



Preparing Optrodes for Extracellular Recording with Optogenetic Manipulation of Cell-Type Specific Neurons

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Background

The construction of optrodes allows for us to combine the worlds of electrophysiology and optogenetics (Deisseroth K, 2011). The electrodes that we construct within our lab consist of two parts: a carbon fiber electrode and a fiber optic. During *in vivo* extracellular recording procedures, we are able to perform electrophysiology recordings using the carbon fiber electrode. In addition, we can use an optic fiber to excite or inhibit specific cells. Cre-dependent adeno-associated viruses can be used to express light-sensitive ion channels or pumps in specific cells in the brain. Thus, only sub-populations of cells are activated or inhibited by the light-activated proteins.

Visceral chronic pain conditions affect millions of individuals across the globe (Klein-hans NM et al., 2016). Urological chronic pelvic pain syndromes (UCPPS) are chronic pain conditions associated with frequent urination, pain during urination and pelvic pain (Berry SH et al., 2011). Despite the prevalence of these conditions, the causative mechanisms are unknown, which does not allow for effective treatment. Evidence from human imaging studies suggest involvement of limbic circuits in the brain of UCPPS (Simons LE et al., 2014). One specific area that has shown robust activation in the context of injury and

pain is the amygdala. Here, we describe the use of optrodes (carbon fiber electrodes with fiber optics) that can be utilized to record and manipulate cell-type specific populations of neurons in the central nucleus of the amygdala (CeA) to identify their role in the modulation and processing of bladder pain.

Materials (see page 8)

Procedure

Step 1: Electrode Production

Each carbon fiber electrode is composed of a single carbon fiber in a borosilicate glass capillary tube (pulled to a point). On the machine interface side, the carbon fiber is attached to a silver wire and male gold connector. On the *in vivo* recording end, the tube is pulled to a point and the carbon fiber is etched back to the start of the tube.

1. Obtain the necessary materials needed for electrode production. This includes: creased piece of paper, carbon fibers, *Kwik-Fil* capillary tubes, gold male connector pins, silver conducting paint, epoxy and a vacuum. **Note:** A 1-centimeter piece of silver wire is soldered to the gold male connector pin prior to electrode production.

2. Cut a 4cm long strand of braided carbon fiber. Tape one end of fiber to creased paper.
3. Isolate a **single** carbon fiber. This can be done by gently teasing the fibers apart on a piece of creased paper by rolling your finger over the braided fiber. Individual fibers will eventually be seen. Single carbon fibers are just visible to the naked eye.
4. Next, put a small droplet of silver paint on the end of the silver wire that will be attached to the carbon fiber. Holding the silver wire from the male gold connector (soldered to the silver wire), gently press the droplet of silver paint to the single carbon fiber. The carbon fiber will stick to the paint. Once the silver paint dries, there will be a stable electrical connection from the carbon fiber to the gold connector pin. **Note:** Complete this step in about 30 seconds to ensure the paint does not dry prior to bonding to carbon fiber.
5. Place the end of a capillary tube to tubing that is connected to the "intake" side of the vacuum. Note: Be sure to place a filter (e.g. cotton ball) in the tubing line between the capillary tube and the intake to prevent any physical objects from being drawn into the vacuum.
6. With the vacuum turned on, carefully place the open end near the free end of the carbon fiber (away from the silver paint/wire). The carbon fiber will be drawn inside of the capillary tube. Push the silver wire into the capillary tube. The gold connector should be flush with the open end of the capillary tube (away from the vacuum).
7. Once the carbon fiber is inside of the glass capillary tube, turn off the vacuum. The capillary tube with fiber and connector can be placed onto a holding rack prior to proceeding to the next step.
8. Repeat steps 3-7 above until the desired number of electrodes are produced.
9. Mix epoxy according to manufacturer's recommendations. Epoxy then should be mixed in a disposable container. A Q-tip can be cut into half and the blunt end is used to epoxy the ends of the electrodes, where the gold male connector is connected to the glass capillary tube. Dry overnight. **Note:** For this step, it is helpful to draw a small amount of epoxy into the capillary tube for a stronger physical connection. Pull out the connector leaving a small amount of the silver wire exposed from the capillary tube. Reconnect the vacuum and apply the epoxy. The vacuum is then used to pull the epoxy into the glass capillary tube.

