# Exploring Neural Systems in a Cockroach Leg

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## II. Methods

### A. Subject Preparation and Experimental Setup

A single healthy lab-grown cockroach is used in this experiment, housed in a 250mL container with a lid to prevent escape. To anesthetize the cockroach, 200mL of ice water was poured into the container for approximately one minute, or until minimal movement was observed. Once anesthetized, the cockroach was removed and placed ventral-side up on a paper towel for leg dissection. Using forceps, the coxa of one posterior leg was secured while the leg was dismembered at the trochanter. The cockroach was then returned to the container without further injury.

The isolated leg was positioned near the edge of a corkboard atop a Backyard Brains Neuron SpikerBox[1], and secured using a needle inserted at the proximal end of the femur. A large reference needle electrode was placed proximally in the femur, close to the pin while a large recording needle electrode was inserted distally in the femur.

The needle electrodes, serving as the reference and recording electrodes, were connected to Channel 1 of the SpikerBox. The SpikerBox was linked to a computer via cable and interfaced with the NCH Tone Generator software. A pair of stimulation alligator clips were attached to the reference and recording electrodes, with the opposite end connected to the audio jack of the computer.

Following the initial experimental sequence, the alligator clips were removed, and the leg was repositioned to the center of the corkboard. Additionally, a second recording channel was connected to the SpikerBox, using another large needle electrode inserted medially in the tibia. This configuration was connected to the Spike Recorder software, instead of NCH Tone Generator software.

### B. Calibration

To verify the experimental setup, an audio stimulus was played through the SpikerBox speakers, resulting in movement in the cockroach leg.

Once the alligator clips were removed, the leg was repositioned, and the third electrode was connected, a test recording was performed using the updated setup. Mechanical stimulation was applied by gently tapping the tarsus, tibia, tibia spine, or femur with a toothpick or forceps, and spike activity was recorded using the Spike Recording software.

The oscilloscope in the Recording Software was adjusted to preferred settings as follows:

- Vertical axis scaling: Adjusted via the +/- buttons.
- Horizontal axis scaling: Adjusted using the scroll wheel.
- Spike detection volume: Adjusted using the volume knob on the SpikeBox.

### C. Experimental Sequence

*Stimulus Response to Electrical Stimulation*

For the first experimental sequence, the NCH Tone Generator software was used to generate a Square Wave pulse. The frequency was varied between 10 Hz and 5 kHz at a specific intervals (10, 100, 500, 1000, and 5000 Hz). The amplitude was adjusted in 25% intervals using the volume slider. The response of the cockroach leg to each combination of frequency and amplitude was observed and recorded.

*Spike Activity in Response to Mechanical Stimulation*

For the second experimental sequence, the Spike Recorder software was used with the event logger function to track stimulus-response events. Two tibial spines were identified that produced visible neural spikes. Each spine was briefly tapped at least 20 times using a toothpick or forceps, with 1-2 second pauses between each tap. The exact timing of stimulus onset was manually recorded for synchronized with neural responses. This procedure was then repeated for the second spine.

*Prolonged Spine Deflection*

In the final experimental sequence, the same two spines (or alternatively, two different spines) were used for prolonged mechanical stimulation. A toothpick attached to a manipulator was positioned to make sustained contact (~10 seconds) with the spine. The start and end of each event were synchronized across at least 10 trials. The process was repeated for the second spine.

## References

[1] "Neuron spikerbox," Backyard Brains, https://backyardbrains.com/products/neuron-spikerbox (accessed Jan. 28, 2025).

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