Video Article

Single Sensillum Recordings for Locust Palp Sensilla Basiconica

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Abstract

The palps of locust mouthparts are considered to be conventional gustatory organs that play an important role in a locust's food selection, especially for the detection of non-volatile chemical cues through sensilla chaetica (previously named terminal sensilla or crested sensilla). There is now increasing evidence that these palps also have an olfactory function. An odorant receptor (LmigOR2) and an odorant-binding protein (LmigOBP1) have been localized in the neurons and accessory cells, respectively, in the sensilla basiconica of the palps. Single sensillum recording (SSR) is used for recording the responses of odorant receptor neurons, which is an effective method for screening active ligands on specific odorant receptors. SSR is used in functional studies of odorant receptors in palp sensilla. The structure of the sensilla basiconica located on the dome of the palps differs somewhat from the structure of those on the antennae. Therefore, when performing an SSR elicited by odorants, some specific advice may be helpful for obtaining optimum results. In this paper, a detailed and highly effective protocol for an SSR from insect palp sensilla basiconica is introduced.

Video Link

The video component of this article can be found at https://www.jove.com/video/57863/

Introduction

Animals have evolved a range of chemosensory organs that sense exogenous chemical cues. In insects, the most important chemosensory organs are the antennae and the palps. On these organs, several types of chemosensory hairs, called chemosensory sensilla, are innervated by chemosensory neurons (CSNs) within the hairs. CSNs in chemosensory sensilla recognize specific chemical cues through signal transduction from chemical stimuli to electrical potentials that are subsequently transferred up to the central nervous systems[1,2,3].

CSNs express various chemosensory receptors [*e.g.*, odorant receptors (ORs)], ionotropic receptors (IRs), and gustatory receptors (GRs) on their membranes, which encode exogenous chemical cues associated with different types of chemosensation[4,5,6]. The characterization of CSNs is key to the elucidation of cellular and molecular mechanisms of insect chemoreception. Now single sensillum recording (SSR) is a widely-used technique for the characterization of insect CSNs in the antennal sensilla of many insects, including flies[7], moths[8], beetles[9], aphids[10], locusts[11], and ants[12]. However, few studies have applied an SSR to insect palps[13,14,15,16,17], because the particular structures of their sensilla make an electrophysiological recording difficult[18].

Swarms of locusts (Orthoptera) often cause serious crop damage and economic loss[19]. The palps are believed to play an important role in the food selection of locusts[20,21,22,23,24]. Two types of chemosensory sensilla are investigated by a scanning electron microscope (SEM). Usually, 350 sensilla chaetica and 7 - 8 sensilla basiconica are observed on each dome of the locust palps[18]. Sensilla chaetica are gustatory sensilla that sense non-volatile chemical cues, whereas sensilla basiconica have an olfactory function, sensing volatile chemical cues.

On locust palps, the diameters of the hair sockets of the sensilla basiconica (ca. 12 µm), are much greater than those of sensilla chaetica (*ca.* 8 µm)[18,25]. The cuticular wall of the sensilla basiconica on the palps is much thicker than that of antennal sensilla[18]. In addition, the dome of the palp has fluid contents within a highly flexible cuticle. These characteristics mean that a penetration with a microelectrode and an acquisition of good electrophysiological signals is more difficult than for antennal sensilla. In this paper, a detailed and highly effective SSR protocol for locust palp sensilla basiconica is presented with a video.

Protocol

1. Preparation of Instruments and Insect

1. **Preparing tungsten electrodes and stimuli solutions**

1. Fix a new tungsten wire (diameter of 0.125 mm, length of 75 mm) into a micromanipulator and sharpen it in a 10% (w/v) sodium nitrite ($NaNO_2$) solution in a syringe at 10 V provided by a power supply for about 1 min under a stereomicroscope (40X magnification).
2. Dip the sharpened tungsten wire repeatedly into the 10% $NaNO_2$ solution, about 4 mm at 5 V in < 1 min (**Figure 1A**).
3. Examine the diameter of the sharpened tungsten tip frequently under the stereomicroscope until it is fine enough to penetrate the cuticle of a locust palp olfactory sensillum (**Figure 1B**).
4. Prepare the stimulus solutions. Dilute each of the chemical stimulus substance in mineral oil. Dilute 1-nonanol and nonanoic acid at 10% dilutions. Dilute E-2-hexenal and hexanal at 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}.
5. Prepare Pasteur tubes carrying the stimuli: insert filter paper strips (length of 2 cm, width of 0.5 cm) into the Pasteur tubes, add the diluted stimulus solutions (each 10 µl) to the filter paper strips, and then plug the Pasteur tubes with pipette tips (1 ml).

