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New Techniques in Stereotaxic Surgery and Anesthesia in the Mouse

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MESSIER, C., S. ÉMOND AND K. ETHIER. *New techniques in stereotaxic surgery and anesthesia in the mouse.* PHARMACOL BIOCHEM BEHAV **63**(2) 313–318, 1999.—Mice are used in increasing numbers in neuroscience research. This increase is linked to the availability of numerous pure genetic lines and the advent of transgenic animals. Many neuroscience techniques can be used in the mouse with success, including stereotaxic placement of cannulae and electrodes. With the recent publication of a mouse brain atlas by Franklin and Paxinos and improvements in surgical procedures for the mouse, stereotaxic surgery in mice can be performed routinely and with accuracy. In the present article, we describe techniques and apparatuses for the surgical implantation of cannulae in the mouse brain. We also present new developments in anesthesia, pain management, and postoperative care that improve survival and recovery times of mice. Using these new techniques, we have gained shorter training time for students, lower mortality rates following surgery, and faster recovery. © 1999 Elsevier Science Inc .

Acetaminophen Cannula Isoflurane Methoxyflurane Mouse Sodium Pentobarbital Stereotaxic instrument Surgery

THE mouse as an experimental animal has been used extensively in numerous applications. The number of pure genetic lines available and the advent of transgenic animals have made the mouse model a very useful tool to understand several human diseases and functions of the brain. Many techniques and surgical practices used for mice are adaptations of procedures developed for the rat. Although many reports describe stereotaxic techniques for the rat, very little has been published for the mouse (3,14,28,30,35). Two brain mouse atlases have been published more than 20 years ago and are now out of print (18,31). With the recent publication by Franklin and Paxinos of a new and more complete stereotaxic brain atlas for the mouse (11), it was appropriate to present new alternatives and updates for the stereotaxic techniques and general surgical practice for the mouse. After using a variant of the Slotnick and Leonard stereotaxic system, we have turned to the new Kopf mouse adaptor for stereotaxic surgery. The Franklin and Paxinos atlas uses the Kopf mouse adaptor, and this adaptor provides the best adjustment systems for flat skull surface coordinates that this atlas uses. In the present article, we describe this new apparatus as well as an update on pain management, anesthesia and postoperative care that are consistent with new standards required by many ethics committees and national bodies regulating animal research.

Apparatus

Most researchers have used either modified commercial stereotaxic instruments or custom-designed ones (3,30,35). Recently, a new adaptor for mouse stereotaxic surgery has been commercially offered (Model 921, David Kopf Instruments, Tujunga, CA: see Fig. 1). This adaptor, which uses temporal bone cup holders instead of the traditional ear bars, has many advantages. It facilitates head placement and significantly reduces the time taken to master the placement technique. It also eliminates the problem of bone damage often produced by ear bars (even blunted ones) in mice. Finally, it is much less painful for the animal, so that lighter planes of anesthesia can be used and recovery from anesthesia can be shortened. The only drawback of the Kopf holder is its cost (around 800 US$).

We use this adaptor in conjunction with an electrode manipulator that has vertical, lateral, and anterior–posterior (A-P) screw-driven scales with a 10-micron resolution and a universal joint for angular settings (Model 1760-761, David Kopf

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Instruments). We mounted the stereotaxic instrument on a 30 × 38 cm Plexiglas rotating table set on ball bearings to allow easy access to the animal from all directions. This equipment allows for excellent precision of either electrode or cannula placements.

Although cannula systems for mice can be purchased commercially (Plastic One, Inc., Roanoke, VA), we use custom-made cannulae made of precut tubing (Small Parts, Inc., Miami Lakes, FL). The guide cannula consists of 8-mm 26-G stainless steel tubing (HTXX-26, Small Parts, Inc.) cut by the manufacturer. The edges of the guide cannula are also smoothened (o.d. chamfered) by the manufacturer. The guide cannula is stereotaxically implanted and fixed to the skull using jeweler screws (screw size: 0.65 mm; head: 1.2 mm; ref: 5125FE68, SARL FOM 2000, Morteau, cedex France). These very small screws minimize damage to underlying cortical structures. For more precise placements, it is also possible to use smaller stainless steel guide cannulae together with fused silica tubing (Polymicro Technologies, Phoenix AZ).

