Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress

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Lesson 1. Introduction

Welcome to the course *Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress*.

The goal of this course is to provide information on how to minimize pain and distress in mice and rats during and after experimental procedures. This course will address:

- Factors that may confound the interpretation of experimental data.
- Methods for monitoring rodents for pain and distress.
- Methods for alleviating or minimizing pain and distress in rodents.
- Systematic documentation of health monitoring for rodents.

**Investigator Responsibility**

Investigators are responsible for minimizing pain and distress in research animals by:

- Judicious use of anesthetics and analgesics
- Refinement of experimental techniques
- Implementation of best practices
- Implementation of humane endpoints

Two critical components in the refinement of experimental techniques are:

- Monitoring animals for pain and distress, and
- Using interventions for reducing pain and distress

Federal animal welfare laws, regulations, and policies mandate the scientist's responsibility for the humane care and use of animals in research. A concise description of the requirements for the humane care and use of laboratory animals is given in the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*.

**Minimizing Sources of Non-experimental Variation**

Maximizing the humane care and use of laboratory animals and minimizing confounders of experimental variation are mutually complementary objectives of research animal management. Both support the integrity of the research data. Achieving humaneness in animal research depends upon the control, and whenever possible, the reduction of animal pain and distress. Minimizing pain and distress also reduces the impact of these extraneous factors on the research, i.e., as sources of non-experimental variation.

For example, in a mouse model of experimental autoimmune encephalomyelitis, implementation of supportive treatment (hydration and nutrition) was shown to protect against loss of body weight and to greatly extend survival of animals on study, from 25 to 60 days. (Ref.: Lab Animal, 29(5): 40-46, 2000.) The enhancement of the well-being of animals in experiments is often best accomplished through a collaboration of scientists and veterinarians. This team approach capitalizes on diverse perspectives for assessing the animal response to the experimental procedures and for arriving at a strategy of humane interventions during a study. Because the behavior of an animal model may be difficult to predict, ongoing efforts are often necessary to refine the supportive treatments used. A dynamic collaboration...
between scientists and veterinarians, involving continuing observations of the animals, will be most productive for developing humane interventions that are beneficial for the scientific outcome of an animal study.

The image below shows normal Sprague Dawley rats on the day sutures were taken out of their head incisions. They appear comfortable. Rats normally sleep stretched out like this with their bodies in contact with one another. These animals have clean haircoats and appear well-groomed. Investigators should be familiar with the causes of animal pain and distress. Pain and distress may be caused by spontaneous or experimentally-induced disease or injury. Many other factors may contribute to an animal's distress or discomfort, including extreme homeostatic challenges. Investigators should try to minimize pain/distress to an extent that is possible and compatible with experimental objectives. 

*Wherever possible, pain/distress should be eliminated.*

Changes in the following parameters may cause or be associated with animal pain or distress:

- Temperature (environmental and body temperature)
- Hypoxia
- Edema
- Blood electrolytes, e.g. hyperkalemia
- Dehydration
- Environment
  - caging
  - cage mates
  - lighting
  - humidity
  - noise
  - vibration

Note - Smaller mammals experience physiologic changes such as starvation (due to high metabolic rate) and chilling (due to large ratio of body surface area to mass) faster than larger animals.

**Systematically Monitoring for Pain and Distress**

A best approach to reducing non-experimental variation caused by animal pain or distress is to systematically monitor animals after a procedure or when illness is expected.

How often the animals should be monitored depends on the severity of the animals' condition, the expected rate of change in the animals' status, and the impact of the procedure on the animals. At a minimum, all animals should be evaluated once daily. However, the nature of the procedure and condition of an animal may dictate that the animal be assessed multiple times a day. As mentioned on the previous screen, smaller mammals may experience physiologic changes such as chilling and starvation faster than larger animals. Therefore, rodents may require more frequent monitoring than
larger animals. Some situations may require hourly or even continuous monitoring during critical periods in which rapid change in an animal’s condition would be anticipated.

This course offers you a systematic daily approach for assessing clinical signs of rodent pain and distress. Some clinical signs may require assessment at a greater frequency to focus on parameters of particular relevance to the specific model and to provide the animals with appropriate intervention to minimize pain/distress.
Lesson 2. Detecting Clinical Signs of Pain and Distress

Signs of pain and distress in rodents are not easy to detect because of their small body size, their tendency to conceal outward signs of pain and distress, and their habit of hiding or freezing when disturbed. Nevertheless, signs of pain or distress can be detected in rodents by carefully observing subtle changes in behavior. The ability to properly assess pain and distress in rodents requires:

- Knowledge of normal rodent behavior and appearance.
- Systematic approach to observing clinical signs in rodents.

