



Investigating sensory processing in the pectines of the striped bark scorpion, *Centruroides vittatus*

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Abstract

Scorpion pectines detect chemical and physical stimuli via thousands of peg sensilla on ground-facing teeth. Each sensillum has multiple neurons that detect stimuli and transmit neural impulses to the subesophageal ganglion (SEG) in the central nervous system. Anatomically, the organization of the pectinal neuropil in the SEG reflects the arrangement of pectinal teeth, suggesting conservation of information about stimulus location in the SEG. In this study, neural impulses from the pectinal nerve of the striped bark scorpion (*Centruroides vittatus*) were recorded in the pecten, abdominal cavity, and SEG using electrophysiology. Recordings from the right pectinal nerve in the pecten showed that right tooth stimulations elicit sensory activity milliseconds before motor feedback, while left tooth stimulations only evoked motor spikes, suggesting no contralateral afferent communication between pectines. In the abdominal cavity recordings, two distinct waveforms (PN1 and PN2) were detected in baseline activity; PN1 seemed to enhance the likelihood that PN2 would fire, suggesting a possible excitatory interaction. Recordings from the SEG showed that mechanically stimulating different pectinal teeth evoked different compound neural activity patterns in the same SEG location. Stimulating the same tooth in succession showed mechanosensory adaptation. These recordings show promise for continued electrophysiological investigations of the scorpion SEG, and, in particular, how information from the pectines is processed and used for orientation.

Keywords Electrophysiology · Neural topography · Subesophageal ganglion · Chemosensory · Mechanosensory

Abbreviations

SEG	Subesophageal ganglion
PN1	Pectinal nerve spike type 1
PN2	Pectinal nerve spike type 2
SE1	Subesophageal spike type 1
SE2	Subesophageal spike type 2

Introduction

Pectines are unique, comb-like appendages (Cloudsley-Thompson 1955; Foelix and Müller-Vorholt 1983) that extend ventromedially from the mesosoma of all scorpions (Fig. 1). The pectines tap or sweep the ground and collect chemical and textural information about the surface below them (Gaffin and Brownell 1997a, b). Each pecten has a series of projections (teeth) that range in number from a

few to nearly 40, depending on the species (Kladt 2007). Each tooth has tens to thousands of minute sensory structures called peg sensilla or “pegs” (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983). Each peg is innervated by around 10–15 neurons, and the peg neurons from each pectinal tooth contribute to a nerve in the pecten (Wolf 2008; Foelix and Müller-Vorholt 1983). Both the right and the left pectinal nerves enter the second mesosomal segment, and from there, each pectinal nerve connects with the subesophageal ganglion (SEG) through the posterior pectinal neuropil (Wolf 2008). In total, Wolf estimates that male *Vaejovis spinigerus* scorpions have about 140,000 afferent neurons that project from each pecten to the central nervous system (Wolf 2008).

Although the pegs on each tooth are chemo- and mechanosensory receptors (Gaffin and Brownell 1997a, b), the chemosensory properties of the pegs have been studied considerably more than the mechanosensory properties. The pegs seem to be functionally redundant when detecting chemicals; that is, most pegs along the entire pecten respond similarly to the same range of chemicals, rather than each tooth having an assortment of chemical-specific pegs (Gaffin and

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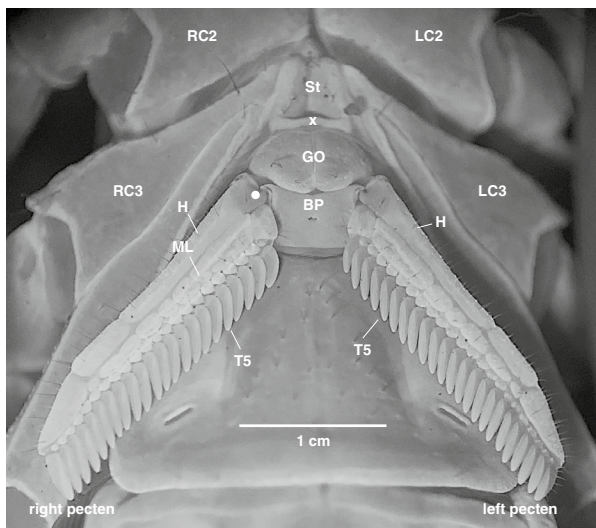


Fig. 1 Photograph of ventral side of a male *C. vittatus* showing pectines and electrode insertion points. The right and left pectines insert in the basal piece (BP) posterior to the genital operculum (GO). Recording electrode insertion point (marked by white dot) is on the right pectinal spine near the point of insertion with the BP. The pectines were stimulated on the right medial lamella (ML), and from a hair sensillum (H) and tooth 5 (T5) on the right and left sides. The electrode placement for subesophageal ganglion recordings (marked by x) was made by lowering the electrode through flexible cuticle just right of midline between the sternum (St) and GO. Other abbreviations: right coxa 2 (RC2), right coxa 3 (RC3), left coxa 2 (LC2), left coxa 3 (LC3)

Walvoord 2004; Knowlton and Gaffin 2011). Although the pegs are also mechanosensory receptors, very few studies have explored this capability. Early studies of scorpion physiology showed the pectines responded to touch (Abushama 1964). Later, pegs were found to contain neurons with similar morphological characteristics to mechanoreceptors of other arthropods (Foelix and Müller-Vorholt 1983).

The ability of the pectines to detect chemical and mechanical stimuli seems to be involved in both prey and mate detections, and perhaps in retracing their paths to a home refuge. In behavioral studies, scorpions increased pectinal tapping after encountering prey (Krapf 1986). Scorpions were also unable to detect and catch prey when their pectines were covered (Mineo and Claro 2006). Additionally, behavioral studies suggest that scorpions use their pectines to detect female pheromones during mating. Male sand scorpions linger, increase the brushing of their pectines across the substrate, and perform pre-courtship behaviors in areas previously exposed to female scorpions or to organic washes of female cuticle (Gaffin and Brownell 1992; Taylor et al. 2012). Further, these pre-courtship behaviors cease when the pectines are removed (Gaffin and Brownell 1992; Gaffin 1994). More recently, it has also been suggested that sand scorpions use their pectines to learn the chemo-textural

nuances around their burrows for homing (Gaffin and Brayfield 2017).