Injections are made through an injection cannula inserted into the guide cannula. The injection cannula consist of a 19-mm 32-G stainless steel tubing cut and o.d. chamfered by the manufacturer (HTXX-32, Small Parts, Inc.). This 32 G tubing is inserted in a 8 mm 26 G tubing. The 32 G tubing is placed so that it protrudes 8.5 mm from the end of the 26 G tubing. It is then glued in place with ethylcyanoacrylate glue (Krazy glue). Once inserted into an implanted guide cannula, this allows the injection cannula to protrude about 0.5 mm from the lower tip of the guide cannula so that minimal damage is done to the tissue surrounding the injection cannula tip before and during the injection. This injection cannula is attached to a flexible plastic tubing (Tygon tubing; i.d.: 0.01 in., o.d.: 0.03 in.; wall thickness: 0.01 in.; material: S-54HL, Cole-Parmer, USA) and a microliter Hamilton syringe (Model 701N, Hamilton, Reno, NV) to perform the injections. When not in use, the guide cannula is closed, using a stylet consisting of a bent stainless steel 0.009 in. wire (Small Parts, Inc.). All cannula parts are disinfected with alcohol and rinsed with sterile saline immediately before use.

Two lengths of stylet can be used. Either the length of the stylet is identical to the one of the guide cannula (8.0 mm), or the stylet is longer (8.5 mm) than the guide cannula, and protrudes 0.5 mm from the tip of the guide. Using a stylet with the same length as the guide cannula means that the injection cannula will protrude 0.5 mm from the guide cannula and destroy some tissue at the time of injection. One consequence is the possible disruption of neural activity at the time of the injection. However, the solution will be injected in unscarred tissue and beyond the tissue damaged during the stereotaxic implantation of the guide cannula.

If a stylet of the same length as the injection cannula is used at the time of surgery, then the potential disruption of neural activity will be reduced at the time of injection. The potential downside is that scar tissue may impede the spread or diffusion of injected solutions. In the mouse, we have not seen any major behavioral disruption using a stylet with the same length as the guide, even with placements in seizure-susceptible structures such as the hippocampus. However, this does not ensure that no disruption of neural activity took place, and experimenters will have to make a choice based on their individual research protocol.

METHODOLOGY

Clean, Aseptic, or Sterile Procedures

In the past, we have operated using aseptic procedures. When done properly and systematically, the aseptic procedure for implanting cannulae did not lead to any infections. An in-depth discussion of the justification of the use of aseptic surgical practice in rodents can be found in a recent text [(39), pp. 153–157). All metallic surgical tools are placed in a 250-ml beaker filled partially with 97% Ethyl alcohol. Other disinfectant solutions commonly used are 2% cetrimide or 0.5% (w/v) chlorhexidine gluconate in 70% alcohol (Hibitane™). After 5 min of soaking, tools are rinsed with saline and placed on a clean paper towel for drying. Surgical materials are cleaned and disinfected between each surgical procedure (i.e., after each animal). Surgical gloves are replaced after each animal. The goal is to minimize crossinfection of animals during surgery to ensure the best recovery possible. If meticulously applied, no preventive antibiotic treatment is necessary.

Preoperative Medication

The animal's weight is recorded for future reference during the postoperative period. We have the best results with mice that weigh 28 g or more. Preoperative pharmacological treatment include glycopyrrolate (0.01 mg/mouse; Robinul, Wyeth-Ayerst, Montreal, Canada). Glycopyrrolate has the advantage of being more potent than atropine sulfate to prevent the accumulation of salivatory and bronchial secretions (24). For neuroscience studies, it has the added advantage of not crossing the blood–brain barrier (29).

