Neural Circuit Recording from an Intact Cockroach Nervous System

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SHORT ABSTRACT:

This article describes the cockroach ventral nerve cord dissection and extracellular recordings from the cercal nerve and connectives. Evoked responses are generated by electrical stimulation of the cercal nerve or direct mechanical stimulation of the cerci.

LONG ABSTRACT:

The cockroach ventral nerve cord preparation is a tractable system for neuroethology experiments, neural network modeling, and testing the physiological effects of insecticides. This article describes the scope of cockroach sensory modalities that can be used to assay how an insect nervous system responds to environmental perturbations. Emphasis here is on the escape behavior mediated by cerci to giant fiber transmission in *Periplaneta Americana*. This intact preparation requires only moderate dissecting skill and electrophysiological expertise to generate reproducible recordings of neuronal activity. Peptides or other chemical reagents can then be applied directly to the nervous system in solution with the physiological saline. Insecticides could also be administered prior to dissection and the escape circuit can serve as a proxy for the excitable state of the central nervous system. In this context the assays described herein would also be useful to researchers interested in limb regeneration and the evolution of nervous system development for which *P. Americana* is an established model organism.

INTRODUCTION:

There are more than 4000 cockroach species but only about 30 are household pests. Perhaps the most recognized is the misnamed American cockroach *Periplaneta americana* which originated in Africa, and is now found nearly everywhere on the planet. In addition to its rapid running speed [1] and evasive behavior, in the tropics *P. americana* is capable of flight [2,3].

The predominant characteristics of the cockroach central nervous system (CNS) are its segmented nature and decentralization of control processes [4,5]. The brain, thoracic and

abdominal ganglia are joined together by paired interganglionic connectives to form the ventral nerve cord (VNC).

The ganglia at each segment are integrating centers. They are composed of an outer, cortical region containing cells responsible for the blood-brain permeability barrier just beneath them, and the somata of neurons originating in that ganglion. These somata may belong to interneurons, modulatory neurons or motor neurons. They supply axons that remain within the ganglion of origin (local interneuron), or axons that project between the ganglia of the CNS (interganglionic interneurons) or that terminate on peripheral muscle cells (motor neurons). Most somata are positioned ventrally or ventrolaterally in the ganglionic cortex [5]. The paired, interganglionic connectives contain only axons and no neuronal cell bodies.

The neuropil of a ganglion contains glial cells (neuroglia), axon tracts, bundles of axons and dendrites (neurites) of neurons. The neuropil is devoid of neuronal cell bodies. This is the region within the ganglion where direct synaptic communication among nerve cells and integration of inputs occur.

The ability of the American cockroach *P. americana* to detect and suddenly respond to an approaching predator (foot, hand, etc.) has been attributed to a reflex circuit that consists of the cerci and giant fiber system [6,7]. The cerci are a pair of horn-like, wind-sensitive structures located on the end of the abdomen (Figure 1). In *P. americana* the ventral surface of each cercus contains about 200 filiform (thread) hairs that are organized into 14 columns. Nine of these columns can be consistently identified in different animals according to the response properties of the associated receptor cell and axon. Each hair is in a socket that allows it to bend most readily in one plane that is column specific. Movement of the hair in one direction along its plane induces a depolarization in the receptor cell and a burst of action potentials (APs) in the sensory neuron. Movement in the opposite direction inhibits any ongoing spontaneous APs [8]. The preferred plane of deflection and directionality of the response is different in each column. Thus, the filiform hair-receptor complexes are responsible not only for detecting the movement of air but also for 'coding', in the form of APs, the direction from which the air current originated. Processing of this information by the CNS results in an 'appropriate' escape response[6,7]. This functional, columnar specificity of the sensory hairs is preserved from animal to animal.

The receptor cell of each filiform hair is responsible for transducing the mechanical deflection of the hair into a neural event (resulting in a burst or inhibition of APs in the receptor cell's axon [9]. The APs travel to the terminal abdominal ganglion (A6) via cercal nerve XI, where they synapse with giant axons of the ventral nerve cord (VNC). The giant axons are believed to be responsible for the transmission and subsequent excitation of motor neurons commanding escape behavior[6,10,11].

The behavioral latency of the escape response of *P. americana* is one of the shortest of

any animal [7]. Behavioral latency is the time between the arrival of a stimulus at a mechanoreceptor and the initiation of an escape response. In experiments using high speed cinematography to record the attempted escape from an attacking toad, the cockroach was observed to begin its turn away from the toad in about 40 ms (time from beginning of tongue extension to cockroach movement [7,12]. Using controlled wind puffs, the behavioral latency could be reduced to 11 ms. Other experiments revealed that a minimum wind puff velocity of 12 mm.s-1 (with an acceleration of 600 mm.(s-2) can evoke an escape response, while even lower velocities (3 mm.s-1) caused slowly walking cockroaches to stop moving [12].

The strong correlation that typically exists between giant fiber systems and escape behavior has been well documented [13,14]. In instances where a particular cell is necessary and sufficient to evoke a particular behavior the cell is referred to as a command neuron [15,16]. Giant interneurons (GIs) in the wind escape circuit of *P. americana* are not necessary for the reflex. Animals that have experimentally ablated GIs still exhibit the escape behavior therefore these GIs are not considered command neurons [17,18]. Severing cervical connectives that are rostral to the sensorimotor circuit also influences the behavior, indicating that descending input from the brain has an effect on the direction of escape [19]. These aspects of fine control and redundancy are paramount to the organism's survival and are complemented by neurochemical modulation via biogenic amines [20].

The *P. americanus* nerve cord preparation has been an elegant model system for neuroethologists over the past many decades starting with the pioneering work of Roeder [21]. It permits students to record, display and analyze primary sensory activity and the resultant responses by giant interneuron to their input. In addition to conveying the idea that identifiable neural circuits underlie behavioral responses to the environment, these exercises should instill an appreciation for the biological contributions made by this common household pest.