When the pectines detect environmental information, the information is inherently spatially organized due to the fixed location of pegs on each pectinal tooth, and this organization appears to be retained in the scorpion SEG. The morphological path of neurons has been traced from the pecten to the SEG, where the pectinal neuropil appears to be topographically arranged by tooth number (Brownell 2001; Wolf 2008). While the topographical organization of the SEG is anatomically evident, it has not been investigated physiologically. The previous electrophysiological studies of scorpions have recorded the neuronal activity in the pegs during chemical and mechanical stimulations (Gaffin and Brownell 1997b; Knowlton and Gaffin 2011) and in the pectinal hairs during mechanical stimulation (Kladt 2007; Kladt et al. 2007). The only documented recordings of neural activity from the scorpion SEG have been from the motor neurons of the walking legs (Bowerman and Burrows 1980). Here, we use electrophysiology to begin to explore the possibility of topographical conservation of pectinal sensory information in the SEG. The following experiments document neural activity from the pectinal nerve and SEG, while the pectines are mechanically stimulated. The mechanosensory properties of the pectines were specifically chosen versus the chemosensory properties to allow finer control of the location of the stimuli on the pectinal teeth. This study shows that mechanical stimulation of the pectines is detectible in SEG recordings and is a crucial first step in exploring how the scorpion SEG processes sensory information from these unique organs.

Materials and methods

Collection and care of scorpions

Adult striped bark scorpions (*Centruroides vittatus*) were located and caught at night near Lake Thunderbird, Oklahoma, using an ultraviolet flashlight and forceps. The body length (prosoma + mesosoma) of a subsample of nine animals was 20.6 ± 1.3 mm (mean \pm SE). The scorpions were subsequently placed in glass jars with sand or coconut mulch and a broken piece of pottery for shelter. The scorpions were acclimated to a reversed light cycle, so electrophysiological recordings of neural activity occurred during the most active part of their circadian cycle. They were fed one cricket every 2 weeks, and they were given water every Tuesday and Thursday. After the experiments, each scorpion was either returned to its jar or euthanized by freezing depending on the amount of hemolymph lost during the recordings. The scorpions were never reused for electrophysiology.

Preparation for electrophysiology

The same preparation was used in all electrophysiological works in this study, with modifications noted as necessary. The scorpion was placed in a freezer ($-20\text{ }^{\circ}\text{C}$) for 1–2 min to temporarily immobilize it. Then, the scorpion's legs and tail were restrained with clay and the animal was placed ventral side up on a glass slide to expose the pectines. Using a dissecting microscope at $40\times$ power, the pectines were secured on double-sided tape adhered to a half-sized cover slip; the cover slip was secured in place with clay. A sharpened silver wire indifferent electrode was inserted into the scorpion's tail to make hemolymph contact; the middle section of the wire was secured to the glass slide with clay, and the free end was later connected to the amplifier. The slide with the prepped scorpion was then taped to a stage under another dissecting microscope inside a Faraday cage on a vibration-free table to minimize electrical and vibrational noise.

Electrolytically carved tungsten electrodes were prepared according to previously established procedures (Gaffin and Brownell 1997b). In preparation for the hooked pectinal nerve recordings in the abdominal cavity, the exoskeleton and genital operculum were removed using microdissection scissors and forceps to expose the entire pectinal nerve in the abdominal cavity (see below for further description of dissection methods). The non-hooked electrodes used in the pectinal spine and pectinal neuropil recordings were sharp enough to pierce the exoskeleton without needing to dissect surrounding tissues. Using a manual micromanipulator, the recording electrode was inserted into the pectinal nerve in the pectinal spine, the pectinal nerve in the abdominal cavity, or the pectinal neuropil in the SEG region, depending on the experiment. The leads from the indifferent and recording electrodes were connected to a differential amplifier (DAM 70, World Precision Instruments), amplified (1000–10,000 times), and band-pass filtered between 300 Hz and 3 kHz. The signal was then relayed to an oscilloscope and to an A–D converter (Micro 1401, Cambridge Electronics Design), which sent the recording to a computer for analysis (Spike 2 software, Cambridge Electronics Design).

Pectinal nerve recordings from pectinal spine

Neural activity was recorded from the pectinal nerve in the pectinal spine in 11 scorpions. The tungsten recording electrode was inserted into the ventral spine of the right pecten, anterior, and medial to tooth 1 and adjacent to the exit point of the pectinal nerve. (The insertion point is marked with a white dot in Fig. 1; also, see Figure 1C in Wolf (2008) for a useful sketch of the relationship of peg sensilla neurons and other sensory units and their confluence in the pectinal nerve.) To verify that the electrode

detected nerve activity, the medial lamella (proximal and medial to pectinal tooth 5; “ML” in Fig. 1) on the right pecten was briefly stimulated with a metal probe; in addition, a hair sensillum was deflected on the lateral lamella, approximately opposite tooth 5 on both the right and left pectines (“H” in Fig. 1).

Mechanical stimulations of the pectinal teeth began after a stable baseline was established. A metal probe was lowered by a micromanipulator to make contact with either right or left pectinal tooth 5 (“T5” in Fig. 1). Two durations of stimulation (“instantaneous” or “prolonged”) were tested. In the “instantaneous touch” stimulations, the probe was withdrawn upon immediate contact with the tooth, with a contact time of a few milliseconds. In the “prolonged touch” stimulations, the probe was lowered to the tooth but was not immediately retracted; the probe stimulated the tooth for 1–2 s. The scorpions used in these recordings had minimal hemolymph loss and were returned to their glass jar after the recordings.

