

Use of a micro-engineered chemical delivery device to evaluate scorpion peg sensillum response to organic stimulants.

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Summary

Scorpions are nocturnal animals that have a rich array of sensory structures that are finely tuned to their environments. In particular, mid-ventral appendages called pectines are complex chemosensory organs that are used in the detection of food and mates. Previous electrophysiological studies have shown that the sensory elements on pectines (thousands of minute peg-shaped sensilla) are sensitive to a variety of volatile organic compounds. However, the crudeness of the stimulus delivery mechanism used in these studies made it difficult to compare response patterns among peg sensilla. We have engineered a new device to allow chemical stimulants to be precisely delivered to a small group of pegs. We tested the efficacy of the engineered device and its dynamics by designing a series of stimulations using 1-hexanol and mineral oil as the control chemical. Peg sensilla on the pectines of *Paruroctonus utahensis* were recorded extracellularly while being stimulated to record the elicited response pattern. We have completed preliminary testing of the delivery device, including optimal duration of stimulus pulse and the best size for the nozzle bore. This device will be used to investigate a basic question of pectine functionality: do all peg sensilla respond with the same pattern of neural activity or are there distinct inter-peg differences? The latter would suggest a segmentation of chemical information at the level of the peg, similar to the olfactory epithelium of mammals.

Introduction

Scorpions use an impressive array of sensory structures to find food, water, mates, and shelter. Scorpions can sense air currents (Hoffman 1967), vibrations (Brownell 1977), humidity (Gaffin et al. 1992), light (Fleissner and Fleissner 2001), and various mechanical and chemical stimulants (Foelix and Schabronath 1983). However, of the many sensory organs, the mid-body pectines are particularly interesting and elaborate. These unique, comb-like appendages function as chemoreceptors and are used in mate- and food-finding activities (Cloudsley-Thompson 1955; Krapf 1986; Gaffin and Brownell 1992).

Each of the paired pectines is composed of a flexible spine and numerous teeth. The ventral surfaces of the

pectinal teeth support thousands of microscopic peg-shaped hairs called peg sensilla (Carthy 1966, 1968; Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983; Swoveland 1978). Each peg has a slit-shaped pore at the tip of a cuticular shaft. The pore gives access to chemicals and opens to a fluid-filled chamber where dendrites of 10 to 15 sensory neurons terminate (Ivanov and Balashov 1979; Foelix and Müller-Vorholt 1983). In some species, the total sensory population from peg sensilla is in the realm of 10^6 - 10^7 neurons (Gaffin and Brownell 2001, Swoveland 1978).

Electrophysiological studies show that peg sensilla respond differentially to various organic stimulants (Gaffin and Brownell 1997a). In addition, synaptic interactions between peg neurons have been shown by cross-correlating the activity of identified peg neurons, suggesting that the pegs play an important role in

peripheral processing of chemosensory information (Gaffin and Brownell 1997b). Taken together, the physiology of individual pegs could provide insights on how information is processed by local networks of synaptically coupled cells.

To fully understand the physiology of individual pegs and how they function within the context of the organ requires a precise, controllable means of delivering chemical stimuli. To date, the methods used to deliver stimulants have been rather crude. Peg sensilla have been exposed to the volatile organic stimulants by blowing puffs of organic compounds across the entire pectine from a distance of about 1 cm (Gaffin and Brownell 1997a). This imprecision makes it difficult to deliver repeatable doses of stimulants and make meaningful comparisons of the response properties of different pegs.

We have engineered a chemical delivery device to provide an efficient means of delivering various stimulants directly to individual pegs. In this study we describe the preliminary testing of this device on peg sensilla of desert grassland scorpions (*Paruroctonus utahensis*). We ultimately hope to use this device to test for variance of response within the peg sensilla population.

