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IACUC GUIDELINE: ASEPTIC RODENT SURGERY AND POSTOPERATIVE CARE

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Introduction:

The Federal Animal Welfare Act Regulations and the Guide for the Care and Use of Laboratory Animals 8th Edition[1] (*Guide*) both set standards that are obligatory for biomedical research involving live, vertebrate animals. The following guidelines augment statements contained in the Guide and further define rodent surgery standards at UCSB. Failure to abide by the standards contained in this guide may result in a revocation of an individual researcher's privilege to perform surgical procedures on laboratory rodents in a research setting.

I. Surgery Facilities

The specific location of the surgical area within the laboratory or vivarium should promote the proper conduct of sterile techniques, and to the extent possible, it should be isolated from other activities in the laboratory or vivarium. The surgical area should be dedicated for that purpose while surgery is performed.[2]

The area where surgeries are performed should be uncluttered to minimize the potential for contamination and facilitate ease of cleaning. The surfaces (e.g., tables) in the surgical area should be impervious to moisture (e.g., stainless steel). The surgical area should be routinely cleaned and disinfected. Animal Resource Center (ARC) staff will conduct regular tests on surgical areas to assess the effectiveness of cleaning and disinfection procedures.

The area where surgeries are performed should be identified in the animal use protocol and will be inspected by the IACUC on a semi-annual basis to ensure that the area is meeting the expectations set forth in the preceding points. Additionally, EH&S should be notified ~~so that they can~~ to monitor isoflurane exposure in these areas to ensure that personnel performing the surgical procedures are not exposed to waste anesthetic gas levels exceeding regulatory limits.

II. Surgeon Training and Qualifications

Personnel conducting surgical procedures must be appropriately qualified and trained in good surgical technique including asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective homeostasis, and correct use of suture materials and pattern.[1,3]

Before hands-on training in any surgical procedure can begin, personnel (e.g., graduates, research assistants/associates, postdocs and PIs) must first complete the Citiprogram.org training course "Aseptic Surgery."

Personnel that are performing survival surgical procedures are required to demonstrate competency to the Attending Veterinarian (AV) for each different surgical procedure that they perform, unless exempted by the IACUC, and must NOT perform any surgical procedure without the assistance and direct supervision of the surgical trainer or AV until they have successfully completed their competency assessment. Personnel will be deemed competent if they can demonstrate to the AV that they possess the necessary knowledge and technical skills to successfully complete the surgical procedure. Anyone who cannot competently perform the surgical procedure (i.e., fails their evaluation) should receive additional training under the direct supervision of a trained and qualified individual.

The AV must be consulted for questions regarding animal health, surgical wound care, animal anesthesia techniques, surgical procedures, and provision of postoperative care. Additionally, surgical outcomes should be continually and thoroughly assessed to ensure appropriate procedures are followed and timely corrective changes are instituted.[1] The AV must be consulted when there is an increased incidence of anesthetic or perioperative deaths or an increased incidence of post-surgical complications.

III. Preparation of Surgical Instruments

Surgical instruments must be sterilized for both survival and non-survival rodent surgery. Several methods (autoclaving or immersion high-level chemical disinfectants) can be used to sterilize instruments and other materials that will come in contact with the animal's tissues. The sterilization method selected will depend on the physical characteristics of the materials to be sterilized. For example, autoclaving is the preferred method to sterilize common surgical instruments. By contrast, delicate implants may be damaged by autoclaving. Alternative sterilization procedures, for example immersion in a liquid chemical sterilant or high-level disinfectant, should be used in these cases. If a liquid chemical sterilant is used, then the manufacturer's recommendations on contact times must be followed, and the sterilized material must be rinsed in sterile water before use to remove any disinfectant residue.

The effectiveness of the sterilization method needs to be verified. The ARC regularly (semi-annually) performs spore testing on all autoclaves.

