vittatus* with *Paruroctonus mesaensis*. The spontaneous spiking frequencies of *C. vittatus* were on average several Hz higher than those of *P. mesaensis*. The recordings from both species show three spontaneously active units in baseline recordings, but the waveforms and activity patterns of these units were distinctly different for the two species. In both, two units (A1 and A2 in both species) were of similar waveform, amplitude, and spiking frequency (Gaffin & Brownell, 1997a). However, the waveforms of these units were distinct for the two species, with *P. mesaensis* having smoothly varying biphasic waveforms and *C. vittatus* having distinctive double-peaked waveforms. The third unit, labelled B in both species, was also very different between the species. The amplitude of unit B in *P. mesaensis* was typically half that of the A1 and A2 units, while unit B in *C. vittatus* was about 1.5 times that of A1 and A2. Also, the shapes of the units were different, with the *P. mesaensis* B unit having a characteristic inflection in its waveform while B units of *C. vittatus* were smoothly biphasic. The B units of *P. mesaensis* fired in weakly bursty patterns, while B units of *C. vittatus* were often strongly bursty.

Both species also had a mechanosensitive unit, which fired with high frequency and was quick to adapt and recover. However, the waveforms differed between the species. In *C. vittatus*, the initial phase of the mechanoreceptor was inverted compared with other units in the record, and compared with the M unit in *P. mesaensis*. This difference may shed some light on the position of this unit relative to other units detected in the recordings. The spike-generating zone of this cell may be at a different level than the other active cells in the sensillum and our electrode tip may have been positioned between them. This finding is consistent with morphological studies that show one of the neurons terminates lower in the peg sensillar chamber than the others, and possesses a tubular
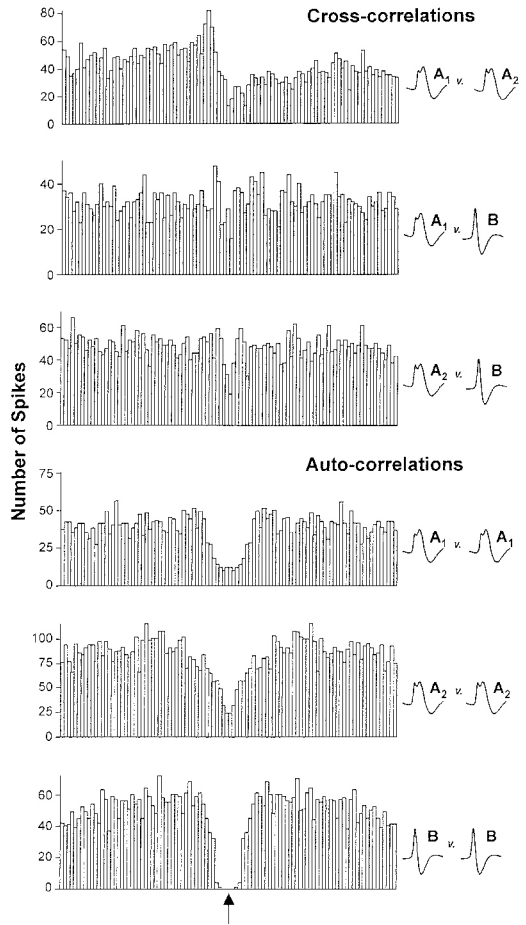
Fig. 4: Correlation analysis of spiking units in a peg sensillum of *C. vittatus*. Histogram profiles of auto- and cross-correlations of spike classes A1, A2, and B are shown for twenty minutes of continuous recording of spontaneous activity from a single peg sensillum. Each histogram displays pairwise comparisons of the spiking activity of defined units in the record. For example, the upper histogram labelled "A1 v. A2" displays unit A1 activity 0.5 s before and after each occurrence of reference unit A2 (centered in the profile at arrow). In this case, the activity of A1 is heightened immediately before the firing of A2, suggesting an excitatory effect of A1 on A2. Cross-correlation of B unit activity with either A1 or A2 shows little to no interaction (second and third histograms). Auto-correlations (bottom three histograms) indicate the degree of certainty that each identifiable spike originates from only one neuron.

body characteristic of arthropod mechanoreceptors (Foelix & Müller-Vorholt, 1983). Also, the difference in M units between *C. vittatus* and

P. mesaensis may reflect a slightly different arrangement of the cells in these two structures.

One additional difference between *C. vittatus* and *P. mesaensis* is the type of interactions observed between peg neurons (Gaffin & Brownell, 1997b). In *C. vittatus*, cross-correlation analysis revealed an excitatory interaction between units A1 and A2. In contrast, only inhibitory interactions were observed between units in *P. mesaensis* (B cells inhibited both A1 and A2).

Many questions remain concerning the physiology of peg sensillar neurons. For example, does the interaction between neurons extend to neighboring sensilla, or is it confined to individual pegs? What is the utility of the enormous population of seemingly identical substrate-directed sensilla? Are they all the same, or are they specially tuned in their chemical specificity? Do sensilla on distal teeth behave similarly to sensilla on proximal teeth? Although we did not detect obvious differences in spontaneous activity of peg sensilla on proximal, central, and distal teeth in this study, their response patterns to chemical stimulants may yet prove different.

Each of the above questions can be approached experimentally, and the pectines of scorpions lend themselves readily to physiological investigation. Both the species examined to date are extremely hardy, and the electrical behaviour of the pegs remains stable in a recorded animal for hours or even days. Pectines thus provide a readily accessible neural network, which should be a useful model for examining response properties of an interacting population of sensory neurons.

Acknowledgements

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