Lab Protocol

Objective: In this lab, you will use extracellular recordings to determine the receptive field of the wind-sensitive giant interneurons of the cricket.

Material: cricket, large Falcon tube, ice bucket, insect pins, Neuron SpikerBox (Backyard Brains), smartphone or laptop with the "Spike Recorder" (Backyard Brains) app installed.

Background

Many arthropods have two projections from their rear-most segment called cerci (singular: cercus) (Figures 1A and 1B). Cerci can serve as sensory organs, weapons, and/or copulation organs. In many insects, cerci are non-functional vestigial[1] organs.

In crickets, the cerci serve as sensory organs. Each cercus is covered by hundreds of mechanosensory hairs. Each hair contains the sensory axon of mechanosensory neurons that respond to wind from a particular set of directions.

All these mechanosensory neurons project to the ipsilateral terminal ganglion[2] (Figure 1C). The terminal ganglion contains giant interneurons that relay the sensory information to rest of the nervous system. Four giant interneurons integrate information from the cerci in an organized manner: each interneuron responds to a preferred wind direction.

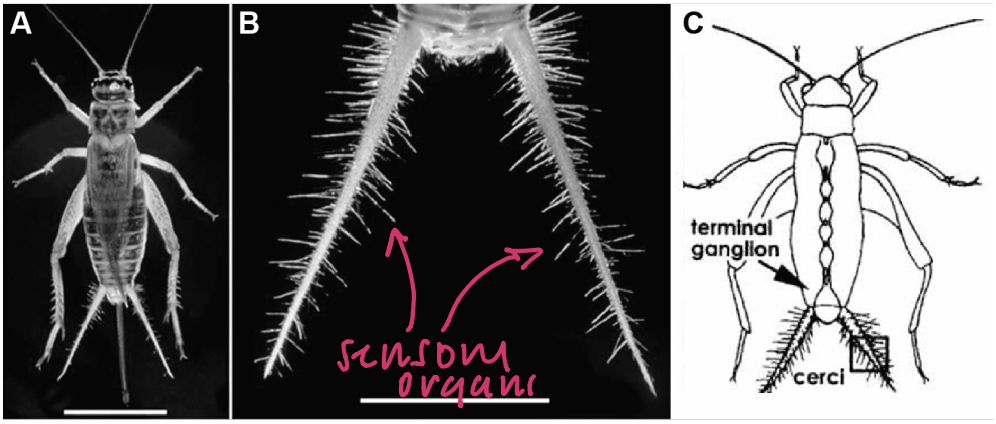

Figure 1: The cricket's cerci. (A) Top view of a cricket. (B) Detail of the cerci. (C) Diagram of the cricket's central nervous system (brain not shown). The arrow shows the position of the terminal ganglion pair.

Today, we will determine the receptive field of the terminal giant interneurons. To do so, we will record their electrical activity under various conditions. Neuron recording techniques can be categorized into two groups:

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[1] Anatomical structures are said vestigial when they have lost some or all of their ancestral function in a species. For example, the wings of ostriches are vestigial.

[2] In insects, the nervous system is organized in neuron clusters called ganglia. The ganglia are found in pairs (one per body side) along the ventral cord. There is at least one ganglion pair per segment. The ganglia of the head form the brain. The terminal ganglia are the rear-most ganglion pair.

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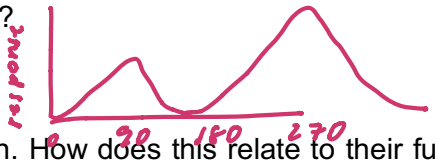
- Intracellular recordings: you record electrical changes (voltage or current) across the cell membrane. The measuring electrode needs to be inserted inside the neuron.

- Extracellular recordings: you measure changes in the extracellular medium induced by neuronal activity. The measuring electrode is placed outside the neuron. This is the technique we will be using today.

Questions:

1. What is (are) the function(s) of the cricket's cerci in nature?
   The hairs sense the direction of wind (can help w/ evading predators)
2. What does the receptive field of each mechanosensory hair look like? Terminal ganglion interneurons show direction preference: what does that tell you about the projections from the sensory neurons to the terminal ganglion?