Alcohol (i.e., ethyl alcohol or isopropyl alcohol) is neither a sterilant nor a high-level disinfectant and, therefore, should not be used to sterilize surgical instruments.[1] However, alcohol at concentrations between 60-90% is rapidly bactericidal against vegetative forms of bacteria and a potent virucidal and fungicidal agent.[4] Its use should be restricted to applications where the presence of bacterial spores is not anticipated, for example, decontamination of autoclaved surgical instruments between serial rodent surgeries.[5]

Following sterilization, surgical instruments and medical or surgical devices (e.g., catheters, cannulas, or osmotic mini-pumps) must be handled using an aseptic technique to prevent their contamination. Further, these sterile instruments and devices must be properly stored. The shelf life of these instruments will depend on how they were prepared and wrapped, how they are stored, and event-related factors contributing to their contamination. Storage of surgical instruments in plastic self-seal sterilization pouches is recommended. Storage in closed or covered cabinets is preferred, but open shelving may be used for storage. Any sterile instrument, device, or sterile package containing these items that has fallen or been dropped to the floor, is exposed to water, or experiences any other event that could contribute to the contamination of the sterile material should be cleaned and re-sterilized.[4]

When performing surgeries on multiple animals (mice or rats), at least 2 sets of instruments should be sterilized by an appropriate method. While the first set of instruments is being cleaned to remove all organic material (i.e., blood) and re-sterilized, the second set should be used on a second animal. If more than two animals will be receiving surgery, and if only the tips of instruments will be in contact with the sterile surgical field or the animal's organs/tissues, then the instrument tips may be cleaned to remove organic material (i.e., blood) and placed in a glass or hot bead sterilizer for approximately 20 seconds (check instrument manufacturer for recommended contact times and temperatures, 240-270 °C). As an alternative to using a glass or hot bead sterilizer in tips-only surgical procedures, surgical instruments may be immersed in a sterile bowl containing 70% isopropyl alcohol for at least 2 minutes.[5] The instruments should be maintained in a sterile holder/container after decontamination (glass or hot bead sterilizer or alcohol), and until used for the next surgery. Decontamination of surgical instruments in this manner during serial tips-only surgeries must be limited in use, and after the 5th animal, fresh sterile instruments must be used.

Certain instruments should not be reused, because they cannot be effectively decontaminated between use. Decontamination of the dental burs commonly used for rodent craniotomy surgeries, for example, is difficult to achieve.[22] Re-use of dental burs needs to be justified in the IACUC protocol. Even new dental burs may not be sterile in their packaging and therefore need to be decontaminated before use. New carbon steel dental burs should be decontaminated using a hot bead sterilizer, as carbon steel will oxidize (rust) when autoclaved.

Finally, remember to clean and maintain glass bead sterilizers according to the manufacturer's instructions, ensuring the heating element can maintain the required temperature and replacing the glass beads periodically.

IV. Preparation of the Animals

All experimental surgical procedures are "elective" procedures and should only be performed on healthy animals without injuries or illnesses. The animals should be receiving ad-lib food and water and acclimated to the housing environment (not just received from the vendor).

Prior to taking the animal to the surgery area, remove all hair over and around the incision site. Hair removal should be performed in a location away from the surgery area to minimize the potential for contaminating the surgery area with loose hair and dander. Hair can be removed with electric clippers, or using a depilatory cream. When using electric clippers, make sure to use an appropriate size blade to avoid causing skin abrasions or nicks that may become infected. It's also important to regularly clean and disinfect the clipper blades to avoid cross contamination. Therefore, make sure to use a small, high-quality, removable, corrosion-resistant blade that can be regularly cleaned and disinfected. When using depilatory cream, remember the commercially available products (Nair® or Veet®) were not designed for rodents (a rodent's epidermis is much thinner); therefore, it's imperative to limit the contact time (<120 seconds)[21], not to rub the depilatory cream into the skin, and to remove all traces of the cream by wiping the area with a water-moistened gauze pad in order to avoid damaging the skin.