PROCEDURE:

1) Dissection

1.1) Select a male cockroach from the holding tank that has robust cerci (Figure 1). The last segments of the male are narrow compared to the female; and containing no ovaries and egg mass, males are easier to dissect.

Figure 1: A cockroach with intact cerci.

1.2) Cut off the wings, legs and head and pin the body, ventral side up, to a Sylgard-coated dish.

1.3) With forceps pick up the ventral plates and cut them off with fine scissors, starting at

the posterior end and working anteriorly. Always keep the internal organs moist with Ringer's while trying to keep the cerci dry. One can use wax or pieces of Sylgard to position the abdomen upwards to prevent the saline from wetting the cerci. If they do get wet, dry them with a pointed piece of Kimwipe. Push to the side the internal organs and the white matter (fat body). The VNC is in the center of the field, runs the length of the abdomen and should be visible between the shiny trachea. The nerve cord is translucent and may initially be difficult to see until the lighting is adjusted properly (Figure 2). DO NOT handle the VNC with forceps or insect pins, rather manipulate it using glass probes.

Figure 2: Ventral view of cockroach nerve cord as seen with the ventral cuticle removed (A). A enlarged view of the segment outlined by arrows is seen in B. In C the connectives were spilt between A4 and A3 with a glass probe. The 6th abdominal ganglion is shown in D with the two cercal nerves leaving at the caudal end. Black paper was placed under the VNC for aiding in contrast for the photographs in C and D.

1.4) Clear away the animal's tracheae system as best as possible from the nerve cord with forceps and with a pair of fine glass needles, very carefully split the VNC connectives longitudinally between A6 and A5 or A5 and A4 ganglia (Figure 3). Cradle the cerci and abdomen upwards out of the saline bath with shortened insect pins (Figure 4). Be extra careful in the last abdominal segment not to damage the cercal nerves that project into the ganglion (Figure 1D and 5).

Figure 3: Schematic ventral view of cockroach nerve cord.

Figure 4: The cerci and are positioned upwards out of the saline bath

Figure 5: The 6th abdominal ganglion with the cercal nerve (outlined by arrows on one side).

2) Extracellular recording

2.1) Setup a Faraday cage which is used to block external, particularly AC, electric fields that could override signals from neurons. The microscope, micromanipulator, and the saline bath will all be set up inside the cage (Figure 6).

Figure 6: The equipment set up

2.2) Position the microscope so that it is overlooking the microscope stage. Once it is placed on the stage, you will need to adjust the position of the high intensity illuminator beam to best visualize the preparation.

2.3) Prepare a Sylgard-lined and place it under the microscope (this is where the dissection preparation will be placed).

2.4) Position the micromanipulator so the attached suction electrode assembly will have easy access to the saline bath and preparation.

2.5) Connect the AC/DC differential amplifier to the Power Lab 26T. Do this by connecting the proper cord from Input 1 on the PowerLab 26T to the output on the amplifier (Figure 7).

Figure 7: Extracellular amplifier used for this lab.

2.6) Adjust the amplifier to the following settings:

CONTROL	**SETTING**
High Pass	DC
Notch Filter	OFF
Low Pass	20kHz
Capacity Comp.	Counterclockwise
DC Offset Fine and Course knob	Counterclockwise
DC Offset (+OFF)	OFF
Gain knob	50
Input (DIFF MONO GND)	DIF
MODE(STIM-GATE-REC)	GATE
ΩTEST	OFF

2.7) Connect the headstage to the input probe on the amplifier.

2.8) Connect the electrical wires from the suction electrode to the head stage with the red (positive) at the top left, green (ground) in the middle, black (negative) at the bottom. This is indicated in Figure 8. The ground wire can be inserted into the abdomen in a convenient out-of-the way spot.

Figure 8: Head stage Configuration

2.9) Connect the USB cord from the PowerLab 26T to the computer. Ensure that both the amplifier and PowerLab 26T are plugged in and turned on before opening the trace capturing software (LabChart7).

2.10) Open LabChart7. The LabChart Welcome Center box will pop open. Close it.

2.11) Click on Setup, then Channel Settings. Change the number of channels to 1 (bottom left of box) and push OK.

2.12) At the top right of the chart set the cycles per second to about 4 kHz. Set the volts (y-axis) to 500 mV (this can be adjusted to optimize visualization of the trace).

2.13) Click on Channel 1 on the right of the chart. Click on Input Amplifier. Ensure that the settings differential, ac coupled, and invert (inverts the signal if needed), and anti-alias are checked.

2.14) To begin recording press start.

2.15) Cut one of the VNC connectives close to A5 and place the cut end attached to A6 into a suction electrode. Be sure to pull some Ringer's into the suction electrode to cover the silver wire inside it before sucking in the nerve. Make sure a ground wire is placed in the fluid held in the abdomen, preferably near A3.

2.16) With a dry pipette blow air on to the hairs located on each cercus. See if stimulating the hairs on the cercus ipsilateral to the recorded connective gives a different response than the contralateral one. Take note of the amplitude of the responses and the number of spikes in a given time interval during the stimulation.

2.17) Move the suction electrode to a cercal nerve for recording. To get a better fit, you may have to switch to an electrode tip with a smaller opening.

2.18) Cut the cercal nerve close to A6 and then suck up the nerve leading to the cercus. There should be spontaneous firing of action potentials. Now, blow air onto the cercus and note the responses.

3) Electrically stimulating the sensory nerves to determine recruitment

3.1) Cut the cercal nerve most distal as possible so that a long nerve root can be pulled into the stimulating suction electrode (Figure 9). The connective between A6 and A5 or another segment more anterior can be used.

Figure 9: Stimulating and recording electrode set up

3.2) Set the recording suction electrode so you can pull up a cut connective into the electrode. Be sure to pull some Ringer's into the suction electrodes to cover the silver wire inside it before sucking in the nerves. Make sure a ground wire is placed in the fluid, preferably around A3.

