Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation

Introduction

Animals can experience pain and distress. It is the ethical and legal obligation of all personnel involved with the use of animals in research to reduce or eliminate pain and distress in research animals whenever such actions do not interfere with the research objectives. The Institutional Animal Care and Use Committee (IACUC) has the delegated responsibility and accountability for ensuring that all animals under their oversight are used humanely and in accordance with a number of Federal Regulations and policies.[2,21,29,30,32] Key to fulfilling the responsibilities for both the principal investigator (PI) and the IACUC are:

- understanding the legal requirements,
- being able to distinguish pain and distress in animals from their normal state, and
- to relieve or minimize the pain and distress appropriately;
- establishment of humane endpoints.

Regulatory Requirements

The IACUC must assure that all aspects of the animal study proposal (ASP) that may cause more than transient pain and/or distress are addressed; alternatives[6] to painful or distressful procedures are considered; and that methods, anesthetics and analgesics to minimize or eliminate pain and distress are included when these methods do not interfere with the research objectives; and that humane end points have been established for all situations where more than transient pain and distress can not be avoided or eliminated. Whenever possible, death or severe pain and distress should be avoided as end points. A written scientific justification is required to be included in the ASP for any more than transient painful or distressful procedure that cannot be relieved or minimized.

The obligation to reduce pain and distress ***does not end*** with the review of the ASP. It is the responsibility of the animal care staff, the research staff, veterinarians and the IACUC, to continue to monitor animals for pain, distress, illness, *morbidity* or mortality during the course of the research study.

If unexpected pain or distress occurs, and is more than an isolated incident, the PI must submit an amendment delineating the unexpected problem and stating their proposed resolution to the issue (e.g. administration of analgesics, lowering the dose of a drug that was administered, etc.). Alternatively, the PI could justify the need for unrelieved pain or distress in the amendment, or in the case of regulated species as a Column E Justification.

If it is necessary to make changes in the ASP procedures, the PI must submit an amendment to the IACUC and receive approval prior to instituting the modification. For example, if unexpected pain or distress was noted following treatment with a specific dose of an experimental agent, the PI must submit an amendment delineating the unexpected problem and stating their proposed resolution to the issue (e.g. administration of analgesics, lowering the dose administered, etc.). Alternatively, the PI could justify the need for unrelieved pain or distress in the amendment, or in the case of regulated species as a Column E Justification.

Recognition of Pain and Distress

Animals must be monitored by trained individuals for pain and distress as appropriate for the species, condition and procedure. Critical to the assessment of the presence or absence of pain or distress is having the ability to distinguish between normal and abnormal animal behavior. This is especially true when dealing with species that often exhibit pain and distress with only subtle changes in their behavior[22] (see Table 1). Therefore, it is critical that the individuals assessing an animal be trained

in the species specific signs of pain and distress, as well as be knowledgeable of the potential outcomes of the procedure, surgery or treatments administered to the animal. Pain and distress scoring is a method to convert subjective animal observations in to objective scoring system which some have found to be helpful in assessing animal behavior.

Whenever more than transient pain or distress is anticipated preemptive measures should be taken to minimize or prevent the development of pain and/or distress. Following the implementation of preemptive or palliative measures, animals must be monitored[1,26] to ensure the efficacy of the measures taken and determine if or when additional treatment will be necessary. The extent and frequency of monitoring will depend on the level of post-surgical/procedural pain and/or distress anticipated and the chosen intervention strategy(s). For example, animals undergoing a procedure known to produce no more than minimal/transient pain or distress may be adequately monitored by the daily observation of a trained animal caretaker. Whereas, the monitoring of an animal undergoing a procedure known to result in severe pain and/or distress may require more frequent monitoring by a team of trained individuals (e.g. trained animal care staff, technicians, veterinarians, investigators, etc.). Animals undergoing pilot studies or procedures new to the investigator or facility may also require a higher frequency of monitoring and a team approach.

