Video Article

Electrode Fabrication and Implantation in *Aplysia californica* for Multi-channel Neural and Muscular Recordings in Intact, Freely Behaving Animals

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Abstract

Recording from key nerves and muscles of *Aplysia* during feeding behavior allows us to study the patterns of neural control in an intact animal. Simultaneously recording from multiple nerves and muscles gives us precise information about the timing of neural activity. Previous recording methods have worked for two electrodes, but the study of additional nerves or muscles required combining and averaging the recordings of multiple animals, which made it difficult to determine fine details of timing and phasing, because of variability from response to response, and from animal to animal. Implanting four individual electrodes has a very low success rate due to the formation of adhesions that prevent animals from performing normal feeding movements. We developed a new method of electrode fabrication that reduces the bulk of the electrodes inside the animal allowing for normal feeding movements. Using a combination of glues to attach the electrodes results in a more reliable insulation of the electrode which lasts longer, making it possible to record for periods as long as a week. The fabrication technique that we describe could be extended to incorporate several additional electrodes, and would be applicable to vertebrate animals.

Video Link

The video component of this article can be found at https://www.jove.com/video/1791/

Protocol

1. Electrode Fabrication

1. To create the electrode, use scissors to cut a piece of enamel-coated 0.001 inch diameter stainless steel wire about 2 feet long. Attach a small ball of putty to each end of the wire, fold the wire in half, and spin the ends to create a twisted pair, called a differential electrode.
2. Tape both ends of the wire to prevent unraveling. Make four differential electrodes, using a different color of tape for each differential electrode for identification purposes (Figure 1A).
3. Twist two of the differential electrodes together, leaving two inches untwisted on one end and 1 inch on the other end. Repeat with the remaining pair of differential electrodes (Figure 1B).
4. Twist both sets of two differential electrodes together, making sure that the ends remain untwisted. Hold up the electrode assembly and apply household silicone glue at the junction of the four differential electrodes, leaving the ends free. Hang and allow the glue to dry for at least 4 hours (Figure 1C).
5. To insulate the branches, lay the electrode assembly down on a flat, movable, surface such a piece of wood. Fan out the ends and secure them with tape. Use a small piece of plastic to elevate the electrode assembly just before it branches, and secure that with tape.
6. At a dissecting microscope, use forceps to coat the branches with silicone glue. The 1-inch long ends will be inside the animal, so they should have a very thin coat of glue. Allow the glue to dry for at least 3 hours.
7. Shorten the 1-inch long end of one differential electrode to two centimeters. On each wire, remove 1cm of silicone and enamel. Curl one bare wire into a hook for recording. Bend the other wire up and away from the hook; this will serve as the ground wire. Repeat for the remaining three electrodes.
8. Select one of the four electrodes on the 2 inch end. Use forceps to scrape silicone and enamel off, exposing 1cm of bare stainless steel wire on each of the 2 wires. Solder a gold connector to each of the two wires. Use a piece of lab tape to secure the end and maintain a separation between the ground and recording pins. Repeat for the remaining three electrodes.
9. Attach a 1-2cm ball of the silicone glue to the cable, about 1.5 inches from the hook end to anchor the electrodes within the animal. Allow to dry for at least one hour (Figure 1D).

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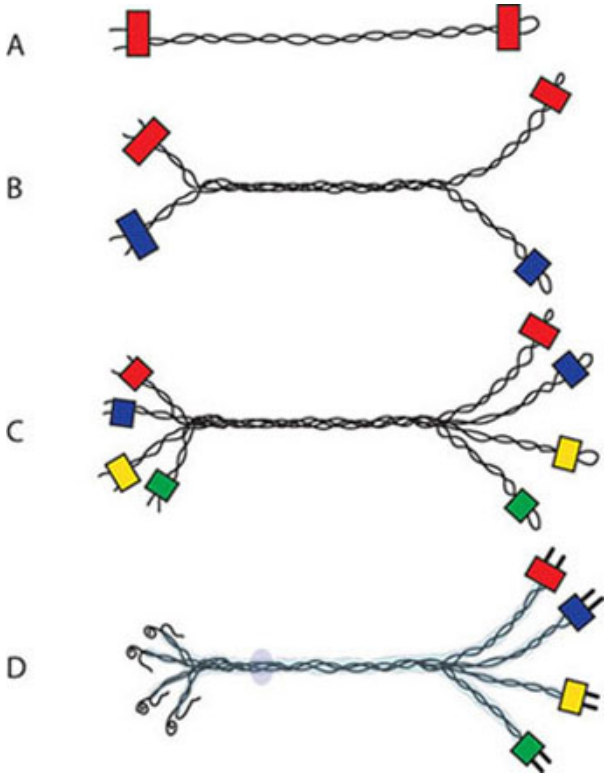