Step 2: Electrode Etching

In this step, the excess carbon fiber is carefully, electrically etched so that the tip of the carbon fiber is just outside the tip of the pulled capillary tube (see below for pulling instructions). **Note: *Danger* Do not turn on current generator until instructed.**

1. A pipette puller (horizontal or vertical) is used to pull the glass capillary tube. For a Sutter horizontal puller, the following settings are used: H: 603; Pull: 45; Vel: 120; Time: 200; Press: 200. Note: Try to pull the glass capillary tube so that the finished electrode is roughly half the length of the original glass capillary tube (length = 5cm).
2. Once the electrode is pulled, cut the free end of the carbon fiber carefully about 0.5 cm from the end of the pulled capillary tube tip.
3. Place electrode in the microscope etching setup like the one depicted in **Figure 1**. The electrode is clamped into the Kite Manual Micromanipulator without base (WPI; KITE-R), which is itself clamped

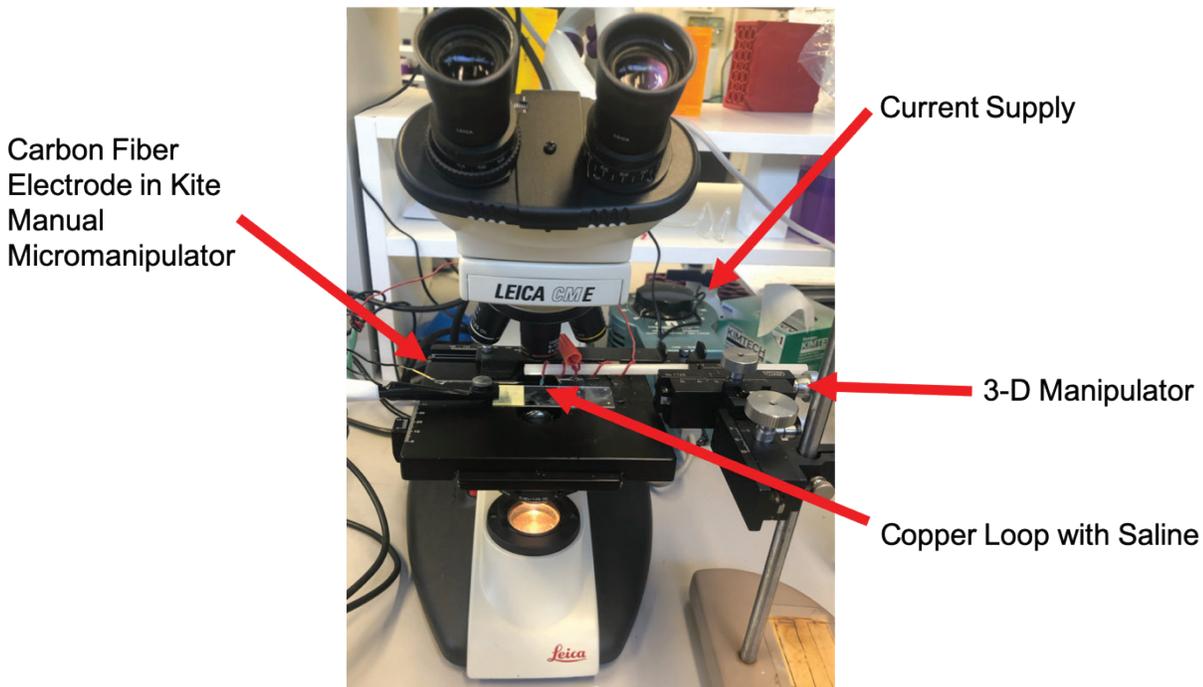


Figure 1: Microscope Set-Up for Electrode Etching

into the Narishige 3-D manipulator. In this setup, a circuit is created between the carbon fiber electrode and current generator. On one side is the pulled carbon fiber electrode connected to the current generator. On the other side is a copper wire loop connected to the current generator. A droplet of saline in the copper loop is utilized to etch back the carbon fiber to the end of the glass capillary tube. The open end of the loop is placed perpendicular to the end of the carbon fiber (like a dog jumping through a hoop).

4. Under the 4X lens, use the Narishige 3-D manipulator to move the carbon fiber underneath the microscope. Look for the junction between the carbon fiber and the end of the glass capillary tube (**Figure 2**). If you cannot find it underneath 4X, you can identify this junction underneath 10X.
5. Once you have identified this junction, note the location of the junction on the 3-D manipulator x/y/z rulers. Move the elec-

trode back away from the center of the field of view (x axis).

