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This issue of the *BRL Bulletin* reviews the rodent anesthetic and analgesic guidelines developed at the University of Illinois at Chicago. While the selection of anesthetic and analgesic agents can be quite complex in animals of compromised health status, there are well established principles which may be applied when developing anesthetic and analgesic regimes for animals undergoing experimental procedures which are otherwise healthy. With this in mind, the *UIC Rodent Surgical Classifications and Analgesic Guidelines* were developed to give investigators a starting point when writing a new protocol, to set minimum standards for postoperative monitoring and analgesia, and to encourage an institution-wide approach to the care of rodent surgical patients.

Selection of Anesthetic Agents

The appropriate choice of an anesthetic protocol is essential for optimal surgical outcomes. Various factors influence the choice of an anesthetic agent. Besides the response of a given species to the anesthetic agent and the potential effect on research results, the experience of the anesthetist, the number of people required for monitoring and maintenance of anesthesia, and the resources and equipment available must be considered. Anesthetic agents used in rodents generally fall into one of two types, injectable or inhalant.

Injectable anesthesia in rodents is usually achieved by a mixture of ketamine and xylazine. This requires less specialized equipment than inhalant anesthesia, but it is more difficult to adjust the depth of anesthesia. This method also allows for some level of analgesia, which is not the case with common inhalant anesthetics such as isoflurane. Inhalant anesthetics offer the advantage of more precise control over the plane of anesthesia and require less time to induce, as well as recover from, anesthesia. In procedures that require the animal to be intubated, as with positive pressure ventilation, inhalant anesthesia may be the most convenient option.

If inhalant agents are used (e.g. isoflurane), an appropriate scavenging system must be in place in accordance with the *UIC Environmental Health and Safety Policy* and the Animal Care Committee (ACC) *Guidelines for the Use of Inhalation Anesthetics for Laboratory Animals*. The depth of anesthesia must be assessed and determined to be appropriate prior to the initiation of a surgical procedure. In rodents, this is achieved by checking that the animal does not respond to a toe pinch. Anesthetic depth and the physiological status of the animal should then be assessed throughout the procedure by evaluating the response to toe pinch or other reflexes, respiratory rate and depth, and the color of skin and tissue.

Selection of Analgesic Agents

The ACC has developed guidelines for minimum levels of analgesia for rodent surgical procedures. The levels are based on increasing invasiveness, the potential for pain caused by the procedure, and the choice of anesthesia. The guidelines serve to contribute to the consistency of care across the institution.

Animals undergoing relatively minor procedures with little potential for pain can be expected to respond well to 12 hours of postoperative analgesia, either as a single dose of a non-steroidal anti-inflammatory drug, such as meloxicam, or an opioid, such as buprenorphine. As the surgical invasiveness or the potential for pain increases, the duration of analgesia and monitoring likewise increase. This is the basis for the classification system used in the *UIC Rodent Surgical Classifications and Analgesic Guidelines*. Analgesia must be given according to the *Guidelines* and the approved animal use protocol. Additional analgesics are indicated if animals appear painful or experience complications during the procedure or recovery period. If complications arise, investigators are encouraged to contact the BRL veterinary staff at (312) 996-7040.

Preoperative analgesia may be necessary depending on the type of anesthesia given. For

procedures performed using inhalant anesthesia, preoperative analgesia (e.g. meloxicam or buprenorphine) must be given. Isoflurane has no analgesic properties, so when used alone for invasive procedures, it can lead to a condition known as “wind-up” in which nerves in the spinal cord become progressively more activated. As a result, the animal recovers from anesthesia with untreated or escalated pain which can make postoperative analgesia less effective, leading to continued pain and a prolonged recovery. Because agents such as ketamine and xylazine block pain in the spinal cord as well as the brain, injectable anesthesia with these agents can prevent wind-up of pain. In this case, analgesics can be administered to the animal at the end of the procedure in all but the most invasive procedures. Highly invasive procedures are assumed to be sufficiently painful that all animals must receive preemptive analgesia in the form of an opioid analgesic. A line block of local anesthetic, such as bupivacaine or a 50:50 mixture of lidocaine and bupivacaine, is also an option. When injected at the site of the incision, pain sensation is blocked at the peripheral nociceptors, and the spinal cord neurons are never activated to initiate the process of “wind-up.” Ideally, multiple forms of analgesia may be combined in a multimodal approach to fight pain more effectively.