Figure 1. Electrode Assembly Fabrication. (A) Single differential electrode consisting of a folded and twisted wire with its ends secured with tape. (B) Two differential electrodes twisted together, leaving untwisted space at the ends. Different colored tape or another marking should be used to identify the ends. (C) All four differential electrodes combined into a single electrode assembly forming a single cable with separate branches of the individual electrodes on each end, marked with distinct colors of tape. (D) Final form of electrode assembly. On the left, there are curled hooks for recordings and ground wires for each differential electrode. On the right, gold connector pins have been soldered to the wires for connection to the amplifier. An anchoring ball of silicone glue has also been placed towards the left side of the assembly.

2. Preparation of Animal For Implantation

1. Select a healthy animal that weighs about 350-450g. In response to small pieces of seaweed, the animal should generate bites at intervals of between 3 to 5 seconds. The complete surgery with electrode implantation should take about an hour.
2. To anesthetize the animal, inject it with 30% w/v isotonic $MgCl_2$. To prevent the loss of hemolymph and $MgCl_2$, the injection site can be coated in superglue before removing the needle.

Figure 2. Schematic diagram showing how the animal is pinned for electrode implantation, and the location of the incision (indicated by a bar).

3. Lay the anesthetized animal out in a dissection tray with its foot down. Facing the animal head on, shift the gill area to your left such that the animal is partially on its side, leaning away. Pin down the anterior tentacles (Figure 2).

4. Place the tray on the stage of a dissecting microscope. Prop up the tray at about a 20 degree angle to elevate the head and collect the hemolymph in the tail, away from the incision.
5. Using dissection scissors, start an incision slightly to your right of the eyespot and extend it 1 to 1.5cm anteriorly. Use retractors attached to the edges of the tray to hold the incision open (Figure 2; note bar near eyespot indicating incision).
6. To expose the buccal mass, use forceps to lift the clear membrane underneath the skin and use scissors to cut an opening matching the main incision. Tuck the edges of the membrane into the retractors.

3. Electrode Implantation

1. Place the electrode assembly on a micromanipulator and move it over the animal's incision.
2. The radular nerve is most difficult to access, and should be approached first. There are two options for accessing the radular nerve.
3. The first option, which is preferable, is to look under the buccal ganglion between BN1 and BN2. If a branch of the radular nerve is visible, use fine-tipped forceps bent into a hook to lift out the branch (Figure 3, loop of RN under ganglion labeled 1).
4. If a branch of the radular nerve is not accessible above the muscle, a second option is to access it via a small incision in I2 next to the connective tissue at the base of the buccal ganglion (Figure 3, RN beneath muscle labeled 2). The incision in I2 must go through two layers of the I2 muscle. Hold onto the edge of the muscle incision with straight forceps and use the hooked forceps to pull out an RN branch.
5. While holding the nerve with the hooked forceps, use a different set of forceps to manipulate the electrode hook onto the nerve.
6. Use the micromanipulator to raise the hook so that the nerve is lifted up away from the buccal mass and clear of any other nerves or tissue. Do not overstretch the nerve.
7. Dry the electrode using a Kim-Wipe or suction off the excess fluid with a syringe. Water tension can cause the nerve to collapse on itself, or a water seal to form under the nerve. In either case, the area should be completely dried before glue is applied.
8. Apply a small dab of superglue where the base of the hook is in contact with the bottom of the nerve. Apply a drop of *Aplysia* saline or hemolymph to set the superglue. When the glue is wet nothing should be moved as getting glue between the wire and nerve would block the electrical connection.
9. Dry the hook again. Mix Kwik-Sil glue on an ice pack.
10. Use the tip of a pin to apply a small amount of the Kwik-Sil glue, completely covering any exposed wire of the hook, but not the ground wire.
11. Allow the glue to set for about 5 minutes, readjusting the glue if it pulls away from sections of the hook. The glue is set when it no longer sticks when touched with the pin (Figure 4 shows the final configuration).
12. Once the radular nerve electrode is set, the I2 muscle can be accessed. In the area between BN1 and BN2, use the hooked forceps to hook and to separate 2 bands of the I2 muscle from the rest of the muscle by gently pulling the bands upwards. Attach the electrode using the same procedure.
13. Buccal nerves 2 and 3 are easily accessed and electrodes are attached with the same procedure.
14. Once all the electrodes are attached, slide the electrode assembly into the animal up to the silicone anchor.
15. Suture the incision closed around the protruding electrode cable, keeping the anchor inside the animal. Superglue the stitches to ensure a good seal.
16. Insert the gold connector tips at the other end of the electrode into a small block of Styrofoam. Return the animal to the main tank in an aerated isolation container for recovery overnight.