Hooked pectinal nerve recordings in the abdominal cavity

For these recordings, the pectinal nerve was exposed in the abdominal cavity and “hooked” with a bent recording electrode. Due to the considerable amount of hemolymph loss from the incision site, only three scorpions were used in these trials. To prepare the hooked nerve recordings, the electrolytically carved tungsten electrodes were bent into hooks using dissecting forceps. Hook diameters ranged from 0.5–1 mm when measured under a dissecting microscope. The scorpions were prepared for electrophysiology as above and then further anesthetized by cooling (at $-20\text{ }^{\circ}\text{C}$) for 2–3 min. Then, the prepped scorpion slide was put in a Petri dish on ice to ensure that the scorpion remained anesthetized, while the right pectinal nerve was exposed in the abdominal cavity. Under a dissecting microscope, the pectinal nerve was uncovered by carefully cutting and moving the right genital operculum to the side with dissecting scissors and forceps. The remaining adhering connective tissue was also removed. The optimal hook size was determined during the dissection of the abdominal cavity by visually comparing the size of the hooks and the diameter of the exposed pectinal nerve. The scorpion preparation was then transferred to the Faraday cage. Using a micromanipulator, the recording electrode was carefully hooked around the exposed pectinal nerve in the abdominal cavity. Baseline neural activity was recorded for approximately 20 min, but responses to mechanical stimulations of the pectines were not possible because of the severe hemolymph loss. After the recordings, the scorpions were euthanized by placing them in a freezer for 24 h.

Subesophageal ganglion recordings

Altogether, ten scorpions were used in the SEG recordings. In most of the recordings, the tungsten electrode was electrolytically carved as usual and then insulated with a layer of nail polish to localize the recording area and minimize noise from the tissue surrounding the shaft of the electrode. The tip was dipped into acetone to expose the tip of the electrode and about 50 microns of the tungsten shaft. The electrode was tested for conductance by running current through it and observing electrochemical activity while dipping the tip into a 1.0 M sodium nitrate solution equipped with a carbon cathode. The insulated electrode was inserted into the SEG region by piercing the animal's midline, anterior to the genital operculum, and posterior to the sternum until high-frequency electrical activity was detected. (Insertion point is marked by "x" in Fig. 1.) The cuticle was soft enough to penetrate with the tungsten electrode when the electrode was nearly perpendicular with the cuticle.

In three initial recordings, selected teeth on both pectines were stimulated with the metal probe to see if the responses in the SEG varied based on pecten location or tooth number. A manual micromanipulator was used to lower the probe and instantly retract it after contact with the distal ventral side of the tooth. The stimulations to each tooth were repeated at least three times before the probe was moved to the next tooth. The set of mechanical stimulations was repeated twice on each pecten.

In six recordings, the coated electrode was lowered deeper into the animal until clear spikes were registered when contacting the right or left pecten. A fine-tipped paint brush was mounted to the head of an electronically controlled micromanipulator (Burleigh step driver PZ-100), which was mounted on a manual micromanipulator. In one useful recording, the brush was initially maneuvered using the manual manipulator and lowered intermittently to the pecten teeth or brushed over various parts of the pecten. Additional stimulations were made by using the manual device to move the brush tip to within 100 microns of the targeted groups of teeth and using the electronic micromanipulator to deliver a sudden 200-micron forward step (at "0" speed setting); the brush was later withdrawn in a single 200-micron step.

In an additional recording, the recording electrode was not coated with nail polish, so compound neural activity could be recorded. As above, the fine-tipped brush and the Burleigh micromanipulator were used to deliver instantaneous 200-micron step mechanical stimulations to selected groups of teeth; the brush was withdrawn in a single 200-micron step after 5 s. The first set of recordings was used to determine if the placement of the recording electrode in the SEG registered different responses as groups of pectinal teeth were mechanically stimulated. Beginning at the

proximal end of the right pecten, four non-overlapping sets of five teeth were stimulated. Each group of teeth received six repeated stimulations in 20 s intervals, and this series of stimulations was repeated twice at each location. A second set of stimulations in the SEG was aimed to test the adaptation and sensory recovery of putative mechanosensory neurons. The electronic manipulator was triggered to advance the brush 200 microns at time 0 followed by immediate withdrawal. Following this initial stimulation, a second 200-micron step stimulation with immediate withdrawal was made at varying time intervals of 2, 4, 6, 8, and 10 s.

