# Chemical Differentiation of Prey and Non-Prey Organisms in *Paruroctonus utahensis*.

*A biology cornerstone manuscript by:*
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## Summary

Scorpions (Arachnida: Scorpiones) can detect various chemicals with their pectines, a pair of chemosensory organs on their ventral mesosomas. These comb-like structures contain a line of teeth, each of which has a dense cluster of peg sensilla on their distal, ground-facing tips. We tested the scorpions' ability to identify and differentiate between prey chemicals and non-prey chemicals using electrophysiological recording techniques to record the number of action potentials caused by stimulants on their pegs. Our data suggests that peg neurons are responsive to biotic substances, and that natural cricket deposits extracted from sand produced particularly vigorous spiking activity.

## Introduction

Scorpions have a pair of unique mechano- and chemosensory appendages called pectines on their abdomens that extend laterally and ventrally. Each of these organs consist of three to forty teeth, depending on species and sex, arranged like the teeth of a comb along the spine of the pecten. The distal face of each tooth contains peg sensilla that detect chemical stimuli (Gaffin 1997, Melville 2000). Previous research shows that the neurons in these pegs produce different patterns of action potential activity when citric acid, ethyl alcohol, and potassium chloride are introduced (Knowlton 2011). This suggests that they could have unique reactions to a much wider variety of substances or to complex natural chemostimulants.

A study on contact chemoreception in scorpion hunting suggests that scorpions can detect motionless prey through chemical stimuli. When placed in an arena with a dead cricket, scorpions could locate the prey even from 10 cm, which suggests the use of chemoreceptors (Krapf 1986). However, studies have yet to investigate whether scorpion pectines respond differently to prey and non-prey chemical stimuli. Therefore, the goal of this study was to electrophysiologically assess the response properties of neurons in scorpion pectines that could be used during chemosensory hunting.

We adapted the mineral oil flood technique (Knowlton and Gaffin 2011) and exposed the peg sensilla of a local species of scorpion, *Paruroctonus utahensis*, to a solution of prey cricket extract, prey cricket sand wash, a non-prey plant extract, and a control solution of potassium chloride. In the mineral oil flood technique, oil is flooded over the scorpion's pectines to control stimulant delivery and action potentials are recorded through an electrode placed in contact with the tip of individual peg sensilla (Knowlton and Gaffin 2011).

We hypothesized that the response frequencies of pectinal neurons would differ in reaction to prey and non-prey chemicals. We expected to observe a high frequency of action potentials in response to the extract from the crickets and a similarly high frequency with the cricket sand wash. Conversely, the plant extract should elicit some increase in action potentials, but notably less than the cricket solutions. In these preliminary studies, we found that the biotic stimuli elicited heightened action potential activity compared to KCl alone and that cricket deposits extracted from sand produced the strongest response.

## Methods

*Animal Care*

We housed our animals *(P utahensis*) in 3.8 L glass jars and fed them one cricket per week and moistened their

sand substrate with water twice per week. A controlled day-night cycle was used, with the lights turning off at 1230 and turning back on at 0130.

*Preparing Test Solutions*

We housed approximately 20 additional crickets in a Tupperware container with sterilized sand on the bottom as a substrate. Four holes were added to the container lid for aeration, covered in tape with smaller holes to prevent escape. Crickets were given seaweed-based solid food and water (soaked into a paper towel) in dishes at the beginning of their storage; we switched to a powder food and water gel two weeks in after depleting the original food supply. Water was replenished weekly, and food every other week. After a month, we made a cricket-sand solution by taking 6.0 grams of sand from the substrate surface. This sand was placed on two Kimwipes EX-L in a funnel over a beaker and 25 mL of distilled water was run through the sand.

For the cricket mush solution, we ground 0.16 grams of frozen crickets using a mortar and pestle in 25 mL of 0.1 M KCl for one minute with approximately thirty hand rotations. This solution was then transferred to an external vial and was filtered through Kimwipes EX-L.

To prepare the plant solution, we thoroughly mashed two painted nettle (*Plectranthus scutellarioides*) leaves, with a mass of 0.57 grams, and 25 mL of 0.1 M KCl, using approximately thirty hand rotations over about a minute. The solution was then transferred to an external bottle and large leaf particles were filtered out.

As a control, 0.1 M KCl was also introduced to peg sensilla. The KCl solution was made on October 26 and was used in the preparation of the other stimulants. The other three stimulants were made on November 9. We froze the three stimulants containing organic compounds between uses at 1.67°C.