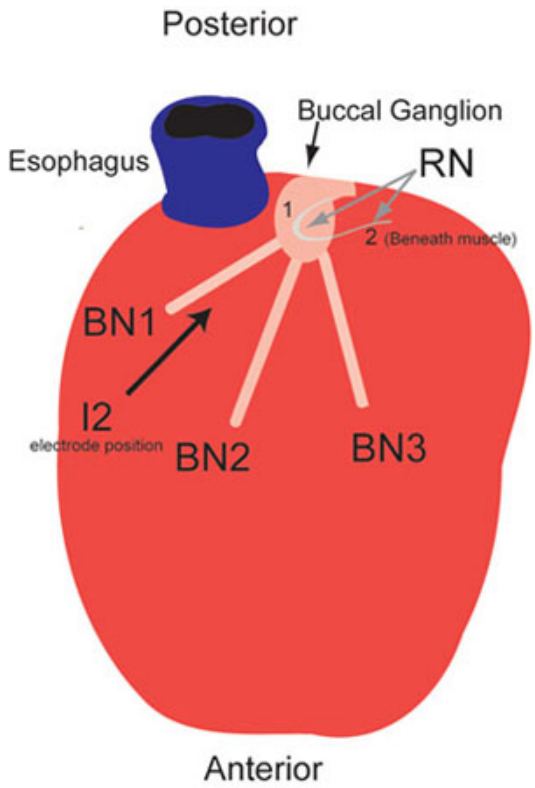

Figure 3. A diagram of the *Aplysia* feeding apparatus known as the buccal mass. Shown are the buccal nerves (BN1, BN2, BN3), the radular nerve (RN) that exits the bottom of the buccal ganglion, then plunges beneath the muscle, and the location on the I2 muscle where the electrode is attached.

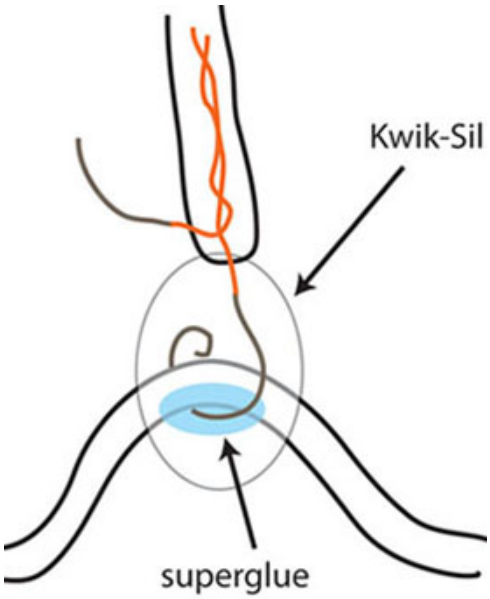

Figure 4. Schematic diagram of single electrode attached to a nerve (or muscle) and glued in place. Note that the de-insulated region of the curled hook is in direct contact with the nerve (or muscle), anchored in place with superglue, and electrically insulated from the fluid medium using Kwik-Sil. Note also that the deinsulated tip of the ground electrode is exposed to the fluid medium.