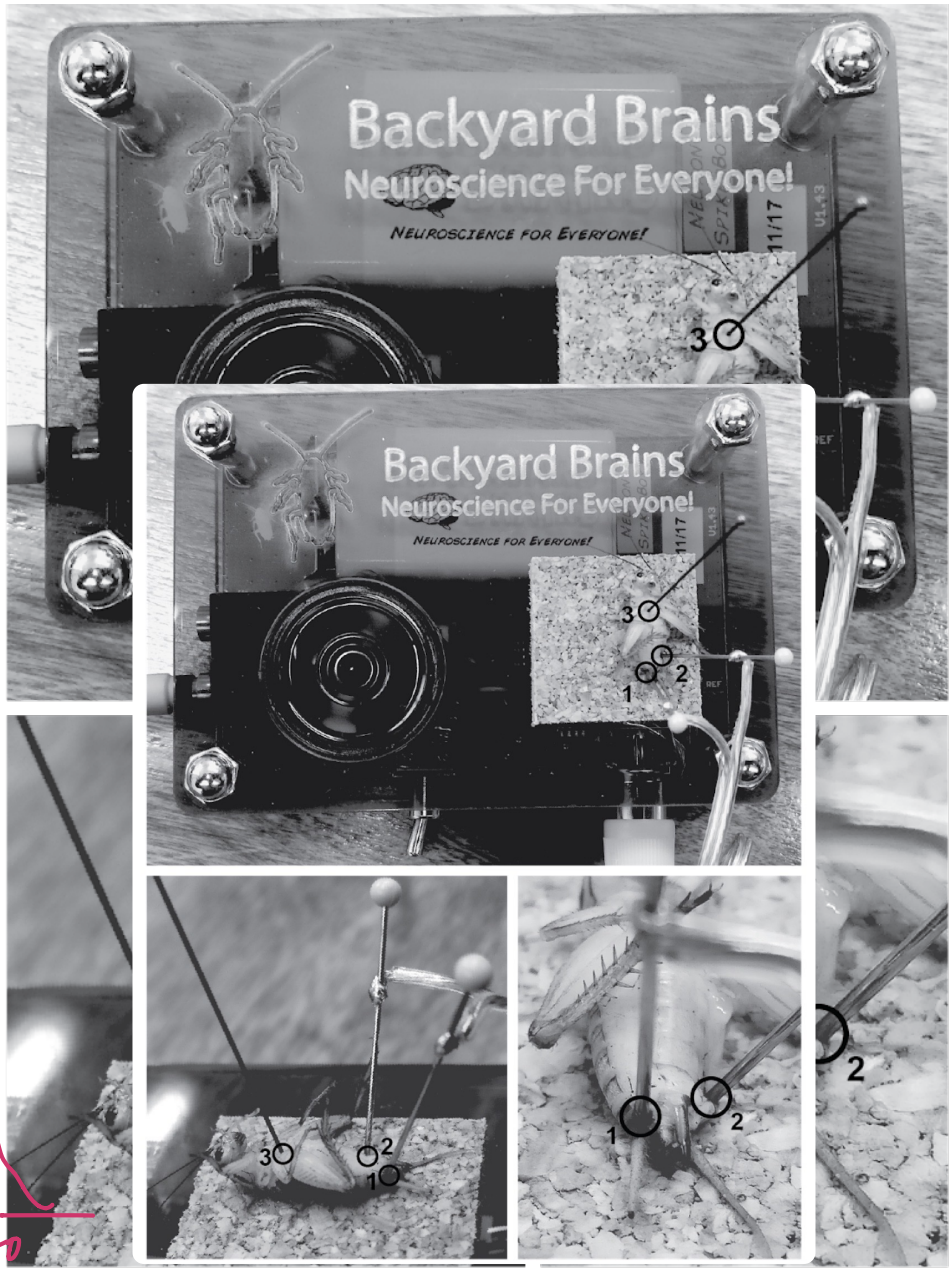


3. The giant interneurons have a thick axon. How does this relate to their function? Do you know another example of thick axon that serves the same purpose in nature? (hint: we talked about it in section 2) larger radius = less axonal resistance = higher velocity
4. What are some advantages / disadvantages of intracellular versus extracellular recording techniques?
   extracellular: multiple neurons, overall activity from a region
   intracellular: more difficult to put electrode inside neuron, one specific neuron, can manipulate current, EPSP/IPSP/Minis visible