Methods

Animals

Several mature *Paruroctonus utahensis*, obtained from Kermit, Texas in April of 2001, were the subjects of this experiment. While in the laboratory, the scorpions were kept individually in 3.8 liter translucent glass jars containing 250 ml of sand collected from the scorpions' natural habitat. These jars were kept in a room where temperature, humidity, and light cycle were kept relatively constant (22° C, RH 55-65%, 2000-0730 h dark 0730- 2000 h light). Each scorpion was fed one cricket and misted with 10 ml of deionized water on a weekly basis.

Electrophysiology

Scorpions used for electrophysiology were briefly anesthetized by cooling (about 2 min at 0°C) and then immobilized ventral side up on a glass slide using clay. 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 pectine were straightened and aligned to facilitate the recording process. The prepared scorpion was then fastened to the microscope stage and a viable peg was located using a high-powered (500-1000x) compound microscope (Olympus BX-50WI) equipped with epi-illumination and long working distance objectives.

A micro-engineered chemical delivery device (fig. 1) was developed to efficiently deliver chemical stimulants to small distinct fields of peg sensilla. The nozzle of this device consisted of 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) to a tip diameter of 40 μm using a designed program patch (1. heat = 440, pull = 40, velocity = 40, time = 150; 2. heat = 400, pull = 125, velocity = 65, time = 150; heat = 305, pull = 55, velocity = 70, time = 150). This pipette was attached to a glass electrode holder, which served as a junction between the pipette and a polyethylene tube (0.38 mm internal diameter, 63.851 μl). This tube was attached to interchangeable syringe filters (Whatman Glass fiber 2.7 μm pore) using a 27 gauge 5/8-inch needle. Each syringe filter was injected with 10 μl of either pure mineral oil or 1-hexanol. The stimuli were administered to the system by passing a steady stream of air through the appropriate filter, the system, and to the peg using a Harvard Apparatus 22 syringe pump and a 20 ml syringe (19.3 mm diameter).

A microscope was used to verify that the chemical delivery device was positioned over the selected peg.

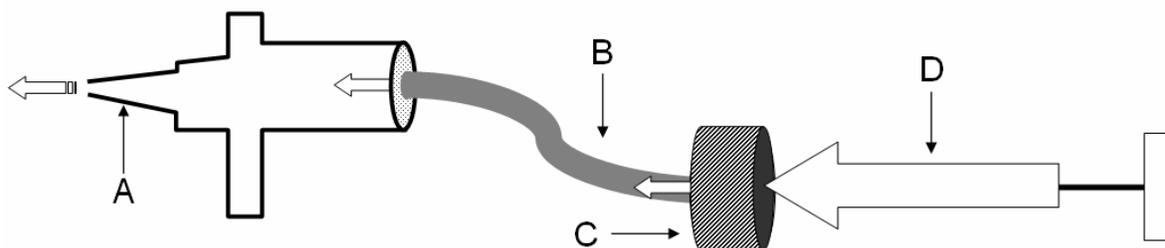


Fig. 1: Delivery system for chemical stimulation of peg sensilla. A glass pipette (A) with nozzle diameter of 40 μm was pulled from a glass capillary pipette and held in place within a plastic pipette holder. The holder was attached to a length of polyethylene tubing (B) which connected to a syringe filter (C) impregnated with the desired chemical. A 10 ml plastic syringe (D) controlled by an automated syringe pump delivered the air flow for the system.

Extracellular recordings were obtained by inserting the electrolytically sharpened tungsten electrode (tip diameter about $1\mu\text{m}$) into the cuticle at the base of the peg (fig. 2). After insertion, the peg was allowed to recover for at least fifteen minutes as baseline activity was recorded. Electrical signals detected by the electrode were amplified 1000 times over a bandwidth of 1-3 kHz, displayed on an oscilloscope, and recorded on audio magnetic tape for subsequent computer analysis.

Chemical stimulation

Once the electrode and chemical delivery devices were in place, the stimulants were methodically introduced to the pegs by passing air current through the impregnated filters and through the rest of the apparatus. In our initial test we used 1-hexanol as stimulant at a flow rate of 7.7 ml/min for two minutes.

