Video Article

Recordings of Neural Circuit Activation in Freely Behaving Animals

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URL: <http://www.jove.com/details.php?id=1297>

DOI: 10.3791/1297

Citation: Herberholz J. (2009). Recordings of Neural Circuit Activation in Freely Behaving Animals. JoVE. 29. <http://www.jove.com/details.php?id=1297>, doi: 10.3791/1297

Abstract

The relationship between patterns of neural activity and corresponding behavioral expression is difficult to establish in unrestrained animals. Traditional non-invasive methods require at least partially restrained research subjects, and they only allow identification of large numbers of simultaneously activated neurons. On the other hand, small ensembles of neurons or individual neurons can only be measured using single-cell recordings obtained from largely reduced preparations. Since the expression of natural behavior is limited in restrained and dissected animals, the underlying neural mechanisms that control such behavior are difficult to identify.

Here, I present a non-invasive physiological technique that allows measuring neural circuit activation in freely behaving animals. Using a pair of wire electrodes inside a water-filled chamber, the bath electrodes record neural and muscular field potentials generated by juvenile crayfish during natural or experimentally evoked escape responses. The primary escape responses of crayfish are mediated by three different types of tail-flips which move the animals away from the point of stimulation. Each type of tail-flip is controlled by its own neural circuit; the two fastest and most powerful escape responses require activation of different sets of large "command" neurons. In combination with behavioral observations, the bath electrode recordings allow unambiguous identification of these neurons and the associated neural circuits. Thus activity of neural circuitry underlying naturally occurring behavior can be measured in unrestrained animals and in different behavioral contexts.

Protocol

Part 1: Recording chamber

1. The recording chamber is of rectangular shape and is made of thin walled glass. Chamber dimensions are 8.5 cm x 2 cm x 5 cm (length x width x height) for animals of 2.5 – 3.5 cm total lengths (measured from rostrum to telson). See Fig. 1 for an example of a chamber used in our experiments.
2. Alternatively, recording chambers can be made from other materials (e.g., non-toxic clear plastic). Chambers size may vary according to experimental procedure and chambers should be customized for each experimental series. For best results, chamber size should be as small as possible without restraining the animals in their natural behavior. As a rule of thumb, the lengths and width of the chamber should not be more than three times the size of the animal.

Part 2: Bath electrodes and ground wire

1. One pair of recording electrodes and a ground electrode are used. Electrodes are made of insulated copper wire (26 AWG with 0.25 mm insulation). One end of the bath electrodes is connected to an extracellular amplifier (A-M Systems 1700; see below) and 0.5 – 1.0 mm of insulation are stripped off the other ends. The ground wire is connected to the ground of the amplifier or any other grounded equipment and 2-3 cm of insulation is stripped off the other end.
2. Bath electrodes and ground wire are attached to the inside walls of the recording chamber with non-toxic glue. Recording electrodes are positioned centrally on both short sides of the chamber and opposite to each other (Fig. 1).
3. Ground electrode is positioned on one long side of the recording chamber perpendicular to the recording electrodes (Fig. 1).
4. Chamber is filled with deionized water. Best results are obtained with water of high resistance (~18 MΩ).

Part 3: Bath electrode recordings

1. Outputs from recording electrodes are amplified (1000x) by an extracellular amplifier (A-M Systems; Model 1700). Signal from the recording electrodes is filtered using a combination of low-frequency (< 100 Hz) and high-frequency cut-offs (> 5 KHz). Signal is then connected to a switch box and a data acquisition board (National Instruments). Digitized data is recorded, stored, and analyzed using data acquisition software (Photron Motion Tools).
2. Alternatively, amplified signal from recording electrodes can be digitized using other analog-digital converters (e.g., MDS Analytical Technologies; Digidata 1440) before digitized data is recorded using other commercially available data acquisition software (e.g., MDS Analytical Technologies; Axoscope).

Part 4: Stimulating probe

1. A stimulating probe is made of a glass pipette (14 cm length) equipped with a pair of fine wire electrodes. Electrode tips are exposed (0.2 mm) to produce an electronic signal when the animal is touched by the probe. This allows to measure the exact time of stimulation.
2. Outputs from stimulating probe are amplified (1000x) by an extracellular amplifier (A-M Systems; Model 1700). The signal is filtered using a combination of low-frequency (< 100 Hz) and high-frequency cut-offs (> 5 KHz). The signal is then connected to a switch box and a data acquisition board (National Instruments). Digitized data is recorded, stored, and analyzed using data acquisition software (Photron Motion Tools).

