Video Article

Survivable Stereotaxic Surgery in Rodents

Brenda M. Geiger[1], Lauren E. Frank[1], Angela D. Caldera-Siu[1], Emmanuel N. Pothos[1]

[1]Department of Pharmacology and Experimental Therapeutics, Tufts University

Correspondence to: Emmanuel N. Pothos at Emmanuel.Pothos@tufts.edu

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Abstract

The ability to measure extracellular basal levels of neurotransmitters in the brain of awake animals allows for the determination of effects of different systemic challenges (pharmacological or physiological) to the CNS. For example, one can directly measure how the animal's midbrain dopamine projections respond to dopamine-releasing drugs like d-amphetamine or natural stimuli like food. In this video, we show you how to implant guide cannulas targeting specific sites in the rat brain, how to insert and implant a microdialysis probe and how to use high performance liquid chromatography coupled with electrochemical detection (HPLC-EC) to measure extracellular levels of oxidizable neurotransmitters and metabolites. Local precise introduction of drugs through the microdialysis probe allows for refined work on site specificity in a compound s mechanism of action. This technique has excellent anatomical and chemical resolution but only modest time resolution as microdialysis samples are usually processed every 20-30 minutes to ensure detectable neurotransmitter levels. Complementary ex vivo tools (i.e., slice and cell culture electrophysiology) can assist with monitoring real-time neurotransmission.

Video Link

The video component of this article can be found at https://www.jove.com/video/880/

Protocol

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 using a gentle toe pinch withdraw reflex demonstrated in Walantus et al.(JoVE, 6, 2007) and Szot et al.(JoVE, 9, 2007). Thermoregulation can be provided through a thermostatregulated 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

1. Set up the stereotaxic instrument and all the materials needed. Make sure the area and instruments are cleaned and sterilized.
2. 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.
3. 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.
4. 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 sterilized hemostats to pinch off the skin and keep the incision open. Using several sterile cotton swabs, dry off the exposed skull surface.
5. 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 with a sterile pencil at this location on the skull; this is where you will be drilling.
6. 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.
7. Next, using a hand drill, make six holes 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.
8. 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.
9. 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 sterile spatula. Make another batch, thicker this time, and completely cover the area and the cannula and anchor screw enough to secure it.
10. 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.
11. 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.
12. Once the animal is off the stereotaxic instrument, inject it with 0.25 ml of penicillin IM (intra-muscular) followed by 1 ml of saline SC (subcutaneously).
13. Place the animal in its own cage and monitor it until it becomes conscious before returning it to its room to recover.
14. Monitor animals 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, supplemental fluid, and antibiotic treatment within 12 hours of surgery, the animal is euthanized.

Discussion

In vivo microdialysis is the tool of choice for measuring multiple neurotransmitters and metabolites in distinct brain sites of a living animal. However, it only monitors extracellular levels of neurochemicals and it does not offer the time resolution to monitor neurotransmitter exocytosis in real time. Through a version of the technique called "net-flux", the actual neurotransmitter concentration at a given site can be calculated, which in turn can give accurate measurements of neurotransmitter rate of reuptake through plasma membrane transporters.

Microdialysis is ideal in illustrating differences in basal extracellular neurotransmitter levels between different groups of animals (i.e. different genotypes) and in deciphering the effects of drugs or other manipulations on neurotransmitter release.

The introduction of assays alternative to HPLC-EC like capillary zone electrophoresis (CZE) coupled with fluorescent detection has increased the time resolution of in vivo microdialysis within a few minutes per sample.

Disclosures

The authors have nothing to disclose.

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