3.3) Next set the parameters on the stimulator to recruit cercal neurons so that the synaptic responses can be recorded in the VNC connectives. One should make a record of the minimal stimulating voltage and duration to recruit a response. Then incrementally increase the stimulating voltage to determine if a maximum response is recorded.

3.4) Close out of the Chart software and Open the Scope software.

3.5) Select Channel1 for Input A (top right of the screen).

3.6) Select Input Amplifier under the Channel A tab. On the screen that appears for Input Amplifier select the following:

CONTROL	SETTING
RANGE	500 mV
AC	CHECKED
LOW PASS	OFF
Differential	CHECKED
INVERT	CHECKED or not

3.7) On the right side of the screen turn off Input B and adjust the time base to 4 kHz by changing Sample to 1024 and Time to 55 msec.

3.8) Navigate to the Settings tab and click Sampling. In the box titled Sweep select the following:

CONTROL	SETTING
MODE	MULTIPLE
SAMPLE	100 SWEEPS
SOURCE	External
DELAY	0 msec

3.9) Connect the stimulating electrode to the output of the SD9 stimulator (Figure 14).

3.10) Adjust the Stimulator to the following settings:
Duration: 0.3 sec
Delay: 10 msec
Frequency: 1 Hz
Voltage: adjust as needed to obtain a signal in the recordings

3.11) Connect the stimulator cable with the two mini-hook leads or clips.

3.12) Connect the BNC trigger output from the stimulator to the trigger input on the PowerLab.

3.13) Next, it might be necessary to change the voltage on the stimulator but you must be
careful not to damage the nerve with a voltage stimulation that is too high.

3.14) Select the Start button at the lower left of the screen. Given the above settings, a clearly defined action potential should appear on the Scope data collection box. Be sure to turn off the stimulator when done collecting the responses of choice.

3.15) Deliver a series of single stimuli of increasing voltage from the software until an action potential appears on the screen. Increase the intensity until a synaptic response in the connectives is observed (**Figure 15**). The large spike (extracellular APs) from the giant axons appears first, then other smaller AP's may also be observed.

3.16) Adjust the Time base for optimum resolution of the responses. Note that the potentials do not grow in response to greater stimulus intensity. Use the single pulse to deliver single stimuli at subthreshold and increasing voltages.

4. REPRESENTATIVE RESULTS:

Stimulation of hairs on the cerci by a puff of air causes discharges of primary sensory neurons that can be recorded using extracellular suction electrodes attached either to connectives between abdominal ganglia or the cercal nerve itself (Figure 9). Spike amplitudes recorded from the two regions are on the order of several micro-volts. Because of sensory integration in the circuit the number of spikes observed in the compound action potential recorded at the cercal nerve is remarkably higher than at the connectives. Also note that there is substantially less noise in the recoding at the connective due to the tighter seal between the electrode and the nervous tissue.

Primary afferent activity in the cercal nerve was evoked by an extracellular stimulating electrode (Figure 10). Using this stimulating paradigm, recordings from the ipsilateral connective between A5 and A6 typically show a large spike characteristic of the giant interneuron(s).

Figure 10: Stimulated cercal nerve produces a spike in the connective. Note the large stimulus artifact preceding the spike.

DISCUSSION:

One of the reasons for exhibiting techniques for this classical preparation is that the cerci system has been and still is an active area of research in addressing questions of the development of neural circuitry as well as questions regarding synaptic repair and regeneration [22-27]. Either paradigm of evoking activity in the cockroach ventral nerve cord can be used to examine the effects of pharmacological agents or insecticides on nervous system function. These experiments are done by simply dissolving neuroactive chemicals into the Ringer's saline. After exchanging this solution with the normal bathing medium, changes in evoked or spontaneous activity may be observed while recording from connectives or a motor nerve to give a consistent readout of the chemical's effect on CNS function.

As in all neurophysiological experiments a common problem is electrical noise. Probably the biggest factor in signal quality for these preparations is the suction electrode seal on

the nerve tissue. A tight seal that does not completely draw in the cercal nerve or connective is ideal. Also a careful dissection is as critical here as in any CNS preparation. Some may find it easier to access the CNS by dissecting the dorsal cuticle. While this reduces the possibility of damaging the ventral nerve cord it can be more difficult to remove all of the viscera using this approach.

It is not described here but this preparation is amenable to intracellular recording in the giant interneurons [28]. The entire nerve cord can also be removed to accommodate several recording and stimulating electrodes simultaneously. In fact exploration of the antennal lobe, mushroom body, and other anterior CNS structures is still in progress [29,30]. While the cockroach CNS continues to shed light on modern neurobiological research this particular preparation is simple enough to be used in undergraduate academic laboratories.

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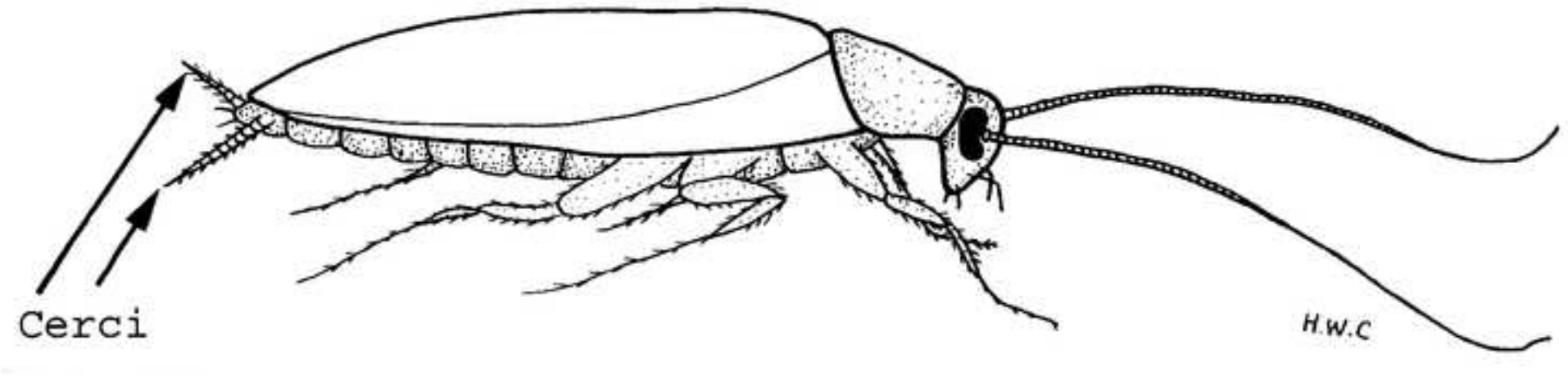

