A new approach to examining scorpion peg sensilla: the mineral oil flood technique

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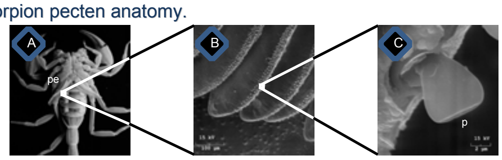
Introduction

All scorpions possess jointed, ventral appendages called pectines, which have patches of ground-directed, chemosensory peg-shaped sensilla that detect substrate-borne chemicals. Previous electrophysiological tests show that peg sensilla respond to alcohols, aldehydes, ketones, and esters with different neural activity patterns (Gaffin and Brownell, 1997). Peg sensilla were stimulated indirectly through puffs of stimuli blown across the peg fields (Gaffin and Brownell, 1997) or by near-range exposure to static clouds of volatile organic compounds (Gaffin and Walvoord, 2004). However, both of these methods suffer because they are using olfactory methods to stimulate gustatory peg sensilla. To mimic the scorpion's natural contact chemical detection system, we have developed a new way to deliver chemical stimulants directly to the terminal pore of individual sensilla through introduction of a polar, liquid substance while recording electrophysiologically under mineral oil. In this study, we evaluated the efficacy of the new mineral oil flood technique. From early indications, we think this new technique offers an improved method of chemical stimulation.

1 Background

When scorpions move in their environment, the ground-facing pectines sweep intermittently against substrate-borne chemicals.

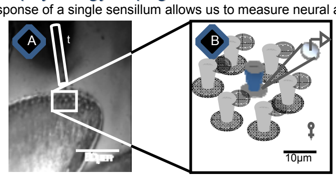
Scorpion pecten anatomy.



A A scorpion pecten (pe) resembles a comb, with teeth extending ventro-laterally from the pectinal spine (here *Hadrurus arizonensis* male). **B** A closer look at the teeth located on a scorpion pecten reveals that the ventral surface of each tooth contains a patch of pegs called peg sensilla. **C** Each peg sensillum has a slit-like opening or pore (p) that faces the ground (*Paruroctonus utahensis* male shown in B & C).

Electrophysiology of peg sensilla.

Chemically stimulating peg sensilla while recording the response of a single sensillum allows us to measure neural activity.



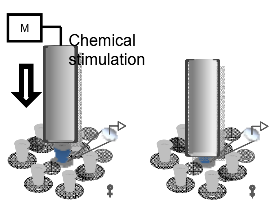
A Pictured is a field of view from a light microscope of the distal region of one tooth under mineral oil (here *Paruroctonus utahensis* female). A glass capillary tube (t) containing a chemical stimulant is introduced to the pore of the sensillum. **B** An electrolytically sharpened tungsten electrode is inserted at the base of a single peg sensillum to capture the neural response during chemical stimulation.

2 The Mineral Oil Flood Technique

The mineral oil flood technique employs mineral oil as a medium through which aqueous chemical stimulants are introduced to peg sensilla.

Animal

A live scorpion is immobilized on a microscope slide, ventral side up to expose the pectines. A cover glass (18 x 18 mm) with walls of wax approximately 1 mm high is positioned slightly below the pectinal joint. A notch in the wax wall allows access of a pecten to the chamber. The pectinal spine and teeth are secured to the cover glass with quick-drying adhesive. Additional wax is added to the area where the pecten spine crosses the edge of the cover slip to complete the wall of the chamber. One drop of mineral oil (~0.05 ml) is placed over the pecten.



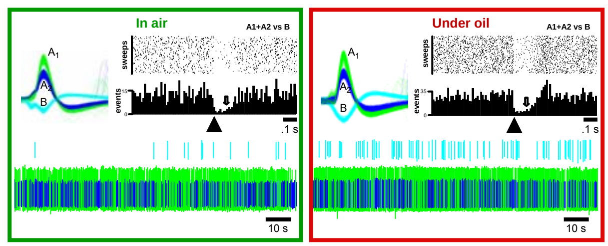
Polar liquids are insoluble in mineral oil; therefore, the pore on the terminal end of a peg sensillum can be directly touched under mineral oil with polar liquids or aqueous stimulants.

A glass capillary tube with a tip diameter of about 10 µm is filled with a polar stimulant and positioned onto a micromanipulator (M), which allows precise control over stimulant delivery to, and removal from, the sensillar pore.

When viewed under the microscope, oil immersion improves the resolution of peg sensilla compared to recordings made in air.

3 Does oil change the neural activity of peg sensilla?

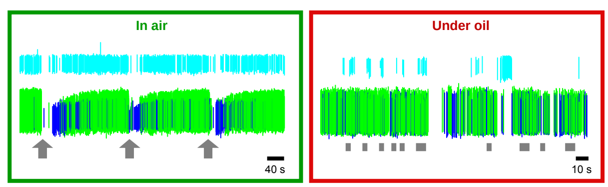
Successful extracellular recordings of chemosensitive neurons (A1, A2, and B) within peg sensilla of *Paruroctonus utahensis* have been made using the mineral oil flood technique.



Extracellular recordings of baseline neural activity. Each colored line at the bottom half of each panel represents a neural impulse from a single cell. In each panel, the upper left image shows superimposed waveforms for A1, A2, and B. Cross-correlograms (upper right in each panel) reveal that the firing of B (black arrow) inhibits the activity of both A1 and A2 for approximately 50 ms (white arrow).

4 How does stimulation of peg sensilla under oil compare to stimulation in air?

Using the mineral oil flood technique, we could confine stimulation to a single peg sensillum and control the onset and removal of the stimulant.



Extracellular recordings of neural activity during chemical stimulation. Peg sensilla were stimulated with 95% ethanol. In air (left panel), the duration of chemical stimulation cannot be controlled. The white arrows indicate when the pipette tip flooded the peg field ethanol. Under mineral oil (right panel), a droplet of ethanol can be applied to a single peg sensillum without flooding the rest of the field. Note that the duration of chemical stimulation, represented by horizontal lines, may be precisely controlled.

5 Future research

- ❑ We expect to quantify the concentration of stimulant reaching the sensillar pore.
- ❑ We expect to refine the mineral oil flood technique for future studies on the Information Enhancement Hypothesis, which suggests that pegs are functionally equivalent and part of a parallel sampling system (Gaffin and Walvoord, 2004).

Conclusions

- ❑ Mineral oil does not change the neural activity of peg sensilla.
- ❑ Unlike previous methods, chemically stimulating peg sensilla under mineral oil allows for precise control over the duration of direct contact between the sensillar pore and the stimulant.
- ❑ Under mineral oil, we can selectively stimulate individual peg sensilla with improved resolution of the peg field.

References

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Acknowledgements

We thank Dr. Mariëlle Hoefnagels and Dr. Don Wilson for their valued suggestions and use of equipment. We also thank Nataliya Popokina for assistance in collecting and maintaining our animals and Josh Bridges for providing the SEMs of peg sensilla. Finally, we thank the *Life Fund* of the University of Oklahoma Foundation and the OU Zoology Adams Scholarship Fund for providing support for this work.