The image to the right shows rats with sleek hair coats that are moving around their cage. Normal feces are present in the bedding. The rats appear relatively normal from this top view. However, the rats in the far left upper corner should be checked a little more carefully as they are hidden and perhaps may be head-pressing, which is a sign of distress.

Performance of a clinical exam should include:

- Observations of animal behavior, appearance, and posture to assess:
  - Signs of pain or distress.
  - Clinical condition and homeostasis.
- Measurements of clinical parameters, e.g., body temperature, clinical chemistries.

The image to the right shows a rat following a neurosurgical procedure. Although he is fairly clean and there is no staining around the eyes (porphyrin staining described later in the course), he is displaying a hunched posture. The hunching of the back is a symptom of abdominal pain that is typically seen in quadripeds. His head is held down and his coat is beginning to have a spiky appearance.

This rat was euthanized and found to have an intestinal ileus from the use of chloral hydrate.
Lesson 3. Appearance and Behavior

Observation
The first step is a gross inspection of rats or mice for abnormalities in appearance and behavior in their home cage. This assessment takes only a few minutes for the practiced observer.

1. From the Cage Exterior.
   Routinely inspect the rodents through the top and sides of the cage. Get in the habit of removing the cage from the shelf and looking through all sides of the cage. Signs of distress may be missed in animals on lower or upper shelves because of low lighting or difficult access. Baby mice and rats can be inconspicuous within piles of bedding or nest boxes.

   These rats below are not having problems after surgery. They are sleeping the way one would expect and they appear comfortable. They are clean, have normal hair coats, good color (skin and mucosa), and normal vital signs.

2. Cage Wire lid Off.
   Lift the cage wire lid to elicit a response to your presence. This disturbance may prompt the animals to move about the cage. Examine the animals' behavior, gait, and hair coat. Normal rats and mice are inquisitive and explore their cage perimeter.

   The image below shows rats that appear alert, inquisitive, and well socialized. They have clean hair coats and are interested in who is on the other side of their cage.

3. Hand Restraint. Examine (and treat) an individual mouse or rat by gently restraining the animal. You can move the animal to a separate examination box for detailed clinical inspection.

Abnormalities
Abnormal mice or rats may huddle in their cage, or they may fail to move around and explore their cage. In addition, rats may vocalize when approached. Inspect an animal's mode and speed of movement. Observe the tail position when the animal moves.
- Is the gait (how it walks) awkward? Observe how all limbs move while walking.
- Does the animal teeter or stumble?
- Is the animal's back hunched and abdomen tucked while walking?
- Is the tail held stiff and upright? Or does the tail drag?

**Tip:** Observe a cage of normal animals for a comparison. Stressed mice and rats commonly display "red tears" or porphyrin staining, which is a discharge from the Harderian gland in the orbit. Porphyrin staining may be seen on the nose, around the eyelids, or on the medial aspect of the forepaws which become stained through grooming of the face. Affected rodents may also fail to groom or they may have piloerection of the hair coat (giving a spiky appearance to the hair).

The image below shows a mouse with porphyrin staining around the eye. Swelling around the eye and muzzle may indicate that these areas are irritated and that the animal has traumatized them by scratching.

**Assessment**

A common approach to assessing animal appearance and behavior is through observation of the following parameters.

**Tip:** It is helpful to have blank forms to use as "score sheets" to enter and track each parameter assessed. (More on this at the end of this course.)

- Activity Level: hypoactivity (hunched, huddled, lethargic); hyperactivity, restlessness, lack of inquisitiveness.
- Attitude: arousal, depression, awareness of surroundings.
- Behavior, Spontaneous: vocalization, self-trauma, isolation from cage mates. These observations are made without disturbing the animal.
- Behavior, Provoked: vocalization, hiding, aggressiveness, minimal response. These observations are made when the animal is disturbed or even prodded.
- Body Condition: emaciation, missing anatomy.
- Food and fluid intake, elimination of feces and urine.
- Fur and skin: unkempt or greasy or dull fur; porphyrin staining around eyes and nostrils; cyanotic, pale, or congested mucous membranes or skin (ears, feet, tail); skin lesions; soiled anogenital area.
• Eyes: clarity/condition of lens, cornea; position of globe (e.g., sunken in orbit or protruding); condition of eyelids, encrustation.