Surgical Classification

Class 1 surgeries are the least invasive and do not involve entry into the thoracic or abdominal cavity. Animals undergoing these procedures must have a minimum of 12 hours of postoperative analgesia. Examples include dental and ocular procedures, subcutaneous implants, cutaneous biopsy or wounding, and simple medical techniques such as tracheal injections and vessel cut-down or cannulation. Craniotomies are also included in this classification, as long as there is minimal manipulation of the periosteum. The periosteum contains pain receptors, but there are no pain receptors in the brain itself. The potential for pain in these procedures is largely due to the skin incision along the scalp, which is comparable to a skin incision at other sites on the body.

Class 2 surgeries are expected to cause more than minor, short-lived pain. They include procedures that enter the abdominal cavity, termed “simple laparotomies,” such as embryo transfer. This class

of surgery does not result in major organ removal or ischemia, which would result in significantly more pain. Animals undergoing Class 2 procedures must have a minimum of 36 hours of analgesia using either meloxicam or buprenorphine. This can be achieved using meloxicam once on the day of the procedure and once approximately 24 hours later. Buprenorphine has a shorter duration of action, so it must be given twice a day. To achieve appropriate analgesia, it must be administered at the time of the procedure and twice on the following day with the goal of once every 12 hours. To avoid the necessity of repeated administration of buprenorphine, there is a slow-release formulation available, *Buprenorphine SRTM-LAB*. This is a specific formulation developed at a concentration suitable for rodents and recommended for use in laboratory mice and rats. It is **critical** that investigators follow the procedures described in *Buprenorphine SRTM-LAB*, found on the BRL website under the Forms tab. This formulation was designed to provide 72 hours of analgesia to laboratory mice and rats. Although this product is a veterinary formulation, the UIC Hospital Pharmacy has agreed to carry it for purchase by investigators who have the appropriate DEA controlled drug license and a prescription from a BRL veterinarian. It is important to note that there is a similar product, *Buprenorphine SRTM* that is intended for use in larger animals and is not suitable for rodents.

Class 3 surgeries have the potential to cause moderate to severe pain. Animals undergoing this class of procedure must receive analgesia for a total of 60 hours, which includes administration at the time of the procedure and for two full days following it. These procedures are more invasive than simply entering the abdominal cavity and involve removing or manipulating a major organ, such as organ transplantation. Procedures that manipulate bones and joints also cause significant pain, so orthopedic procedures are also included in this class.

Class 4 surgeries are the most invasive and have the potential to produce the most severe pain. As with Class 3 surgeries, these procedures must also be accompanied by a minimum of 60 hours of postoperative analgesia. Examples of Class 4 procedures include hindlimb ischemia and

thoracotomy. Since the intensity of pain is expected to be great, these procedures require preemptive analgesia in the form of an opioid or an incisional block using a local anesthetic. Care must be taken when administering local anesthetics. These drugs can have severe adverse effects as they are distributed systemically following absorption. Central nervous effects can ultimately lead to seizure due to depression of inhibitory pathways in the brain. Cardiovascular effects are largely due to action on conductance within the heart. At sufficiently high doses they may even cause cardiac arrest. The maximum dose of bupivacaine is 2 mg/kg, and the maximum dose of lidocaine is 4 mg/kg, but only as much as needed should be injected subcutaneously to be sure that the incision site has been infiltrated. The local anesthetic should be diluted in saline to provide sufficient volume to infiltrate the desired area if necessary. The use of a local anesthetic must be done preoperatively to achieve the desired effect, and a minimum of five minutes must pass between injection and incision to allow the local anesthetic to take effect.

Postoperative Monitoring

The *Guidelines* also describe the minimum postoperative monitoring period that is required for each class of surgery. Animals undergoing procedures of all classes must be monitored for a minimum of two weeks following the procedure. As the intensity or duration of pain increases, the postoperative monitoring must also be more frequent for two reasons. The first is to ensure that signs of pain are recognized as soon as possible and measures are taken to treat this pain. The second reason is that more invasive procedures have the potential for increasing postoperative complications. Many complications may not arise at the time of surgery, so the animal must be monitored until the healing process is complete. The duration of postoperative monitoring is designed to continue beyond the last administration of analgesia to ensure that the animal does not experience pain or complications once analgesia is discontinued. For this reason, animals undergoing all classes of surgery must be observed for signs of pain or complications no less than once a day for two days immediately following the procedure. For Class 3 and 4 surgeries, the duration increases to three days postoperatively. These time periods allow for observation of the animal as the final dose of analgesia is wearing off and allows for identification

of animals that need further analgesia. For all procedures, animals must be monitored twice during the second week with three to four days between observation periods.