A small dose of sodium pentobarbital (1 mg/mouse; Somnotol, MTC Pharmaceuticals, Cambridge, Ont, Canada) is used as a preoperative medication to facilitate induction. Preoperative analgesia is provided by adding a solution of acetaminophen (80 mg/5 ml; children's Tempra, Mead Johnson) to the drinking water at a dose of 1 ml of Tempra in 100 ml of water (2,26,37). Acetaminophen, also known under the generic name paracetamol, is a nonsteroidal antiinflammatory drug. A review of the use of nonsteroidal antiinflammatory drugs for the relief of pain and effective doses for rats and mice can be found in a recent review (19). Acetaminophen is made available to the mice for 3 days before the operation so that neophobic reaction are decreased. This ensures that the mice start drinking the acetaminophen solution immediately following recovery from anesthesia. The acetaminophen premedication also ensures that tissue levels of the drug are high during and immediately after the operation, thus allowing some immediate analgesia during the time the mice are unable to drink.

TABLE 1
INJECTABLE ANESTHETICS FOR STEREOTAXIC SURGERY IN MICE (9)

Compound	Dose	Duration of Anesthesia	Duration of Loss of Righting Reflex
Hypnorm (fentanyl/fluanisone) + diazepam	0.3 ml/kg IM, 5 mg/kg	45–60 min	2–4h
Ketamine + xylazine	100 mg/kg, 10 mg/kg	20–30 min	2–4hr

TABLE 2
MAC 50: MINIMUM ALVEOLAR CONCENTRATION (4,13,23)

	Methoxyflurane	Isoflurane	Halothane
Rat	0.27	1.38	1.13
Mouse	0.22	1.41	0.95

TABLE 3
ANESTHETIC CONCENTRATION FOR MICE (9)

	Concentration (%)	
Anesthetic	Induction	Maintenance
Halothane	3–4	1–2
Isoflurane	3.5–4.5	1.5–3
Methoxyflurane	3.5	0.4–1

Choice of Anesthetics

Three general options are possible for anesthesia for mouse stereotaxic surgery. The first one is the use of injectable compounds. The major problem with injectable anesthetic in the mouse is that it is impractical to use the intravenous route. Because the intraperitoneal, subcutaneous, or intramuscular route has to be used, this leads to variable absorption rates of the drugs and inconsistent anesthesia. Various injectable coumpounds have been used as anesthetic in the mouse for stereotaxic surgery. The most common ones are pentobarbital or thiopental. Although these agents have been shown to be effective sedative, they are not reliable anesthetics when used alone (6,32,40). The most reliable and effective injectable regimen for stereotaxic surgery in mice are presented in Table 1. A complete discussion of the various merits of these compounds and other regimen can be found elsewhere (8,9,12).

Another issue in the use of injectable is the interstrain variability of efficacy. For example, there are a threefold variation within strain for the duration of unconsciousness produced a standard dose of pentobarbital (20–22). This has a few practical implications. First, it is best to use an agent with a wide safety margin. Second, initial anesthesia trials should be conducted when using an agent for the first time or when changes in strain, sex, age, or supplier are made. The main advantages of injectable agents is low cost and ease of administration. This may be the best option for occasional users. However, when fast recovery and consistent survival is important, inhalation agents should be used.

Three volatile anesthetics can reliably be used with mice: halothane, isoflurane and methoxyflurane. Isoflurane and halothane vaporizers can be readily obtained, and commercial adaptors for the fastening of vaporizer outlet to the stereotaxic table are available (Stoelting, Wood Dale, II). In Table 2, the minimum alveolar concentrations in mice and rats for these anesthetics are presented. Table 3 presents the concentration for induction and maintenance for these agents. Isoflurane produces faster induction and recovery than halothane (7,34). Isoflurane is practically not metabolized and may be preferred in studies involving drug metabolism (5).

Although, it is possible to use vaporized isoflurane or halothane (27,35), we have been using methoxyflurane (Metofane, Janssen Pharmaceutica, North York Ont, Canada), a volatile anesthetic that does not require vaporizer (even though it can be used with one). This is the main advantage of methoxyflurane over halothane and isoflurane. If a vaporizer is available, isoflurane or halothane should be preferred for increased precision and for lower toxicity for the surgeons.

