Sep 15, 2022

Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice

Mark Bevan[1]

[1]Northwestern university

dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

divya.darwinarulseeli

ABSTRACT

Step by step protocol for headplate surgery for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake mice

DOI

dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

PROTOCOL CITATION

Mark Bevan 2022. Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice. **protocols.io** https://protocols.io/view/headplate-surgery-protocol-for-in-vivo-electrophys-cgputvnw

CREATED

Sep 15, 2022

LAST MODIFIED

Sep 15, 2022

PROTOCOL INTEGER ID

70100

MATERIALS TEXT

All animal procedures follow the policies of the National Institutes of Health and have been approved by the Institutional Animal Care and Use Committee of Northwestern University.

Reagents

•Alcohol prep pads
•Bupivacaine
•Filtered HEPES-buffered synthetic interstitial fluid (HBS)
•Isoflurane
•Ketamine
•Kwik-Sil silicone adhesive
•Meloxicam
•Metabond adhesive luting cement
•Nolvasan surgical scrub
•Ocular lubricant
•Povidone-iodine prep pads

Instruments and Materials

•Anesthetic induction chamber
•Autoclaved fine science surgical tools, including scalpel, scissors, forceps
•Cotton tipped applicators
•Dry glass bead sterilizer
•Epidural reference electrode
•Headplate holder
•Laboratory scale
•Lightweight stainless-steel head plate
•Micromotor drill
•Micro precision screwdriver
•Needles and syringes
•Small animal anesthesia machine
•Surgical cellulose eye spears
•Surgical drape
•Stereotaxic frame and instruments for mice
•Surgical clippers for rodents
•Thermal heating pad
•Upright microscope

Surgical preparation

1 Before and after any procedures, spray down working lab surfaces and equipment with 5% Nolvasan to disinfect the surgical area.

2 Autoclave all surgical instruments and ensure that sterility is maintained throughout the surgery. If multiple surgeries are required, a micro bead sterilizer can be used between procedures.

Citation: Mark Bevan Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice https://dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

3 Ensure at least 1 mL of filtered HEPES-buffered synthetic interstitial fluid (HBS) has been thawed. Fill sterile syringe with HBS, label, and store on ice.

Anesthesia induction and placement into stereotaxic frame

4 Weigh the mouse and record its weight.

5 Place the mouse in an anesthetic induction chamber and anesthetize with 3-4% isoflurane.

6 Once the mouse is immobile, remove from the induction chamber and perform an intraperitoneal injection of ketamine (100mg/kg in 0.9% saline solution). If mouse regains mobility during or after this process,place back into induction chamber until the animal is fully anesthetized.

7 Using a rodent trimmer, shave the fur off the dorsal surface of the head from approximately the back of the neck to the back of the eyes.

8 Apply ophthalmic ointment to the animal's eyes to prevent corneal drying.

9 Transfer the anesthetized mouse to the stereotaxic apparatus and place on a thermal heating pad (set to 37 °C) covered in a sterile surgical drape. Firmly secure and fix the animal's head in the stereotaxic frame with ear bars. If done properly, movement of the animal's head should be restricted in the lateral axis. Next, secure the mouth in the incisor adaptor of the jaw cuff holder taking care that the tongue is not pinched or blocking the airway. Gently slide the mouse's nose into the nose cone connected to the isoflurane anesthesia system.

10 Follow with 1-2% isoflurane for anesthesia maintenance during the surgery. Frequently monitor the mouse to ensure consistent levels of anesthesia throughout surgery. Depth of anesthesia can be assessed by toe pinch throughout the procedure.

Craniotomy

11 Disinfect the surgery area by wiping the skin over the scalp with three alternative wipes of 10% povidone-iodine solution and 70% isopropyl alcohol. Use each antiseptic prep pad only once.

12 Provide local anesthetic by injecting bupivacaine (2mg/kg in 9% saline solution)

Citation: Mark Bevan Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice https://dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

subcutaneously under the scalp. Wait 5 min for the drug to be absorbed into the tissue.

13 Use forceps to gently apply a toe pinch to ensure that the animal remains areflexic and properly anesthetized.

14 Lift up the skin near the midline of the scalp with forceps. Make a small horizontal incision with fine surgical scissors to remove the skin over the top of the skull. The cranial suture points between bregma and lambda should be exposed. Trim the skin further if the surgical area needs to be extended. Using cotton tipped applicators, push the remaining skin aside so that the skull becomes visible.