Procedure

1. Put a cricket in a Falcon tube.
2. Put the tube in the ice. After a few minutes, the cricket will be anesthetized.
3. While your cricket is chilling, download the Spike Recorder app on your smartphone/laptop. Make sure the device you are using has a headphone jack.
4. When your cricket is immobilized, pin it to the cork pad of the SpikerBox with an insect pin (Figure 2).
5. Insert one pin into the cricket abdomen (the reference electrode) and the other into one side of the terminal ganglion (the measurement electrode) (Figure 2).
6. Connect the SpikerBox to your phone. Turn on the SpikerBox, open the Spike Recorder app, and observe the basal neuronal activity.
7. Gently stimulate the cerci by blowing on it with either your mouth or compressed gas. How did the neuronal activity change?

8. Try stimulating cerci at different angles. What do you see?



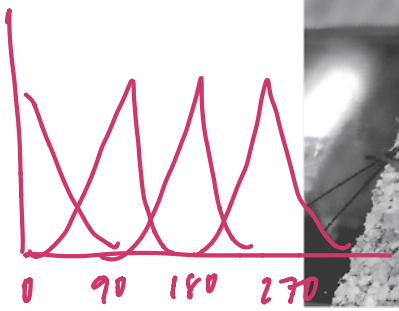


Figure 2: Position of the different pin. 1: Measuring electrode. 2: Reference electrode. 3: Insect pin.

↳ terminal ganglion

Questions:

1. Define the term "receptive field".
   Region of stimulus space where the stimulus changes the firing rate of a neuron.
2. How would you precisely determine the receptive field of the terminal giant axons?
   Blow wind from different directions. For one hair, its the perpendicular direction
3. Figure 3 illustrates a successful recording. How would you make sure that all the extracellular action potentials isolated belong to one neuron only?
   - only one AP pattern (similar amplitude)
   - periodicity in the extracellular recording (same interval)

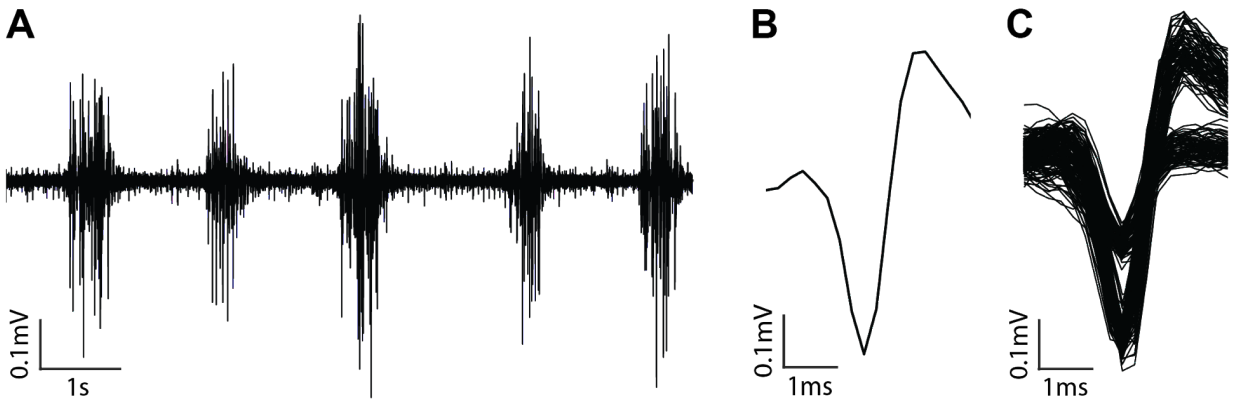


Figure 3: Exemplar extracellular recording of the cricket's terminal ganglion. (A) Recording as it appears on the Spike Recorder app screen (acquisition rate: 44,000Hz - online bandpass filter: 300Hz - 10,000Hz). (B) Detail of one extracellular action potential. (C) The 100 first extracellular action potentials extracted from the recording, stacked together. You can distinguish two units.

Definitions

1. Rate coding: intensity of signal = more frequent APs
2. Temporal coding: signal of AP determines AP firing
3. AP rate: frequency (# of APs / second)