METHOD OF FABRICATION FOR NERVE CUFF ELECTRODES FOR USE IN ANIMAL MODELS

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METHOD OF FABRICATION FOR NERVE CUFF ELECTRODES

FOR USE IN ANIMAL MODELS

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This work is dedicated to my sister Lisa and her husband Christopher Yates, who helped me realize my dream of coming to one of the best technical institutes in the world, my brother Nathan and his wife Aimee Pulley, who helped me find a home in Atlanta and who have been pillars of my support system, and my sisters Jennifer Miller, Tonya Patten, and April Pulley who I love very much and wish were closer. Finally, I dedicate this work to my sister Aurora Pulley and my father John Michael Sanner who are no longer with us.

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SUMMARY

Many electrophysiological experiments require the recording, stimulating, or both in the peripheral nervous system. There are many electrodes currently on the market, but they are either not designed for implantation or are not robust enough to be used multiple times *in situ*. The cost of buying these electrodes from a manufacturer can be prohibitive and many labs prefer to make their own. This introduces variability between studies, as different techniques and configurations in the design and fabrication of electrodes can create variance in electrical impedance, spatial arrangement, or other factors. This paper presents a detailed methodology for the construction of electrodes that are robust, have uniform impedance values of $Z = 2.38 \pm 0.906$ kΩ. at 1 kHz alternating current (AC), and can be used in multiple *in vitro* or *in situ* experiments, or for chronic implantation *in vivo*. This method will reduce the amount of time and material needed to construct electrodes for experimental studies in animals.

CHAPTER 1

INTRODUCTION

This paper will detail a method of fabrication for nerve cuff electrodes to interface with the peripheral nervous system (PNS) for purposes of physiological experimentation. To conduct PNS experiments over periods of time greater than one day, it is necessary to have biocompatible electrodes that can remain chronically implanted for weeks or months without degrading in electrical characteristic. For such technology to be used in humans, electrodes will have to be implanted for years. It is reasonable to expect that electrodes must have similar electrical characteristics between individual electrodes in order to reduce variance in signal, and that they must not physically fail at any point during or after implantation so the experiment can continue to completion, meaning they must be fabricated to be both precise and robust.

A detailed methods paper on fabrication of electrodes was very much needed. There are many techniques for spot welding—some of which are discussed in section 4.3—but only resistance welding techniques are addressed in this paper; laser welding and additive welding were not examined in this paper. Trade journals and technical books and manuals on welding do not offer much insight, as they are meant for working in larger scales which do not translate well to micro scales where too much current can vaporize the small amount of metal available to form the weld. The sentence, “The pad was welded to the wire,” which is often the only description provided on the method of adhering a pad to a wire, therefore encompasses a process that could take months to understand without training. This means each lab that wishes to begin PNS stimulation would have to work out a means to interface with the nerve on their own, rather than having a set method.

The methods within this document provide detailed instruction on the fabrication of nerve cuff electrodes using spot welding techniques to adhere platinum – 10% iridium (Pt-Ir) pads to stainless steel wire. This provides an in-house method to create customizable, robust, reliable electrodes that can be used repeatedly for in situ animal testing, or implanted in vivo for long term studies. The electrodes are characterized by testing impedance (Z) with a 1 kHz sinusoidal AC signal at 1.75 V_{pp}. The mean impedance calculated for the sample (n = 16) of electrodes created through these methods was then compared by two-sample T-test with unequal variance of mean Z to a sample (n = 18) of electrodes created with a previous method used in the lab which involved adhering Pt-Ir pads to wire with silver conductive epoxy. The hypothesis is that electrodes fabricated by the method within this paper will have both a lower mean impedance value and lower variance between electrodes than those fabricated using conductive epoxy when tested with a 1 kHz sinusoidal AC signal at 1.75 V_{pp}. Stated formally:

$$H_0: \mu_Z \text{ of sample 1} = \mu_Z \text{ of sample 2}$$

$$H_1: \mu_Z \text{ of sample 1} > \mu_Z \text{ of sample 2}$$

Where sample 1 are electrodes created with silver conductive epoxy and sample 2 are electrodes created via the methods in this paper.

The confirmation of the alternate hypothesis would indicate that the methods herein are robust and repeatable. This would enable electrophysiologists to create electrodes in-house that are customized to their purpose and via the same methods across labs, which would eliminating a potential source of error and aid in repeatability and verification of experiments.

CHAPTER 2

LITERATURE REVIEW

The PNS is sub divided into the autonomic and somatic nervous systems. The autonomic nervous system regulates unconscious activities, such as breathing, heartrate, hormone regulation, etc. The somatic nervous system is responsible for the translation of the physical environment into neural signals through sensory input—afferent signals—and translation of motor signals from the brain into activity of the muscles—efferent signals. Much of the work exploring the function of these systems was completed decades ago and has since passed into classical knowledge. There is a resurgence of interest in this area in the last two decades, however, as more labs are exploring techniques to stimulate and record from the PNS for purposes of neuromodulation with a wide range of clinical uses, including enabling those with incontinence to control their bladder function, regulating hormone levels through the autonomic nervous system, and controlling and receiving sensation through prosthetic limbs utilizing the somatic nervous system. This section will explore some of these uses and the means by which researchers are utilizing nerve cuff electrodes to interface directly with the PNS.

As early as 1926 it was shown that receptors throughout the body are responsible for encoding that occurs in the peripheral nervous system (PNS) before information is transmitted to the central nervous system (CNS) [1]. More recently, in [2] they found that the frequency of vibration experienced in skin scanning across surfaces with different spatiotemporal characteristics—i.e. variations in roughness—produced different firing patterns of action potential that encoded unique information about the nature of the surfaces. This same sort of encoding takes place in the autonomic nervous system as baroreceptors on the aortic arch, for example, can send information about pressure [3], or through vision as different wavelengths of light excite different receptors in the retina of the eye, and in auditory stimulation as different wavelengths of sound reach different regions of the cochlea. Individual axons attached to these receptors are eventually mapped to different areas of the brain, which enables spatiotemporal encoding to occur without the need for conscious thought.

The mechanoreceptors in the skin encode information which must then be sent through the PNS before it is decoded and interpreted centrally. In [4] two amputees using neuroprosthetic limbs that utilized nerve cuff electrodes to directly stimulate the PNS were able to feel sensation and by using different stimulation patterns researchers were able to evoke multiple sensations that enabled the users to perform fine motor tasks. It was found that modulating the amplitude of the stimulation signal in a sinusoidal waveform evoked a response that subjects reported as a solid touch rather than a tingling sensation. By implanting on the radial, ulnar, and median nerves, researchers were able to evoke sensation in several different spatial regions and place sensors on the prosthetic limb accordingly.

In this lab, it has been shown that kilohertz frequency alternating current (KHFAC) can be used to selectively block the fast or slow components of the compound action potential (CAP) in the frog model [5] and have unpublished data that shows success in the rat model as well. The use of such neural modulation has many potential uses, though further research is necessary to determine the mechanism of KHFAC conduction block. Future clinical could include modulation of pain while still allowing motor and sensory signals to be passed for those who experience chronic PNS pain or untreatable pain such as those suffering from fibromyalgia, blocking motor signals without blocking sensory and vice versa, and in the autonomic system, modulating afferent and efferent signals from visceral organs independently to evoke different physiological responses.

In order to study neuromodulation through the PNS a nerve interface is required, prompting the need for nerve electrodes. There are several models of nerve cuff electrodes being tested by various labs. The spiral cuff electrode uses different layers of polymers to create a naturally curling spiral shape that does not require sutures to remain closed and in contact with the nerve. It is also flexible and enables the nerve to swell and contract while still maintaining contact [6]. The longitudinally implanted intrafascicular electrode (LIFE) is, as its name implies, implanted longitudinally between the fascicles of the nerve [7,8]. The flat interface nerve electrode (FINE) capitalizes on the oblong shape of most nerves to press the nerve flat along the long axis of its cross section in order to have more pads in contact with more individual fascicles [9]. Currently, the FINE is implanted in two human subjects for chronic trials that have already extended beyond two years and has been shown to enable stimulation to the PNS to modulate the somatosensory cortex and enable the sensation of touch that subjects report feels true-to-life [10]. The purpose of these last two examples is to create better spatial resolution in an attempt to tune which axons are being recorded or stimulated since, as discussed previously, individual axons map different areas of the body to different neurons in the brain.

Nerve cuff electrodes are used for whole nerve recording and stimulation and are limited in this respect since they are not capable of stimulating or recording selectively. They record CAPs and stimulation evokes a response in every fiber in the nerve that is exposed to the stimulus. However, much can still be learned from experiments involving whole nerve recording and stimulation since the different fiber types, classified as A, B, and C in 1941 by [11], have different propagation speeds. This allows different components of the CAP to be analyzed.