*Scorpion Preparation*

First, we immobilized the scorpion by placing it in a pre-frozen glass jar in a freezer for at least two minutes. Then, we removed the scorpion and placed it onto a microscope slide with its pectines facing upwards. We applied moldable clay to the scorpion's tail, pedipalps, and legs, holding them in place on the microscope slide, and left the pectines exposed.

*Pecten Chamber Preparation*

We constructed a platform for the scorpion's pectines out of a microscope cover glass, custom made based on pecten size. Dimensions were approximately 10 x 18 mm. Double-sided tape was placed on the upper side of the platform where the pectines were located. We placed one edge of the platform just behind the point of pectinal intersection on the scorpion. Stabilization of the platform was done using additional clay applied below the microscope slide. We used forceps to place the pectines on the platform. Then we used a small spatula to apply beeswax (melted on a hot plate) around the pectines, forming a dam that later was used to hold mineral oil. We inserted a silver wire in between two of the tail segments to form an indifferent electrode connection with the hemolymph.

*Testing*

Most of our data are from a male *P utahensis* scorpion that was tested during its active hours (1230-0130) on

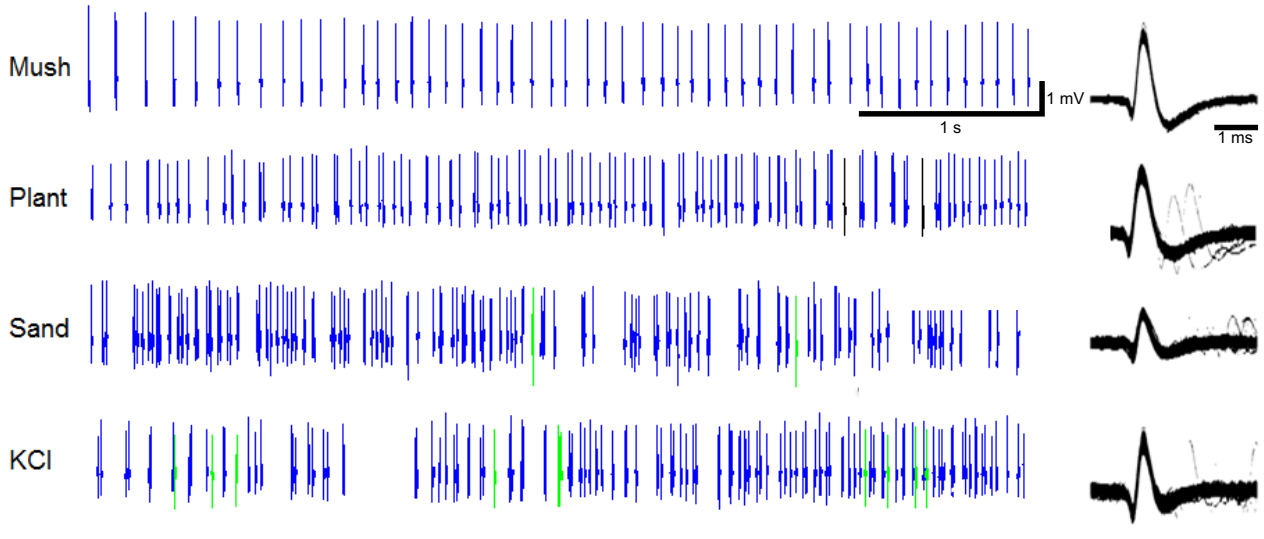


**Fig. 1:** All the recorded spikes for the most representative recording of each substance. The recorded spikes are superimposed in a time-expanded window at the left of each record.

November 16. Before testing, we filled the wax chamber with 5 µL of mineral oil, or enough to submerge the pectines. To apply stimulant to the sensillum, we used custom-made micropipettes (made with a micropipette puller) with a tip diameter roughly 2 µm greater than that of the sensillar pore. We used a micropipette filler to insert the stimulant solution into the micropipette.

After we inserted the stimulant solution into the micropipette, we inserted the recording electrode into the blunt end of the pipette so that it contacted the solution. Once this was complete, we moved the scorpion under the microscope and lowered the pipette tip over sensilla on four different teeth, one tooth for each stimulant. Each tooth's sensilla were tested five times, each time for approximately five seconds with approximately 45 seconds between recordings. We tested our control 0.1 M KCl solution on the third right tooth, the cricket extract solution on the fourth, plant extract on the sixth, and substrate rinse on the seventh. Testing was performed close to the central region of each tooth.

*Analysis*

We used the *Spike 2* program (Cambridge Electronics) to analyze our recordings. Each 5s frame of action potentials was sorted by waveform and then graphed. We also counted the number of action potentials in the first 0.5 seconds of each trial.