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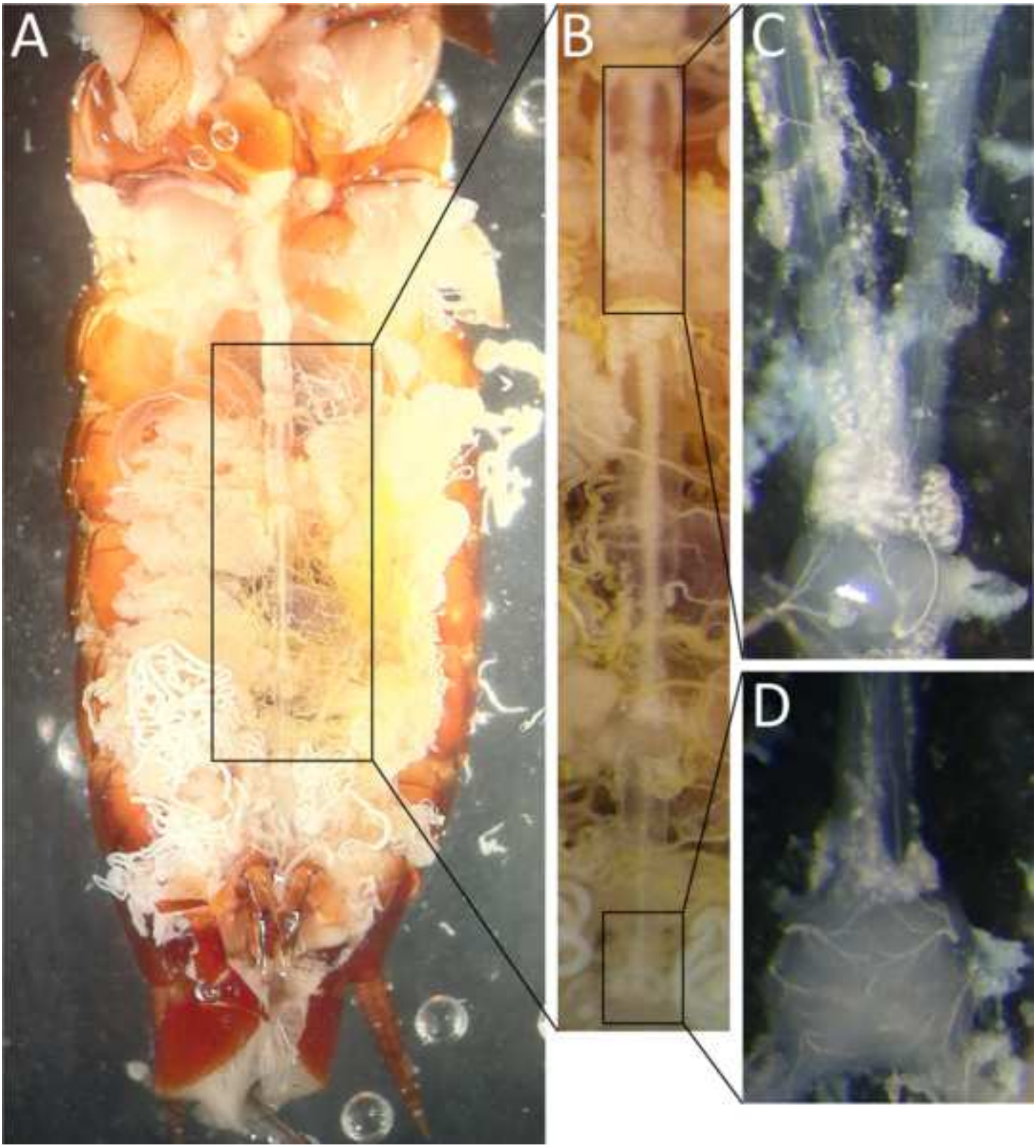