CHAPTER 3

MATERIALS

List of Materials

DESIGNATOR	ITEM	DESIGNATOR	ITEM
100 mL Glass beaker	Fisherbrand FB-102-100 Beaker	Microscope	Nikon SMZ645 Light microscope
Aluminum Stock	6061 Aluminum 2” wide, 0.04” thick	Needle	#3 Crewel sewing needle
Bolt	#5-40 3/8” 8.8 Stainless steel bolt	Parafilm	American National Can Parafilm “M” laboratory film
Calipers	Mitutoyo Absolute Digimatic CD-8” CS	PDMS	Sylgara 184 Silicone elastomer (polydimethylsiloxane)
Clamp	Fabricated aluminum cuff clamp	Pt-Ir stock	ESPI Metals Knd2877 Platinum 10% Iridium foil 1”x1”
Connector	PlasticsOne 8MS363 Pedestal 2298 6 pin	Putty knife	Hyde 01440 Putty knife
Crimp pins	PlasticsOne E363-0 Socket contact skewed	Rat Ringer’s solution	135 mM NaCl, 5.4 mM KCl 5.4, 1 mM $MgCl_2 \cdot 6H_2O$, 1.8 mM $CaCl_2 \cdot 2H_2O$, HEPES 5 mM, and NaOH to adjust to 7.2 pH
Cut pads	Pads cut from Pt-Ir stock	Razorblade	Stanley 11-921 Razorblade
Dental cement	Henry Schein Natural Elegance 101-9306 A1 Flowable composite	Scissors	Value™ stainless 458-612 Scissors
Double-sided tape	3M 410M Double-sided tape 1 ½”	Silicone tubing (cuff)	A-M Systems silicone tubing Rat sciatic: 808200 0.062” x 0.125” x 0.315” Rat vagus or mouse sciatic: 807600 0.058” x 0.077” x 0.0095” Cat sciatic: 809400 0.125” x 0.250” x 0.625”
Electrode	Tungsten Sparkle pulse welder electrode	Silicone tubing (lead)	A-M Systems silicone tubing 806400 0.020” x 0.037” x 0.0085”
Fine felt pin	Sharpie® Fine 12E 23	Small plastic	VWR 12577-005 Small

		weigh boat	weighing dish
Forceps	FST by Dumont 5/45 Forceps	Straight-edge	Stainless steel L600-12 ruler
Grounding pliers	Xuro Grip 475 with ground attached	Tap	#5-40 Tap
Impedance tester	FHC Inc. ICM Neurocraft impedance tester	Tray	Perforated circuit board (perf board)
Insulated wire	A-M Systems 793500 Stainless steel 7 strand 0.002” bare 0.0090” coated wire	UV PPE	UV protective safety glasses
Insulin needle	Terumo ½ cc 28 G x ½”	UV wand	LY-A180 Light curing unit
Lab tape	VWR ½” Lab tape	Welder	200 W Sparkle pulse welder
Male crimp pins	TE Connectivity 205089-1 20 G Crimp pin	Wires welded to pads	Fabricated wire leads with Pt-Ir pads
Micro-scissors	FST 15008-08 25° Micro-scissors		

Table 3.1: List of Materials. This table lists all materials used in the methods section of this paper as well as the short version used to describe them throughout the paper. These methods are meant to be adaptable for use with the tools available to the fabricator. Those listed within this chapter are those used in the making of the electrodes used as examples. In all cases, materials that fulfill the same function can be substituted.

CHAPTER 4

METHODS

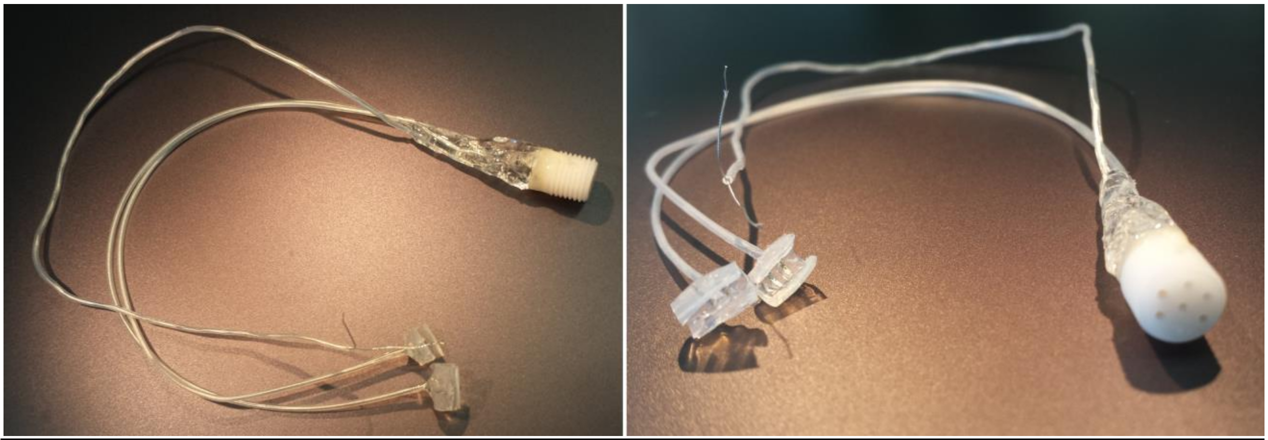
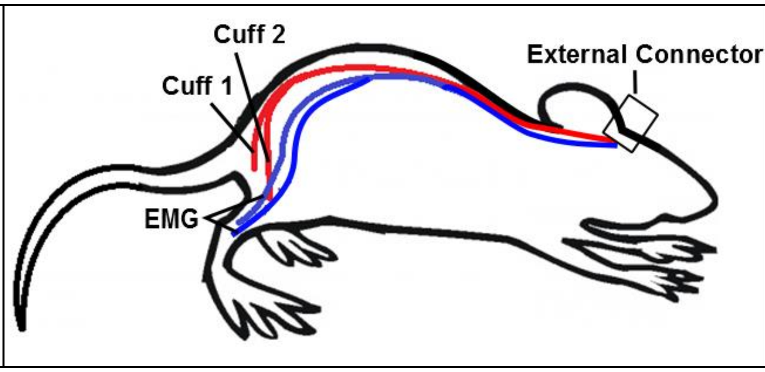


Figure 4.0.1: Two views of the same finished electrode. This is an example of an electrode with two bipolar cuffs and a bipolar EMG electrode in one package with a 6-pin PlasticsOne connector. It is ready for electrical characterization followed by gas sterilization in preparation for implantation on the sciatic nerve in the rat model.

The electrode shown in figure 4.0.1 is an example of just one type of electrode that can be fabricated through these methods. Areas of customization include the number of pins in the connector, the number of electrode leads, the number of pads in each electrode, the type of electrodes used, the length of the electrode leads, etc. Figure 4.0.2 shows how this electrode would be implanted in an animal using the right rear sciatic nerve of the rat model. Figure 4.0.3 provides a schematic of the different components of the electrode.

Figure 4.0.2 (right): Diagram of an electrode implanted in the rat model. Cuff electrodes are red, EMG is blue. The cuff electrodes are implanted on the sciatic nerve.



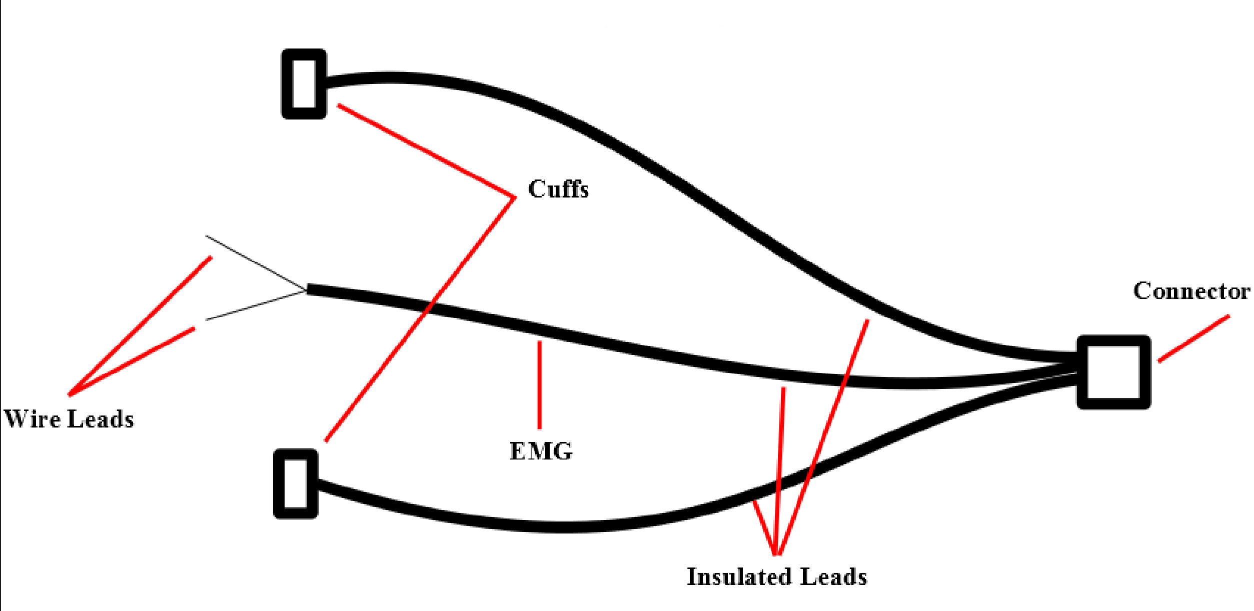


Figure 4.0.3: Schematic of electrode with labels. Each component of the electrode is represented and labeled. The methods section will use these terms to describe the components listed. *Note: The term wire leads merely refers to any wire not enclosed in silicone tubing.*