It is ultimately the responsibility of the PI and the personnel conducting the procedure to ensure the timely and adequate identification, monitoring and documentation of the animals undergoing potentially painful or distressful procedures. Investigators may request the assistance of institute and facility veterinary and technical personnel when monitoring their animals, but all individual(s) responsible for monitoring an animal must be identified prior to conducting the procedure and their accountability clearly delineated and accepted.

Animals should be observed a minimum of once daily or more often based on professional judgment and the research being conducted. The animals should be monitored for expected and unexpected signs of more than transient pain or distress and, if observed, appropriate intervention strategies implemented (e.g. non-pharmacological approaches, analgesics, anesthetics, euthanasia, etc.), unless the withholding of treatment is scientific justified. Observations and actions taken to relieve pain or distress must be documented.

The documentation of monitoring of the animal is important and required. The nature and the frequency of the documentation are dependent on the species and the potential for pain and/or distress. For example, the identification of cages containing an animal which has under gone a potentially painful or distressful procedure with a “special observation” cage card has proven helpful in drawing special attention to the animal during the caretaker’s daily health check. Cages containing animals requiring more intensive monitoring should also be appropriately identified and their monitoring and/or treatments documented either at the room, cage or animal level (e.g. room log, cage card, medical record, etc.), in addition to the investigator’s notations in their laboratory notebook. Documentation must be available to all personnel monitoring the cage or animal (i.e. IACUC, veterinarians, animal care staff, etc.).

Intervention Strategies

Strategies for the management of pain and distress may include non pharmacological considerations (e.g. modified housing and husbandry practices, dietary modifications, surgical approaches[8], desensitization and acclimation strategies, etc.), pharmacological interventions or euthanasia. The chosen strategy will vary with the species, the procedure(s) being performed, duration of action needed, route of administration preferred, degree and type of analgesia required and research being conducted (see Table 2). It is strongly suggested that PIs consult their IC veterinarian during the development of an animal study protocol, prior to its submission to the IACUC. This approach has been demonstrated to

expedite the protocol approval process.

Excellent resources[3,4] and formularies[7,10,12,14,15] are now available which provide extensive information on the recognition and alleviation of pain and distress in laboratory animals (See Plumb Veterinary Drug Handbook[26] and other references below). These resources, coupled with trained and skilled animal care personnel and the professional judgment of your IC and animal facility veterinarian provides each investigator and their IACUC with powerful tools for the recognition and alleviation of pain and distress in laboratory animals.

Preemptive measures should be taken to minimize or prevent the development of pain and/or distress. For example, a skilled surgeon can often minimize tissue trauma which in turn minimizes post-operative pain and distress. The use of ketamine or opioids preemptively, even in low doses has been demonstrated to prevent the development of some forms of pain.[24] In addition, the use of a single dose of a non-steroidal anti-inflammatory agent (NSAID), sustained-release formulations,[13] or other analgesic agent can have a positive effect on the speed with which animals return to normal behavior.[16,17,36,37,38] It has been repeatedly demonstrated in humans that the provision of effective analgesia reduces the time taken for post-operative recovery.[11]

There are also many pharmacological intervention strategies for the management of pain and distress. Traditional analgesics include local or regional anesthetics, opioids and NSAIDS. Using two or more of classes of these analgesics together, or combining these analgesics with nontraditional analgesics such as NMDA antagonists,[20,40] alpha$_2$-agonists,[35] tramadol,[5] and even the antiepileptic drug gabapentin[27] has been shown in both human and veterinary patients to enhance analgesia and allow a reduction in the use of more powerful analgesics. This approach, called multimodal analgesia also has the advantage of providing even analgesic dosing thus promoting the animal's well being.

For procedures in which the pain intensity is anticipated to be high, techniques such as constant rate infusions of local anesthetics and or opioids either systemically, locally at the surgical site or via an epidural catheter and transdermal preparations of drugs provide uninterrupted analgesia and are being used successfully in larger laboratory animals.[9] The analgesic regimen chosen should always be made in consultation with your veterinarian.