Results

Pectinal spine recordings

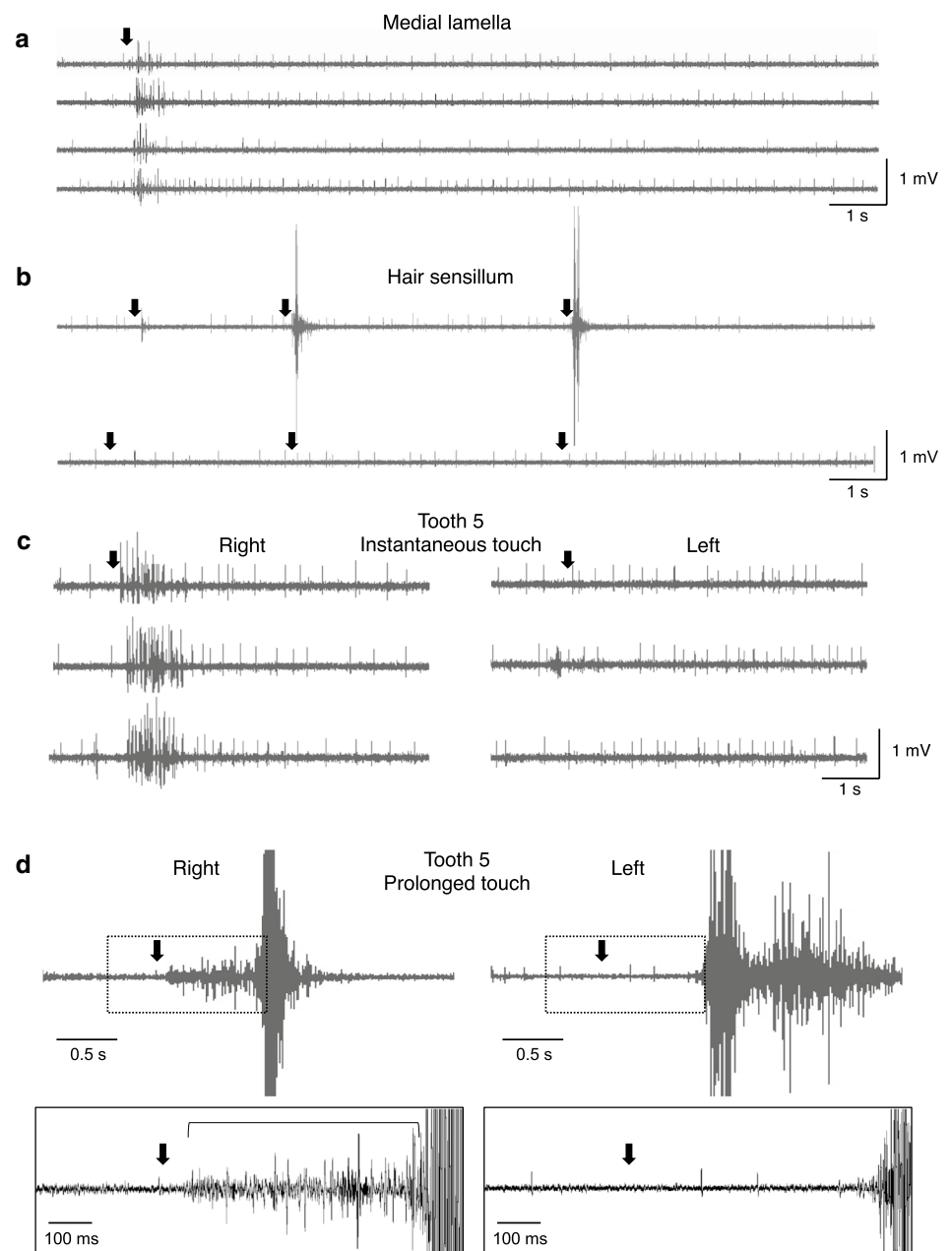
The pectinal spine recording preparation was minimally invasive and stable, so spontaneous baseline neural activity could be recorded for days with very little hemolymph loss. Intermittent muscle activity was also readily recorded and would mask the neural action potentials. Mechanical contact with the medial lamella generated a brief, but consistent increase in the number of putative sensory spikes detected (Fig. 2a). Next, a hair sensillum on the lateral spine across from tooth 5 was individually stimulated on both pectines. After stimulations of the right hair sensillum, there was a brief, compound sensory neural response detected by the right pectinal electrode (Fig. 2b, upper trace); no neural activity was detected in the right recording electrode after repeated stimulations of the left hair (Fig. 2b, lower trace). Then, pectinal tooth 5 on the right and left pectines were stimulated for a brief amount of time. The instantaneous stimulations of the right tooth 5 evoked apparent sensory neural activity (Fig. 2c) resembling the medial lamella response, but with more vigorous spiking. Sensory activity was not detected by the recording electrode in the right pecten when the left pectinal tooth 5 was instantaneously touched.

Following the instantaneous touches, the pectines were stimulated with the probe for 1–2 s. Immediately after contact with the right pectinal tooth 5, the recording electrode detected a brief period of apparent sensory activity followed by large spikes of motor activity (Fig. 2d). When the left pectinal tooth was stimulated by the prolonged touch, motor activity was detected in the right pectinal nerve, but the smaller sensory spikes were absent before the large motor spikes.

Abdominal cavity recordings

In these recordings, the pectinal nerve was exposed in the abdominal cavity and the hooked electrode was placed

Fig. 2 Electrophysiological recordings from an electrode in the right pectinal spine. **a** Four recordings from the right pectinal spine while stimulating the medial lamella on the right side. **b** Upper trace: recordings from the right pectinal spine while stimulating a hair sensillum on the right side. Lower trace: recordings from the right pectinal spine while stimulating a hair sensillum on the left pecten. **c** Left traces: three recordings of neural activity from the right pectinal spine while stimulating tooth 5 on the right pecten with an instantaneous touch. Right traces: three recordings from the right pectinal spine while stimulating tooth 5 on the left pecten with an instantaneous touch. **d** Recordings from the right pectinal spine during a prolonged mechanical stimulation of tooth 5 on the right and left pectens. The regions within the dotted boxes are expanded below each recording; the bracket indicates apparent sensory activity before motor feedback in the right tooth stimulation



around the right nerve. These recordings were of spontaneous baseline activity without stimulations to the teeth. Baseline activity consisted of repetitive spikes throughout the recording; the abbreviated record in Fig. 3a is a representative example showing spontaneous spiking of about 1 Hz. Analysis of the record with Spike 2 software revealed two spike waveforms, pectinal neuron 1 (PN1) and pectinal neuron 2 (PN2); PN1 is the larger spike type, and PN2 is shorter and broader (Fig. 3b). In this record, PN1 consistently fired throughout the recording, whereas PN2 fired less frequently. The record was expanded to show the time relationship between PN1 and PN2 (Fig. 3c). PN1 was not always followed by PN2, but

if PN2 was detected, a PN1 spike immediately preceded it by 20–40 ms (Fig. 3d).

Subesophageal ganglion recordings

The initial SEG recordings were collected using an insulated recording electrode, while individual teeth were stimulated by a metal probe on the right and the left pectens. A dramatic increase in individual nerve cell spikes occurred immediately after stimulation of tooth 1 and 15 on the right pecten (Fig. 4a). This response was highly repeatable, although the first stimulation produced a longer response than any subsequent stimulations. A Spike 2 wavemark analysis showed

Fig. 3 Baseline electrophysiological recordings while recording electrode is hooked around the right pectinal nerve in the abdominal cavity. **a** Upper trace: full, unprocessed record from the pectinal nerve while hooked with the recording electrode, which shows a series of two consecutive spike types (PN1 and PN2). Middle trace: isolated spike type PN2. Lower trace: isolated spike type PN1. **b** Overlay of expanded spike types PN1 and PN2. **c** Expanded record from pectinal nerve showing PN1 spiking before PN2. **d** Cross-correlation histogram showing the time of PN2 spiking after PN1 has fired