4.1 Fabrication of Clamps

In order to fabricate nerve cuff electrodes using the methods detailed within this paper, clamps must first be fabricated to help secure the cuff of the electrode in an open, flat position. Exploded view and top orthographic diagrams of the custom clamps, along with detailed instructions on manufacturing them are displayed on the following page (Fig. 4.1.1). These instructions can, and should be, modified to fit individual cuff sizes as customized for different purposes. To determine the necessary dimensions the inner circumference of the tubing used for the cuff will need to be calculated, as well as the overall width. The clamp designed in this chapter is for a rat sciatic electrode using silicone tubing with an inner diameter of 1.42 mm, which yields a circumference of 4.46 mm.

Materials required: 2" x 0.040" 6061 Aluminum, 4 x Bolts, Drill press, 2-Axis CNC, 1/8" end mill, Tap appropriate for bolt size, Double-sided tape, Putty knife [Table 3.1]

The top and bottom plates were cut from 2" wide by 0.040" thick stock 6061 multipurpose aluminum. The two plates were sandwiched together and clamped before drilling four holes, one in each corner, for the bolts. The bolt size used is arbitrary, though a thread count greater than or equal to 20/inch is recommended as the material to be tapped is very thin. Double-sided tape was used to adhere the bottom plate to a larger piece of stock aluminum of at least 1" thickness, which was then clamped into the 2-axis CNC machine. The reason for this

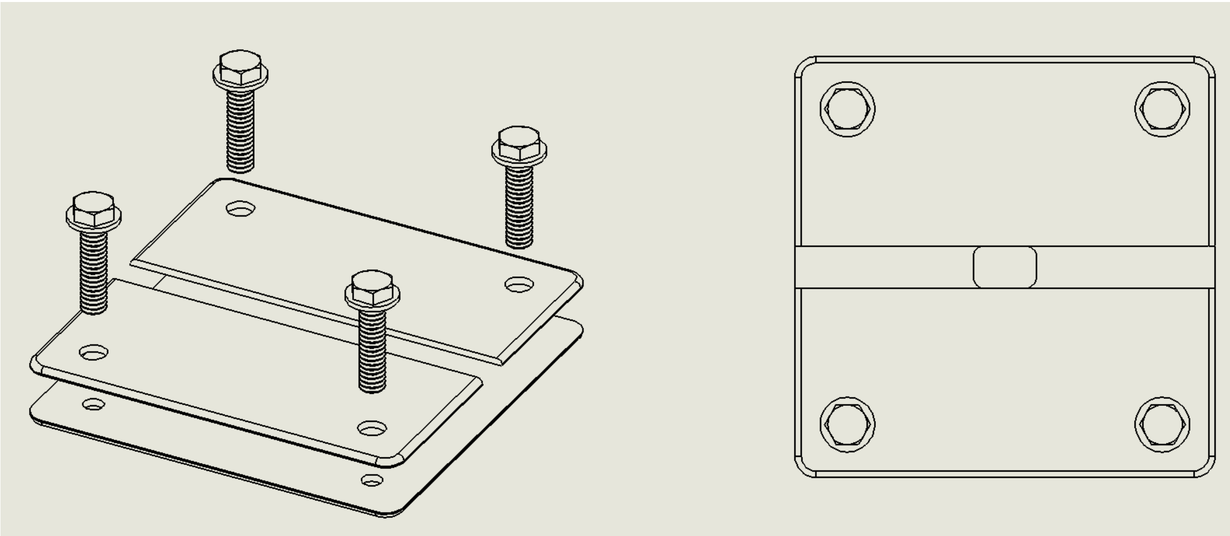


Figure 4.1.1: Diagram of custom clamp for fabrication of nerve cuff electrodes. Shown are the exploded view (left) and top orthographic view (right). The bottom plate and two top plates are made of 6061 aluminum alloy with ANSI 1/2" #5-40 bolts. Dimensions are adjustable as needed. This image was made with a bottom plate of 2" x 2" x 0.04" and a square pocket cut in the center at 4 mm x 8 mm.

step is the 0.040" thickness of the aluminum is too thin to clamp in the vice without bending and would vibrate while attempting to mill the pocket. A 1/8" end mill was used to cut a center pocket of 5 x 8 mm. This will leave rounded corners with a 1/8" radius, which needs to be considered when deciding the dimensions of the pocket, as too tight a pocket will leave rounded corners, which can interfere with the work area. A putty knife was used to detach the plate from the aluminum block after the pocket was milled. Mineral spirits were used to remove any residue left from the double-sided tape. The holes drilled in the bottom plate earlier were then tapped. In this case, #5-40 bolts were used. The holes in the top plate were then bored out with a larger drill bit so that the bolts were able to slide freely through them without catching. The top plate was then cut into two pieces, 2.5 mm from the centerline, in order to mate with the edges of the hole milled in the bottom plate. The four bolts were then used to secure the two top plates to the bottom plate. Repeat this process to make as many clamps as needed. Instructions on using the clamp to secure a cuff can be found in section 4.5.

Note: For cuff electrodes that do not require pads and instead use naked wires, the pocket does not need to be milled in the center of the bottom plate. Otherwise, the procedure for making a clamp is identical.

4.2 Cutting Electrode Pads

Electrode pads are needed for two reasons: 1) To increase the surface area in contact with the nerve and 2) to use a material that is more conducive to contact with the nerve. It would be cost prohibitive to use wire made entirely of gold, or platinum 10% iridium (Pt-Ir), for example. The reason for using these metals is to tune signal to noise ratio and biocompatibility. In [12] it was shown that the best metals to use are gold, platinum, iridium, and tungsten. Pt-Ir was chosen for use in these methods.

The best method for cutting electrode pads is to use a CNC laser cutter (or engraver) as this will give the most accurate, precise results. However, most laser cutters can be damaged when cutting metal as the beam reflects off the surface and machines capable of cutting metal can cost hundreds of thousands of dollars. The technique discussed in this paper, however, can be performed with a pair of scissors and yield results with a tolerance better than +/- 0.01 mm when cutting pads 0.5 mm wide by 3.3 mm long. For detailed instructions see Appendix I: 4.2 Cutting Electrode Pads.

Materials required: Microscope, Scissors, 25° Micro-scissors, Lab tape, Fine felt pen, Straight-edge, Calipers, Forceps [Table 3.1]

4.3 Spot Welding Techniques

Methods for spot welding appropriate for welding pads to wire at these scales include laser welding and resistance welding. The cost of a laser welding machine is prohibitive, however, as they start at several thousand dollars and increase to hundreds of thousands for industrial grade machines. Capacitive discharge resistance welders can be made for roughly one hundred dollars in parts, or purchased for a few hundred up to several thousand dollars. Those in the three to five thousand dollar range typically include a built in scope and have actuators that control the duration and force exerted by the cathode electrode. For the purpose of this paper, techniques will be discussed using a 200 W Pulse Sparkle Welder. It includes a welding pencil electrode made of tungsten, a stand, a foot-pedal, a pair of grounding pliers, and a pair of welding glasses. This same model can be purchased for half the price through suppliers in China.

In resistance welding the weld is formed by heat created by inducing a large charge that passes between the two metals to be bonded according to the formula:

$$\mathbf{Q = I^2Rt}$$

Where Q = heat generated; I = current; R = total resistance;

t = total duration of heat.