Each two minute stimulus was followed by a one minute waiting interval in which no stimulus was being administered, allowing time to affix the next impregnated filter to the apparatus. Each stimulant and waiting period sequence was followed by the use of a mineral oil charged filter to flush the system and remove any trace molecules of previous stimulants. The flow rate was held at 7ml/ min for two minutes to ensure that the system was clean. After the deployment of the first series of 1-hexanol and mineral oil, an additional series of stimulation was administered to the same peg.

To test the effect of altering the stimulus parameters on the elicited response, we devised a series of stimulation pulses. Once the electrode and chemical delivery devices were in place, the stimulants were deployed using a 10 ml syringe (14.5 mm diameter) with a 7 ml/ min flow rate. For the first series, 1-hexanol was administered to the target peg for 30 seconds, followed

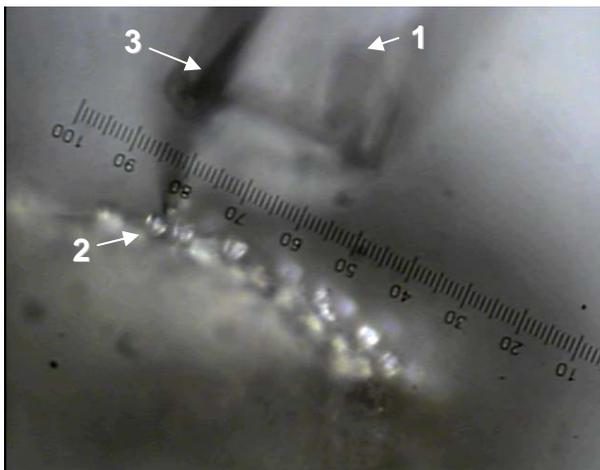


Fig. 2: Pipette and electrode configuration. The pipette (A) was positioned directly above the pegs (C) while the electrode (B) was inserted into the peg cuticle.

by a 30- second recuperation period in which no stimulus was administered. This was repeated five times. 1-Hexanol was then administered to the target peg for 45- second pulses followed by a 45- second recuperation period. This process was repeated five times. The final stimulation series consisted of deploying 1-hexanol for 60- second pulses followed by a 60- second recuperation period. This stimulation process was repeated three times.

Results

Several variables had to be considered during the construction of the stimulus delivery device. Of considerable importance was the diameter of the nozzle tip, as this greatly affected the flow of stimulant to the peg. Nozzles with large tips ($> 50\mu\text{m}$) placed close to the peg field obstructed the recording electrode. Nozzles with very small tips created high resistance to stimulant flow and resulted in backpressure into the delivery system. Our devices were tested under a water bath for leaks and general flow characteristics. Ultimately, we found a tip diameter of $40\mu\text{m}$ enabled the tip to be maneuvered within $10\text{-}15\mu\text{m}$ of the peg sensilla while still allowing electrodes access to pegs.

We resolved the dynamics of the engineered apparatus by using it to stimulate a peg sensillum of a *P. utahensis* (fig. 3). When stimulated with 1-hexanol, the frequency of the action potential firing rapidly increased at an average time of 13.8 seconds (range 5.0s-21.0s). The frequency continued to increase without reaching a plateau until the stimulus was terminated. The maximum frequency obtained during the stimulation period was 19.1 action potentials per second (range 17.5-20.7). Upon removal of the stimulus, the action potential frequency returned to pre-stimulus values (baseline) after an average of 7.8 seconds (range 6.9s-8.6s). When the paraffin oil was delivered it took 7.1 seconds on average to elicit an increase in action potential frequency (range 5.6s-9.4s). The maximum frequency obtained was during the mineral oil stimulation period was 13.9 action potentials per second (range 12.5-15.2). The maximum frequency occurred on average 33.0 seconds after deploying the mineral oil stimulant (range 23.0s-41.7s). After the maximum frequency was obtained, an average of 56.6 seconds passed prior to the returning of the frequency to baseline levels (range 48.9s-67.0s)