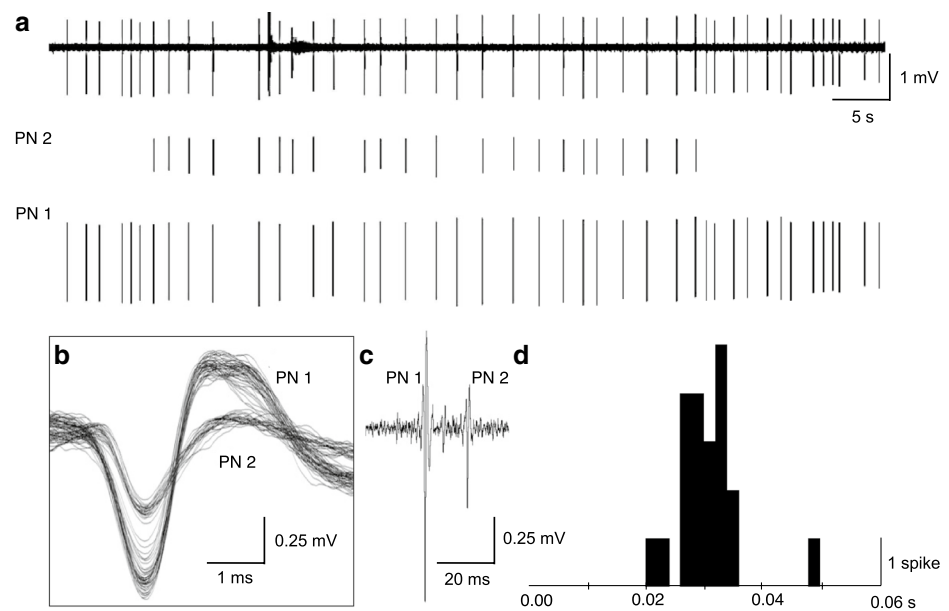
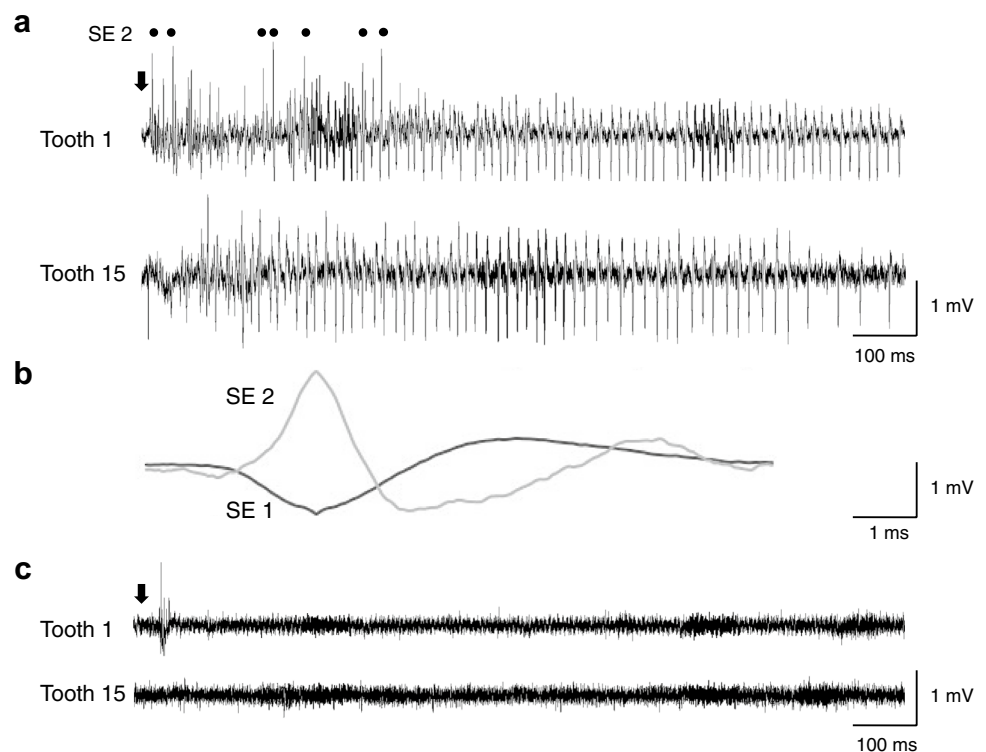


Fig. 4 Electrophysiological recordings from the SEG while stimulating teeth 1 and 15 on the right and left pectines. **a** Neural response to stimulations of tooth 1 (upper trace) and tooth 15 (lower trace) on the right pecten. Two spike types (SE1 and SE2) were detected during tooth 1 stimulations, while only one spike type (SE1) was detected after stimulations of tooth 15 on the right pecten; black dots above the top trace denote firings of spike type SE2 after right tooth 1 stimulations. **b** Average waveforms of spike types SE1 and SE2. **c** Recordings from the pectinal neuropil while stimulating tooth 1 (upper trace) and tooth 15 (lower trace) on the left pecten



that the response to stimulations of tooth 1 consisted of two different spike types: subesophageal 1 (SE1) and subesophageal 2 (SE2). The majority of the spikes in the right tooth 1 record are SE1, and the SE2 spikes are denoted with black dots above the record (Fig. 4a, top trace). The SE2 spikes from stimulations of the right tooth 1 were recorded within the first 400 ms after stimulations, whereas the SE1 spike type was detected consistently for approximately 1 s. The

record from tooth 15 had only SE1 spikes; SE2 was not detected in this record (Fig. 4a, lower trace). The averaged waveforms of SE1 and SE2 are overlaid in Fig. 4b. The SE1 spikes following tooth 1 stimulation had the same waveform as the SE1 spikes following tooth 15 stimulation (Fig. 4b). There were no neural responses to stimulations of the other right pectinal teeth or the left pectinal teeth with the metal probe (Fig. 4c).

In several recordings, a coated electrode was lowered deeper into the animal to record responses of individual neurons after stimulating the right or left pecten. Figure 5 shows an SEG recording taken slightly to the left of the animal's midline, again just posterior of the sternum. The electrode tip was judged to be about 250 microns below the cuticle surface; this measurement was taken by painting the exposed portion of the inserted electrode with black acrylic paint and measuring the length of the unpainted electrode tip under a dissecting scope after withdrawing it from the animal. The upper trace in Fig. 5a shows spiking activity in response to repeated contact with a fine brush to mid-pectinal teeth. The averaged spike shapes are shown to the left of the trace, and the occurrences of the separated waveforms are indicated in the two traces below. Systematic brush contacts using the Burleigh micromanipulator (Fig. 5b) produced similar response patterns when groups of teeth within a pecten were stimulated, which were different when compared to responses from the other pecten. Repeated stimulations at varying time intervals showed apparent adaptation of the response (Fig. 5c). Also, contact of the lateral lamella on the left pecten induced the same types of spikes as seen in the contacts with the left pectinal teeth (Fig. 5d).