There are three main types of resistance welds (Fig. 4.3.1) [13]. Opposed, or direct, welding involves having the two pieces of material between the anode and cathode so the current makes a straight, direct path between them. Step, or indirect, welding is used when both sides of the pieces cannot be accessed, typically when a smaller piece is being welded to a larger piece [13]. The cathode is placed atop the smaller piece and the anode atop the larger piece so they are offset at different elevations. In series welding, two welds are formed at the same time; however, since there are multiple paths for the current to take through the materials, it is less predictable [13,14]. Also, when welding two different materials, if the material furthest from the cathode has a higher resistance than the material in contact with the electrodes, it is likely that no current will pass between them and no weld will form [13].

This paper will focus on opposed, or direct welding. Rather than use a pencil-type electrode for the anode, the pads are placed on the side of a pair of grounding pliers with the wire atop them and the cathode pressed into the top of the wire. The electrode geometry used in this paper is conical with an approximately 20° angle of incidence and a flat point. *Note: It is important when filing the welding electrode to do so parallel to the shaft so the current will follow the grooves rather than in rings. The cross sectional tip is then filed flat to ensure a perpendicular surface. The cone helps direct the current to a point, while the flat allows it to disperse over the contact area.*

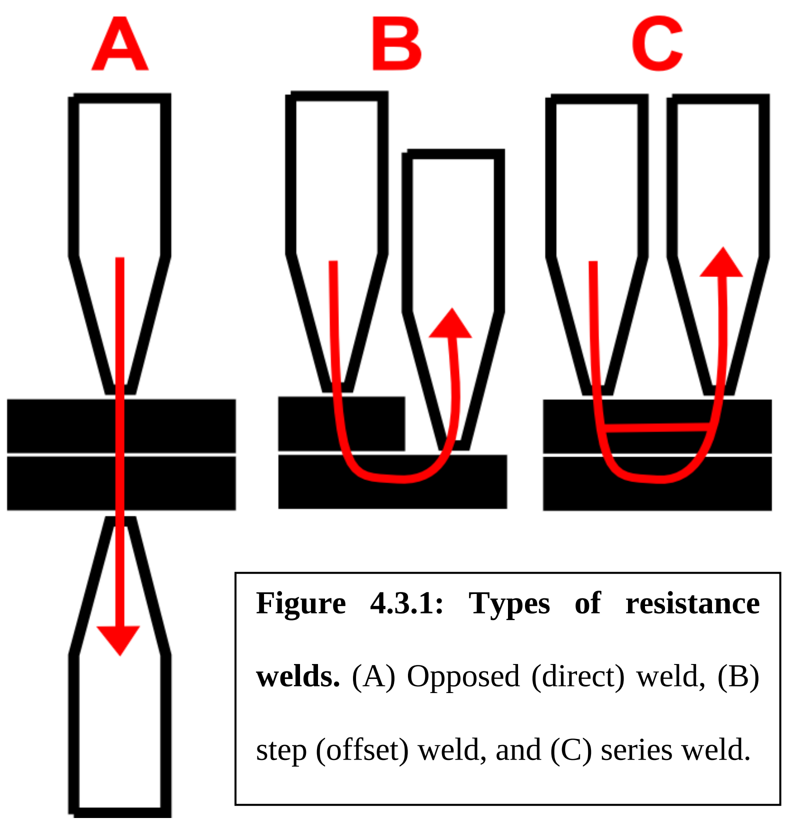


Figure 4.3.1: Types of resistance welds. (A) Opposed (direct) weld, (B) step (offset) weld, and (C) series weld.

4.4 Spot Welding Pads to Wires

The following modifications were made to the 200 W Sparkle Pulse Welder: The tungsten cathode electrode was removed from its stand by removing the screw that holds it in place, and pulling the wire out through the guide. The tungsten electrode was then covered in modeling clay to a thickness of approximately 3 mm to create an insulating barrier so the electrode could be held in the hand like a pencil. *Note: It is very important to ensure the electrode is completely covered at all times to avoid electrical shock. This shock is not harmful, though it can cause pain.* For detailed instructions see Appendix I: 4.4 Spot Welding Pads to Wires.

Materials required: 200 W Sparkle Pulse Welder, 2 x Micro forceps, 25° Micro-scissors, Lab tape, Microscope, Insulated wire, Cut pads, Tungsten pencil electrode, Grounding pliers [Table 3.1]

4.5 Preparing the Cuff

Many commercial cuff electrodes, or fabrication methods, create a cuff by molding or laminating polymeric materials such as PDMS into the desired shape. The method discussed in this paper requires the use of silicone tubing and can easily be customized to fit any size nerve simply by changing the inner radius and thickness of the tube. For most applications, the thinnest possible tube for the particular inner radius is desired as nerves are pliable and likewise need pliable cuffs to avoid mechanical damage. For the electrodes with pads, the rat sciatic nerve will be the basis for the explanation. For detailed instructions see Appendix I: 4.5 Preparing the Cuff.

Materials required: Calipers, Needle, Microscope, Scissors, 25° Micro-scissors, Clamp, Silicone tubing (cuff) [Table 3.1]

4.6 Fixing the Wires and Pads in the Cuff

Here the process splits into two parts, electrodes with and without pads. For certain applications, such as the rat vagus nerve, electrodes that use bare wire with no pad are desired because the cuff is too small to allow the use of pads. The method for making bipolar electrodes without pads will be discussed in the next section. While this section discusses bipolar electrode fabrication, the same methods are used to make tri-polar electrodes by adding another lead. For detailed instructions see Appendix I: 4.6 Fixing the Wires and Pads in the Cuff.

Materials required: Cuff previously prepared (Sec. 4.5), Calipers, Sewing needle, Microscope, Scissors, 25° Micro-scissors, Clamp, Wires welded to pads, 28 G insulin needle, Small plastic weigh boat, PDMS [Table 3.1]

4.7 Bare Wire Electrodes

There are two forms of bare wire electrodes that this paper will discuss. The first is for one use electrodes to use for in vivo experiments. These can be used for short experiments or when an electrode is needed in just a few minutes, such as if an electrode were to fail during an experiment. The second type of electrode is reusable and can even be used in chronic implantations, typically those that involve very small nerves such as the vagus.

Materials required: (One-use electrodes): Wire, Parafilm, Scissors, Micro-scissors, Insulin needle, 2 x Forceps, Calipers (Additional for reusable/chronic electrodes): PDMS, Silicone tubing (cuffs) [Table 3.1]

One-use electrode fabrication is a simple process that can be explained without images. Cut two pieces of parafilm to a width of 2 cm and a length of 3 cm. Lay one down beneath the scope so the paper is down and the paraffin wax side is up. For bipolar electrodes cut two piece of wire to a length of 10 – 20 cm depending on personal preference. Strip the insulation from a 0.5 – 1 cm segment approximately 1 cm from the end of each wire using the method illustrated in figure 4.4.3. Lay the wires parallel along the long axis of the parafilm so they are each 0.5 mm from the center on opposite sides. One end of the wires should be between 0.5 and 1 mm from the short end of the parafilm . Use the calipers to measure 1 mm between the wires in several places along their length to ensure equal spacing and the wires are parallel. Tape the wires down near where they meet the parafilm. Cut a window of the desired length and width in the center of the second piece of parafilm using the sharp edge of an insulin needle like a scalpel. For a bipolar electrode a width of 2 mm is sufficient to expose both wires without removing too much insulation. The circumference of the nerve will dictate the height of the electrode, and should be less than the circumference to ensure that when the wire wraps around the nerve it will not short. For a rat sciatic nerve, 3.5 mm, and a rat vagus nerve, 2 mm. However, for the vagus, this is much larger than the circumference and care should be taken when positioning the electrodes to ensure no shorting of the wires with themselves. Next, lay the second piece of parafilm over the first so the exposed part of the wires is within the window and the parafilm faces are touching. Press the two pieces of parafilm together. Remove the tape holding the wires in place. When the electrode is ready for use, remove the paper on the side that has the window cut in it. First, however, connectors will need to be soldered to the leads, which is covered in section 4.9.

Reusable electrodes are a bit more involved and follow much the same process as electrodes with pads for the steps proceeding this one. The main difference is in the fabrication of the cuffs, which is detailed in Appendix I: 4.7 Bare Wire Electrodes.

4.8 Insulating the Wires

Insulating the wires is important because the insulation on the small wires that will be used is extremely thin. If that insulation be compromised in any way it can lead to shorts, or introduce noise from electrical activity of nearby muscles. Bundling all of the lead wires within a single silicone tube also makes them easier to handle during implantation, and provides a single surface for fibrous encapsulation once implanted. The methods in this section are the same for every type of reusable or chronic cuff. For detailed instructions see Appendix I: Insulating the Wires.