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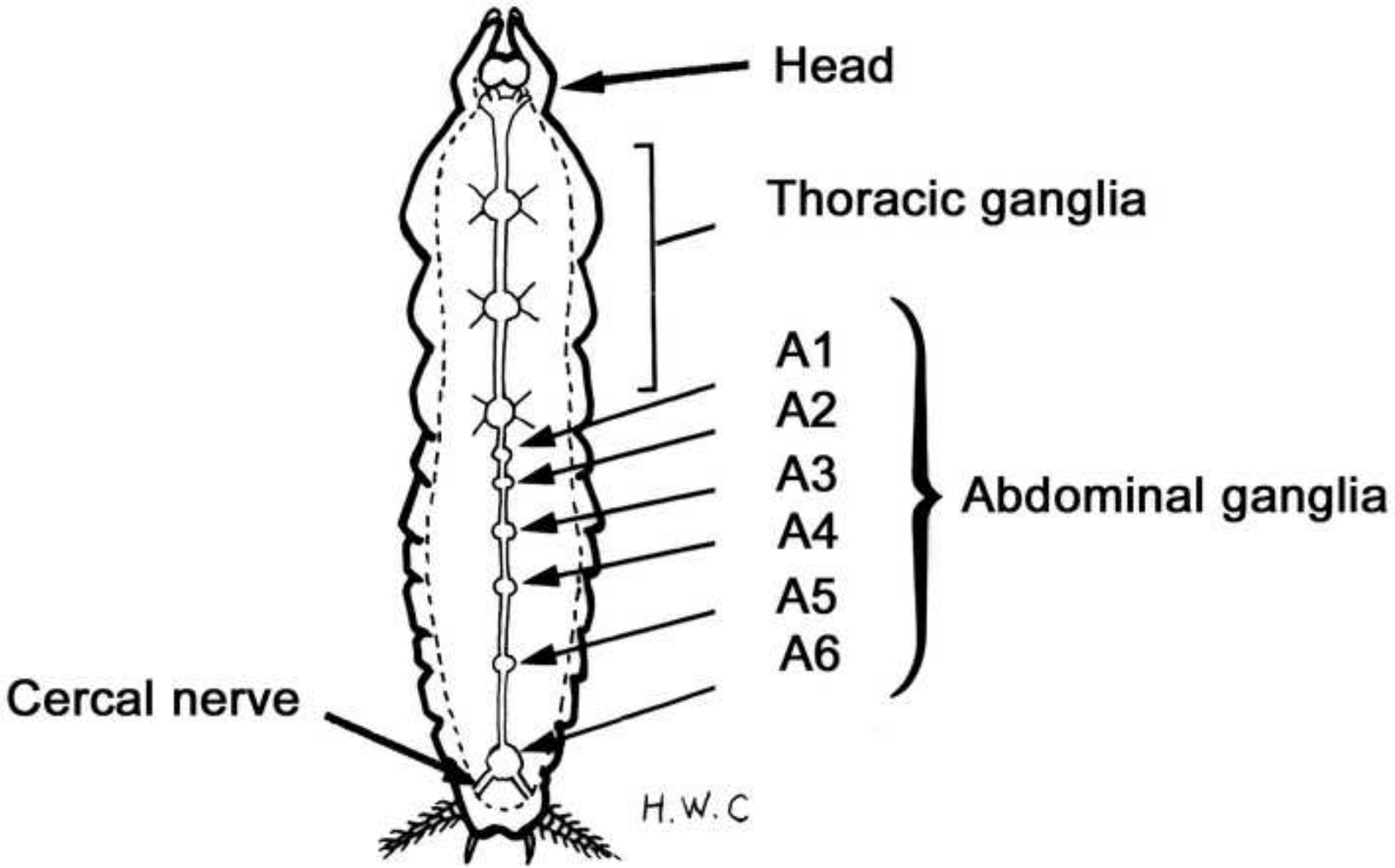

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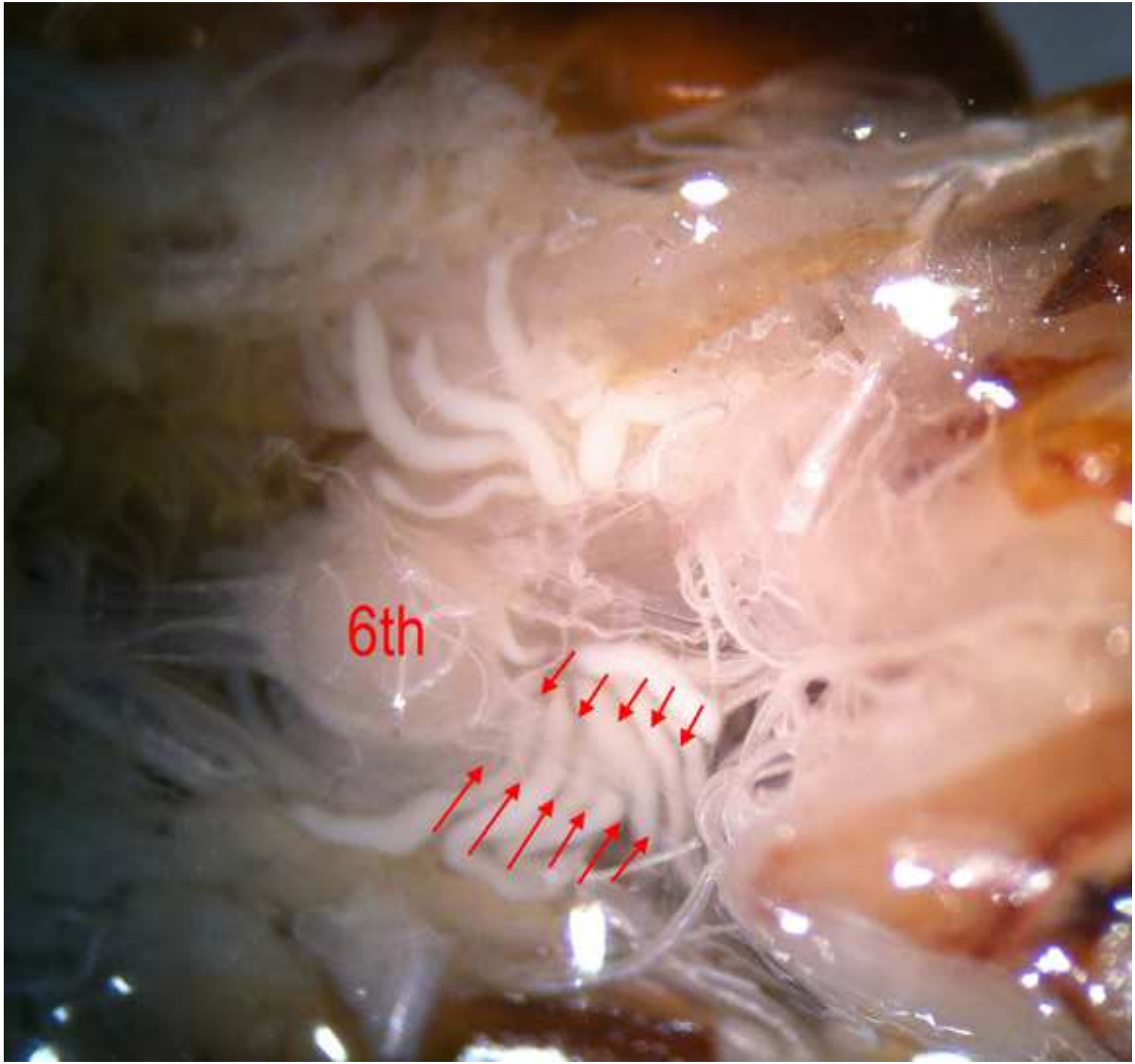