Summary

The relief of pain and distress in research animals is ethically sound, humane, and promotes good science. The establishment of clear lines of responsibility coupled with appropriate endpoints, monitoring and intervention strategies are key to the prevention, minimization and/or alleviation of pain and distress in laboratory animals. Several excellent references and formularies are available to the researcher, veterinarian and husbandry personnel to facilitate their ability to recognize and modulate pain and distress in laboratory animals. Experience has demonstrated that a dynamic, interactive team approach to the recognition and alleviation of pain and distress in laboratory animals yields results that protect animal-welfare while promoting good science.

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TABLE 1: POTENTIAL SIGNS ASSOCIATED WITH PAIN OR DISTRESS IN RATS, MICE AND RABBITS

	Mice	Rats	Rabbits
Decreased Food and Water Consumption	X	X	X
Weight loss	X	X	X
Self-imposed isolation/hiding	X	X	X
Self-mutilation, gnawing at limbs	X	X	X
Rapid Breathing	X	X	X
Opened-Mouth Breathing	X	X	X
Abdominal Breathing	X	X	X
Grinding Teeth		X	X
Biting/Growling/Aggression		X	X
Increased/Decreased Movement	X	X	X
Unkempt Appearance (Erected, Matted, or Dull Haircoat)	X	X	X
Abnormal Posture/Positioning (e.g., Head-pressing, Hunched Back)	X	X	X
Restless Sleep			X
Tearing (including Porphyria), Lack of Blinking Reflex		X	X
Dilated Pupils			X
Muscle Rigidity, Lack of Muscle Tone	X	X	X
Dehydration/Skin Tenting/Sunken Eyes	X	X	X
Twitching, trembling, tremor	X	X	X
Vocalization (Rare)	X	X	X
Redness or Swelling Around Surgical Site	X	X	X
Increased Salivation	X	X	X

TABLE 2: POST PROCEDURAL PAIN POTENTIAL [a,b]

Minimal to Mild [c]	Mild to Moderate [d]	Moderate to Severe [e]
Catheter implantation	Minor laparotomy incisions	Major laparotomy/organ incision
Tail clipping	Thyroidectomy	Thoracotomy
Ear notching	Orchidectomy	Heterotopic organ transplantation
Subcutaneous transponder placement	C-section	Vertebral procedures
Superficial tumor implantation	Hypophysectomy	Burn procedures
Orbital sinus venotomy	Thymectomy	Trauma models
Rodent embryo transfer	Embryo transfer in non-rodents	Orthopedic procedures
Multiple injections	Bone marrow collection	
Non-corneal ocular procedures	Corneal procedures	
Intracerebral electrode implantation		
Vasectomy		
Vascular access port implantation		
Craniotomy (periosteal pain)		
Superficial lymphadenectomy		

[a] Table adapted from "Guidelines for the Assessment and Management of Pain in Rodents and Rabbits". 2006, American College of Laboratory Animal Medicine

[b] The analgesia and monitoring required may vary due to a number of factors; such as the invasiveness of the procedure, degree of tissue trauma, surgical time, skill of the surgeon, and the tissues or organs involved.

[c] Post procedural pain relief for minimal to mild pain may be adequately addressed with preemptive analgesia, tissue infiltration with a long acting local anesthetic, and a single dose of a long acting NSAID or mixed opioid agonist-antagonist, or other agent.

[d] Post procedural pain relief for mild to moderate pain may be adequately addressed with tissue infiltration with a long acting local anesthetic combined with one or more doses of a long acting NSAID and/or an opioid or other agent in addition to pre-emptive analgesic administration.

[e] Post procedural pain relief for moderate to severe pain should encompass multimodal analgesia (e.g. combining a pure opioid agonist with a NSAID, tissue infiltration with a long acting local anesthetic, etc.)