Materials required: Leads secured in cuff, Silicone tubing (leads), PDMS, Insulin needle, Forceps, Micro-scissors, Lab tape, Tray [Table 3.1]

4.9 Connectors

In order to use the electrode it must have a connector on the wire lead. For in situ work with the parafilm or reusable cuff electrodes, simply insert the wire into the cup of a male crimp pins and solder it in place, then use heat shrink tubing to go around each pin and wire, then a larger piece around both pins and wires. It is recommended that different colors of heat shrink tubing are used in order to differentiate which electrode was used in what experiment. For chronic implantable electrodes, there are typically more than one electrode being implanted and they all require a single connector. For the purpose of this paper the connector discussed will be the MS3D3 6-channel pedestal connector from Plastics One. For detailed instructions see Appendix I: 4.9 Connectors.

Materials required: Connector, 6 x Crimp pins, Scissors, Lab tape, Crimpers, Dental cement, UV wand, Microscope [Table 3.1]

4.10 Finalizing Electrodes

The final stage of the process involves ensuring all leads are insulated with silicone tubing and PDMS and trimming the cuffs.

Materials required: Cuff electrode with leads attached to the connector with dental cement, Silicone tubing, PDMS, Micro-scissors, Razorblade, Insulin needle, Calipers [Table 3.1]

Cut a piece of silicone tubing of sufficient inner diameter to surround the bundle of exposed wire at the base of the pedestal connector, and of sufficient length to cover all of the wire from the pedestal to the beginning of the silicone tubing of the leads. It may be necessary to trim some of the lead tubing so they are all the same length. Slit the piece of tubing lengthwise and wrap it around the bundle of wires. Use an insulin needle to drip PDMS within the slit, at both ends of the tube, and to cover the outside of the tube to seal the slit and connect the pedestal and the lead tubing at each end. Cure at 120°C for 20 minutes. Multiple applications of PDMS may be required to attach the tubing and cover exposed wires. Finally, use a razorblade to cut the cuff tubing to the length desired, ensuring a minimum of 1.25 mm from the edge of the electrode contacts. For a bipolar rat sciatic nerve with 0.5 mm contact pads, this is a 4.5 mm. Cut by placing the cuff on a hard surface and, leaving the cuff curled, measure by looking through the cuff (the pad should be visible) and press the center of the razorblade through the cuff keeping it perpendicular to the surface, and the outer edge of the cuff.

4.11 Characterizing Electrodes

Test each pad/wire of each lead for continuity with a standard multimeter to ensure there are no shorts and each pin is properly mapped to the desired pad/wire. To test the electrode for quality, this paper tests impedance with a 1 kHz sinusoidal AC signal at 1.75 V_{pp} using an ICM neurocraft impedance conditioning module by FHC Inc. Impedance values were recorded four times at thirty second intervals for each electrode to account for drift. The mean and standard deviation of these values were calculated over all of the electrodes fabricated by this method to test for variance. This data was then compared with a one-tailed, two-sample with unequal variance t-test of the means and direct comparison of variance to data recorded from an earlier method of fabrication within the lab that used conductive epoxy to adhere pads to wires for cuff electrodes. Signal to noise ratio (SNR) was also calculated for each sample. The hypothesis is that electrodes fabricated by the method within this paper will have both a lower mean impedance value and lower variance between electrodes than those fabricated using conductive epoxy when tested with a 1 kHz sinusoidal AC signal at 1.75 V_{pp}.

Materials required: 100 mL glass beaker, 50 – 80 mL rat Ringer’s solution, Impedance tester [Table 3.1]

4.12 Sterilization

Before electrodes may be implanted, they must be sterilized to ensure biocompatibility with the animal. This method of fabrication uses ethylene oxide (EtO) gas sterilization. After the sterilization process, the electrodes are encased in gas sterilization pouches that can be stored safely until used. However, it is recommended that the gas sterilization takes place as close to the experiment as possible to ensure no contamination in the interim. Sterilization should be complete a minimum of 72 hours before the implantation procedure to allow any excess EtO to dissipate.

Survival surgeries for implantation should take place within a sterile field following standard surgical practice as well as any additional measures enacted by particular institutions.

CHAPTER 5

RESULTS

Two methods of fabrication were tested against each other using a two-sample t-test with unequal variance. The first method used silver conductive epoxy to adhere Pt-Ir contact pads to the wire. The second used direct welding resistance spot welding techniques to adhere Pt-Ir contact pads to the wire. All electrode leads used in Table 5.1 were tri-polar. The SNR of each sample is 0.98 and 2.62, respectively.

	Epoxy Z (kΩ)	Weld Z (kΩ)
	6.74	1.73
	26.42	2.50
	8.02	1.93
	65.60	2.00
	3.70	1.70
	13.18	1.23
	4.83	1.80
	4.98	3.20
	1.88	3.95
	45.10	1.50
	35.46	4.35
	38.12	2.70
	13.06	1.80
	8.95	2.90
	30.00	1.70
	7.78	3.03
	2.38	
	2.20	
Mean	17.69	2.38
STD	18.29	0.91
Variance	334.41	0.82

Table 5.1 (left): Electrode Impedance Values (kΩ). The mean of four tests for impedance of each lead fabricated are shown for electrodes created with silver conductive epoxy (Epoxy Z) and spot welding techniques contained within this paper (Weld Z). The mean, standard deviation (STD) and variance for each sample are also shown. Values are rounded to the second decimal because the impedance tester used was accurate to that place.

5.1 Statistical Analysis

A one-tailed, two-sample with unequal variance t-test was performed between sample 1, electrodes fabricated with silver conductive epoxy, and sample 2, electrodes fabricated with spot welding techniques discussed in this paper.

$$H_0: \mu_Z \text{ of sample 1} = \mu_Z \text{ of sample 2}$$

$$H_1: \mu_Z \text{ of sample 1} > \mu_Z \text{ of sample 2}$$

It was found that electrodes created with silver conductive epoxy have a μ_Z = 17.69 +/- 18.29 kΩ and a σ^2 = 334.4, while the electrodes created using these methods have a μ_Z = 2.375 +/- 0.9058 kΩ and a σ^2 = 0.8205, yielding a p-value of 1.23×10^{-3}, which is less than $\alpha = 0.01$ (Figure 5.1). This allows rejection of the null hypothesis and confirms the alternate hypothesis. A direct comparison of variance also shows $\sigma_1^2 = 334.4 > \sigma_2^2 = 0.8205$, meaning the methods used in this paper are more repeatable.

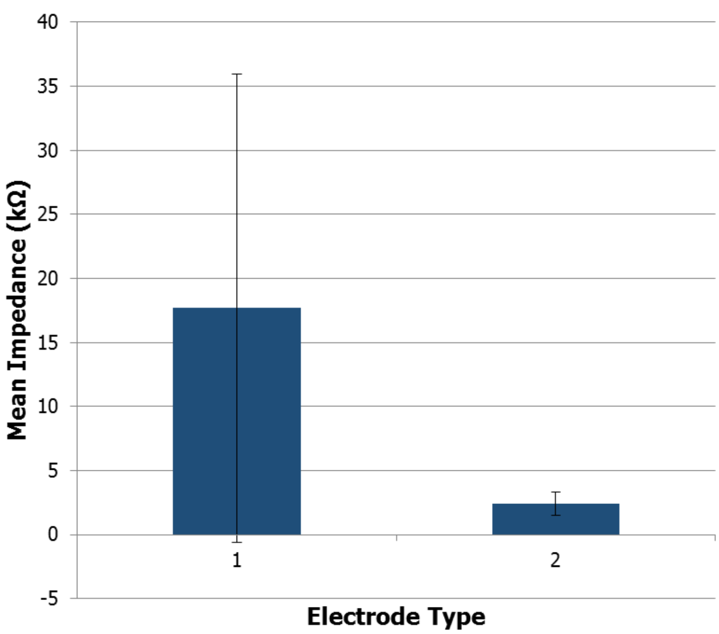


Figure 5.1 (left): Results of one-tailed t-test with unequal variance comparing μ_Z of samples 1 and 2. Sample 1 is electrodes fabricated with silver conductive epoxy μ_Z = 17.69 +/- 18.29 kΩ, sample 2 is electrodes fabricated through the methods in this paper μ_Z = 2.38 +/- 0.91 kΩ, with a p-value = 1.24×10^{-3}.

CHAPTER 6

CONCLUSIONS AND FUTURE IMPLICATIONS

A method paper on the fabrication of nerve cuff electrodes was very much needed. Most devices used are proprietary and are either not customizable, or customizing can be cost prohibitive, meaning experiments must be designed around the electrode rather than the other way around. For those who wished to fabricate their own electrodes, there was no definitive guide on doing so, meaning the method would have to be worked out by reading journal articles or by trial and error. Most method sections of articles do not go into detail and simply state, “the pad was welded to the wire.”