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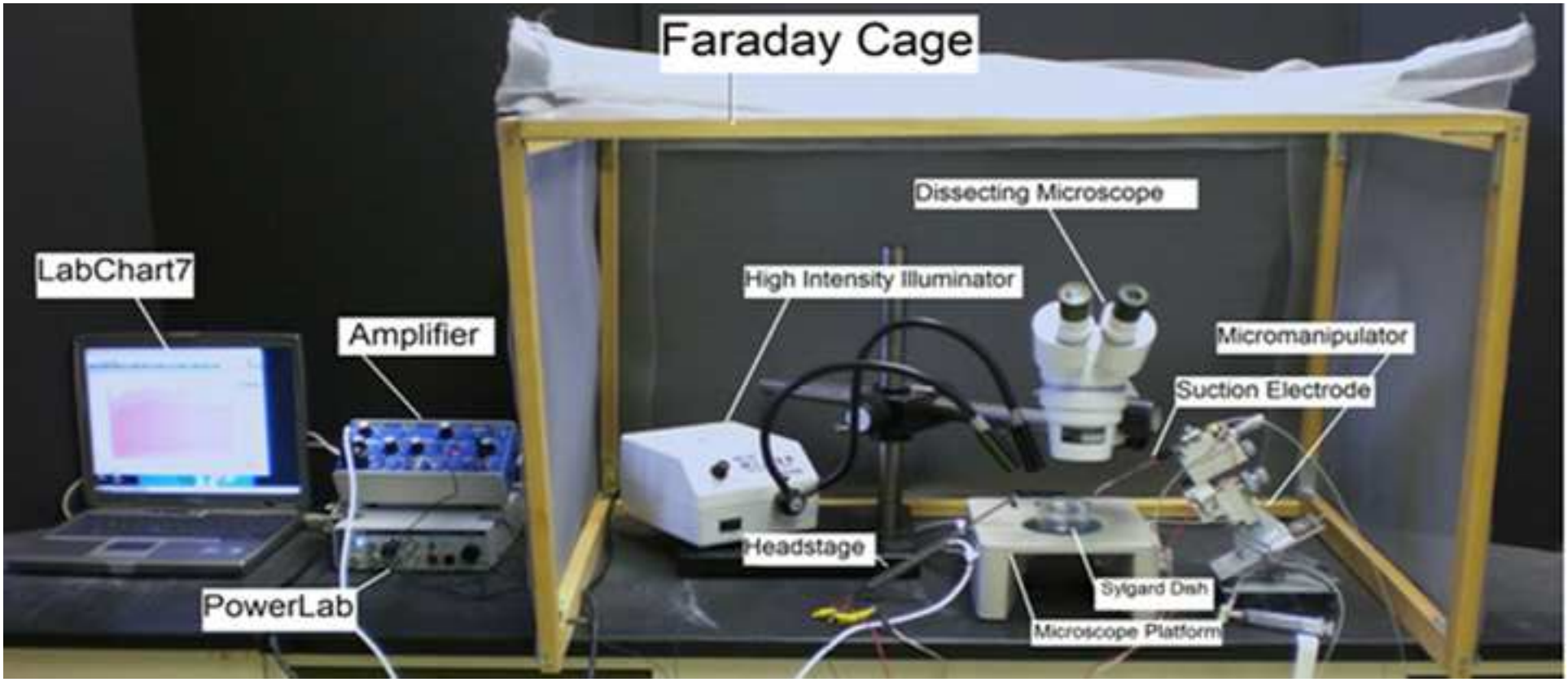