2. **Prepare the insect**
   1. Rear locusts (*Locusta migratoria*) with fresh wheat seedlings under crowded conditions at a relative humidity of 60%, a temperature of 28 - 30 °C, and a photoperiod of 18:6 h (light:dark). Choose 1- to 3-day-old 5th instar locust nymphs and remove the antennae with fine scissors to avoid any interference when recording.

3. **Preparing the locust maxillary palp holder**
   1. Use a glass slide (25 mm x 75 mm) as the base of the maxillary palp holder (MPH). Attach a plastic piece (1 mm in height, 10 mm in width, 35 mm in length) to a corner of the glass slide with double-sided adhesive tape, and finally fix a cover glass (18 mm x 18 mm) on top of the plastic piece with double-sided adhesive tape. Place a small piece of red rubber tape onto the cover glass as a non-slip layer. The plastic piece and the cover glass constitute the platform for the locust palp. The height of the platform is approximately 1.5 mm.
   2. Install a tungsten wire (diameter of 0.125 mm, length of 36 mm) at a distance of 1.5 mm parallel to the inside edge of the platform. Fix the two ends of the wire onto the platform with double-sided adhesive tape.

2. Preparation of Locust Maxillary Palps

1. Cut a centrifuge tube (1.5 ml) vertically in half and cut off the bottom. Place the locust into the prepared tube. Leave the ventral region and the head of the locust exposed. Fix the assembly to the glass slide with double-sided adhesive tape (**Figure 2A**).
2. Pull the right maxillary palp onto the platform.
3. Put the tungsten wire at the fourth segment of the palp. Place adhesive putty on each side of the tungsten wire, about 2 mm from the maxillary palp (**Figure 2A** and **2B**).

3. Single Sensillum Recordings

1. Place the locust maxillary palp preparation under a microscope at a low magnification (100X). Adjust the position of the preparation until the palp is perpendicular to the recording electrode (**Figure 3A**).
2. Insert the reference electrode (tungsten electrode) into the locust eye using a micromanipulator. Move the recording electrode (tungsten electrode) close to the maxillary palp with the micromanipulator (**Figure 3B** and **3C**).
3. Adjust the odor delivery device to about 1 cm from the maxillary palp (**Figure 3B**).
4. Open the recording software Auto Spike 32. Set the recording parameters as follows: the recording scale on 500 µV; the high cutoff of the filter on 300 Hz, the low cutoff on 200 Hz; and the pretrigger on 10 s.
5. Connect the recording electrode to a 10x universal AC/DC amplifier.
6. Switch the microscope to a high magnification (500X). Insert the recording electrode into the base of a basiconic sensillum on the maxillary palp and delicately adjust the recording electrode to obtain good spontaneous spikes (**Figure 3D**).
7. Open the stimulus controller to deliver a continuous air stream at 20 ml/s. Set the stimulation time to 1 s. Record signals for 10 s, starting 10 s before the onset of the stimulus pulse.
8. Use a 10x universal AC/DC amplifier to amplify the signals. Feed the signals into the IDAC 4. Analyze the signals with the Auto Spike 32 software. AC signals are band-pass filtered between 200 to 300 Hz. Use Auto Spike 32 to distinguish peak-to-trough amplitudes from noises. Calculate the responses of the neurons as the increases in action potential frequencies (spikes per second) over the spontaneous frequencies. Perform a statistical analysis using GraphPad Prism 7.