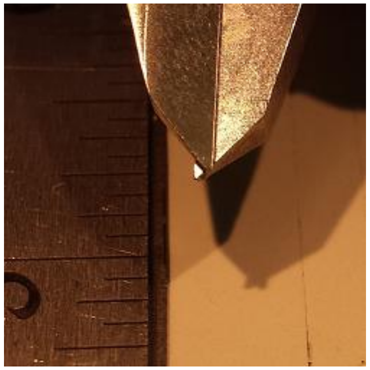
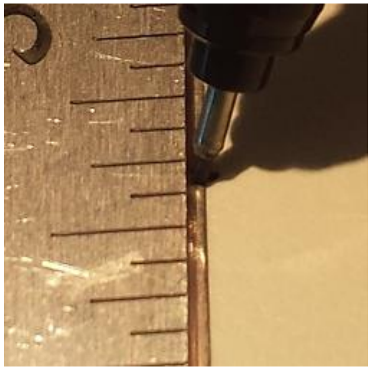
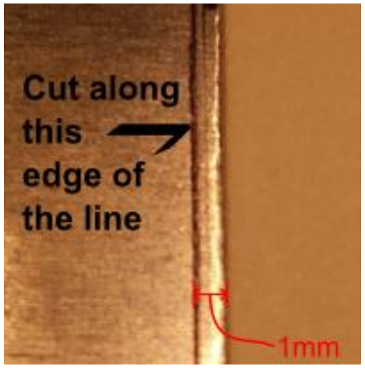
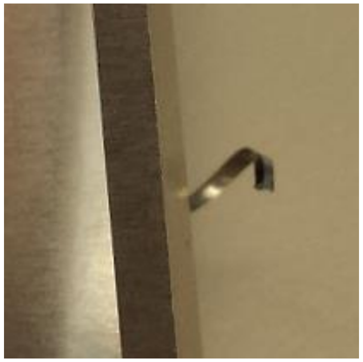
The electrodes fabricated for this study were originally meant for use in a study to determine the selective block ability of KHFAC stimulation on sensory and motor fibers in awake, freely moving rats. The length of time to create a viable electrode was longer than anticipated as the first method with silver conductive epoxy failed. Also, different connectors were used that were not secured to the skull and the animals were able to pull them out and chew through the leads before data could be collected. The study was also limited in that undergraduates at the Georgia Institute of Technology are not allowed unsupervised access to animals, meaning surgeries and experiments could only be performed when a graduate student had time to assist. This slowed the study considerably, so it was unable to be completed before the end of the Petit Scholar program.

The experiment has since been redesigned to use the new connectors detailed in section 4.9 and secure them to the skull with dental cement, as well as use a real-time x-ray video capture device to monitor gait before and after block stimulation is applied, which should yield more quantitative and conclusive results than the previous method used, the Plantar Test, which involved the application of an infrared heat stimulus to the paw and timing the rate of withdrawal. That test was rather subjective, as the reason for withdrawal could not definitively be attributed to feeling the heat sensation with 100% certainty, and methods of judging pain response are subjective.

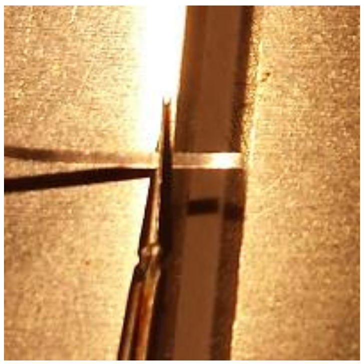
The methods herein create a unified source of information for multiple means to fabricate electrodes and will enable future students in this lab, as well as others, to learn the fabrication process with much less effort and expense in both time and materials. For many electrophysiology experiments the interface is one of, if not the, most important factor in acquiring quality, representative data. The electrodes created by these methods have been shown to have electrical characteristics that are both within viable range for impedance and show a small variance between electrodes which will help reduce signal to noise ratio and variability between data sets taken with different electrodes. This will increase the quality of experiments, as well as produce verifiable results which can be tested by independent sources, which is an important step in the scientific process.

APPENDIX I: METHODS

4.2 Cutting Electrode Pads

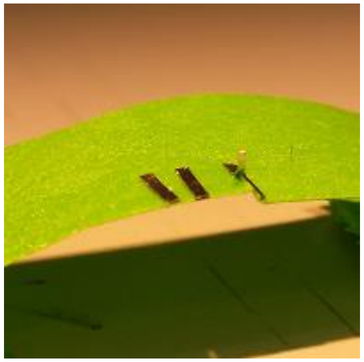
	*Note: The metal used in the images is stainless steel for demonstration purposes, not Pt-Ir*. Set the calipers to 1.00 mm. Using the small jaws of the caliper, set first one end, then the other of the straight edge (left 4.2.1). Mark a line with the felt pen (right 4.2.2).	
	Use the large paper scissors to cut the strip of metal. This should be done under the scope. *Note: Always use the highest magnification setting possible to keep tolerances tight.* The goal is to cut along the edge of the line that was against the ruler (left 4.2.3). The strip will begin to curl (right 4.2.4).	

Once the strip is cut the curl can be removed manually and then pressed flat with a sheet of metal or other hard, flat item (left 4.2.5). Set the calipers at the desired length of the pad (3.30 mm). Measure by pressing the strip down atop one of the large jaws of the calipers and butting it into the other, ensuring it is perpendicular to the edge (right 4.2.6).



While cutting (left 4.2.7) it is important to keep the scissors perpendicular to the strip. It can also be difficult to cut precisely on the edge of the jaw because the strip must be raised slightly which creates parallax. It is best to offset the strip the width of the scissors so the strip overlaps the far jaw, enabling both the strip and the scissors to remain on the same plane. This avoids parallax.

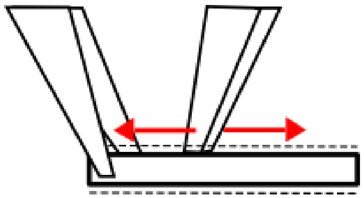
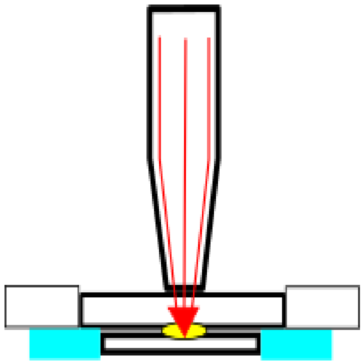
This results in pads that are the same size with a tolerance of +/- 0.01 mm (left 4.2.8). Next, the pads need to be cut to half the width to get 0.50 mm. Adhere them to a piece of lab tape, fold it, and adhere it to the work surface beneath the scope as shown (right 4.2.9). Ensure there is a gap underneath.

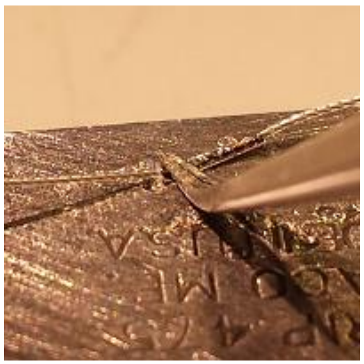
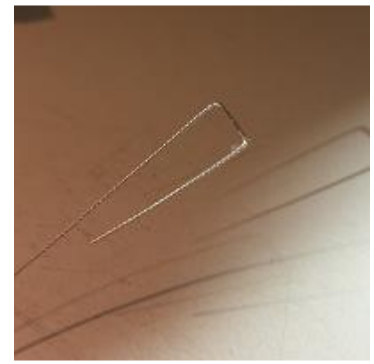
	Using the 25° micro scissors at 5x magnification, cut the pad in half (left 4.2.10, right 4.2.11). This will have to be estimated as it is too small to measure accurately; however, it should yield pads within the range of 0.49-0.51 mm, well within tolerance. Repeat this process as many times as needed.	

4.4 Spot Welding Pads to Wires

Tips on Spot Welding: Ensure all surfaces are clean with 70% ethanol and the electrode tip is filed to a cone with a flat tip. If a weld fails or the tip sticks to the weld, file down any carbon or metal deposits. Ensure the ground surface is clean, flat, and making full contact with the pad. The 200 W Sparkle Pulse Welder is not very precise with its settings. Some experimentation should be done to properly tune the current. For this paper the dials were both set to 3. The dials seem to be additive rather than “course” and “fine” as indicated. The foot pedal should be placed somewhere easily found without looking, but where it will not be triggered accidentally.