6. Now, move the copper loop underneath of the microscope and orient so that the loop is perpendicular to the carbon fiber and the carbon fiber is aimed directly at the center of the open loop.
7. Place a droplet of saline in the copper loop.
8. Now, attach the male gold connector to the female connector that is connected to

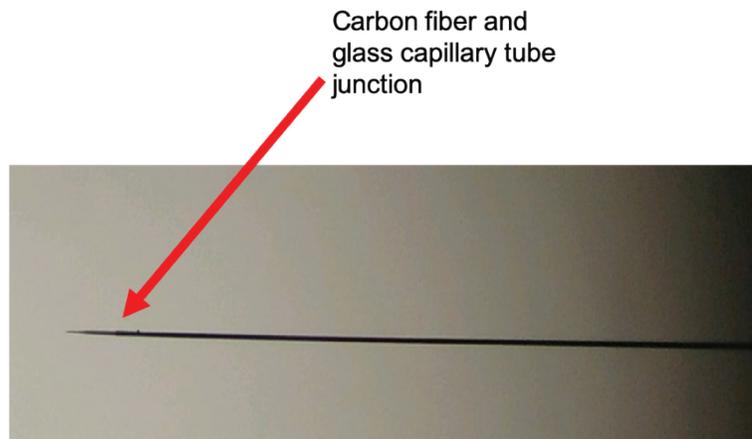


Figure 2: Carbon Fiber Electrode Underneath 4X

the same current generator that the copper loop is connected to.

9. ***Danger***. Prior to turning on power supply ensure that there are no metal-to-metal shorts in the circuit. The gold connectors should not be touching the 3-D manipulator. The copper loop should not be touching the stage of the microscope. Once the power is turned on, extreme caution must be used to avoid the potential for a painful or lethal electrical shock.
10. After ensuring a safe workspace, the power supply can be turned on to 120 V. Under the 4x lens, the electrode can be advanced slowly into the saline on the copper loop. As the fiber is etched, you should see small bubbles. The electrical current slowly “cuts” back the carbon fiber and “sharpens” the end of the fiber to a point. Go slowly with this process, gradually etching from the free end of the fiber back towards the junction of the capillary tube and the fiber. Note: If no etching occurs there is likely no electrical circuit due to a break in the carbon fiber (in the capillary tube). Discard electrode (gold connectors can be reused).
11. Continue to advance the electrode until the carbon fiber is etched back approximately 0.1-0.2 mm from the end of the glass capillary tube.
12. Place finished electrode into an electrode holder.
13. Repeat steps 1-12 for each electrode to be etched.

Step 3: Optical Fiber Preparation

1. Prior to building the optrode, you must prepare your fiber optic.
2. Cut a piece of yellow Kevlar tubing to approximately 1 foot using the Kevlar scissors. Slide fiber optic connector cover over the Kevlar tubing.

3. Soak the connector in isopropyl alcohol for 1 minute.
4. Blow out inside of connector with compressed air to dry it.
5. Use wire stripper to expose 1 inch of sheath.
6. Score fiber optic cable with the diamond knife and pull both ends to break.
7. Use fiber optic stripper to strip the outer layer off of roughly 10mm of cable.
8. Slide the fiber optic cable through the yellow Kevlar tubing.
9. Test to verify that the connector fits with stripped cable.
10. Mix epoxy.
11. Fill a 3 mL syringe with blunt needle with the epoxy. Remove any air bubbles.
12. Put the syringe into the back of the connector and fill until there is a small bubble on the front end. Remove the syringe.
13. Slide the fiber optic cable into the connector until it stops. Slide gold connector onto back of the connector.
14. Use the 0.178 hole on a crimper to crimp the big part of the gold connector. Then use the 0.128 hole on the crimper to crimp the small part of the connector.
15. Put epoxy on the interface between the gold connector and the yellow Kevlar tubing. Slide the black boot over the connector.
16. Allow the epoxy to cure overnight.

Step 4: Optrode Production

1. Using a diamond pen, score a new glass capillary tube at an angle. This should be done approximately 7.5cm down the

capillary tube. This needs to be done for each new optrode that is made.