4. Recording

1. Acclimate the animal in an aerated container while attached to the recording equipment for at least 1.5 hours. Monitor the signal while the animal is acclimating to verify that all electrodes are working and that noise levels are low.
2. Use a video camera to record feeding behavior while using the computer to record signals from the neural and muscular electrodes. Synchronize the video and electrode recordings using a square wave signal that controls a digital counter in the camera view while it is simultaneously recorded as an additional channel on the computer in Axoscope.
3. Feeding behaviors are elicited by the techniques described in Morton and Chiel 1993.

Although invertebrate animals do not require formal approval by an institutional animal use and care committee, we have ensured that all treatments of *Aplysia* minimize harm and suffering to the animal, and that all surgical techniques are done while the animal is fully anesthetized.

Representative Results

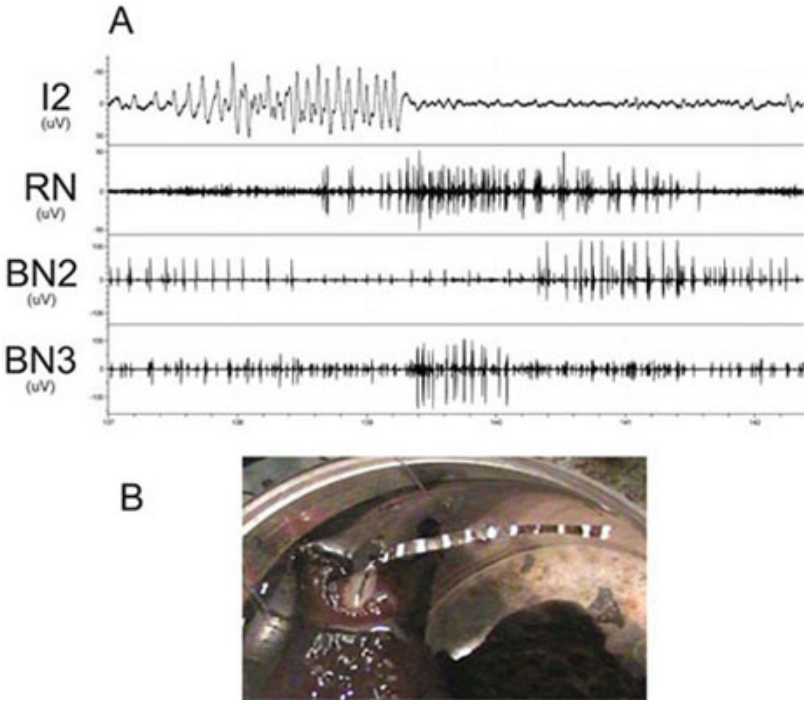

Figure 5. (A) Simultaneous four channel recordings from a muscle (the protractor muscle I2), and from three buccal nerves (the radular nerve, RN, buccal nerve 2, BN2, and buccal nerve 3, BN3) during a swallowing response. (B) A still frame from the simultaneous video recording of the freely behaving animal as it swallows a precut seaweed strip (0.5 cm in width), which has been marked at 0.5 cm intervals with white bands so that its movements can be readily visualized as the animal feeds.

Discussion

A key innovation that has made these recordings successful is combining the electrodes into one cable. Two electrode recordings have been used before, but expanding to four individual electrodes resulted in the formation of surgical adhesions that often prevented the animal from making normal feeding movements. Combining the electrodes into one cable reduces the bulk of the electrodes inside the animal, which in turn reduces the immune response and allows the animal to move more freely.

The other innovative aspect of this procedure is the combination of Superglue and Kwik-Sil to attach the electrodes to the nerve or muscle. Previously, only Superglue had been used, but the combination of the two glues works more reliably and can last several days longer than Superglue, which breaks down in about 3 days when exposed to water.

We have shown that it is possible to implant extracellular electrodes in intact, behaving animals for recording or stimulation from the cell bodies of individual identified neurons [Warman and Chiel, 1995; Lu et al., 2008]. The multiple electrode technique that we have described in this paper could thus be used to simultaneously record from and manipulate the activity of four different identified neurons, or a combination of neurons, muscles and nerves.

We have been using the technique with four electrodes, but it is likely that it would work for several additional electrodes. Furthermore, in an unpublished experiment, these electrodes were used to record from the pudental nerve of the rat, indicating that aspects of this technique could be useful in vertebrate animals and in humans.

Acknowledgements

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