Video Article

Electrophysiology of Scorpion Peg Sensilla

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Abstract

We describe a modification of an existing tip-recording technique[1,2] for electrophysiologically investigating short, peg-like sensory sensilla[3,4]. On the mid-ventral surface of all scorpions are two appendages called pectines, which have dense fields of mechano- and chemosensory peg sensilla[5,6]. One method for assessing chemoresponsiveness of these sensilla uses a tungsten electrode for extracellularly recording neural activity within a sensillum as a volatile odorant is introduced to the sensory field[5,7]. The limitations of this method include slow data collection and uncontrolled stimulant introduction to, and removal from, the peg field. To overcome these limitations, we developed a new tip-recording technique that uses nonpolar mineral oil as a medium through which to deliver water-based tastants to individual peg sensilla[8,9]. We have successfully applied this method to obtain sensillar chemoresponses to citric acid, ethanol, and salt. Here we describe the experimental protocol for such a study[9]. We think this new method may be useful for studying the response properties of other arthropod chemosensory systems, including those of insects[10, 11] and crustaceans[12].

Video Link

The video component of this article can be found at https://www.jove.com/video/2642/

Protocol

1. Pre-recording, Live Animal Preparation

1. Remove the scorpion from its home jar and place it in a pre-chilled glass container; next, place the jar containing the scorpion into a freezing environment (-5 °C) for a minimum of two minutes. This time may vary according to the age, size, species, etc. of the specific scorpion under study. But generally, the freezing environment phase is complete once the animal is motionless.
2. After two minutes, remove the immobilized scorpion from the cold glass container, and place it ventral side up on a microscope slide. Secure the stinger, tail, legs, and pedipalps with moldable clay (oil-based, Van Aken Plastalina).
3. Make a platform on which to put the scorpion's pectines. Adjust the platform's length and width according to the size of the pecten. Typically, our platforms are constructed out of microscope cover glass and are approximately 10(L) X 18(W) mm.
4. Adhere double-sided adhesive tape to the pecten platform, trimming the tape (if necessary) to fit.
5. To make a pectinal chamber, put three of the platform's four sides into pre-melted wax (we use 100% beeswax chips) to a depth of 3-4 mm. We melt the wax by placing the chips within a glass petri dish, located on a hot plate. Once the wax on the edges of the pectinal chamber is cooled to room temperature, this should create walls of wax about 1 mm high.
6. On the animal, locate the point of pectinal insertion, and place the un-waxed edge of the platform (tape side-up) just posterior to it. Afterward, stabilize the platform (so that it will not move later on) by gently pressing its right and left peripheral edges against the clay used to restrain the animal's legs.
7. Use fine-tipped forceps to carefully place the pecten or pectines inside the chamber. Specifically, using forceps, hold the pecten along the pectinal spine, and gently bring the pecten out from underneath the cover glass and lower it onto the adhesive platform floor. Establish a pecten-to-tape bond by carefully applying pressure to the pectinal spine.
8. Now apply melted wax (we use a pre-heated metal spatula) to the un-waxed edge of the platform. The purpose is two-fold, to secure the pecten and to fully enclose the chamber for future mineral oil introduction. Use caution when applying the wax over the animal's pecten, as it is possible to damage the pecten with wax that is too hot.
9. The pecten chamber construction is complete. Next, establish an indifferent electrode connection with the animal's hemolymph by inserting a silver wire between two tail segments.

2. Simultaneous Extracellular Recording and Chemical Stimulation

1. We prepare the animal 24 h prior to conducting extracellular, tip-recordings, which, among other reasons (i.e. animal adjustment to fixed position), allows time to custom-make stimulant pipettes of a specific (sensillum-dependent) tip-diameter. We use a micropipette puller for achieving such specifications. Ideally, the diameter of the pipettes is ≈ 2 µm larger than the sensillar pore diameter. We adjust our micropipette specifications accordingly for each animal.