Part 5: Video recordings

1. A high-speed video camera (Fastcam-X 1280 PCI, Photron) is positioned perpendicular to the recording chamber to provide a side view. Level of brightness inside the recording chamber must be adjusted to provide best results, e.g. by using a goose-neck illuminator or other focusable light sources.
2. High-speed videography is combined and synchronized with electronic recordings using a breakout box & data acquisition board (National Instruments). Amplified signal from the bath electrodes is connected to the breakout box and data acquisition board using BNC cables. An external hand-switch trigger starts the synchronized video and data acquisition.

Part 6: Experimental procedure

1. A single animal is introduced into the chamber and allowed to acclimate for 5 minutes. Escape tail-flips are elicited by single taps of different intensity to the head or the abdomen, respectively. The intensity of the taps is controlled by the experimenter. Each escape tail-flip is recorded with high-speed video at a frame rate of 1000 f/sec, and data points of electronic field potentials are recorded at 25 kHz. Start of the bath and video recording is initiated by manual switch on a trigger box. Recording time is determined by chosen frame rate (e.g., 1000 f/sec = 4 sec total recording time). Post- and pre-trigger recording times can be selected.
2. **Note:** Bath electrode recordings should be verified once for each tested species by combining bath electrode recordings with other available recordings methods. In crayfish, a pair of silver electrodes can be surgically implanted around the ventral nerve cord. Stimuli eliciting escape tail-flips can be applied and recorded electronic traces of implanted and bath electrodes can be compared.
3. Escape tail-flip behavior and corresponding neural activity can be recorded in a variety of different contexts, e.g. in response to visual threats, during predator attacks, or during agonistic encounters of two crayfish. Chamber size, recording mode, etc. has to be adjusted accordingly (see *Discussion*).

Part 7: Data analysis

1. Single video frames are analyzed using motion tool software (Photron). Electronic traces are used to identify type of neural circuit that was activated by each stimulus. Video data is compared to synchronized physiological recordings to determine the movement of the animal and the activated neural circuit. Each activated circuit produces a characteristic electronic signature (see *Representative results*).
2. Latency between probe contact and neural/muscular response are calculated for each experiment by measuring the delay between the onset of the probe signal and the onset of the signal recorded with the bath electrodes.

Part 8: Representative results

A series of single high-speed video frames and corresponding electric field recordings for escape tail-flip in response to a tactile stimulus delivered to the head or tail of a juvenile crayfish (Fig. 2).

Fig. 2A: Strong tactile stimulus to the head evoked a tail-flip controlled by the medial giant circuit. The recorded spike of the giant neuron (asterisks) and the large phasic deflection that follows enables non-ambiguous identification of the tail-flip as mediated by giant neuron activity. The backward movement shown in the video traces determines the identity of the activated neural circuit (MG).

Fig. 2B: Tail-flip mediated by the lateral giant circuit after a strong tactile stimulus was applied to the tail. Upward and forward motion seen in the video traces together with the synchronized electronic trace displaying the giant spike and the large, phasic initial deflection determines the identity of the activated neural circuit (LG).

Fig. 2C: Tail-flip controlled by non-giant circuitry. A more gradual tactile stimulus was delivered to the thorax of the animal. While the movement captured on video does not allow unambiguous identification of the activated circuit, the electronic recording lacks a giant spike and consists of much smaller deflections identifying the activated circuit (Non-G).

Fig. 3: Latency measurements for all three types of escape tail-flips. Time between probe contact and physiological response was measured for seven animals. Giant-mediated tail-flips are elicited significantly faster than non-giant tail-flips.

FIGURE 1, Herberholz

Figure 1: An example for a recording chamber used in our experiments for animals of 2.5-3.5 cm in total lengths. The bath electrodes are glued to opposite sides of the chamber while the ground wire is attached to the long side of the chamber and perpendicular to the bath electrodes.

Figure 2: Single video frames recorded at 1000 f/sec and corresponding electronic recordings for three different types of stimulation.