## Results

We looked for the presence of different spike shapes in response to different stimulants. The action potentials were classified for each of the four stimulants and no difference was observed between the four waveforms, which most likely indicated the same cell firing (Fig. 1). However, there is a noticeable difference in firing rates between the substances as well as in how they changed th rough the duration of the recordings (Fig. 2). In the first two seconds of the recording, the sand wash, plant, and cricket extracts had a higher frequency than the KCl. Throughout the rest of the recording, the plant and cricket extracts had a greater frequency than the other stimulants. The KCl increased at a greater rate than the other stimulants throughout the entire recording.

## Discussion

In the initial seconds of the recordings, the biological stimulants appeared to induce a greater rate of action potentials than natural minerals. It seems possible that the strong response to the sand wash reflects the possibility that scorpions in their natural habitat would be more likely to encounter this stimulus compared to cricket mush. This response also seems in line with an account of some scorpions' ability to rediscover previously stung

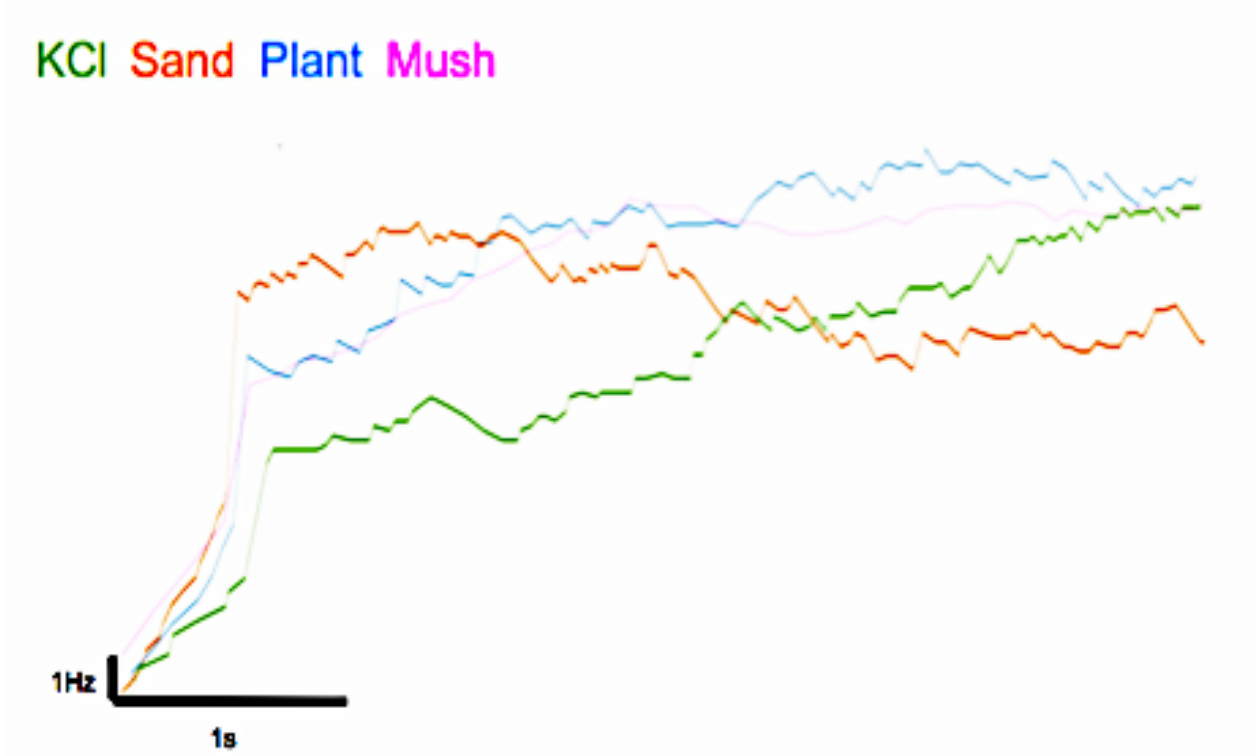


Fig. 2: Instantaneous spikes per second for the representative recordings shown in Fig. 1.

prey if they did not roam too far away before they died (Skutelsky 1995).

It is interesting that the scorpion responded slightly more to the plant extract than the cricket mush during most of the recording period. This may have happened because 0.57 grams of plant matter was used in the plant extract compared to the 0.16 grams of cricket matter in the cricket mush.

These recordings were taken from the same animal, on the same pecten, within an hour of each other, although they were on different teeth. This provides a measure of consistency in circumstances, but creates a lack of diversity. It is possible that the chosen subject was an anomaly, or that the results would have been different if the experiment was performed at a different time of the year or at a different point in the animal's feeding cycle.

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