Video Article

Spinal Cord Electrophysiology II: Extracellular Suction Electrode Fabrication

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Abstract

Development of neural circuitries and locomotion can be studied using neonatal rodent spinal cord central pattern generator (CPG) behavior. We demonstrate a method to fabricate suction electrodes that are used to examine CPG activity, or fictive locomotion, in dissected rodent spinal cords. The rodent spinal cords are placed in artificial cerebrospinal fluid and the ventral roots are drawn into the suction electrode. The electrode is constructed by modifying a commercially available suction electrode. A heavier silver wire is used instead of the standard wire given by the commercially available electrode. The glass tip on the commercial electrode is replaced with a plastic tip for increased durability. We prepare hand drawn electrodes and electrodes made from specific sizes of tubing, allowing consistency and reproducibility. Data is collected using an amplifier and neurogram acquisition software. Recordings are performed on an air table within a Faraday cage to prevent mechanical and electrical interference, respectively.

Video Link

The video component of this article can be found at https://www.jove.com/video/2580/

Protocol

Electrophysiological recordings of isolated spinal cords can reveal genetic and developmental changes to neural circuitry [1]. We previously demonstrated a method to dissect neonatal mouse spinal cords [2]. Here we present a method to prepare suction electrodes useful in recording fictive locomotion in isolated spinal cords [3].

The tip of a plastic tubing electrode can be drawn to a very fine tip by hand using a low-temperature alcohol lamp [3-5]. The plastic tubing (PE90, Clay Adams Intramedic™) is held over the flame to soften the tubing into a malleable form. As the tubing begins to melt and becomes more translucent it is removed from the heat source and the ends are gently pulled apart. The timing of removing the tube from the flame is important to insure the plastic does not collapse or split while being drawn. The thin portion of the tubing is cut with a razor blade according to the desired internal diameter, as dictated by the age of the specimen or the segmental level of the cord. For ease of placing an o-ring on the tubing, a 45° cut is made at the thick end of the tubing with a razor blade. A ferrule and o-ring are installed on the thick end to attach the electrode tip to the barrel of the electrode.

To allow for specifically sized electrodes and avoid the difficulties of hand-pulling electrode tips, electrodes can be constructed using small tubing inserted into a thicker tubing (PE90). The lengths of the tubing are specific to the requirements of the recording setup and are variable. We present the measurements used in our experiments [1-3,5]. A 1cm length of PTFE fine tubing (Zeus, Small Parts) and a 10cm length of PE90 tubing are cut with a razor blade. The ends of the small tubing should be examined to be sure they are not crushed or closed. They can be opened using an insect pin (Fine Science Tools). A drop of adhesive (JB Weld) is placed onto the midpoint of the PTFE tubing. The PTFE tubing and adhesive are drawn into the PE90 tubing using mouth suction on the PE90 tubing. A 45° cut is made at the thick end of the tubing and a ferrule and o-ring are attached, as previous.

The ferrule and BNC connector are unscrewed from a commercial suction electrode. The BNC connector is soaked in xylene for one hour to remove the wax seal. The connector is then rinsed in water and dried.

A lower angled side port must be made on the side of the barrel. A metal rod is heated using a Bunsen burner and pressed into the existing hole on the side of the barrel at a low angle. A 15cm length of 0.010 inch silver wire (A-M Systems) is cut and soaked in bleach for fifteen minutes. This is done to create a silver chloride coating that aids in signal conduction [6]. The wire is rinsed with water, dried, and then soldered onto the BNC connector. The BNC connector and silver wire are inserted into the suction electrode barrel with enough room for the BNC connector to remain to the side of the barrel. Suction tubing is inserted into the side port 2-3cm. Silicone sealant is injected into the barrel approximately 2-3cm. The BNC connector is threaded into the barrel of the electrode pushing the sealant forward.

The tip of the electrode is now threaded into the front end of the electrode. Shrink tubing can also be added to reinforce the suction line from the side port.