FIGURE 2A, Herberholz

A) A strong tactile stimulus was delivered to the head of the animal and elicited a medial giant (MG) mediated tail-flip. Six video-frames are displayed on the left. Recording trace from the probe used to touch the animal is shown in gray; the point of contact is indicated by the black arrowhead. Recording trace obtained with the bath electrodes is shown in blue. The inset shows the small giant axon spike that precedes the large phasic deflections. Grey bars correspond to the video-frames shown on the left. The first notable movement of the crayfish's tail occurred at frame #3, seven milliseconds after the contact with the probe.

FIGURE 2B, Herberholz

B) A strong tactile stimulus was delivered to the tail of the animal and elicited a lateral giant (LG) mediated tail-flip. Six video-frames are displayed on the left. Recording trace from the probe used to touch the animal is shown in gray; the point of contact is indicated by the black arrowhead. Recording trace obtained with the bath electrodes is shown in red. The inset shows the small giant axon spike that precedes the large phasic deflections. Grey bars correspond to the video-frames shown on the left. The first notable movement of the crayfish's tail occurred at frame #3, eight milliseconds after the contact with the probe.

FIGURE 2C, Herberholz

C) A weak and gradual tactile stimulus was delivered to the head of the animal and elicited a non-giant (Non-G) mediated tail-flip. Eight video-frames are displayed on the left. Recording trace from the probe used to touch the animal is shown in gray; the point of contact is indicated by the black arrowhead. Recording trace obtained with the bath electrodes is shown in black. The trace lacks the giant spike potential, the large initial deflections and consists of potentials of much smaller amplitude. Light gray bars correspond to the video-frames shown on the left. The first notable movement of the crayfish's tail occurred at frame #6, 115 milliseconds after the first contact with the probe.

FIGURE 3, Herberholz

Figure 3: Response latency measurements for seven different animals of both sexes and similar sizes (mean lengths $\pm$ stdv: 3.2 cm $\pm$ 0.2 cm, measured from rostrum to telson). MG (blue bar) and LG (red bar) tail-flips have significantly shorter response latencies than tail-flips mediated by Non-G (black bar) circuitry. Means and standard deviations are shown. Bars with the same letter did not differ significantly from each other (Wilcoxon Signed Rank test for pair-wise comparison, $p < 0.05$).

Discussion

Non-invasive recordings of single neuron activity or neural circuit activation are difficult to obtain in unrestrained animals. The method described here provides a means to identify patterns of neural activation underlying naturally occurring behavior.

In the past, we successfully used this technique to measure patterns of activity in neural escape circuits of juvenile crayfish during the formation of social dominance hierarchies¹, during attacks from natural predators², and more recently, in response to visual threats³. Currently, we use synchronized bath electrode recordings and high-speed video recordings to measure the importance of movements of head appendages during the execution of escape behaviors in crayfish.

While this technique has only been used in two different invertebrate species (crayfish and dragonflies) and in two different laboratories⁴, it seems likely that it can be applied to other animal model systems, including vertebrates, some of which are aquatic and express behaviors that are controlled by large neurons. For example, fast escape responses of many teleost fish are controlled by Mauthner cells, large identifiable neurons⁵. Escape behavior mediated by the Mauthner cells has received much attention in the literature and has been studied on several levels of analysis; yet, there is growing evidence that Mauthner cells control rapid body turns in situations that are unrelated to escape^{6,7}. The evidence, however, is mostly derived from comparing kinematical variables of the behaviors and not from direct measurements of Mauthner cell activity. It may be feasible to use bath electrode recordings in combination with high-speed videography to measure field potentials generated by the Mauthner cells or associated muscular activity.

In addition to its scientific value, the technique described here is also ideally suited for educational purposes (e.g., undergraduate teaching laboratories) due to its overall simplicity and inexpensiveness.

Acknowledgements

The bath recording technique was first used by Fricke (1984)⁸ and Beall et al. (1990)⁹ to measure electric fields generated during tail-flips. The technique was later modified and improved in the laboratory of Dr. Donald Edwards (Georgia State University) by his former graduate student Dr. Fadi A. Issa and his former postdoctoral associate Dr. Jens Herberholz. Further refinements have been made and new research applications have been tested in the laboratory of Dr. Jens Herberholz at the University of Maryland. I would like to thank my colleague Dr. David Yager for letting me use his high-speed video system and my research assistants David Rotstein and William Liden for help with the experiments.

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