15 Using a cotton swab, rub the surface of the skull to remove the periosteum. Use HBS to wash the site. If bleeding occurs during this or subsequent steps, place a surgical cellulose eye spear over the area to absorb the blood while gently applying pressure.

16 Locate bregma and lambda points on the skull surface. Align the z-coordinates of both bregma and lambda to ensure that the head of the animal is leveled and “flat skull” position is achieved. A Hamilton syringe with a sterile injection needle can be mounted directly onto the stereotaxic arm to facilitate this step. Head positioning can be realigned by adjusting the nose bar.

17 When the mouse is correctly positioned, use stereotaxic coordinates to locate your target position for in vivo recording and using a sterile needle or scalpel blade make a small indentation on the surface of the skull. This will be used to mark the location for drilling. Make a separate small indentation on the surface of the skull directly above the cerebellum. This site will be used to mark the drilling location for the epidural reference electrode.

18 Using a handheld micromotor drill carefully drill burr holes in the skull at the marked locations identified for in vivo recording and reference electrode implantation. Dedicated drill bits have been labelled and calibrated to generate the appropriate dimensions for each of these craniotomies. Be careful while applying pressure, as drilling through the bone will likely cause damage to the brain parenchyma. Stop when almost through the bone, use a bent injection needle to remove the thinned layer of bone tissue and open the burr hole. Use HBS to wash the site and irrigate the burr holes.

Headplate implantation

19 19. To enhance the bonding of the dental cement to the skull, gently score the surface of the skull with a handheld drill. During this process, ensure that the drill does not penetrate through the skull into the brain.

20 Irrigate the surface of the brain with HBS to wash away any blood or debris.

Citation: Mark Bevan Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice https://dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

21 Draw away excess HBS from the reference electrode craniotomy. Using a bent injection needle carefully remove the dura within the craniotomy site. Removal of the dura requires extreme care and patience to avoid unnecessary complications. When performed correctly the brain will appear to be unblemished with distinct blood vessels.

22 Using fine point forceps firmly grasp the reference electrode by holding onto its stainless-steel neck and hold in place above the craniotomy site. Using a micro precision screwdriver thread and secure the reference electrode into the burr hole. Fix in place carefully so that the bottom surface of the reference electrode is in direct contact with the surface of the cerebellar cortex without creating tissue damage.

23 Use surgical cellulose eye spears to ensure that the surface of the skull is completely dry and devoid of debris.

24 Draw up Kwik-Sil silicone sealant with a transfer pipette and apply a thin layer directly over bregma and the in vivo recording craniotomy. This will retain a stereotaxic reference point on the skull and prevent the dental cement from penetrating under the skull. Wait for 1-2 min for the Kwik-Sil to cure.

25 A custom-designed stainless-steel headplate (2.5 mm length x 1 mm width x 0.1 mm thickness) with a 1 mm × 0.6 mm oval opening is used for head-fixation procedures. Attach the headplate to the headplate holder and mount onto a stereotaxic arm. Use the horizontal and vertical axes of the stereotaxic frame to ensure that the headplate is orientated in a level plane.

26 Lower the headplate holder so that it is positioned 5-10 mm above the skull. Center the headplate directly over the skull, ensuring that it doesn't collide with the reference electrode.

27 Apply Metabond dental cement around the edge of the exposed skull to create a base for the headplate. Wait for 5-10 min for the dental cement to dry and harden.

28 Lower the headplate holder so that the headplate is positioned directly over the surface of the skull. Ensure that the in vivo craniotomy is directly within the headplate window. Apply dental cement around the skull, base, and reference electrode, creating a mount covering the skull. Continue to fill the opening space between the skull and the headplate with dental cement leaving only the craniotomy area exposed. Wait for 5-10 min for the dental cement to dry and harden. It is important that the dental cement dry out completely before removing the mouse from the stereotaxic apparatus.

Citation: Mark Bevan Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice https://dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1

29 Detach the headplate (now affixed to the skull) from the headplate holder.

30 Perform a subcutaneous injection of meloxicam (20mg/kg in 0.9% saline solution).

31 Gently remove the mouse from the stereotaxic apparatus and return to its home cage.

32 Place the home cage on an electric heating pad to keep the mouse warm until it is awake and ambulating.

33 Allow 72 hours for the animal to recover and acclimate to headplate fixation before conducting any experimental procedures.

Citation: Mark Bevan Headplate surgery protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice https://dx.doi.org/10.17504/protocols.io.bp2l694z5lqe/v1