

Survivable Stereotaxic Surgery and *In Vivo* Microdialysis in Rodents

Brenda M. Geiger, Lauren E. Frank, Angela D. Caldera-Siu, Emmanuel N. Pothos

Tufts University School of Medicine

Department of Pharmacology and Experimental Therapeutics

Program in Neuroscience

Materials Needed:

Ketamine and Xylazine

Heat Sterilizer

Electric Shaver

Povidine/Iodine

Cotton swabs

Scalpel

2 forceps

5% Ethanol

Self-tapping screws

Drill and drill bit

Rat guide cannula (from Bioanalytical Systems, Inc. or other suppliers)

Rat or mouse microdialysis probe (from Bioanalytical Systems, Inc. or other suppliers)

Anchor screws

Dental Cement powder and mix

Spatula

Bacitracin

Needle and 1cc syringe

Saline

Penicillin

Microdialysis Cage (from Instech Inc. or other suppliers)

Microdialysis Swivel (from Instech Inc. or other suppliers)

Microdialysis Pump

Gas-Tight Syringe

Summary

Two-month old average age C57BL/6J mice or equivalent or three-month old average age Sprague Dawley rats or equivalent are anesthetized with ketamine (60 mg/kg i.p. for rats; 100 mg/kg i.p. for mice) and xylazine (10 mg/kg, i.p. for either species). Sedation is monitored by withdrawal reflex from a tail and toe pinch, corneal reflex, and lack of spontaneous movement. Thermoregulation can be provided through a thermostat-regulated heating pad (ALA Instruments Inc.) and monitored through a rectal thermometer. Head is shaved of fur and cleaned with iodine before incision. After skin incision (2 cm long for rats, 1 cm long for mice) and removal of all soft tissue from the surface of the skull, placement of the guide cannula is determined in relation to bregma. A 6 mm hole is drilled through the skull with a battery-operated driller designed for rodent surgery (Fine Science Tools, Inc.). Care is taken so that the drill bit does not penetrate through meningeal membranes or blood vessels. Skull is implanted with bilateral 5 mm 21 gauge stainless steel guide shafts leading to the posterior nucleus

accumbens, dorsal striatum or prefrontal cortex. The stereotaxic coordinates are established as per Franklin and Paxinos, 1997 (The Mouse Brain in Stereotaxic Coordinates, Academic Press) or Paxinos and Watson, 2006 (The Rat Brain in Stereotaxic Coordinates, Academic Press). Implants are secured by dental cement. A bolus of Lactated Ringers of the 0.9% saline is given at the end of surgery (5mls SC in rats and 1 ml SC in mice after fluids are warmed to normal body temperature) to prevent dehydration. Buprenorphine (0.1-0.5mg/kg SC) is administered twice daily and then on an as needed basis, if animal appears to be in pain. Local antibiotic treatment (bacitracin ointment) and systemic antibiotic treatment (penicillin 100,000 IU/kg IM every 12 hours for the first 48 hours post-op) are administered if post-operative infections occur.

Following surgery animals are individually housed with food and water available *ad libitum*. At least one week is allowed for recovery before microdialysis and euthanasia. Following recovery from surgery the animals are transferred to a microdialysis cage and microdialysis probes are inserted and cemented in the guide shafts that have been installed during surgery. Probe insertion does not cause pain or discomfort because the probe is bypassing skin, muscle and meningeal tissue through the guide shaft. Therefore, probe insertion is done without anesthesia and any anesthesia-induced effects on neurochemistry or behavior are avoided. We let the probes stabilize for 12 hours and then we start sampling every 30 minutes for another 8-12 hours depending on the experiment. We monitor locomotor behavior of the animal through photocells or manual recording of movement by the experimenter. Microdialysate samples are injected into a High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC) instrument for neurochemical detection and analysis. We look for effects on basal neurochemistry and locomotor behavior. At the end of the experiment the animal is euthanized by an overdose of systemic ketamine (200 mg/kg i.p.) and xylazine (20 mg/kg, i.p.). Then the heart is perfused with 0.9% saline followed by 4% paraformaldehyde. The brains are removed, frozen and cut along the microdialysis probe tract to verify accurate probe placement.

Procedure:

Set up the stereotaxic instrument and all the materials needed. Make sure the area and instruments are cleaned and sterilized.

Shave off fur with electric razor. Go from the ears to just in-between the eyes, move razor in different directions to effectively clean area of fur. Apply povidine/iodine to shaved area but protect the eyes from it.