2. The length of the capillary tube should be measured and recorded. This beveled capillary tube can be epoxied to the side of a finished electrode. The bevel should be approximately halfway up the tip of the electrode (~7 mm from the tip). The epoxy should be allowed to dry for at least 20 minutes.
3. The fiber optic must be checked with the laser. Connect the fiber optic to the laser and shine the fiber optic towards the wall. The fiber optic should form a perfect circle. If there is not a perfect circle, you must polish the fiber optic using sandpaper. First, use black sand paper (small figure 8 patterns), then brown sand paper (slightly larger figure 8 patterns), and finally, pink sand paper (largest figure 8 patterns).
4. Continue testing the fiber optic and polishing the fiber optic until it produces a clear circular pattern.

5. Test the power of the laser for each fiber optic to reach 10.5-11 mW and record the setting for each fiber optic.
6. The fiber optic will be inserted into the beveled capillary tube (from Step 1) after it has been lowered into the brain's surface with the carbon fiber electrode that it is attached to (approximately 2,500 microns from the brain's surface). **Note:** This would be the same as lowering the electrode into the brain as reported in Step 5, Numbers 17-18.

Step 5: Surgery and Recording

This section explains the craniotomy to open a cranial window over the region that our lab records from, the CeA. The surgery allows for a cranial window that an electrode will be lowered into to record from the specific region of interest with the above optrodes. All mice used in our experiments are female mice between the ages of 9-13 weeks. They weigh approximately 20 grams each. **Figure 3** shows an overview of the surgical set-up.

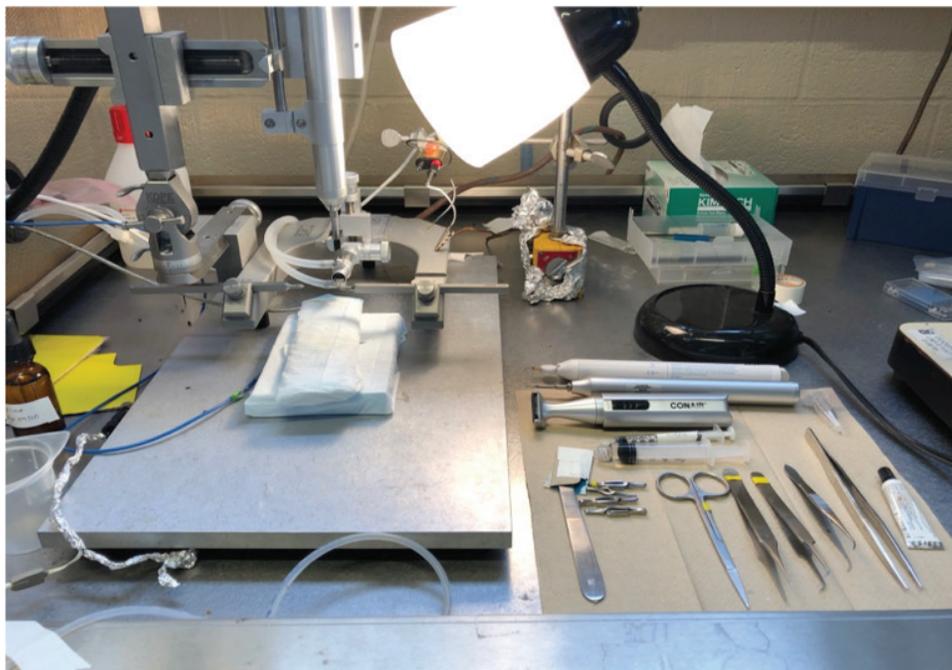


Figure 3: Overview of Surgical Set-Up.