Clean and aseptically prepare the surgical site. Alternate between a topical antiseptic solution (e.g., Betadine® Solution) and 70% alcohol to carefully clean and disinfect the skin surface. Use a surgical sponge, cotton swab, or cotton-tipped applicator to apply the topical antiseptic solution and alcohol. Clean in a gradually enlarging circular pattern from the center of the proposed incision to the periphery. For surgeries in mice, the surgical site is often very small, making the above cleaning technique impractical. In these situations, focus on cleaning the surgical area and avoid bringing the sponge or swab back from the contaminated periphery to the clean surgical area. Repeat the skin preparation cycle for a total of three times, each time beginning at the center and proceeding to the periphery. End the preparation of the surgical site with an application of antiseptic solution (i.e., Betadine® Solution).

To prevent hypothermia, try not to wet the animal any more than necessary.

Care should be taken to prevent contamination of the sterile surgical field during subsequent handling and positioning of the animal. Glad Press'n Seal freezer wrap provides an uncontaminated (i.e., free of microorganisms and organic material), inexpensive and effective method to cover the sterile surgical field.[6]

Place the animal on a clean, absorbent surface (e.g., disposable under pads) and maintain its body temperature using a water-circulating heating pad or equivalent external heat source, taking care not to overheat the animal (**i.e., maximum pad temperature should be 42°C**). If you're using a homeothermic temperature controller (e.g., https://www.ezsystemsinc.com/product/ez-hpm-mouse-heating-pad/) to monitor and control the animal's body temperature, make sure that you're familiar with its operation (i.e., **read the instruction manual carefully and ensure correct over-temperature safety settings are set)**. Regardless of the brand and type of heating device, measure and ensure an even surface temperature (i.e., no hot spots; maximum surface temperature of 42°C) on the heating pad and always place a towel, drape or disposable under pad between the animal and the heating device to prevent a thermal injury to the animal.

Administer the dose of pre-operative analgesic and local anesthetic before starting the surgical procedure. If you are using buprenorphine, remember that it will exacerbate isoflurane's respiratory suppression; therefore, reduce the concentration of isoflurane when both drugs are administered **concurrently**, or administer the buprenorphine at the end of the surgical procedure (after the isoflurane has been turned off). Finally, apply sterile lubricating ophthalmic ointment (such as Puralube®) on the anesthetized animal's eyes to prevent drying of the cornea.

V. Preparation of the Surgeon

Surgeons should wash their hands before surgery.

Surgeons must wear a mask, sterile gloves, and a clean scrub shirt or clean lab coat to prevent contamination of the sterile surgical field. A new pair of sterile surgical gloves should be used for each animal. Alternatively, if only the tips of the surgical instruments come into contact with the surgical field or animal's organs/tissues, then the same pair of surgical gloves may be decontaminated between animals and used on up to 5 animals.[5] To decontaminate the gloves, either spray them with 70% isopropyl alcohol and rub the wet gloved hands together for 30 seconds, or dip the fingertips of the gloves in a sterile bowl containing 70% isopropyl alcohol for 30 seconds.

VI. Anesthesia and Surgery

The surgical field must be kept sterile throughout the procedure. Sterile instruments must be prevented from contacting non-sterile surfaces. Catheters, probes, or cannulas must not contact non-sterile surfaces, for example, the animal's fur or paws. In most cases, sterile drapes are required for the maintenance of the sterile field.

Most protocols employ multimodal anesthesia/analgesia, which combines a local anesthetic, NSAID (non-steroidal anti-inflammatory drug), opioid analgesic, and inhalant anesthetic. This approach reduces the overall dosage of a single drug (enhancing safety) and targets distinct pain pathways (increasing effectiveness).

Isoflurane gas anesthesia is the most used anesthetic, but it lacks analgesic properties (blocking peripheral nociception). However, controlling the dose of isoflurane is easier compared to injectable anesthetics. If injectable anesthetics are employed, provide the anesthetized animal with oxygen via a

face mask and administer a reversal agent at the end of the surgery. It's crucial to note that injectable anesthetics, such as the Ketamine/Xylazine cocktail, should not be used in older rodents, obese rodents (rats exceeding 400 g; mice exceeding 35 g), or Nile rats due to potential health risks.

If isoflurane is used to anesthetize the rodent patient, then please make sure to follow the SOP for the isoflurane anesthesia machines for instructions on how to use and maintain the anesthesia machine.