Representative Results

Two sensilla subtypes (pb1 and pb2) on the locust maxillary palp are identified based on different response dynamics to chemical odorants (10% 1-nonanol and 10% nonanoic acid). The neurons in pb1 produce significantly more spikes to 1-nonanol than to nonanoic acid while the neurons in pb2 are significantly less activated by 1-nonanol compared with nonanoic acid (**Figure 4**). Hexanal and E-2-Hexenal can evoke a locust palp opening response (POR)[26]. Hexanal is an abundant host plant green leaf volatile which may contribute to a further confirmation to the food source[26]. The spikes elicited in the pb1 neurons last longer than those of pb2 when they are stimulated by E-2-hexenal (**Figure 4**). The neurons in pb1 and pb2 exhibit similarly robust responses to hexanal (**Figure 4**). Comparing the mean changes of all spikes between the periods 5 s before and 5 s after the stimulation indicates that the response to 1-nonanol is significantly higher than to nonanoic acid in pb1, but contrarily in pb2 (**Figure 5**). The neurons in these two subtypes of sensilla respond dose-dependently to E-2-hexenal and hexanal, and their response patterns to these two aldehydes differ (**Figure 6A** and **6B**).

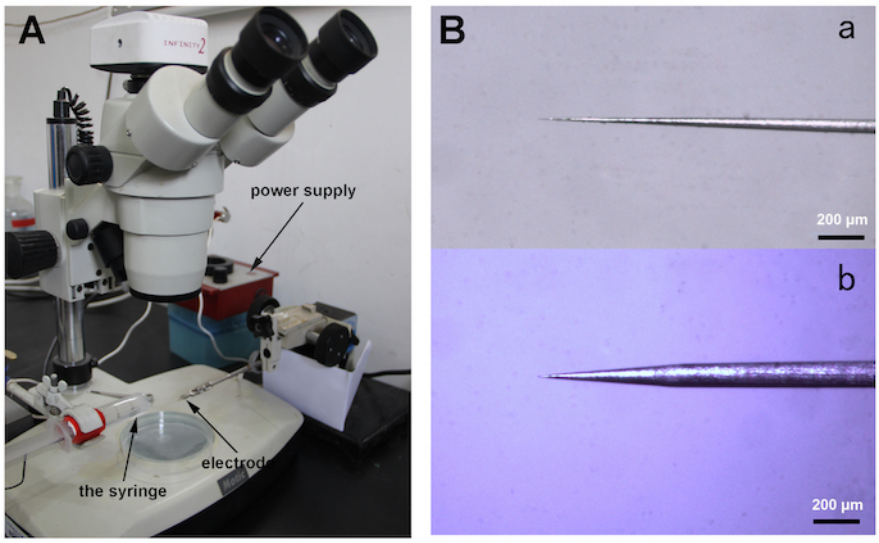

Figure 1. Electrode preparation. (**A**) This panel shows a general view of the electrode sharpening apparatus. The syringe containing 10% $NaNO_2$ (left) is used to sharpen the electrode (right). (**B**) This panel shows a close view of the electrode tip (a: suitable; b: unsuitable). Please click here to view a larger version of this figure.

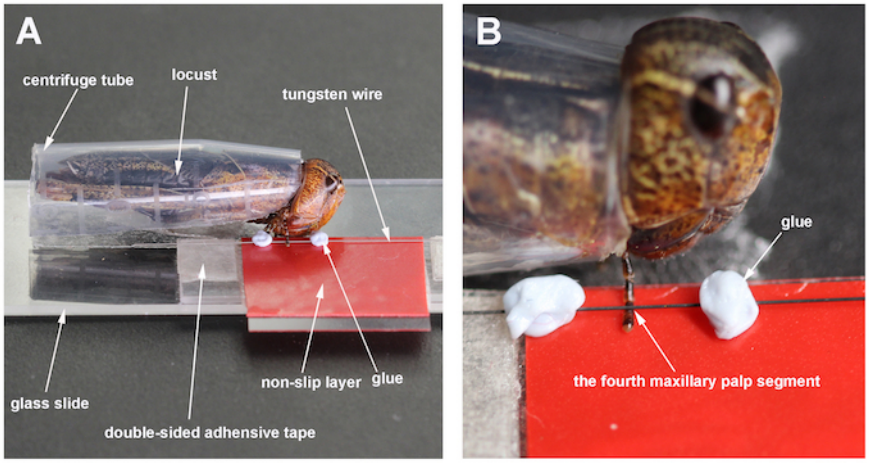

Figure 2. Locust maxillary palp holder (MPH). (**A**) The MPH and a locust are mounted on the glass slide before positioning it under the microscope. (**B**) This panel shows a close-up of the locust maxillary palp, fixed by tungsten wire on the platform. Please click here to view a larger version of this figure.