1. Turn on heating pad, temperature monitor and the Kopf Microdrive so that the heating pad can heat prior to the beginning of surgery. It is vital to monitor the mouse's temperature (approximately 36-37°C) throughout surgery. The mouse's temperature is monitored via the WPI heating pad and temperature monitor. The heating pad is set at 40°C and the temperature monitor is placed underneath the mouse's skin through a small incision made in its back with a pair of surgical scissors.
2. Knock out the mouse in a knock out container with isoflurane.
3. Transfer and secure the mouse in the Kopf stereotaxic frame. The isoflurane is set at 2% with the oxygen flow rate set at 1. The mouse's teeth are secured in the bite bar and the ear bars are tightened, so that they sit right in front of the mouse's ears (ie their cheek bones). The nose cone is tightened.
4. Shave mouse's head with the electric razor.
5. Make an incision on the top of the mouse's head to expose the skull. The incision should be made from between the mouse's eyes to the back of the skull.
6. Apply Awka Tears to the eyes of the mouse. Use buffalo clamps to pull the incision from step 5 open (**Figure 4**). The suture lines should be visible, including bregma and lambda.
7. Use Q-tips to clean the surface of the skull.
8. Using a pulled glass pipette (an old, used electrode) the skull is leveled for the region of interest with the stereotaxic frame. Using the Allen Brain Atlas, identify the amygdala coordinates. Bregma and lambda can only be different by <10 microns.
9. Mark the AP and lateral amygdala location on the skull with a Sharpie marker.
10. Drill around the Sharpie dot in a donut shape. It is important to drill slowly because you don't want to drill into the brain and cause damage. Once the skull is thin enough, use the syringe with a needle to slice around the drill marks. Once the skull is sliced through, one side of the donut hole can be lifted with the needle. Forceps can be used to remove this skull flap (**Figure 5**).
11. Use small cotton pieces to stop the bleeding.
12. Use the needle to remove the dura from the surface of the brain. Use small cotton pieces to stop the bleeding. If bleeding does not subside, you can use a cauterizer around the edges of the cranial window.



Figure 4: Skull incision being pulled apart with buffalo clamps to make sutures visible

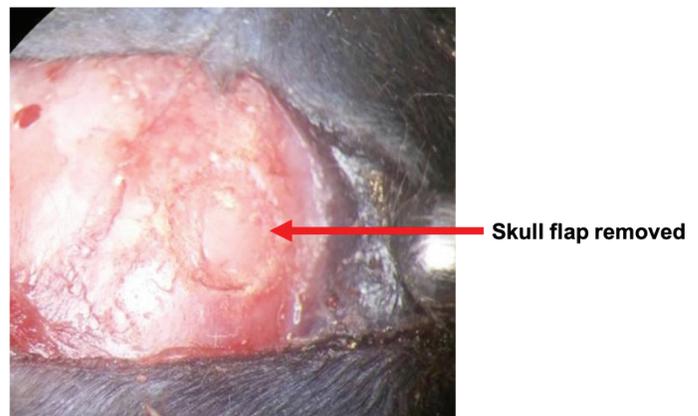


Figure 5: Skull flap removed to expose cranial window.

13. Once the bleeding is controlled, mineral oil should be applied to the surface of the brain.
14. A 24-gauge catheter coated in surgilube is inserted into the ureter of the mouse.
15. Isoflurane is turned down from 2% in 0.125% intervals every ten minutes until the isoflurane is at 0.875%. This is so that recording takes place under low doses of isoflurane.
16. An electrode is connected to the ISO-180 amplifier and the ground is connected to one of the buffalo clamps.
17. Lower the electrode slowly to the brain's surface using the stereotaxic frame and the microscope.
18. When the electrode is at the brain's surface, all of the plugs should be unplugged, and the lights should be turned off to reduce noise.
19. The Kopf Microdrive can be used to lower the electrode into the brain. The burst is set at 2 microns and the run is set at 100 microns for our experiments. The electrode is lowered to the appropriate amygdala depth coordinates (about 3,800-4,000 microns from the brain's surface).
20. In the region of interest, search for neurons. Using Spike 2, the wave pattern of the neuron can be viewed. A neuron with a strong action potential can be monitored for 5 minutes. Following the 5 minutes, a test bladder distention set is performed. This is 15-60 mmHg urinary bladder distentions with compressed air. Distentions have a 20 second pre-distention phase, 20 second distention at 60 mmHg, followed by a 1-minute post-distention phase.
21. If the neuron is responsive (ie. inhibited or excited), a full distention set can be completed. If the neuron does not respond, continue to search for a neuron that does respond to painful stimuli (ie. urinary bladder distention). Once a responsive neuron is found, initiate light activation of optrode. For excitatory optogenetics, you should see a firing rate during light pulsing that is similar to the frequency of light stimulation (an increase in firing).
22. At the conclusion of recording, 50 microliters of Euthasol is administered. Once the mouse is non-responsive, the marker switch on the ISO-180. The region of the brain that was recorded from is lesioned for 3 minutes at 20 V.
23. The brain is removed from the mouse following euthanasia. It is stored in 4% paraformaldehyde for 48 hours and then moved to 20% sucrose for 3-5 days. The brain is frozen and H&E staining is completed to verify the recording site.