Methoxyflurane is not hepatotoxic for rats or mice for short surgical procedures. However, it is potentially toxic for chronically exposed or susceptible humans (1,16). Three measures can reduce exposure to metoxyflurane during surgery. Inducement is preferably done under a fume hood. The surgery should take place in a well-ventilated environment, and the stereotaxic table should be fitted with a down-draft ventilation scavenger system (see Fig. 1) to prevent inhalation by the surgeon (33). This system should be vented directly outside or to a fume hood. For induction, a few drops of methoxyflurane are placed on cotton balls in the bottom of a 250-ml beaker. The cotton is covered with a piece of loose-fitting cardboard to prevent any direct contact between methoxyflu-

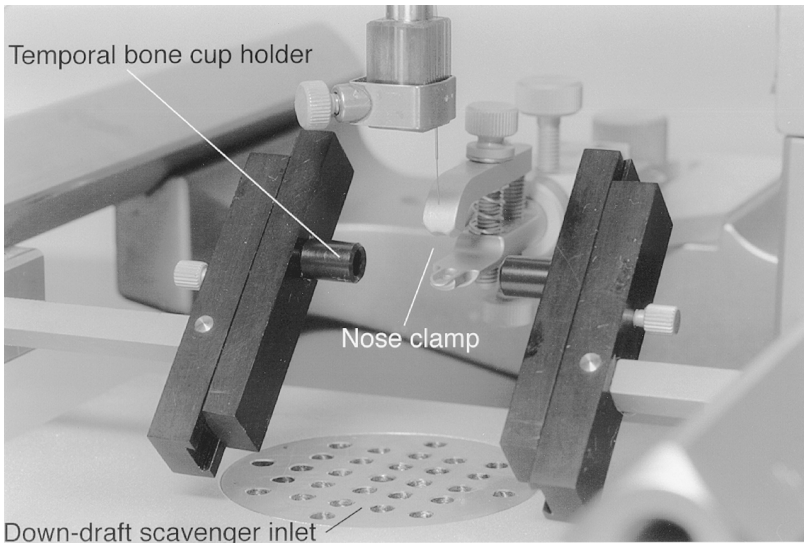

FIG. 1. Stereotaxic table and down draft setup.

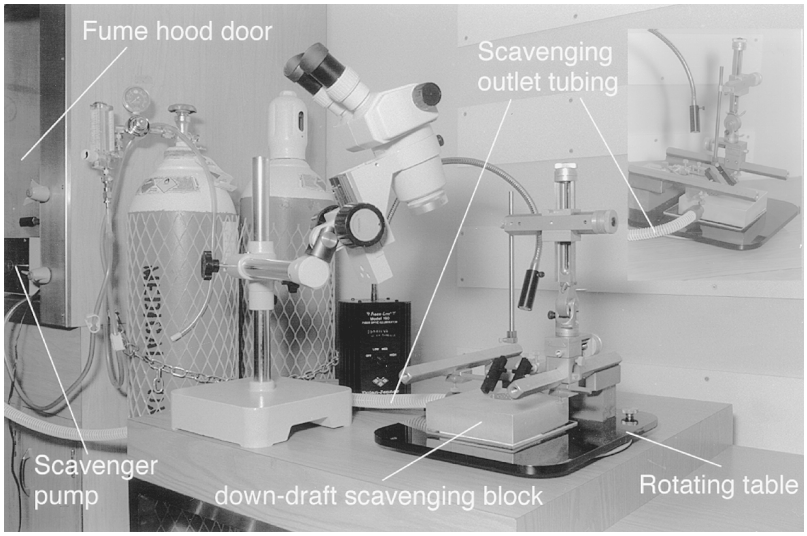

FIG. 2. Kopf mouse adaptor for stereotaxic surgery.

rane and the animal. The animal is placed in the beaker, and a piece of card board is placed on the beaker to prevent the animal from escaping (methoxyflurane is heavier than air and will stay at the bottom of the beaker). Every minute the animal is checked to assess anesthesia until it has reached the required stage. To increase anesthesia during surgery, a cotton-tipped applicator soaked in methoxyflurane is placed in front of (but not touching) the mouse's muzzle. The cotton swab is placed inside a small nose cone to minimize dispersion of anesthetic fumes.