In general terms, we administer anesthesia to render an animal unconscious and prevent them from experiencing or remembering the pain from the surgical procedure. However, if we fail to control the dosage of anesthetic administered, it can lead to adverse effects. The most critical parameters that we can and should carefully monitor in our rodent patients is ventilation (respiratory rate) and body temperature.

The surgeon or the anesthetist must pay continuous and close attention to the animal's anesthetic depth and adjust the anesthetic dose accordingly. At a minimum, the following vital signs should be monitored at regular intervals (15 minutes) and controlled during all rodent surgeries:

- The animal's response to painful stimuli (incision, toe/tail pinch) - there should be no withdrawal response or movement. Please note that there are anatomical differences in responsiveness/sensitivity: hind feet > front feet > tail. Make sure that you consistently test the same anatomical area, and that you don't use too much force to avoid causing trauma. In this respect, consider using a Touch Test Sensory Evaluator (6.65 filament), which facilitates consistent and objective testing of an animal's pain withdrawal response. By pressing this filament on the foot pad of one of the hind feet until the filament bends, you'll have applied a consistent force on the foot pad that will not cause trauma but is sufficient to elicit a pain withdrawal response if the animal is not fully anesthetized. If there is a response, increase the isoflurane concentration gradually, wait for the anesthetic to take effect, and re-check the animal for a response.
- The animal's body temperature – it should be 36.5 - 38°C for mice and 35.9 - 37.5°C for rats. Monitor the animal's body temperature by using a lubricated (sterile single-use lubricant) rectal temperature probe of appropriate size for mice or rats. If you observe a body temperature below 34°C, turn down the isoflurane concentration. Hypothermia can exacerbate isoflurane's effects (e.g., both cause hypoventilation but by different mechanisms). A general rule of thumb, if the animal is hypothermic, the concentration of isoflurane should be decreased by ~25%.
- The animal's respiratory rate (i.e., breaths per minute, BPM) and character - the rate should not be too low (<50 BPM) and there should not be agonal or deep abdominal breathing, or a hunched posture (in mice), which indicate that the animal is too deeply anesthetized or experiencing an airway or breathing obstruction. If the respiratory rate is too low, decrease the isoflurane concentration, wait for the anesthetic change to take effect, and re-check the animal's respiration rate and pain withdrawal response before proceeding. It may also be necessary to increase oxygen flow rate (to 1 L/min). The minimum oxygen flow rate should be 0.5 L/min. However, this assumes that there is not a problem with the non-rebreathing anesthetic circuit (i.e., mixing inspiratory and expiratory gases). Finally, the animal's body position also matters, sternal recumbency is less restrictive (better ventilation) than dorsal recumbency.
- Check the animal's cardiac output and tissue perfusion - the color of mucous membranes should be pink and moist; the color of the eyes (albino rodents) should be red; and/or the color of the skin (ears, paws) should not be pale or blue. If an abnormal (pale or blue) skin or mucous member color is observed, immediately lower the isoflurane concentration and the increase oxygen flow rate to 1L/min (in case there is a problem with the anesthetic circuit; mixing inspiratory and expiratory gases).

- Using a pulse oximeter to monitor heart rate and oxygen saturation of the blood (%SpO2) is strongly recommended. Saturation levels <90% are of concern; immediately lower the anesthetic (isoflurane) concentration and the increase oxygen flow rate.

Control any intraoperative bleeding by placing sterile gauze (e.g., 2 x 2) or a sterile cotton-tipped applicator over the bleeding area and applying gentle pressure. Blot, do not rub or wipe, the area to remove or soak up the blood. Alternatively, sterile absorbable gelatin sponges (e.g., Gelfoam) may be used to control bleeding.

Use the appropriate material and method to close the surgical incision. Stainless steel wound clips are recommended for closing most skin incisions in rodents. Tissue adhesive (glue; Vetbond™ or Dermabond™) should only be used to close easily approximated skin edges (i.e., no tension) created by a sterile surgical incision. Tissue adhesive must be used sparingly (only one or two drops) and applied only on the cut edges of the skin and not on the deeper tissues or the skin surface. The applicator tip should not contact the tissue, and if it does, it should be discarded and replaced.