In one additional SEG recording, an uncoated tungsten electrode was placed between the genital operculum and sternum and groups of pectinal teeth on the right pecten were systematically stimulated with the electronic micromanipulator and a fine paint brush. Compound neural activity was strongest during stimulations of the proximal group of teeth (1–5) and degraded toward the distal teeth (Fig. 6a). The responses were highly repetitive throughout the six stimulations at each location. In varying time interval stimulations (Fig. 6b), the mechanosensory response adapted quickly, as evidenced by the compromised response to a repeated stimulus within 2 s but recovered fully when the second stimulus was delayed by 8 s.

Discussion

This study has accumulated electrophysiological recordings from multiple points along the pectinal neural pathway of *C. vittatus* while mechanically stimulating various parts of the pectines. The recordings in this study were from the pectinal nerve in the right pectinal spine, the abdominal cavity, and the SEG. These recordings show that it is possible to detect electrophysiological responses to pecten stimulation in the scorpion SEG and set the stage for further investigation of topographic order and sensory processing.

Pectinal spine recordings

Neural activity from the pectinal nerve in the right pectinal spine was recorded before and after mechanical stimulations of the right medial lamella, the right and left hair sensillum, and the right and left pectinal tooth 5. Stimulations of a long pectinal hair on the right pecten produced a response detected by the recording electrode, while there was no change from baseline after stimulations of a long hair in a similar location on the left pecten. The difference in responses between the right and left long pectinal hair records confirmed that the changes from baseline activity after mechanical stimulations were due to a neural response from the stimulation location and not from general noise induced into the system. Brief mechanical stimulations of the right pectinal tooth also evoked bursts of small sensory spikes lasting approximately 1 s, which is consistent with previous findings that peg sensilla on the pectinal teeth of *C. vittatus* contain mechanoreceptive neurons (Gaffin 2001).

While the recording electrode was in the right pectinal nerve, sensory spikes were detected after all stimulations of the right pecten, but no sensory spikes were detected after left pecten stimulations (Fig. 2). Based on these results, it is evident that the right pectinal nerve contains fibers that carry mechanosensory information from the hair sensillum and pectinal teeth on the ipsilateral side to the pectinal neuropil. The right pectinal nerve does not, however, receive sensory information from the contralateral pecten. These electrophysiological records are consistent with the anatomical findings of Wolf's backfill experiments, which showed that afferent projections to the pectinal neuropil from the right and left pectinal nerves may touch but do not interconnect (Wolf 2008). After the prolonged stimulations to both the right and the left pectines, motor activity was detected in the right nerve, which shows that the pectinal nerve also contains efferent fibers from the SEG that can be activated after longer mechanical stimulations (Fig. 2d).

The recordings in the pectinal spine also provided evidence that the duration of mechanical stimulation affects the neural response. In the pectinal spine recordings, the response to prolonged stimulations was drastically different than the response to instantaneous touch. Instantaneous stimulations of the right tooth induced small sensory spikes immediately after the stimulations, but there was no change from baseline when the same stimuli were presented to the tooth on the left pecten. The prolonged stimulations of the right and the left pectinal teeth both induced large motor spikes in the right pectinal nerve 600–700 ms after the stimulation. The major difference in the right and left prolonged stimulations was what occurred during the time between the stimulations and the motor response. The prolonged stimulation on the right tooth initiated smaller sensory spikes before