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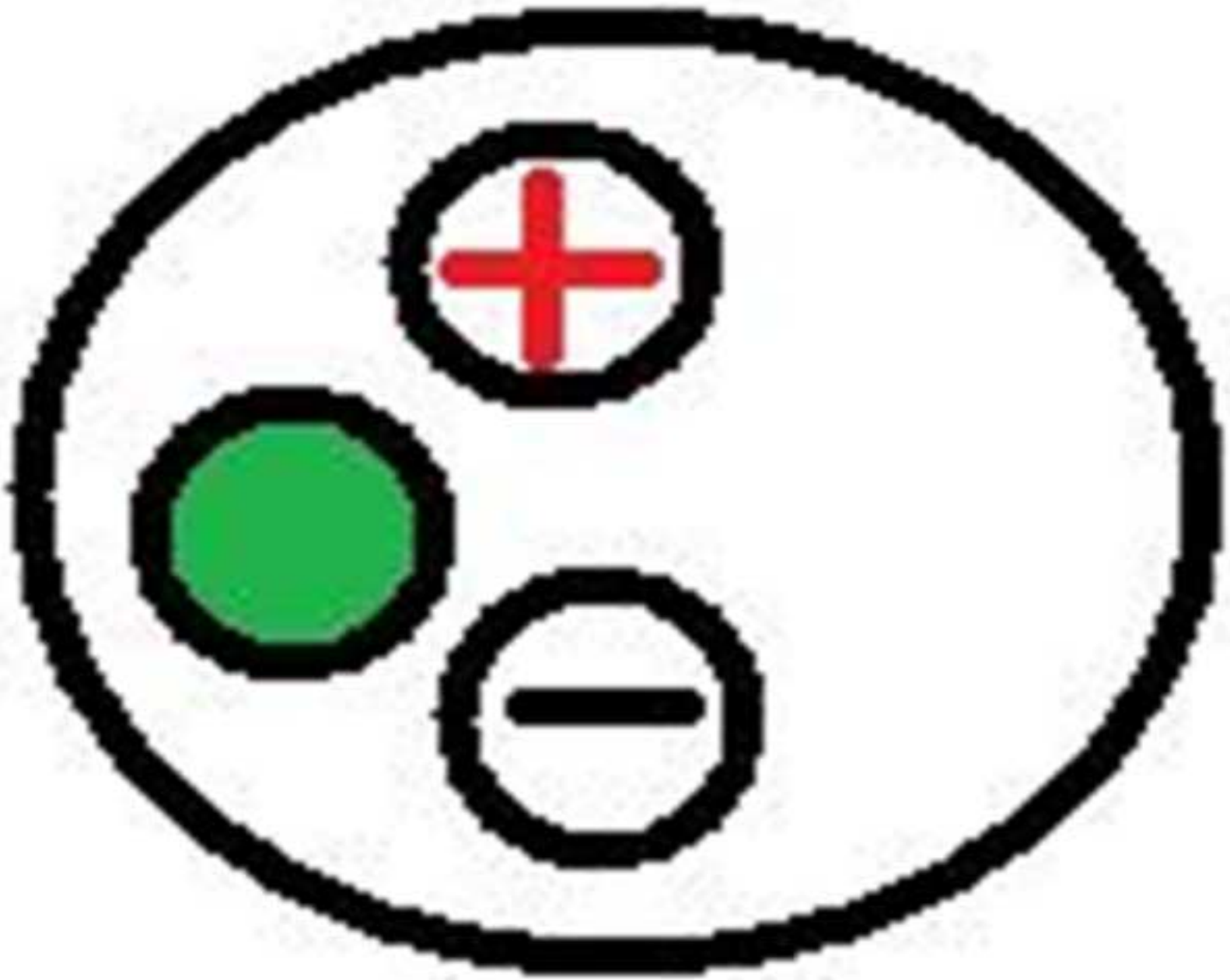

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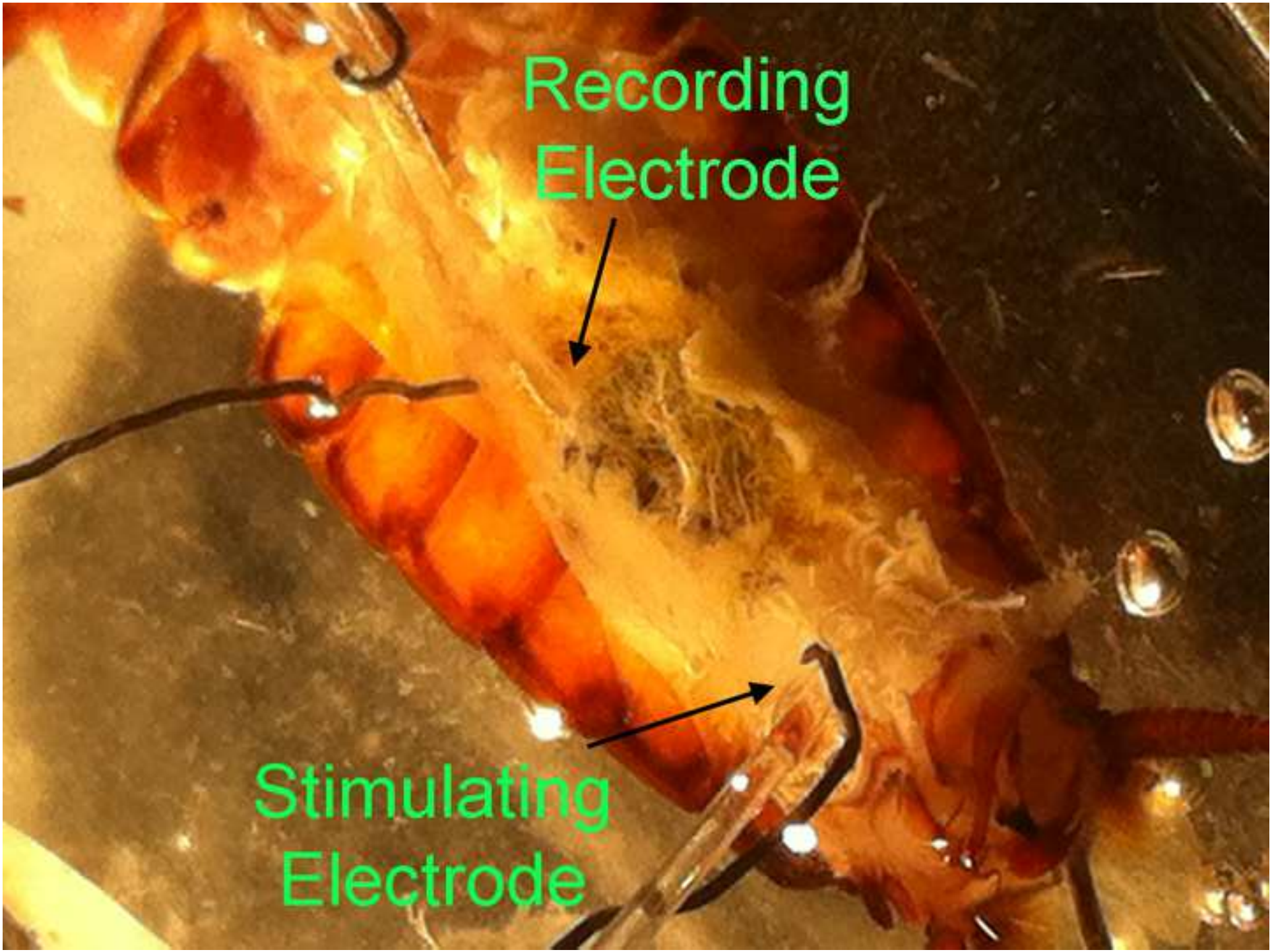