Electrodes are mounted on micromanipulators that are attached to magnetic stands, and placed near the recording dish. BNC coaxial cables are attached to a headstage that is wired to an amplifier. The amplifier is connected to an adaptor (Polyview) that is connected to a analog to digital card (National Instruments) in a PC computer with data acquisition software (Polyview).

When recording electrophysiological data, it is necessary to eliminate all outside interference. The recording dish, amplifier, and head stage are placed on an air table within a Faraday cage to prevent electrical and mechanical interference, respectively.

Representative Results:

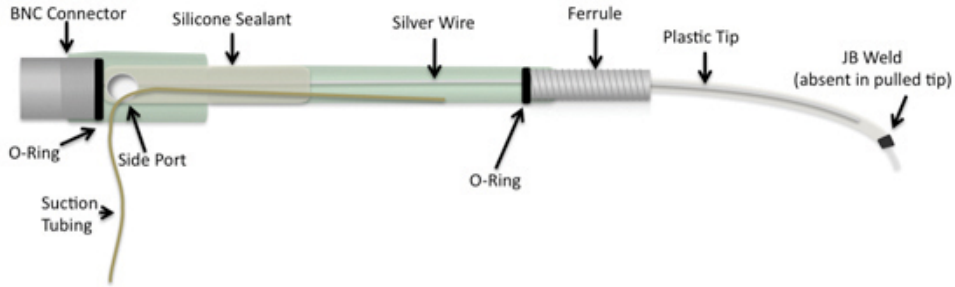

Figure 1. A commercially available suction electrode can be modified by adding a heavier gauge silver wire and a plastic tip that is hand drawn on an alcohol lamp, or constructed with commercially available plastic tubing of specific sizes. In addition, the paraffin sealant is replaced with more durable silicone caulk sealant.

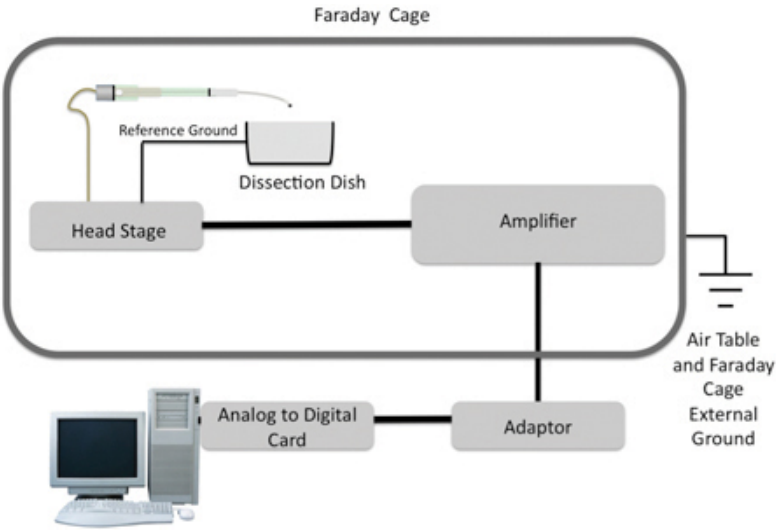

Figure 2. A schematic of the suction electrode in relation to the air table (for mechanical isolation), Faraday cage (electrical isolation), amplifier and computer. It should be noted that there is a ground referenced to the recording dish and a second ground that is referenced to the air table and Faraday cage.

Discussion

Nervous system development can be studied using isolated rodent spinal cords. In the presence of neurotransmitters, fictive locomotion can be generated from the spinal cord in the form of patterned electrical activity [1,3]. These rhythmic bursts are produced at 0.2 to 0.5 Hz and are patterned in left-right and flexor-extensor alternations. At different developmental stages, the robustness and patterns of this activity varies [1]. Genetic mutations can also disturb the patterning of this activity [3,5,7]. Transgenic and developmental studies of this activity provide insights into the organization of central pattern generating circuitry and inform studies of neural development, generally.

Disclosures

No conflicts of interest declared.

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