Provide analgesic treatments to alleviate the pain caused by the surgical procedure. The evolving standard of veterinary care for rodent surgical procedures is to provide multimodal analgesic treatments combining a local anesthetic (i.e., 0.5% lidocaine with 1:200,000 epinephrine in a multi-dose vial) administered by irrigation of a surgical opening or local infiltration, a non-steroidal anti-inflammatory analgesics (NSAID; meloxicam) administered by SC injection, and an opioid analgesic (e.g., buprenorphine) also administered by SC injection at the end of the surgical procedure. The timing of the NSAID treatment is important to consider. If the anesthesia (isoflurane) and surgical procedure duration (e.g. long duration) may cause hypotension and hypovolemia (even acutely), then the NSAID should be administered during the postoperative phase (just after the isoflurane is turned off) to minimize the risk of an adverse effect. Additional doses of the non-steroidal and opioid analgesics should be administered at prescribed dosing intervals during the postoperative period as described in the approved IACUC protocol. Please see the tables below for specific drug and dose recommendations.

If an emergency evacuation occurs during surgery, and the animal being operated on has an exposed body cavity (e.g., craniotomy), an open incision(s), or exposed cannulas or catheters, then that animal should be euthanized by administering an overdose of anesthesia prior to evacuating the area if time and circumstances permit. If the animal is anesthetized but no or only minor surgical manipulations have been performed, then leave the anesthetized animal to recover in a confined space (e.g., a cage in the surgery room) and evacuate the area. If a live animal was left in a procedure area during an emergency evacuation and the animal's welfare is at risk due to prolonged building access restrictions, notify the AV or ARC staff immediately. Employees should never put themselves at risk during an emergency evacuation to care for animals.

VII. Postoperative Care

The animal should be continuously monitored until it is conscious and has stable vital signs (e.g., regular breathing pattern and rate).

The animal should be housed in the recovery cage/enclosure by itself (i.e., not housed in a cage with other animals) on a clean and dry surface (not bedding), and it should be provided with supplemental heat during the recovery period. Specifically, the animal should be placed on a clean disposable absorbent pad in a clean and empty rodent cage that is resting on a heating pad. Check the surface temperature of the heating pad to ensure that it is ≤42°C.

The animal should be given fluid replacement therapy: sterile **warmed** physiological fluids. Sterile saline, lactated Ringer solution (LRS), or Dextrose 5% in LRS are suitable fluid replacement options.

Subcutaneously (SC) administer the fluids at a rate of 1 ml/100 g (10 ml/kg) for rats or 0.5 to 1 ml per animal (20-40 ml/kg) for mice. For rodent surgeries lasting less than an hour, the SC fluids can be administered at the end of the surgical procedure. If the surgery is going to exceed 1 hour in duration, administer the warmed fluids before the surgery commences. If there is substantial blood loss during surgery (for mice that would be a blood volume ≥0.2 ml, and for rats a volume ≥2 ml), immediately administer additional warmed fluids of at least the same volume as the blood loss, and then at rate of 10 ml/kg/hr.

The animal should not be returned to the animal room until it can stand and move about the cage (i.e., has fully recovered from anesthesia). It is recommended that the animal be placed in a cage with clean bedding with access to food and water in its cage. If necessary, provide food/water in a container (e.g., paper cup or ceramic bowl) on the bottom of the cage in the form of moistened pellets or a gel-based food supplement. Continue to house the animal individually, if they have exposed (i.e., not protected by a metal cover) external catheters or ports.

A member of the research team or other individual to whom postoperative care has been delegated must monitor each animal at least once a day and document all observations and treatments in the postoperative recovery log. Each animal must be carefully observed for signs of pain and surgical complications, including infection or dehiscence of the surgical site(s) at least once a day. Pain assessment guidelines are included below.