After resolving the relative efficacy of the designed apparatus, we studied the effects of altering the stimulation parameters. Figure 4 indicates the response of the sensillum to each of the impulse series. Stimulation of the peg sensillum with 1-hexanol resulted in an abrupt decrease in action potential amplitude within the 30 second, 45 second, and 60 second impulse

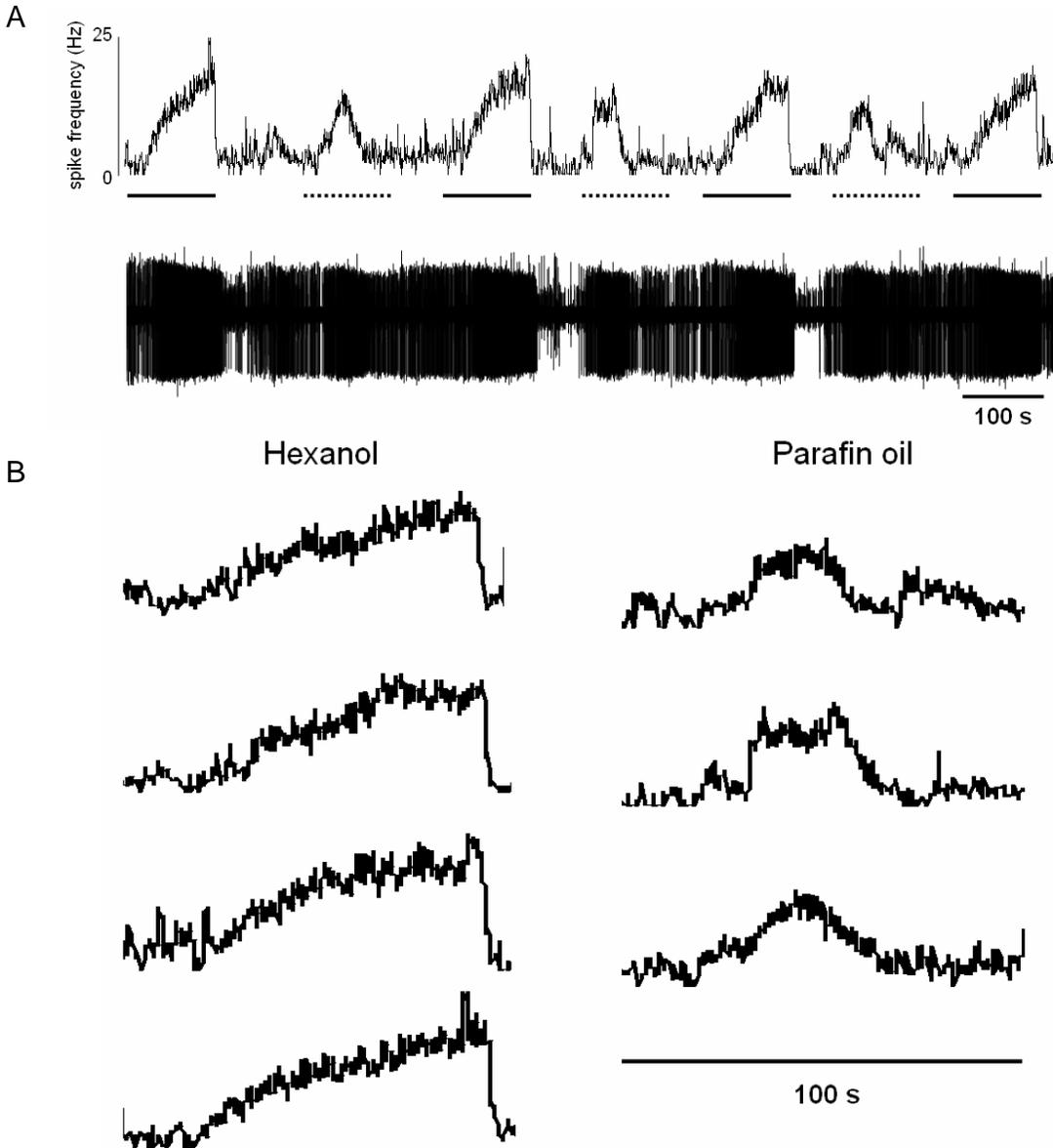


Fig. 3: Chemosensory response to organic stimulus. (A) Raw record (below) and mean spiking frequency (above) for peg sensillum stimulated by hexanol (solid line) and paraffin oil (dotted line). (B) Expanded views of changes in mean spiking frequency in peg sensilla to stimulation with hexanol and paraffin oil.

series. The amplitude of the action potentials continued to decrease until cessation of stimulation. Once the stimulus was removed, the action potential amplitude began increasing to pre-stimulation values. In the 30 second and 45 second impulse series, the amplitude was unable to fully return to baseline amplitude levels due to the onset of the next stimulation impulse, which resulted in the subsequent reduction of action potential amplitude. Within the 60 second waiting period which followed each 60 second impulse, the amplitude of the action potentials attained pre-stimulation values.

Discussion

The results illustrate that the engineered apparatus was effective in delivering a stimulus to a selected region of pegs. That the action potential frequency of the stimulated peg increased within an average time of 13.8 seconds not only illustrates that the apparatus is a viable way to deliver a stimulant directly to a selected target, but also shows that the delivery process is highly reproducible. The rapid decrease in action potential frequency upon the cessation of 1-hexanol stimulation indicates that the flow of the stimulant is controllable.