Stereotaxic surgery. The guide cannula to be implanted is held using an injection cannula (attached to a small piece of tubing) that is clamped to the Kopf electrode holder (model 1778). For this use, the injection cannula only protrudes 8 mm from its sleeve so that its tip is flush with the tip of the guide cannula to be implanted: this prevents damage to the targeted nervous tissue during the implantation procedure. A guide cannula is slipped onto this injection cannula. Verticality is verified, and the mouse is placed into the stereotaxic instrument. The mouse is held by an incisor nose clamp and by temporal bone cups (Fig. 1). The temporal bone cups are placed so that they are fastened firmly to the parietal bone of the skull (just in front of the ears). The flatness of the skull is verified by taking measurements with the tip of the cannula holder.

Once the animal is placed in the stereotaxic instrument, a small amount of BNP antibiotic cream (a mixture of Bacitracin, Neomycin and Polymixin; Vetcom, Inc., Upton, Que, Canada) is smeared on its eyes to prevent infection and corneal damage during surgery. Additionally, a small (5 mm^2) piece of paper is placed over the antibiotic cream to provide additional protection from corneal damage (white cornea) produced by the intense light of the fiber optic illuminator (Fiber Lite model 190, Woburn, MA). A strip of soft fabric is placed over the mouse's body and tail to prevent excessive heat loss during surgery. The use of isolation is very important because mice become hypothermic very quickly. Other alternative methods include a thin sheet of Styrofoam under the mouse in the stereotaxic instrument or wrapping the mouse in bubble packing material.

The fur is shaved, and a chlorhexidine gluconate 4% disinfectant solution (Hibitane, Ayerst, Montreal, Canada) is smeared over the shaved area. This is followed by wiping with a 1:1 solution of 70% alcohol and a mixture of 1,5% of chlorhexidine gluconate and 15% of cetrimide (Savlon, Ayerst, Montreal, Canada). A topical anesthetic is applied over the cleared skin to block pain during and after surgery (2% Xylocaine Jelly; Astra Pharma, Mississauga, Ont., Can).

The skin is cut to expose the skull, and the periosteum, the shiny membrane overlying the skull, is scraped with a tartar dental scraper. The skin is retracted using 28-mm serrefine forceps (Model 18050-28, Fine Science Tools, Vancouver B.C., Canada). The skull is dried with a cotton tipped applicator and an industrial electronics air blower (Ungar, model 6966C, Asbestos, Canada). Two holes for the screws are made using a small handheld trephine (high speed twist drills, dia: 0.6 mm, Mascot, West Germany, part No. 73). The 0.65-mm screws are inserted and left to protrude about 1 mm from the skull surface so that the dental cement can flow beneath and around it.

The coordinates of Lambda or Bregma are measured and recorded. If bregma is too low (or too high) relative to lambda, then it is aligned with lambda by raising (or lowering) the nose clamp. (Bregma should not differ from lambda by more than 0.0050 cm.) The height of the skull is measured at 0.2500 cm to the left and to the right of bregma and recorded. If the height of the skull is uneven laterally, the sliding bar of the bone cups can be adjusted.

The cannula holder is then positioned over the appropriate structure. The mouse atlas provides the approximate location of the desired structure, but these coordinates will first have to be verified and adapted for the mouse strain used, because there can be significant genetic variations in the size and location of brain structures (38).

The skull is then trephined to allow the cannula passage using a larger hand-held trephine (flat pivot drills, dia: 0.04 in. Grobet, USA part No. 28.400). This operation is easier if a smaller hole is first made using the small trephine, which is then enlarged using the larger trephine. The dura is then perforated using a small needle, and the measurements for

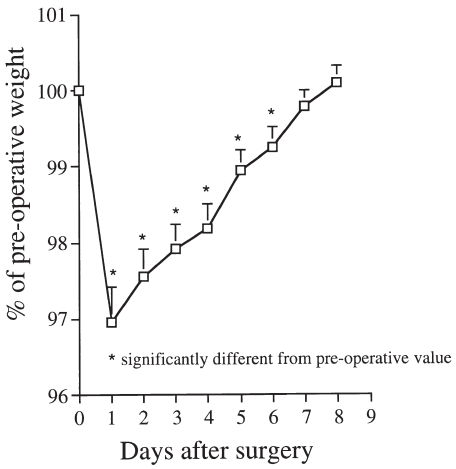

FIG. 3. Percentage of weight loss following cannula implantation surgery (mean ± SEM). Preoperative weight is 100%.

bregma are verified because the skull may have moved when drilling the hole.