All surgical wounds must receive appropriate wound care. It is recommended that skin incision sites be cleaned with an antiseptic solution or that a topical antimicrobial gel containing Povidone-Iodine Complex be applied. For cranial implants, use a 5% povidone-iodine ophthalmic solution to clean and maintain the skin-implant margins. Try not to get any solution in the eye, but the cited solution is used in ocular surgery and should be safe. If you get any in the eye, flush it with sterile saline. External wound clips or sutures should be removed 7-14 days after the surgery.

Subcutaneous and cranial implants are generally well-tolerated in mice and rats but curing any wound or infection that develops can be difficult without removing the implant, so prevention (routine cleaning) and early detection and treatment are critical. In some cases, cage mates or the rodent itself may cause self-directed (through scratching) skin wounds around their surgically implanted devices, such as subcutaneous vascular access ports or cranial implants (e.g., headplate). Trimming their nails, separating the animal from any cage mates, and daily treatment with topical antiseptics (Vetericyn or Povidone) are the usual conservative/first line approaches to resolving this problem. If the surgical wounds do not heal or if there is any swelling or discharge from the implant site, please notify the AV.

The AV must be contacted if there are any unexpected complications.

VIII. Pain Assessment and Alleviation

As defined by the International Association for the Study of Pain (IASP): Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Further, the fourth principle of the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training states: “Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.”[3]

Technical proficiency in aseptic surgical technique, especially gentle tissue handling, is very important in reducing pain from surgery because: "As a rule, pain is likely to occur in proportional terms as a result of tissue injury—more extensive tissue damage results in greater pain and thus a need for a stronger

analgesic regimen."[7] Furthermore, "the intensity of acute pain following a tissue insult is greatest within the first 24 to 72 hours after the insult [surgery]."[8]

The response of animals to pain, and hence any behavioral signs of pain, will vary depending on the animal species and the surgical procedure. There is no simple cage-side evaluation of pain in rodents. A nuanced interpretation of animal behavior is required to make a reasonable assessment of animal pain and distress. The best way to identify signs of pain is to closely observe the appearance and behavior of the animal for at least two days prior to surgery and note any changes after surgery. However, this behavioral assessment only works well if the animals have a varied repertoire of normal behaviors, which only exists when animals are housed in an enriched environment.

The following signs are associated with persistent pain or distress in rodents.[7-10] This information is also included on the back page of the IACUC's Rodent Postoperative Care Log. **If any of these signs are observed, notify the AV.**

- Animals in pain or distress will demonstrate little or no spontaneous movement. For example, rats that are in pain or distress will be reluctant to rise on their hind limbs and explore the outside of the cage when the lid of the cage is removed. If the animal is also reluctant to move even when disturbed, this is a sign of increased severity of pain or distress.
- Absence or reduced frequency of normal behaviors. Mice in pain or distress may show decreased or no nest building behavior. Rats in pain or distress may show decreased foraging, and little to no interest in gnawing enrichment.
- Increased grooming or licking directed at the injured area
- Partially closed or squinted eyes, also known as the facial grimace response
- Piloerection (hair standing up)
- Increased aggressiveness or vocalization when handled
- Abnormal posture (hunched posture in mice; arched backed and/or tucked-in abdomen in rats)
- Reduced food and water intake resulting in rapid weight loss or dehydration (skin tenting and sunken eyes). A delay in recovery of body weight following surgery can reflect a residual pain/distress or postoperative complication. Therefore, postoperative body weight monitoring is a critical severity assessment. Any animal that is dehydrated or experiences a weight loss during the recovery period that is >10% of the preoperative body weight should be reported to the veterinarian.

IX. Guidance on the Selection and Use of Non-Pharmacological and Pharmacological Postoperative Support[10-13]

The following categorical examples of the potential pain caused by different surgical procedures may be useful to investigators in determining the appropriate pharmacological analgesic support for their animals. **However, with appropriate justification the IACUC may approve analgesic regimens and postoperative durations that differ from these examples.**

The specific anesthetic and analgesic plans for the rodent surgical procedure(s) described in approved IACUC protocols were developed in consultation with the AV. They were based on the predicted pain intensity of the surgical procedure and the expectation that the surgical procedure would be performed by a member of the research team who is technically competent in the surgical technique. **Researchers are therefore expected to follow the anesthetic/analgesic regimen and postoperative duration in their approved protocol.**

Members of the research team are expected to assess, at least once daily, the effectiveness of analgesic treatments and must notify the AV if the analgesic treatments are not effectively alleviating the animal's pain.