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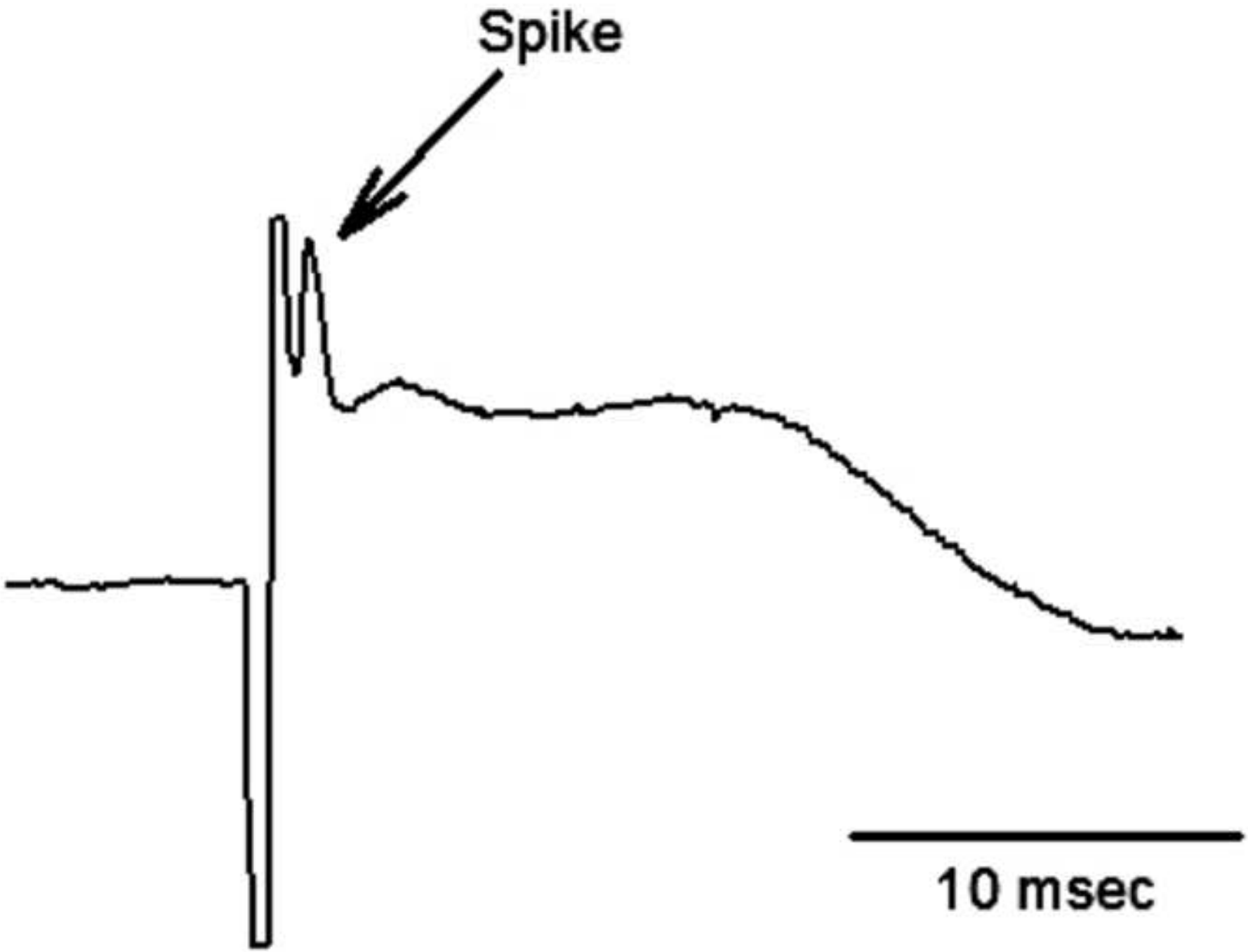

*Table of Reagents/ Materials Used

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Name of the Reagent	Company	Catalogue Number	Comments
Sylgard	Dow Corning	182 silicone kit	182 silicone elastomer kit
NaCl	Sigma -Aldrich	**S7653**	
KCl	Sigma -Aldrich	P9333	
CaCl2	Sigma -Aldrich	C5670	
NaH2PO4 2H2O	Sigma -Aldrich	71505	
Na2HPO4 7H2O	Sigma -Aldrich	S9390	
NaOH	Sigma -Aldrich	221465	To adjust pH
HCl	Sigma -Aldrich	H1758	To adjust pH

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Material Name	Company	Catalogue Number
Dissecting tools	World Precision Instruments	assortment
Insect Pins	Fine Science Tools, Inc	26001-60
Dissecting micrscope	World Precision Instruments	PZMIII-BS
Glass electrodes	Sigma -Aldrich	CLS7095B5X
Micromanipulator	World Precision Instruments	MD4-M3-R
Silver wire (10/1000")	A-M Systems	782500
Computer	Any company	
AC/DC differential amplifier	A-M Systems	Model 3000
PowerLab 26T	AD Instruments	27T
Head stage	AD Instruments	
LabChart7	AD Instruments	
Electrical leads	Any company	
Glass tools	Make your self	
Cable and connectors	Any company	
Pipettes with bulbs	Fisher Scientific	13-711-7
Beakers	Any company	
Wax or modeling clay	Any company or local stores	
Stimulator	Grass Instruments	SD9 or S88
Plastic tip for suction electrode	Local hardware store (Watt's brand)	¼ " OD x 0.170" ID

Comments
Box of 200, Suction electrodes
Can fix for base or on a metal rod
Comes with AC/DC amplifier
For manipulating nerves
Box of 500
Cut in small pieces. Pull out over a flame and cut back the tip to the correct size

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Title of Article: Neural Circuit Recording from an Intact Cockroach Nervous System

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