Concluding Remarks

Optrodes are a valuable tool that one can use to explore a neuron's electrical properties, while also providing the ability to simultaneously manipulate specific cell populations through the use of light (Haubensak W et al., 2010; Sadler KE et al., 2017). This technique allows for scientists to have a better understanding of the mechanism used by cells that play differing roles in various neurological and pain conditions.

References

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Materials

Electrode Production

Gold Connectors (Male Connector Pins from *A-M Systems*, Cat #: 520200; Lot #: 559310)

Silver wire (*Warner Instruments*, AG8W;

Cat #: 64-1318)

Carbon Fibers (T0W 1M7-12K)

Creased piece of standard printer paper

Silver Conducting Paint (*Ladd Research*, ZJP067)

Kwik-Fil Capillary Tubes (*World Precision Instruments*; Borosilicate Glass Capillary Tubes #TW150-4)

Vacuum line or vacuum pump

Tubing

Epoxy (*Gorilla Glue Brand Epoxy*)

Pipette puller (e.g. vertical – *Kopf Instruments*; Model 730; horizontal – *Sutter Instrument Company*; Model P-97)

Saline (RMBI, Cat #: Z1376)

Basic compound light microscope (*Meyer Instruments*; Leica CME)

Current Supply (*Staco Energy Products*, Type 2PF1010; Output: 0-120/140V, 10A, 1.4 KVA)

Note: “12 Amp” fuse is replaced with a 1¼ Amp fuse to reduce danger.

3-D manipulator (*Narishige* Cat #: 2077)

Kite Manual Micromanipulator without base (*World Precision Instruments*; KITE-R)

Electrode Storage Jar (*World Precision Instruments*, Cat #: E210)

Optrode Production

Kwik-Fil Capillary Tubes (*World Precision Instruments*; Borosilicate Glass Capillary Tubes Cat #: 1B100F-4)

Kevlar Fiber Sleeve (*Thor Labs*; Cat #: FT030)

Kevlar Scissors

Connectors Set (*Thor Labs*; Cat #: 30126G2-230)

Compressed Air

Isopropyl Alcohol (*Miscellaneous*)

Diamond Pen (*Thor Labs*; Cat #: S90W)

Fiber Optic (*Thor Labs*; Cat #: BFL37-200)

Cable Stripper (*Thor Labs*; Cat #: T12S18)

Syringe for epoxy application (*Thor Labs*; Cat #: MS403-10)

Crimper (*Thor Labs*; Cat #: CT042)

Black, brown, and pink sand paper (*Thor Labs*; Cat #: LFG03P, LFG1P, LFG3P, and LFG5P)

473 nm DPSS laser (*Shanghai Laser and Optics Century Company*; Cat #: BL473T3)

Laser Power Supply (*Shanghai Laser and Optics Century Company*; Cat #: ADR-700A)

Surgery and In vivo Extracellular Recording

Surgical Tools

3 pairs of forceps (1 blunt end pair,

1 sharp end pair and 1 curved sharp pair)

1 pair of scissors

4 buffalo clamps

Scalpel with a blade

Q-Tips

Cotton Balls

Electric Razor (*Conair*, Cat #: NE163N)

Heating Pad/Temperature Monitor (*World Precision Instruments*, Cat #: ATC-2000)

24 gauge IV catheter (*BD Insye-N*)

Surgical Lube (*Savage Laboratories*, Cat #: J760)

Stereotaxic Frame (*Kopf Instruments Model 963, 923-B, 922*)

Isoflurane Vaporizer (*Drager*)

Oxygen Tank (*Airgas*) and tubing

1cc syringe with needle

Mineral Oil (*Sigma*, Cat #: M5904)

Akwa Tears (*Akorn*)

Ideal Microdrill (*Cell Point Scientific*,
Cat #: 4458)

Bovie High Temperature Cauterizer (*Bovie*, Cat #: DEL1)

Kopf Microdrive (*Kopf Instruments*, Model 2660)

ISO-180 Signal Amplifier (*World Precision Instruments*)

Micro3 1401 Analog/Digital Converter (*Cambridge Electronic Design*)

Spike 2 Software (*Cambridge Electronic Design*, Version 8.03)