Non-pharmacologic postoperative support for mice and rats:

- House singly until ambulatory
- Provide supplemental heat
- Provide soft, absorbent bedding
- Provide modified food and water access by placing gel-based food supplements or moistened food pellets in a cup/bowl on the bottom of the cage

Pharmacologic support for mice[15-20].

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (0.05 ml), irrigation of a surgical opening or local infiltration AND Meloxicam, 2-5 mg/kg, SC immediately postop, and then at the same dose and route (SC) once per day for 2 days OR Carprofen**, 10-20 mg/kg, SC immediately postop, and then 25 mg/kg/day PO*** for 2 days	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (0.05 ml), irrigation of a surgical opening or local infiltration AND _____ Meloxicam, 2-5 mg/kg, SC, immediately postop, and then at the same dose and route (SC) once or twice per day for 2-3 days OR Carprofen**, 10-20 mg/kg, SC immediately postop and then 25 mg/kg/day PO*** for 2-3 days _____ OR Buprenorphine extended-release (Ethiqa XR) 3.25 mg/kg, single dose immediately postop	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (0.05 ml), irrigation of a surgical opening or local infiltration AND _____ Buprenorphine, 0.1-2 mg/kg, SC, immediately postop, and then at the same dose and route (SC) every 6-8 hours for 1-3 days AND Meloxicam, 2-5 mg/kg, SC, immediately postop, and then at the same dose and route (SC) twice per day for 3 days _____ OR Buprenorphine extended-release (Ethiqa XR) 3.25 mg/kg, immediately postop, and a second dose 48 hours later

*Care should be taken to calculate and use an appropriate safe dose of the local anesthetics in mice due to their small size. The dose (% x volume of administration) provided here is the maximum dose for a 25g mouse (10 mg/kg).[11] Lidocaine formulations with a concentration >0.5% lidocaine (5 mg/ml) may be used but will need to be diluted. The most common method for diluting and buffering is by alkalinization of the lidocaine with sodium bicarbonate just prior to the injection. Buffering with sodium bicarbonate (NaHCO3) 8.4% in a 10:1 or 9:1 ratio (10 or 9 parts lidocaine-epinephrine containing 1 or 2% lidocaine to 1 part sodium bicarbonate containing 8.4 g/l) more closely resembles the neutral pH (~7.4) in human and animal tissues. Buffered lidocaine solutions are known to cause less pain than unbuffered lidocaine in humans. Use only as much volume as need to irrigate/infiltrate the incision site.

** Recent studies (15, 20) have confirmed the efficacy of high-dose carprofen analgesic treatment (20 mg/kg SC and 25 mg/kg/day PO) for mice undergoing neurosurgical procedures (craniotomy). However, they also highlight

the narrow therapeutic index of this therapy: mild erosive lesions in the stomach of 24% of carprofen-treated mice, which must be considered. For mice treated with high dose carprofen, postoperative body weight is a critical severity assessment, and any mouse failing to recover its presurgical body weight should be promptly referred to the AV for evaluation and treatment.
*** When analgesics are provided PO, start offering the unmedicated gel or tablet to the animal 2 days before surgery and check to make sure that the animal is eating enough of the medicated tablet/gel to receive the full calculated dose. If the mouse does not consume the full oral dose, then the difference (what was consumed versus the prescribed dose) will need to be administered SC.

Pharmacologic support for rats[17-19].