• Posture: hunched back, tucked abdomen; prostrate; head tucked down.

• Locomotion: gait, ataxia, lameness, action of each limb, position of tail when ambulating.

• Neurological: tremor, convulsion, circling, paralysis, head tilt, coma.

• Vital Signs: respiratory distress (open mouth breathing, pronounced chest movement).

• Other clinical parameters that are relevant to your study: presence and status of tumors, infection, or surgical wounds.

Even though this mouse is eating, he has a terribly rough hair coat, mottled appearance, is underweight and hunched.
Lesson 4. Physical Exam for Clinical Condition  

After assessing the animals' appearance and behavior (preceding lesson), conduct a physical exam using methods that are appropriate to the species and experimental model.

Performing a clinical exam on rodents is somewhat limited compared to larger animals due to the greater difficulty in venous access and the smaller sampling size of biological fluids. Nevertheless, specific methods and equipment for rodents allow a clinical exam to provide information on animal well being.

In the image to the right, the rats appear distressed. The investigators on this study believed that this was normal for day one postoperatively because the animals were moving. However, one can see head-pressing, no evidence of grooming, and red tears in these rats. One rat (bottom) does not move his tail in a normal way. A physical exam of this animal revealed low body temperature, hind limb weakness, anemia, pain, and weight loss.

In conducting a physical exam, use quantifiable characteristics whenever possible. These can be tracked over time and compared to a starting baseline or to normal, untreated animals. Such measurements are not only helpful for clinical assessments, but they can also be useful when compiling research data and writing manuscripts. Later in this course, simple record-keeping methods will be discussed to help utilize this information.

You may evaluate:
- Behavior
- Body weight
- Surface lesions (wounds, masses)
- Hydration status
- Body temperature (telemetric methods)
- Blood parameters (Blood collection can be difficult/stressful in mice; may be used to confirm disease or failed treatment.)

Specific physical exams may be added to list on the preceding screen to facilitate the detection and monitoring of illness, pain, and distress that result from your study procedures. For example,

- A neuromuscular exam can be conducted with simple techniques to measure hindlimb or forelimb strength and neurological deficits.

- Abdominal palpation (gentle) of the abdomen may detect pain due to peritonitis. (In rats ? listen for vocalization or grunting or breath-holding by placing the animal close to your ear.)

Later lessons describe a systematic approach for a typical physical exam. Methods to treat abnormalities are included in this discussion.
Lesson 5. Body Weight  

Assessment
Measuring body weight is a rapid way to determine whether an animal is eating and drinking. Body weight changes are a sensitive indicator of rodent health, and a baseline weight measurement allows monitoring of the impact of the experiment on the animal. Reduction in body weight may reflect starvation, dehydration, or a combination of both. Failure of young animals to gain weight is equivalent to a loss of body weight. Most rodents used in research are still growing. Therefore, body weight changes should be interpreted in terms of both actual loss of weight and lack of expected growth. It is helpful to compare body weights of treated animals with those of normal controls.

Body weight of mice and rats can vary dramatically depending on stock or strain. Refer to the weight curves on each strain or stock available from the animal vendor.

In addition to measuring body weight, you should assess body condition. This was briefly mentioned in a previous lesson (Appearance and Behavior: Assessment). Rodents can be assessed for emaciation or cachexia (body wasting) by examining and palpating the lumbar spine and iliosacral areas. A scoring system can be applied to the progressive loss of fat and muscle mass to gauge the severity of emaciation. Approaches for nutritional supplementation will be described in this lesson. For treatment of hydration, refer to a later lesson Fluid and Electrolyte Balance: Treatment.

In the image below, the mice are huddled. The mouse on the left has piloerection and a poor body condition. This animal has a generalized loss of muscle mass, making the spine prominent. One can palpate along a mouse's back and pelvic area to determine the extent of loss in the muscle mass.

Nutritional Support
Animals recovering from surgery develop a negative nitrogen balance as do human surgical patients. Young rodents are especially vulnerable to starvation because they lack long term fat and glycogen stores. Rodents typically have a reduced food (and water) intake 1-2 days post surgery. Low food intake may be more severe and more prolonged if animals are experiencing pain and distress (e.g., if pain alleviation is inadequate).