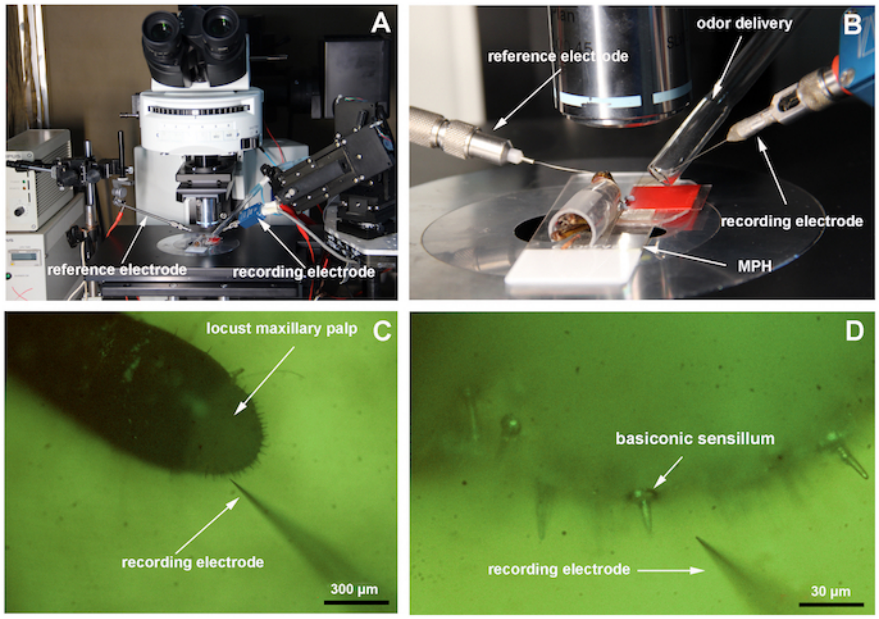

Figure 3. Single sensillum recordings. (**A**) This panel shows a view of the electrophysiology setup. (**B**) This panel shows a close view of the locust preparation mounted on the microscope. (**C**) This image shows the locust maxillary palp at 100X magnification. (**D**) This image shows the palp at 500X magnification. The arrow indicates a basiconic sensillum. Please click here to view a larger version of this figure.

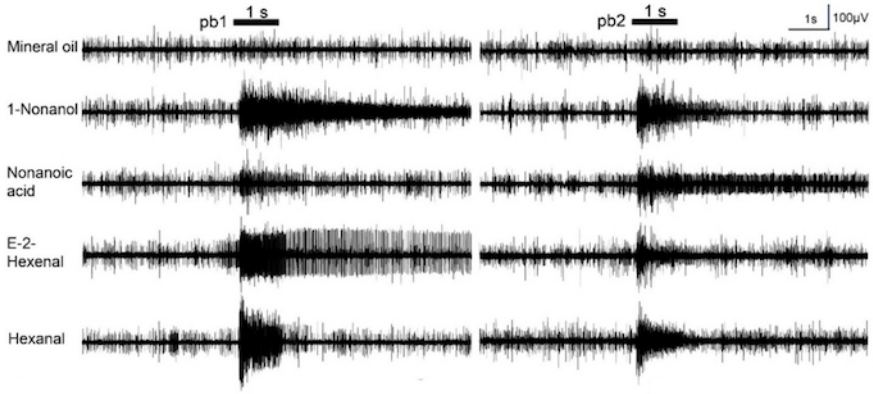

Figure 4. Response traces of single sensillum recordings of the locust maxillary palp. In this panel, pb1 stands for subtype 1 of the palp sensilla basiconica; pb2 stands for subtype 2 of the palp sensilla basiconica. The bars above the traces indicate the stimulus duration (1 s). For these recordings, all odors are used at 10% dilutions except for E-2-hexenal and hexanal, which are diluted to 1%. This figure has been modified from Zhang *et al.*[26]. Please click here to view a larger version of this figure.