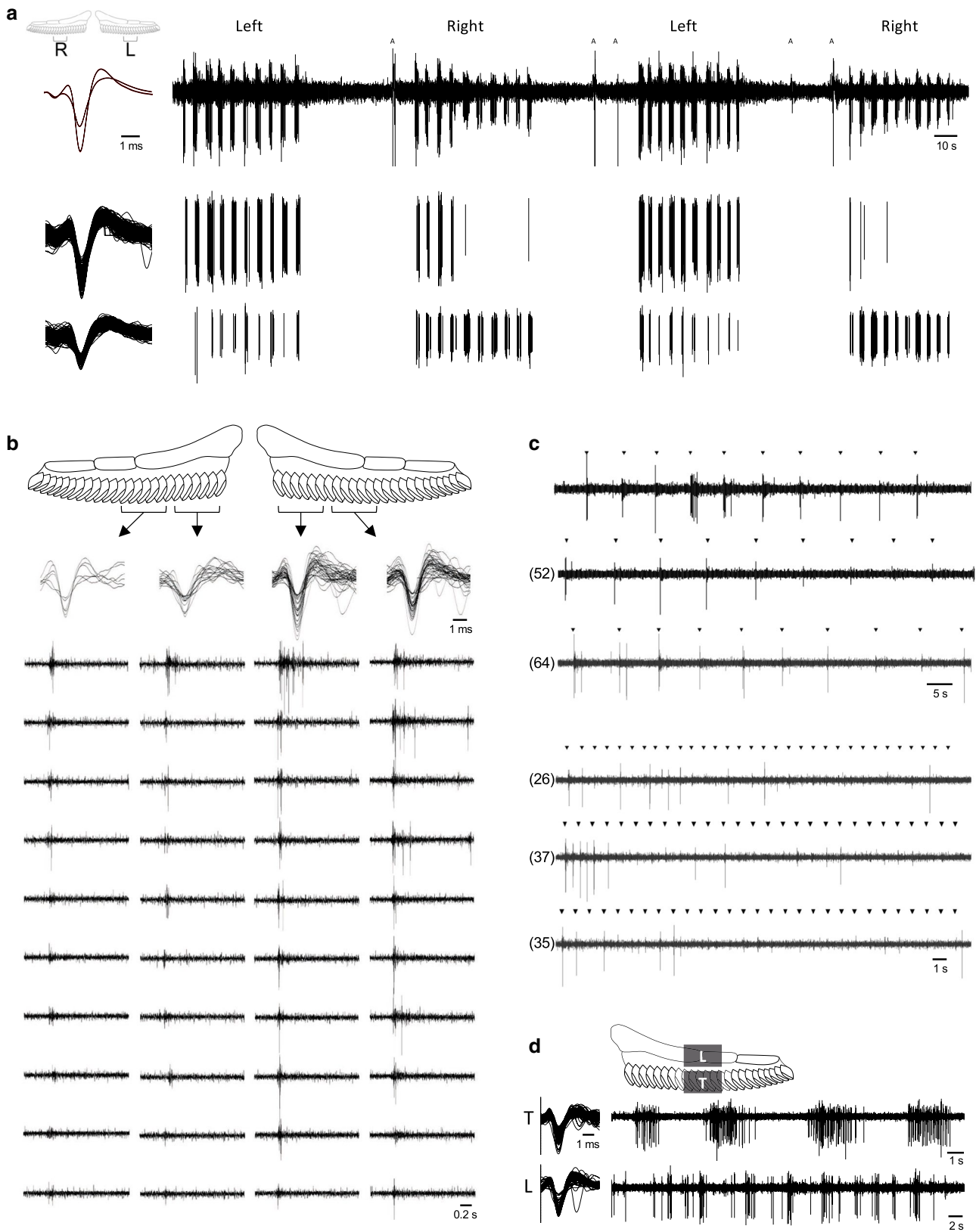


Fig. 5 Responses in SEG recording to various pectinal mechanostimulations. The recording electrode was inserted slightly to the left of the midline just anterior of the genital operculum. **a** Neural response to stimulation of a right and left mid-pectinal teeth to manual lowering and retracting of a fine-tipped brush. Top trace shows raw record. (Electrical artifacts are indicated by “A.”) Expanded waveforms at left are averaged shapes of spikes induced by mechanical stimulation. These waveforms are sorted in the bottom two traces. **b** Neural responses to instantaneous mechanical stimulation (200 μm step) from two ranges of teeth on right and left pectines. Shown are ten stimulations for each site; superimposed waveforms corresponding to the stimulations are indicated at the top of the traces. **c** Indication of adaptation in neural response to instantaneous mechanical stimulations (200 μm step) at varying time spacings. The arrowheads at top of each tracing indicate time of forward step (followed by immediate retraction). The numbers in parentheses to the left of traces two through six indicate time delay in seconds from the previous stimulation. **d** Manual stimulations of teeth (T) and lateral lamella (L) generate similar spikes. Tracings of responses to several brushes across left mid-pectinal teeth and lateral lamella are shown along with the superimposed spikes to the left

the motor spikes, but after the prolonged stimulation on the left pecten, there were no smaller spikes before the motor activity. The difference in responses between the instantaneous and prolonged touch shows that varying durations of the same mechanical stimuli can evoke different neural responses.

Abdominal cavity recordings

The hooked electrode recordings consisted of baseline activity of the pectinal nerve in the abdominal cavity. The original intent of this set of experiments was to repeat the same stimulations from the pectinal spine recordings to provide continuity between the recordings in the pecten and the SEG, but due to the limited amount of time before the animal lost a significant amount of hemolymph, only baseline activity was recorded from the three animals in the hook dissection recordings. Still, the reported firing patterns may be useful to future studies on how information is relayed to the SEG. Two distinct spike types were detected: PN1 and PN2. A histogram of the timing of PN1 and PN2 (Fig. 3d) shows that when PN2 fires, it always fires 20–40 ms after PN1, which suggests possible excitatory interaction between PN1 and PN2 spike types. The variable latency of PN2 may indicate a chemical interaction between the two neurons rather than an electrical communication, but more research, particularly with intracellular recordings, would be needed to propose a mechanism for this possible excitatory synapse. Future studies may also consider supplementing the hemolymph loss by perfusing a saline solution to extend the time available for these recordings.

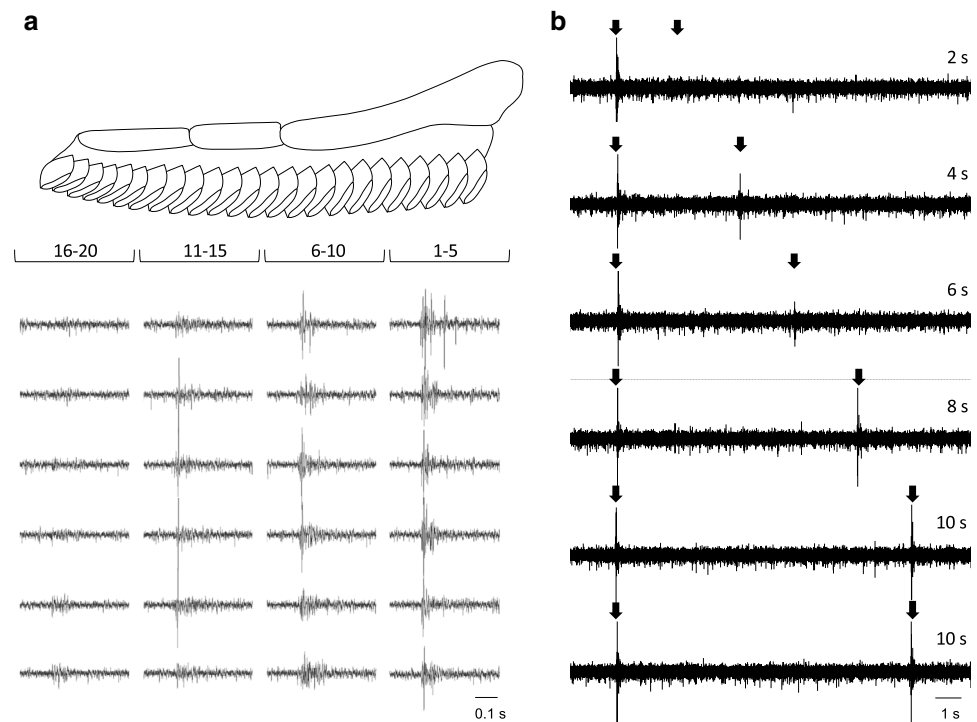
Subesophageal ganglion recordings

In the initial SEG recordings, the recording electrode was insulated with nail polish, so the recording site was restricted to the tip of the electrode. This allowed extracellular recording from a few neurons, and it minimized the noise from surrounding neural tissue. The electrode pierced the flexible cuticle in the midline of the animal just anterior to the genital operculum as denoted by the “x” in Fig. 1. Dissection of the abdominal cavity was not necessary for the electrode placement, but the micromanipulator and electrode angles were crucial to pierce the cuticle in the correct location. The SEG was not visualized for electrode placement in this method, which made correct electrode placement more difficult but also involved a lower risk of damage to surrounding neural tissues or excessive hemolymph loss. An alternative approach would have been to remove the tissue above the SEG (Bowerman and Burrows 1980), but this approach was not used because of the risk of significant hemolymph loss.