Mount the animal onto the stereotaxic apparatus by placing the ear bars into the ear canal and tightening into place. First mount one ear bar in the ear canal, and then hold it in place and slide in the other ear bar. You know you are in the correct location when the head can no longer be moved side to side. Secure the mouth with the anterior mount of the stereotaxic and make sure that the head is level with a ruler. Put the ruler in a vertical position with respect to the stereotaxic instrument platform and check for a 90° angle

between the ruler and the middle of the animal's scalp). Confirm this through the stereotaxic instrument if it offers such capability.

Make an anterior/posterior incision on the scalp with a sterile scalpel extending from the lambda to just in-between the eyes of the animal. Use hemostats to pinch off the skin and keep the incision open. Using several cotton swabs dry off the exposed skull surface.

Put the guide cannula onto its mount, find bregma on the skull, and position the guide cannula right over this location. Write down the anterior/posterior and lateral coordinates. From bregma find the correct coordinates needed for the placement of your probe with the aid of the stereotaxic atlas. Position the guide cannula to the correct coordinates by adding or subtracting from bregma. Bring your guide cannula down until it is touching the skull, and then record this ventral coordinate. Make a pencil mark at this location on the skull; this is where you will be drilling.

Remove the guide cannula and sterilize your drill bit. Carefully drill a hole at the pencil mark until you get through the width of the skull. Check with the guide cannula to see if it would clear the hole without touching the sides. Keep drilling and checking until the cannula can clear in a straight path. Once the hole is made, use a sterile needle to gently punch the meninges in order to allow unobstructed insertion of the cannula.

Next, using a hand drill, make six hole for skull screws, two anterior to the cannula hole, two lateral to the cannula hole and two posterior to the sides. Sterilize six screws and place them onto the skull until they are tightly anchored on.

Clean the guide cannula with ethanol and saline, mount, and lower it slowly to the proper ventral coordinate. Make sure that the sides are not touching and that it is going in perfectly vertical.

Place the anchor screw medially and behind the posterior skull screws and hold it in place with tweezers. Mix a thin batch of liquid dental cement and cover the guide cannula, screws, and the rest of the skull with a spatula. Make another batch, thicker this time, and completely cover the area and the cannula and anchor screw enough to secure it.

As the cement becomes thicker and before it solidifies, separate the skin from the cement cup and mold the cement cup with the spatula to make sure the cement cap is smooth all around and does not irritate the skin later.

Allow the dental cement to completely dry before removing the animal from the apparatus. Remove the hemostats. Apply bacitracin all the way around the cement cap.

Once the animal is off the stereotaxic instrument inject it with 1 ml of penicillin IM (intra-muscular) followed by 1 ml of saline SC (subcutaneously).

Place the animal in its own cage and monitor it until it become conscious before returning it to its room to recover.

Animals are monitored until they recover from anesthesia on the day of surgery and daily post-op until the end of the experiment for signs of infection and evaluation of pain/distress. This includes weekends and holidays. Low spontaneous movement, distress vocalization upon handling, hunched posture, diarrhea, swelling and discharge in the area of the headmount and lack of feeding/drinking are all signs of pain and distress. Buprenorphine (0.1-0.5mg/kg SC) is administered twice daily and then on an as needed basis, if animal appears to be in pain. Local antibiotic treatment (bacitracin ointment) and systemic antibiotic treatment (penicillin 100,000 IU/kg IM every 12 hours for the first 48 hours post-op) are administered if post-operative infections occur. If any of these symptoms persist following administration of buprenorphine and supplemental fluid and antibiotic treatment within 12 hours of surgery, the animal is euthanized.

Microdialysis

Following 1-week recovery from surgery, a concentric microdialysis probe is implanted into the guide cannula 15 hours prior to the start of the microdialysis session. A spring connecting the microdialysis swivel to the animal's head cap is mounted via an alligator clip on the support anchor screw. Artificial cerebrospinal fluid (ACSF; 149 mM NaCl, 1 mM NaH₂PO₄, 3 mM KCl, 1 mM MgCl₂ and 1.4 mM CaCl₂, pH 7.4) is pumped through the probe at 0.5µl/min overnight and 1 µl/min during sampling via a gas-tight syringe mounted on a microdialysis pump. Samples of the interstitial fluid are taken every 30 minutes and analyzed using HPLC-EC until the end of the experiment.

At the end of the experiment the animal is euthanized by an overdose of systemic ketamine (200 mg/kg i.p.) and xylazine (20 mg/kg, i.p.). Then the heart is perfused with 0.9% saline followed by 4% paraformaldehyde. The brains are removed, frozen and cut along the microdialysis probe tract to verify accurate probe placement.