Tape each side of a pad (as cut in 4.3) to the top of the grounding pliers in the field of the scope at maximum magnification. Roughly 0.2 mm of each side of the pad should be covered (left 4.4.1). Next, lay the insulated wire over the center of the pad so one end overhangs by ~2 cm. Tape it ~1 mm back from the edge of the tape on the pad (right 4.4.2).

	To strip the insulation from the wire, grasp one side of the wire with one pair of forceps, with the second pair closed, place the tip against the insulation of the wire and move it rapidly back and forth along the wire 1-2 mm in each direction (left 4.4.3). This will cause the insulation to stretch, bunch up, and eventually split down the middle. Pull the split insulation perpendicular to the wire until it breaks. Then grasp the broken insulation near the stationary forceps and break it off at each end of the pad.
With the wire stripped for the length of the pad it is ready to weld. First weld in the center of the pad. Hold the electrode as near perpendicular as possible in the center of the pad with light pressure (right 4.4.4). The pressure should be firm enough that there is no air gap between the grounding pliers, the pad, or the wire. Increased resistance from air or insulation will cause the spark to arc, vaporizing the metals in a flash of light. *Note: Tinted goggles should be worn, or the eyes closed, when welding.*	
	Next move the welding electrode to the edge, near the tape, and press firmly as the wire needs to bend to close the gap created by the tape. Using the correct pressure will take practice. After each end is welded, weld toward the center from both sides until 10-12 welds have been completed along the entire length of the wire (left 4.4.5). *Note: When welding, it is best to use one pair of forceps to hold the wire steady ~2 mm from the point of the weld to avoid the wire rolling from beneath the electrode.*

<table>
<tr>
<td colspan="2">Remove the tape from the short side of the wire (right 4.4.6). Note: The layer between the wire and the pad should be pulled horizontally outward to avoid bending the wire or breaking the welds. Once the tape is removed, hold the wire and pad down with the forceps and weld the wire to the end of the pad. Place a piece of tape over the wire and pad near the center of the pad and remove the tape on the left to repeat the process on the far side.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With welding complete on the lead side, place the back of the closed forceps atop the wire just past the end of the pad (left 4.4.7). Bend the long side of the wire (lead) until it remains at an ~90° angle. Do the same at the short end (tail). This should make a square “U” shape from the wire (right 4.4.8). Repeat this process to create all the leads needed.</td>
<td></td>
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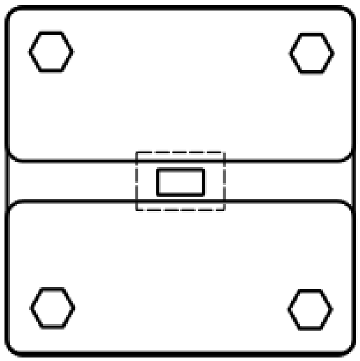
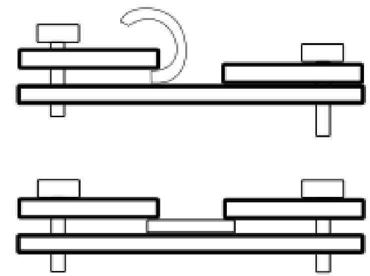
4.5 Preparing the Cuff

	The goal is to center the cuff over the hole of the clamp and secure it in place in a flat, open position (left 4.5.1). First, cut the cuff to the desired length. For a rat sciatic cuff, ~1.5 – 2 cm. Slit the cuff along its axis with the micro-scissors. Position it as shown (right 4.5.2 top) and tighten the screws. Then flatten it on the other side and clamp (right bottom).	

4.6 Fixing the Wires and Pads in the Cuff

<table>
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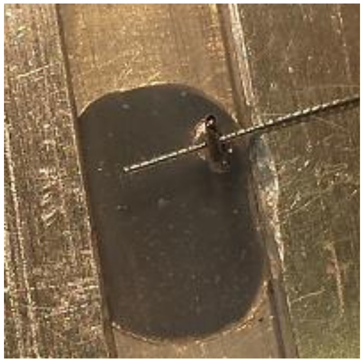
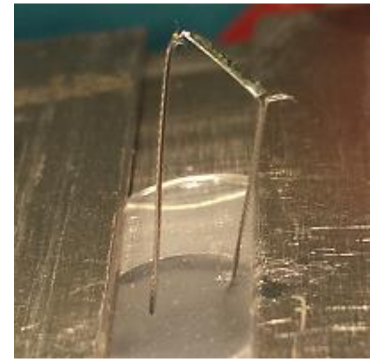
<td></td>
<td>Push a standard sewing needle through the top of cuff until only the eye remains, then thread the needle with the long end of the wire so 2 – 3 mm are through the needle (left 4.6.1). Pull the wire through until the short end of the lead is touching the top of the cuff (right 4.6.2).
Note: The top of the cuff is what would be the inner surface if curled.</td>
<td></td>
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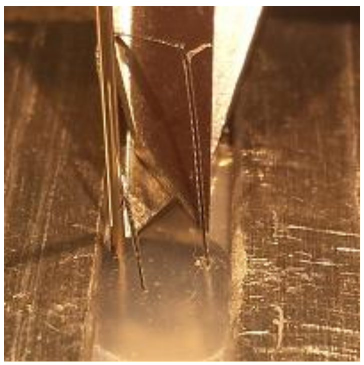
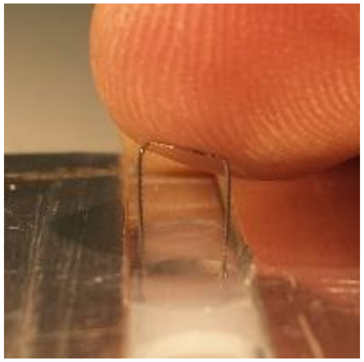
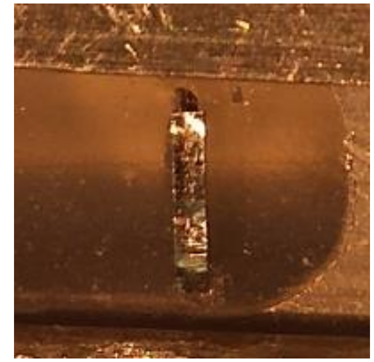
<tr>
<td></td>
<td colspan="2">Use the small jaws of the calipers to measure the distance between the wires and push the needle all the way through the cuff (left 4.6.3). It is best to go slightly over. For example, for the 3.3 mm pad, a distance of 3.4 mm is measured. This is because the wire curls past the end of the pad and if it were too narrow it might cause the pad to bend upward creating separation between the pad and cuff.
Note: Ensure the distance you are measuring is parallel to the edge of the cuff.</td>
</tr>
</table>

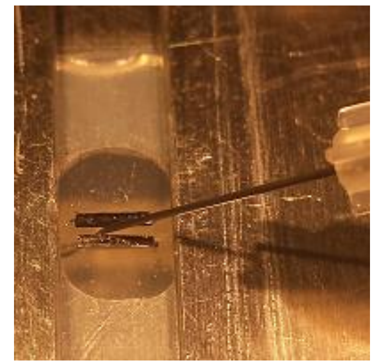


Use forceps to guide the short end of the lead into the hole made by the needle. Then pick up the clamp and use a finger to apply gentle, even pressure downward on the pad until it rests on the surface of the cuff (left 4.6.4, right 4.6.5). The back of closed forceps can be used to ensure it is flat and make minor adjustments.



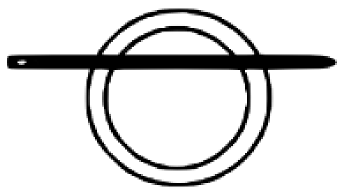
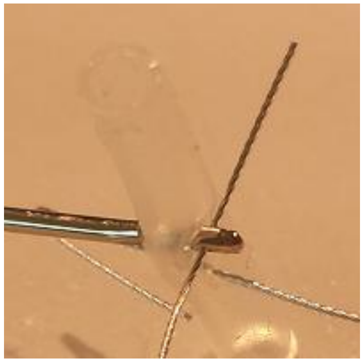
Measure 1.5 mm from the center of the pad then push the needle through and thread it as in figure 4.6.1. Repeat the steps up to this point to place a second pad, ensuring there is a 1 mm gap between the pads. Next, prepare 2 – 3 g of PDMS in a small weigh boat. Use an insulin needle to scoop a drop of PDMS and apply it to the surface of the cuff on each side of the pads and in between them (right 4.6.6).

Note: Do not attempt to draw PDMS into the syringe, just use the needle to scoop drops one at a time.



When a thin layer of PDMS has been lain around all of the pads and spread evenly, place the clamp assembly on a portable, heat resistant tray and tape the wires down. A 15 cm x 15 cm blank circuit board makes a good tray. Place this in an oven and cure at 120°C for 20 minutes. When it is done, use the back of the forceps to scrape off any PDMS that may have covered the pads. Finally, remove the clamp.