2. 1 h prior to recording, we add 5 µl of mineral oil to the pectinal chamber, submerging the pecten under oil. Then, we position the animal beneath a high-powered microscope, which has long working distance objectives and epi-illumination.
3. To chemically stimulate peg sensilla, we introduce aqueous tastants (i.e. citric acid and salt) through the oil medium. First, we use a micropipette filler to inject the stimulant into the stimulant pipette. Note: all stimulant solutions should contain an electrolyte for electrical conductance.
4. Next, after positioning the stimulant pipette on a micromanipulator (in the recording rig), insert the recording electrode into the blunt, open end of the stimulant pipette. It is important to ensure contact between the metal electrode and electrolytic stimulant solution.
5. Once the preparation is electrically grounded (via the indifferent electrode), simply lower the pipette through the oil medium and onto a sensillar pore. The duration of chemical stimulation per pectinal sensillum can vary from as much as thirty minutes to as little as one second. Generally, we can sample multiple pegs (> 30) per pipette. In the event that the stimulant pipette becomes clogged with minute debris, simply exchange the clogged pipette for a new, unused pipette.

3. Preparing an Animal for Multiple Days of Experimentation

1. If the animal is to be used over an extended time period, we recommend reusing the pectinal chamber and replacing the drop of mineral oil for each recording session. After a given day's recording session is complete, remove the animal from the recording set-up and perform a mineral oil wash.
2. Specifically, add another drop (5 µl) of mineral oil to the chamber, and remove all oil. This helps minimize the presence of residual stimulant, which may have leaked out of the pipette during the recording session.
3. Before the next session, add one drop of oil as described previously.

4. Representative Results:

Depending on the recording equipment used (e.g. the particular amplifier, digitizing hardware, etc.), a representative result is an extracellular recording with a S:N ratio of at least 3:1.

Successful construction of the mineral oil chamber is necessary for isolating chemical stimulation to individual sensilla, as it provides a medium for delivering polar (water-based) chemical tastants[8]. If there is too little oil or none at all, the stimulant solution will spread from the recorded sensillum to its neighboring sensilla, which is likely to cause a prolonged, uncontrolled bout of chemical stimulation[8].

Discussion

The protocol above describes how to prepare a desert grassland scorpion (*Paruroctonus utahensis*) for electrophysiological study. Specifically, we show how to build a chamber for controlled extracellular tip-recordings of pectinal chemosensory neurons under oil. Because oil and water do not mix, it is possible to isolate chemical stimulation (with water-based stimulants) to single sensilla. It should be stressed that *P. utahensis*, is a relatively small animal (≈ 2.5-5 cm) and applying this protocol to larger animals may require many size adjustments, such as the size of the animal's platform, chamber, and amount of oil applied. We recommend conducting pilot studies to test the minimum amount of oil necessary for controlled delivery of water-based chemical stimulants.

Furthermore, we found no effect of oil on the baseline neural activity within pectinal sensilla[8]. This should be confirmed for other model systems as well, prior to assessing chemoresponsiveness.

This method may be used in conjunction with another method to test for chemoresponse interaction effects among sensilla in the sensory field. For example, it is possible to tip-record (under oil) from one sensillum as we base-record (via a tungsten electrode) from a neighboring sensillum[9]. Such a recording configuration may be used to assess whether or not chemically stimulating one sensillum affects the neural activity of the base-recorded sensillum. To date, no one has tested this for scorpion pectinal sensilla, and it remains an open question for other chemosensory systems as well.

To summarize, we think the tip-recording method under oil advances the scope of electrophysiological investigations into the neural basis of arthropod gustation. In this manuscript, we provide a protocol for preparing animals for this method, and we hope it provides a firm foundation for further study of peripheral sensory nervous systems.

Disclosures

We have previously published these methods in two journals, Knowlton and Gaffin (2009) and Knowlton and Gaffin (in press).

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