Mild to Moderate Pain	Moderate Pain	Moderate to Severe Pain
Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.5 ml), irrigation of a surgical opening or local infiltration AND Meloxicam, 1-2 mg/kg, SC, or Carprofen, 5-10 mg/kg, SC immediately postop, and then Meloxicam at the same dose and route (SC) once a day or Carprofen at the same dose (5-10 mg/kg) but PO*** once a day for 1-2 days	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.5 ml), irrigation of a surgical opening or local infiltration AND Buprenorphine, 0.05 mg/kg, SC, immediately postop AND Meloxicam, 2 mg/kg, SC or Carprofen, 5-10 mg/kg, SC immediately postop, and then Meloxicam at the same dose and route (SC) once a day or Carprofen at the same dose (5-10 mg/kg) but PO*** once a day for 1-3 days OR Buprenorphine extended-release (Ethiqa XR)** 0.65 mg/kg, single dose immediately postop.	Local anesthesia*: 0.5% lidocaine with 1:200,000 epinephrine (≤0.5 ml), irrigation of a surgical opening or local infiltration AND Buprenorphine, 0.05 mg/kg, SC, immediately postop, and then at the same dose and route (SC) every 8-12 hours for 1-3 days AND Meloxicam, 2 mg/kg, SC, immediately postop, and then at the same dose and route (SC) once a day for 3 days OR Buprenorphine extended-release (Ethiqa XR)** 0.65 mg/kg, immediately postop, and a second dose 48 hours later

*Care should be taken to calculate and use an appropriate safe dose of the local anesthetics in mice due to their small size. The dose (% x volume of administration) provided here is the maximum dose for a 250g rat (10 mg/kg).[11] Use only as much as needed to irrigate/infiltrate the incision site.
**Do not house rats on wound chip bedding after administration of Ethiqa XR. Pica involving wood chip bedding can lead to the death of the animal.
***When analgesics are provided PO, start offering the unmedicated gel or tablet to the animal 2 days before surgery and check to make sure that the animal is eating enough of the medicated tablet/gel to receive the full calculated dose. If the mouse does not consume the full oral dose, then the difference (what was consumed versus the prescribed dose) will need to be administered SC

IX. Records[2,14]

Anesthesia, surgery, and postoperative care records are required for all surgical procedures in order to document the appropriate performance of anesthesia and surgery and to demonstrate compliance with the surgical, anesthetic/analgesic, and post-operative plan in the approved protocol. The AV will regularly review these records. The records should be kept in the room where the animals are housed. Having the records in the room accomplishes several functions. 1) It explains the condition of the animals to animal care staff (a sedated animal may otherwise be thought to be ill). 2) It assures animal care staff and USDA Animal Welfare inspectors that the animal is being cared for. 3) It informs animal care staff how recently the investigator has seen the animal; this knowledge helps them decide whether or not there is a need to contact the investigator to inform him or her of the present condition of the animal.

Although individual records are desirable, a composite record may be used for a group of rodents. The record (individual or composite) should include the surgery date, name of the surgeon(s), a brief description of the surgical procedure, any drugs or treatments that were administered, and a note of any complications or previous surgical procedures that may have been performed on the animal. The latter is required so that the IACUC or other regulatory agency (e.g., USDA) can assess whether or not an animal has undergone more than one survival surgical procedure. Repeated failure to maintain clear, concise, and complete surgery or postoperative records may lead to a revocation of an individual researcher's privilege to perform surgical procedures on laboratory rodents in a research setting. If a researcher did not perform the surgical procedures but is responsible for providing postoperative care for that animal, their repeated failure to maintain clear, concise, and complete records may lead to a revocation of their privilege to work with animals.

The record should document that the animal was appropriately anesthetized prior to making the first surgical incision, and it should identify the intraoperative assessments of the animal's vital signs that were performed at regular intervals (e.g., every 15 minutes). Additionally, it should include notations of any variations in vital signs and anesthetic depth that were observed and actions that were taken to correct such variations.

The record should include a notation for each time the animal was examined postoperatively. After all wounds have healed and all sutures/wound clips have been removed, the postoperative record requires no further entries but should continue to be kept in the area where the animals are housed. The record should be removed from the animal room when the animal is no longer housed in the room (i.e., at the experimental endpoint).

This IACUC website offers templates for a rodent anesthesia and surgery record and a postoperative care record recommended by the AV. Alternatively, the PI may use a different template for their lab, but it should capture and document important information with comparable detail. Please consult with the AV to ensure that the level of surgical and postoperative documentation in your template is appropriate for your surgical procedure and species.

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