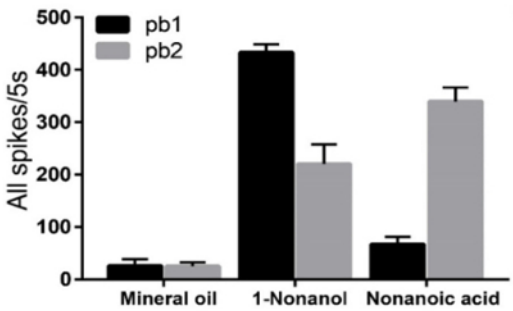

Figure 5. Comparison of mean numbers of spikes in neurons in pb1 and pb2 stimulated by nonanoic acid and 1-nonanol. The mean numbers of the spikes are calculated in the periods 5 s before and after stimulation. In pb1, the mean numbers of the spikes in the neurons responding to 1-nonanol increase significantly higher than those of the spikes in neurons responding to nonanoic acid (n = 11 palps; ANOVA with *post hoc* t-tests; $p < 0.0001$), in contrast to pb2 (n = 10 palps; ANOVA with *post hoc* t-tests; $p = 0.0110$). The error bar represents SEM. This figure has been modified from Zhang *et al.*[26]. Please click here to view a larger version of this figure.

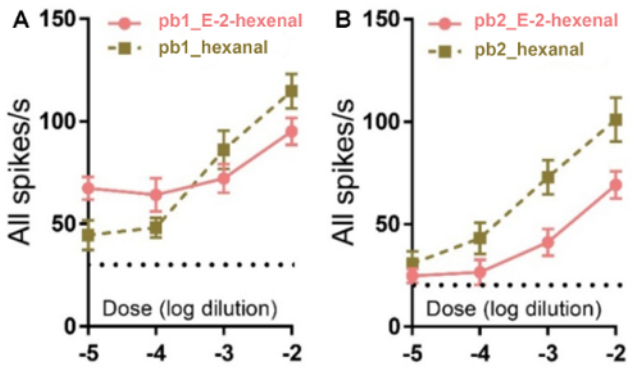

Figure 6. The patterns of neurons in pb1 and pb2 responding dose-dependently to E-2-hexenal and hexanal. (**A**) This panel shows the patterns of the neurons in pb1 (± SEM; *n* = 12 palps). (**B**) This panel shows the patterns of the neurons in pb2 (± SEM; *n* = 10 palps). This figure has been modified from Zhang *et al.*[26]. Please click here to view a larger version of this figure.

Discussion

Insects rely on palps to detect food odors, and their palps are believed to play an important role in speciation[13,27]. The palps are simple olfactory organs and are receiving increasing attention as an attractive model for the exploration of the neuromolecular networks underlying chemosensation[28].

Insect labellar and palp SSRs have been successfully performed on *Drosophila melanogaster*, *Anopheles gambiae*, and *Culex quinquefasciatus*[13,14,15,16,17] but have rarely been reported in the form of a video presentation[16,29]. In contrast, video data on antennal SSRs are available for *Drosophila*, the navel orangeworm moth (*Amyeloistransitella*), *Schistocerca Americana*, and the bed bug (*Cimex lectularius*)[16,30,31,32,33].

Locust palp sensilla basiconica have a particular structure that differs from that of locust antennal sensilla and many other insect sensilla. Using the method described here, action potentials generated by locust palp sensilla basiconica subtypes pb1 and pb2 could be recorded and discriminated (**Figure 4** and **Figure 5**).

The critical step is the penetration of the recording electrode. The recording electrode should be inserted into the base of the sensillum and advanced until good signals are acquired. In addition, it is important to prevent the dome of the palp from collapsing when the recording electrode is inserted into the base of the sensillum. To achieve this, we set up a platform including a special locust maxillary palp holder (MPH) and used a tungsten wire to compress the fourth segment of the palp. Many repetitions of this procedure demonstrate that this is effective. Based on the response patterns of the neurons in the sensilla to several odorants, we have, for the first time, identified two subtypes of sensilla basiconica on the locust maxillary palp, namely pb1 and pb2.

The limitation of the technique outlined in this publication is that it could be used to record big insects (*e.g.*, moths, beetles, and locusts) while not to record small insects (*e.g.*, flies and mosquitoes), which have their own platforms and techniques[13,14,15,16,17]. This technique is complementary to existing methods.

In conclusion, a highly effective protocol of an SSR from insect palp sensilla basiconica is described in detail. This protocol could provide researchers with a useful technique in the study of molecular and cellular mechanisms of insect olfaction on the mouthpart. This method linked with gas chromatography could be used to identify natural electrophysiologically-active ligands in extracts of favorable food resources.

Disclosures

The authors have nothing to disclose.

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