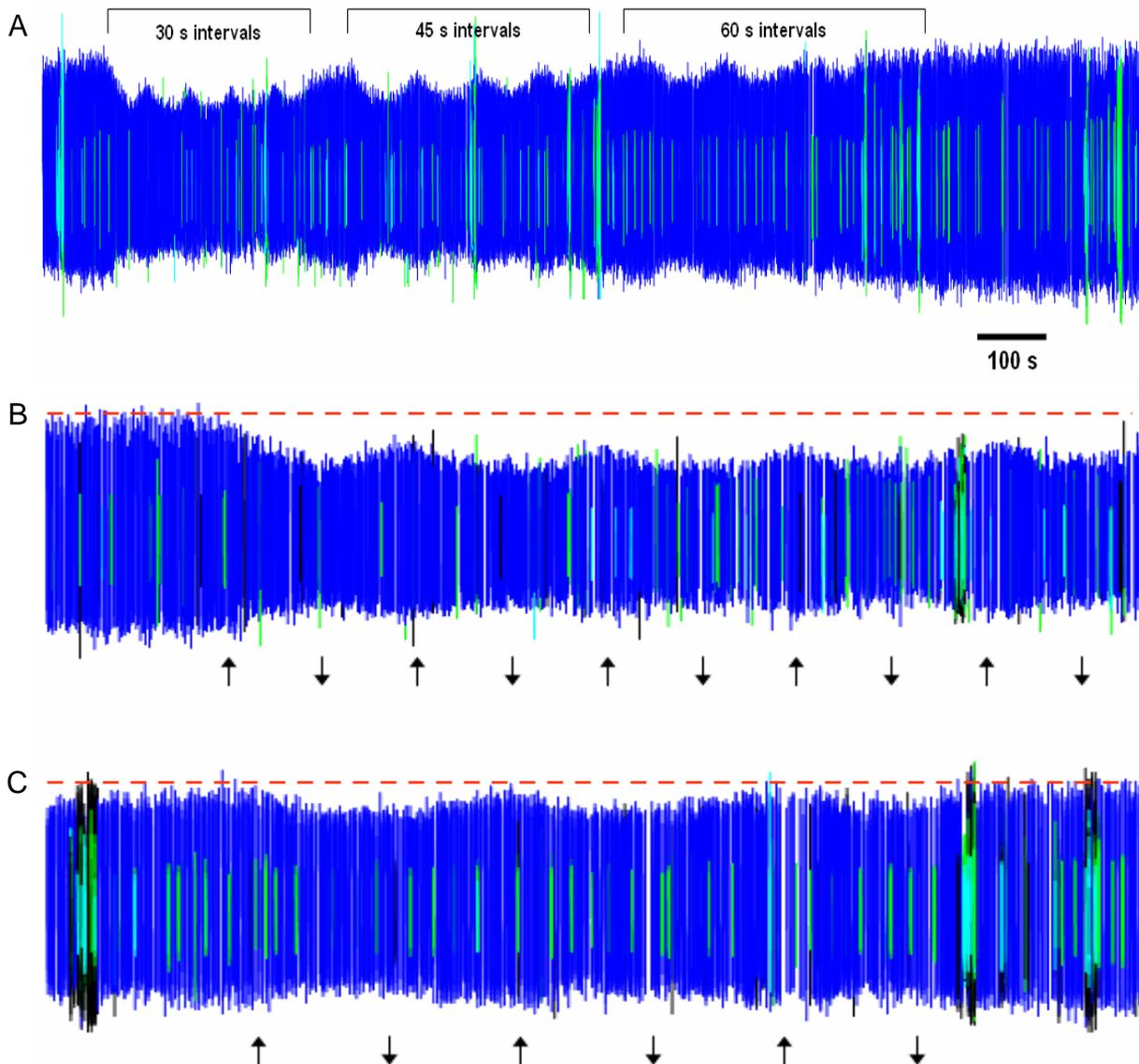


Fig. 4: Electrical response of peg sensillum of *P. utahensis* to variations in impulse duration (A). The response was expanded to show the 30s series (B) and the 60s series (C). Up arrows indicate initiation of stimulation while down arrows indicate termination of stimulation. The amplitude of the action potentials decreased when stimulated with 30s, 45s, and 60s pulses of organic stimuli. When the stimulus was removed, the action potential amplitudes returned to

This response also illustrates the sensitivity of the peg sensillum to the stimulant.

Stimulation with mineral oil elicited a response similar to that of the 1-hexanol, but with several fundamental differences. An increase in action potential frequency was observed at an average time of 7.1 seconds after mineral oil delivery, as opposed to the 13.8 seconds prior to a noticed increase with the 1-hexanol stimulation. This is because the 1-hexanol stimulation occurred prior to the mineral oil stimulation and this resulted in the charging of the apparatus. Since the

stimulus had to move from the tip of the sharpened pipette to the peg rather than all the way from the impregnated syringe, the onset time was significantly reduced. The fact that the mean frequency of action potentials did not increase throughout the stimulation period as it did in the 1-hexanol series, is attributed to the clearing of the apparatus. At a point during the stimulation period, the 1-hexanol that was responsible for inducing the increase in action potential frequency was flushed from the apparatus. This resulted in the

frequency of action potentials returning to baseline values.

The documentation of the various dynamics of our delivery device is imperative for future studies investigating the functionality of individual peg sensillum. For example, we would like to use this system to test for variations in response across the peg population. In addition we hope that better stimulus control will allow a complete description of synaptic interactions of peg neurons during chemical stimulation.

By investigating the effect of altering various parameters of the stimulation we were able to make some conclusions about the attributes of the engineered device. Because the action potential amplitude reduction occurs precisely upon stimulation with 1-hexanol within all of the impulse series, and that the amplitude repression reverses upon cessation of stimulation, we conclude that this response is due to the stimulation. This recurring oscillation of action potential amplitude further illustrates the reproducibility of response patterns obtainable using the apparatus. Future studies should use the 60 second impulse timing because this duration allows the amplitude to return to full baseline values. This would be beneficial in reducing any confound variables from concealing other important responses. Although we are confident that 1-hexanol is responsible for the amplitude decrease, we are unable to resolve the mechanism for the amplitude suppression. This response may be due to a peg sensillum membrane effect or by other mechanisms that we have not studied yet.

Acknowledgments

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