The investigator must plan surgeries so the postoperative care and monitoring can be performed as required. If a situation arises in which the investigator cannot fulfill his or her postoperative monitoring responsibilities and/or administer treatments, the investigator must notify the veterinary staff. Should the veterinary staff be required to perform postoperative monitoring and/or administer treatments, incurred costs will be charged to the investigator's BRL account.

Record Keeping

Keeping written records of anesthetic and analgesic administration and monitoring is important. This information may be useful to help explain unexpected experimental data or visualize significant trends that could explain anesthetic complications.

Record keeping is also an important part of the program, as written documents may be requested by the ACC, the veterinary staff, or groups such as AAALAC International, during site visits. For laboratory mice and rats, the *UIC Small Animal (Rodents/Frogs/Birds) Surgical Record* is used to document perioperative care. The information that must be recorded includes general laboratory and protocol information, as well as the specific anesthetic and analgesic agents used, along with the concentration, dose, volume, route, and times of administration for each. Furthermore, postoperative monitoring and analgesic administration must be recorded until the animals are released from monitoring at a minimum of fourteen days, assuming there are no postoperative complications. At this time, any skin sutures or wound clips must be removed, and the form closed out by recording the date that the animals were released from monitoring. The form is then removed from the animal room, filed in the laboratory, and retained for at least one year. An exception to this rule is when the procedure was performed in a biohazard or chemical hazard room, in which case the forms should not be removed from the room to avoid possible exposure to personnel in the laboratory to these substances. One form may be used for each cage of mice or

rats, but individual animal weights must be recorded for each animal on the form.

Furthermore, all institutions that receive funding from the NIH to support animal research are required to have a program of perioperative care that complies with the *Guide* and *PHS Policy*. However, all investigators at UIC, regardless of the funding source, are required to follow the *Guidelines for Survival Surgery in Rodents and Lower Vertebrates*. This includes notification of the veterinary staff when a surgical procedure is performed and appropriate documentation of perioperative care on the *UIC Small Animal Surgical Record*. With proper planning, a successful outcome to anesthetic and analgesic procedures can be obtained which will support the collection of high quality data. The veterinary staff at the BRL can be contacted at any time to help with problems that may arise or to discuss the development of future projects.

For more information please see:

Guidelines for Survival Surgery in Rodents and Lower Vertebrates (http://www.brl.uic.edu/media/PDF/guidelines_rodent_survival_surgery.pdf) and *Procedure for Maintaining Small Animal (Rodent/Frogs/Birds) Surgical Record* (https://www.brl.uic.edu/media/PDF/procedure_maintain_small_animal_surgical_record.pdf).

Further Reading

Anesthesia and Analgesia in Laboratory Animals, 2nd edition, 2008 (Wiley-Blackwell)

Bennett, B. T., Brown, M. J. and Schofield, J. C. *Essentials for Animal Research Personnel*. National Agricultural Library, Beltsville, MD, 1990.

Ferrets, Rabbits, and Rodents, 3rd edition, 2012 (Elsevier Press)

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Flecknell, P.A. *Laboratory Animal Anesthesia: An Introduction for Research Workers and Technicians*. Academic Press, Ltd., London, 1987.

Hampshire, V., Gonder J.C., *Research Animal*

Anesthesia, Analgesia, and Surgery. Scientists Center for Animal Welfare. 2007

Hughes, H.C., *Anesthesia of Laboratory Animals*. Lab Animal, Sept., 1981, p. 40.

Mathews, Karol. *Veterinary Clinics of North America Small Animal Practice Management of Pain*. W.B. Saunders Company. 2000

National Research Council. *Recognition and Alleviation of Pain in Laboratory Animals*. National Academies Press. 2009

Plumb's Veterinary Handbook, 7th edition, 2011 (Academic Press)

ANNOUNCEMENTS

AAALAC is Coming - Representatives from the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) will be at UIC November 4-6th as part of the institution's triennial accreditation site visit. Representatives from AAALAC will assess the many facets of the animal care and use program including the functionality of the Animal Care Committee, animal facilities and animal care, the veterinary care program, and investigator laboratories. The site visit team often requests to visit laboratories (last time they visited 26 laboratories) that conduct survival surgery, food and water restriction, or use hazardous agents. Laboratory staff can help prepare for the site visit making sure the laboratory is clean and well organized and a hard copy of currently approved protocols and rodent surgery forms are readily available. In addition, drugs should be in date, controlled substances should be stored in a secure cabinet or lock box, and records of use should be completed and available for review.

USDA Annual Report – On October 9th, PIs will receive their annual animal usage form. This form needs to be completed and returned as soon as possible in order to facilitate submission of UIC's Annual USDA Report, which is due December 1st.