4.7 Bare Wire Electrodes

<table>
<tr>
<td></td>
<td>First, cut the desired silicone tubing the length of a cuff (~1 – 1.5 cm). Pierce the cuff with a sewing needle as shown (left 4.7.1). Push the needle through until only the eye remains, and then thread a wire 3 mm through (right 4.7.2). Finally, pull the wire through until the bent portion emerges from the far side of the tube.</td>
<td></td>
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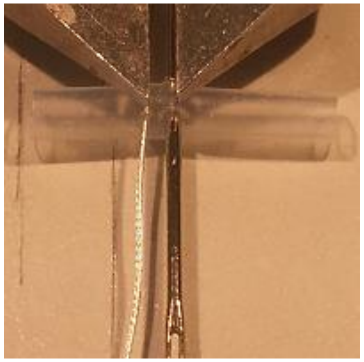
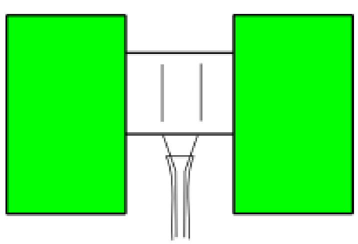
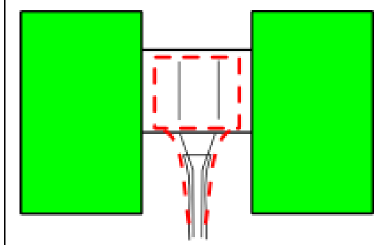
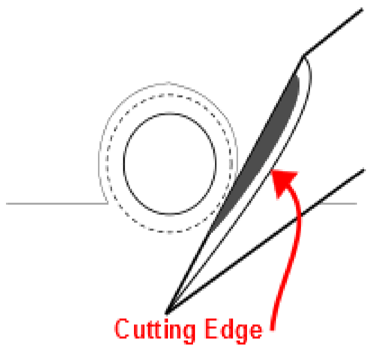
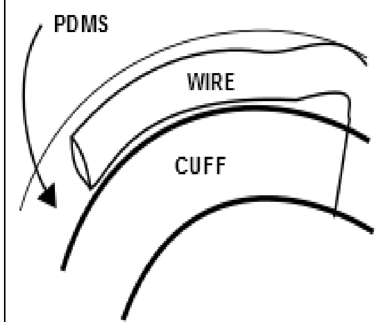
<td></td>
<td>Use the calipers to measure 1 mm from the first wire (left 4.7.3), then repeat the process to thread a second wire through. Pull the wires back so the bent portion is lain over the back of the tube (right 4.7.4). Note: The back is the bottom of the cuff in figure 4.7.1.</td>
<td></td>
</tr>
<tr>
<td colspan="3">Next, slit the tube lengthwise with the micro scissors in the center of the segment that is above the needle in figure 4.7.1. This will give the wires maximum exposure to the nerve. Note: Be careful not to cut the wires. Insert the cuff and wires into the clamp as in section 4.5. Lay down a thin layer of PDMS and cure it in the oven, as described in figure 4.6.6. Finally, expose the wires and remove the insulation using the technique in figure 4.4.3. From section 4.8 Insulating the Wires onward, the process is the same.</td>
</tr>
</table>

4.8 Insulating the Wires

Hold the cuff with one hand, pull the wire leads taut, and cut them to the same length with the scissors. Cut a length of silicone tubing (leads) that will extend from the cuff to 2 – 3 cm before the end of the lead. Run the wires through the tubing. It is typical for the wires to start to bend after 5 – 10 cm. When this happens, pinch the tubing to the wire at the end of the tubing with one hand. With the other, pull the tubing taut, then pinch the tubing to the wire 5 – 8 cm from the first hand. Release the tube with the first hand. This will cause the tube to elastically return to its original shape. Repeat this process, incrementally moving the tube down the wire until it is exposed. Essentially, use the fact that the tube stretches and returns to its original shape to pull the wire through the tube once it is no longer possible to push the wire into the tube. If the tube begins to bunch up further down the wire, maintain the pinch at the far end of the tube and drag two fingers down the length of the tube to smooth it out.



Once the tube is in place so one end is tight to the back of the cuff and the other has ~2 cm of wire coming out the end, tape the tube slit-side down to the tray with a piece of tape on each side of the cuff holding it in place, and another over the lead tube. Use the micro-scissors to cut off the tails of the leads that are bent over the tube, leaving 2 -3 mm so it looks like the figure to the (left 4.8.1).

<table>
<tr><td colspan="2">Next, apply PDMS liberally to the entire back of the cuff. Allow PDMS to drip between the wires and the tubing. It will seep into the tube for 1 – 3 cm, which is desirable. Apply PDMS to the back of the tube as well. The dotted red line shows the area to which PDMS should be applied (right 4.8.2). Cure the electrode in the oven at 120°C for 20 minutes.</td><td></td></tr>
<tr><td>
</td><td>Use the insulin needle like a scalpel to cut the layer of PDMS directly beneath the wire tails (left 4.8.3). Use the tips of the closed forceps to push the wire down into the slit made in the PDMS so the end is not exposed, as the exposed end needs to be covered (right 4.8.4). Finally, seal the slit with PDMS and cure at 120°C for 15 minutes.</td><td>
</td></tr>
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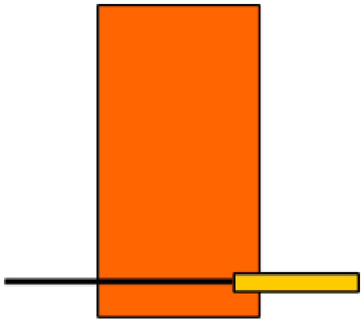
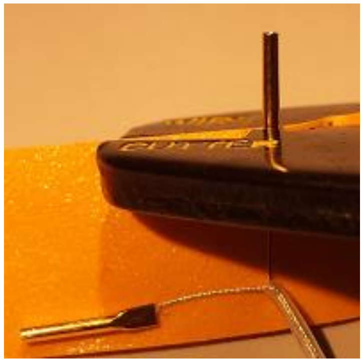
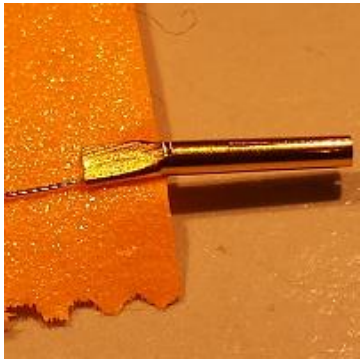
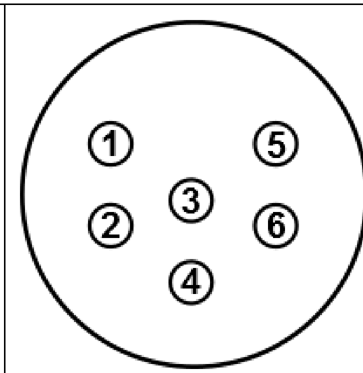
4.9 Connectors

	Press a crimp pin into the adhesive side of a piece of lab tape so just the portion to be crimped is covered by the tape. In this example, ~2 mm. Strip the insulation from the end of the wire lead and insert into the pin then press it into the adhesive (left 4.9.1). Grasp the tape and pin at the edge of the tape with a flat part of the crimpers and squeeze (right 4.9.2).	
	This should yield a flat crimp with little to no bare wire exposed (left 4.9.3). The edge of the tape acts as a great guide for where to place the crimpers, use it advantageously. When crimping, squeeze as hard as possible. It is best to get a flat crimp in the first attempt as further attempts are likely to open some portion of the crimp.	

<table>
<tr>
<td>Insert the pins into the back of the 6-channel pedestal. To the (right 4.9.4) a pin-out schematic is as follows. 1 and 2: first bipolar electrode. 3 and 4: second bipolar electrode. 5 and 6: bipolar EMG electrode. Note: An EMG electrode is just a wire lead encased in silicone tubing (leads) with ~2 – 3 cm of insulated wire exposed at the far end. Use PDMS to seal the end of the tube to avoid fluids wicking inside. Before surgery, expose a length of bare wire sufficient for contact with the desired muscle.</td>
<td>
</td>
</tr>
<tr>
<td colspan="2">With all of the pins in place, use UV curing dental cement to fill between the backs of the pins and cover them to just past the junction with the wire. Cure with the proper wavelength UV light as per the specifications of the dental cement chosen. This will create a solid, resin, insulating back. Note: Before curing the dental cement, ensure all the wires are tightly bundled so they may be covered by a single tube and cured in PDMS.</td>
</tr>
</table>

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