Although the exact location of the electrode tip in Fig. 4 is unknown, it was most likely in the right pectinal neuropil, based on its placement slightly to the right of midline and the observation that only stimulations of the right pecten induced changes from baseline neural activity. Additionally, anatomical studies showed that neuropils do not receive contralateral afferent information (Wolf 2008). In future electrophysiological studies, it may be useful to visualize the exact electrode site after the recordings. This could be done by passing an anodic current through the electrode to ablate the tissue after electrophysiological recordings and then preparing histological slides of the pectinal neuropil to mark the exact location of the electrode, but this methodology was out of the scope of these initial SEG recordings.

There was a neural response detected in the SEG after stimulations of the right tooth 1 and right tooth 15, but there was not a response to stimulations of any individual teeth on the left pecten. Spike type SE1 occurred in the responses from both teeth, while SE2 was only seen in the beginning of the response from right tooth 1. Because the waveform of SE1 was the same in the responses to tooth 1 and 15, it is most likely that this is a recording from a single neuron. The stimulations did not evoke movement in the animal or the pectines, which suggests the neural activity was not a motor response descending to the pectines. The response in the SEG to stimulations to both teeth 1 and 15 is perplexing, but not implausible. Anatomical data suggest that the posterior pectinal neuropil receives chemosensory information based on the location of the tooth on the pecten, although each section of the neuropil has a few axons from each tooth (Wolf 2008). Wolf also found that the interneurons from the posterior pectinal neuropil to the anterior pectinal neuropil leave as an unorganized, single tract-like structure (Wolf 2008). This initial SEG recording could be from an interneuron

Fig. 6 SEG response during mechanosensory stimulation of groups of pectinal teeth with uncoated electrode. **a** Compound neural responses obtained via tungsten micro-electrode inserted slightly to the right of midline through flexible cuticle between the sternum and genital operculum (see Fig. 1). Shown are six repetitions of mechanical stimulations with fine paint brush to groups of pectinal teeth (roughly from proximal to distal teeth 1–5, 6–10, 11–15, 16–20). **b** Apparent adaptation and recovery of mechanosensory response. Shown are consistent mechanical stimulations of teeth 6–10 spaced at 2 s, 4 s, 6 s, 8 s, and 10 s (two examples)



that received and integrated information from right tooth 1 and right tooth 15, which then ascended to the anterior pectinal neuropil. The response to stimulations of tooth 1 lasted longer and contained more SE1 spike types than the response to stimulations of tooth 15, so the interneuron may be coming from the region in the posterior pectinal neuropil that is responsible for processing the majority of afferent information from tooth 1. It is unclear why the SE2 spike type is recruited in stimulations of tooth 1 and not tooth 15.

The recordings generated from the deeper penetration shown in Fig. 5 were interesting in that there were distinct, large amplitude action potentials detected in response to various pecten contacts. These recordings were different in that there was not a difference in the response patterns when various parts of each pecten were contacted (e.g., teeth or lateral lamella), but the spike composition in the responses was different between the two pectines. We speculate that the electrode may have been picking up on neurons relaying general information about pecten contact from both pectines to motor centers. The activity of these neurons showed signs of adaptation with higher frequency stimulations.

In subsequent SEG trials, an uninsulated recording electrode was inserted into the SEG and groups of pectinal teeth were stimulated with a fine brush. The electrode was uninsulated so that compounded neural activity would be recorded from multiple teeth being stimulated at the same time. In these recordings, the electrode was inserted in a similar manner as the initial SEG recordings. There were neural responses to stimulations of multiple groups of teeth,

but the proximal groups had the largest change from baseline (Fig. 6a). Based on these results and Wolf's description of the pectinal neuropil structure, the recorded neural activity is probably from the afferent axon bundles that enter the neuropil from the pectinal nerve (Wolf 2008). Since the electrode was uninsulated, it could detect electrical activity from multiple afferent axons along the tip and shaft of the electrode. The ability to detect varying amounts of sensory information from multiple teeth while recording in a single location in the SEG is consistent with the expected results of recording from an afferent axon bundle. After the response to mechanical stimulations was well documented and the neural activity returned to baseline, the groups of teeth were stimulated in succession in multiple different time intervals to test sensory adaptation and recovery. A second stimulation, 1 s after the first stimulation, did not produce a neural response. The response began to recover at 2 s and was fully recovered at 8 s after the initial stimulation (Fig. 6b).

Although the results from the SEG recordings are preliminary, they offer promising expectations for further research into the organization of the scorpion SEG. First, these extracellular recordings show that it is possible to study the mechanosensory properties of the pectines within the SEG using electrophysiology. The only other successful recordings from the SEG were intracellular recordings from the motor neurons of scorpion walking legs (Bowerman and Burrows 1980). The methods used in these two studies can be the basis of further research on the SEG in living scorpions. Specifically, these methods introduce great potential for

learning about the complete neural pathway of the pectines, which are a highly innervated and are unique organs to the scorpion (Gaffin and Brownell 1997a).

Although these initial experiments could not provide a map of the sensory innervation in the pectinal neuropil, they did reveal that neurons in the SEG are selective for mechanical stimuli from specific teeth. This is the first step in analyzing the physiological organization of the pectinal neuropil. Future studies could systematically record from multiple locations in the pectinal neuropil while mechanically stimulating the teeth. The role of chemoreception and neural activity must also be explored in future studies. Overall, knowing more about the neural organization and function of the SEG in processing pectinal information will be crucial to understanding the role of the pectines in navigation and other behaviors.

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Compliance with ethical standards

Conflict of interest None.

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