The cannula holder is then lowered to the desired depth. The dental acrylic is prepared by mixing Pink Freeflow Powder and Methyl Methacrylate solution (both from Dentsply, York, PA) and used to secure the cannula to the anchor screws and skull. A faster-curing resin is also available (Jet dental repair; Lang Dental Manufacturing, Wheeling IL). Because the uncured dental acrylic is an irritant, it is preferable to avoid contact with the skin of the animal. Finally, the surface of the dental acrylic should be smooth so that, after surgery, the asperities (rough edges or surfaces) do not irritate the inside of the skin or wound the animal's paws as it tries to groom. If this is not done, the animal will groom excessively, and infection will result, shortening the life of the implant.

Postoperative care. The mouse is removed from the stereotaxic instrument and placed in its home cage and covered with a strip of paper to prevent hypothermia during the recovery period. The home cage is placed on a water circulation heating pad (39°C) for half an hour. Then the animal is returned to the holding room. BNP antibiotic is again applied over the mouse's eyes. The mouse receives a 1-ml SC injection of Lactated Ringer's injection to replenish fluids. Sutures are placed ahead and behind the dental acrylic if necessary using 3/8-circle Kalt needles (size 3, Fine Science Tools). The stylet is inserted into the guide cannula to keep it patent.

The animals are usually kept in single cages with nesting material available (Nestlets, AnCare Corp., North Bellmore, NY). This allows the mouse to build a protective nest. This prevents heat loss particularly during the early recovery period.

Postoperative analgesia is provided for 3 days by adding a solution of acetaminophen (80 mg/5 ml; children's Tempra, Mead Johnson) to the drinking water at a dose of 1 ml of Tempra in 100 ml of water. This results in a solution of drinking water containing 0.16 mg/ml of acetaminophen. If animals drink about 5 ml/day, they ingest more than the ED_{50} dose of acetaminophen for low to moderate pain, which is 17 mg/kg (37). Doses of acetaminophen of up to 200 mg/kg can be administered by mixing a more concentrated solution of acetaminophen in water. These doses have been shown to be effective for more severe pain (36). Acetaminophen treatment is prolonged only if obvious signs of pain, marked weight loss, or poor grooming are noted. The length of the acetaminophen treatment was established on the basis of the time during which animals show reduced activity and poorer fur condition in our laboratory. This is also the time period recommended by current veterinary practice (9,10).

If severe pain is suspected in an animal, more effective pain control may be obtained with buprenorphine (a mixed opiate agonist/antagonist at μ-receptors: 0.05–0.1 mg/kg SC every 12 h; Temgesic, Reckitt & Coleman) or butorphanol (kappa and sigma opiate agonist: 1–5 mg/kg SC every 4 h; Torbugesic, Ayerst). We rarely use these drugs in stereotaxic surgery unless obvious signs of pain, marked weight loss, or poor grooming are apparent. These drugs have been shown to be effective and safe for mice (15,17,25,26). Butorphanol, a mixed opiate agonist/antagonist that has little addictive or rewarding properties (25) and is not a controlled substance, could be a more useful drug in some studies.

Recovery. The use of this collection of techniques and practices have allowed us important productivity gains as well as improving overall care and health of mice. We have achieved a postoperative mortality of 2.7%. As shown on Fig. 3, animals recover their preoperative weight around 8 days following surgery. The average weight loss is 3% (less than 1 g in a 33-g mouse). Finally, in one experiment, in which mice carried a cannula for a period of 9 months, we had no instance of implant loss.

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