Returning animals to a physiological plane that is as near normal as possible is nearly always consistent with the scientific objectives of the study. Thus, the impact of surgery on the experimental model should be minimized. Nutritional support (as well as fluid and electrolyte therapy) is important for enhancing an animal's recovery post surgery.

Nutritional support can also be important for nonsurgical studies in which morbidity and reduced food intake occurs. If you have included weight loss as a humane endpoint, you can actually generate false negative findings simply by failing to provide adequate nutritional support during the peak impact of a study. This is detrimental in research on interventions designed to help animals overcome sickness.
Stimulating appetite to increase food intake is helpful to promote a more rapid recovery in rodents as in other species. Something that tastes different and better than the normal every-day diet may be appealing to rats and mice and so may stimulate their appetite. Although some studies may have restricted nutrient requirements, the provision of a home-made or sterile commercially prepared supplement can be helpful to increase food intake and to maintain homeostatic controls such as caloric intake, electrolyte balance, and insulin/glucagon ratio. Commercial rodent surgical recovery diets may be used for balanced nutrition and fluid source, e.g., Surgical Transgel® (Charles River Laboratories). In addition, peanut butter has been used to tempt rodents to eat.

A high protein and high fat diet, which may coax an inappetant rodent to eat, can be prepared as follows:

- One cup hot water
- One package raspberry Jell-O
- 30ml STAT VME High Calorie Liquid® (by PRN)
- 20ml Pediasure® (by Abbott Laboratories)
- 2 scoops Designer Protein™ (by Next Proteins International)
- Blend well
- Pour into ice cube trays
- Refrigerate

Feed the above diet at a rate of:

- ¼ cube per rat per day.
- 1 cube per cage of mice (5) per day
Lesson 6. Fluid and Electrolyte Balance  Top of Page

Maintaining normal homeostasis is greatly dependent on osmotic pressure between tissue spaces. Fluid and/or electrolyte imbalance resulting in dehydration or edema may produce discomfort and add to pain and distress resulting from other causes. Also, animals in pain and distress are likely to have reduced fluid and food intake and so may develop dehydration secondarily. Rodents commonly become dehydrated due to experimental procedures that affect their water intake. Therefore, scientists and caregivers must be able to assess and control hydration.

Performing the exam:

- Observe the animals' behavior. Rodents that are dehydrated may be sluggish.

- Assess the animals' appearance. Skin turgor, hair coat, eye clarity, and the shape and position of the eye within the orbit are useful indices of hydration. To assess skin turgor, tent the skin. Grasp, lift, and twist a fold of skin over an animal's back and watch the skin fall downward into normal position. Compare the response in a normal animal. In a dehydrated animal, the skin is less elastic and may remain tented longer and return more slowly to normal position.

- Blood may be collected (in rats) for measuring total serum protein and electrolytes.

Over-hydration
In conditions of diuresis and low specific gravity, urine may be collected for measuring urine specific gravity on a refractometer.

Since rodents often urinate when picked up, you can be ready with a tube to collect a sample. You may also gently express the bladder. To locate the bladder, gently palpate the caudomedial abdomen while the animal is hand-restrained. The bladder will feel like a pea-sized structure. Be careful to avoid traumatizing the bladder! Excessive force will cause the bladder wall to hemorrhage, and blood will appear in the urine.

A clinical refractometer is an inexpensive hand-held device that measures specific gravity and total protein. Rodent urine typically has a high specific gravity and so a small animal instrument should be used rather than one designed for humans. Although here, too, rodent urine specific gravity is likely to be above the scale. Therefore, the use of a refractometer will be more useful in conditions associated with diuresis and low specific gravity.

Commercial urine dip sticks also measure urine specific gravity as well as urine creatinine, blood, leukocytes, protein, ketones, pH and bilirubin.

Treatment of Imbalance of Fluids and Electrolytes
Rodent discomfort and morbidity can be minimized with:

- Adequate administration of fluids.
- Monitoring for clinical dehydration.
Providing supplemental fluids during experimental studies where there is predictable morbidity is often helpful for optimizing well-being in rodents. There are two common approaches for maintaining hydration status:

1. Administering fluids proactively without assessing hydration status, based on the assumption that most animals in the study will have a similar degree of dehydration.
2. Assessing hydration status and then formulating a fluid dosage to normalize hydration. This approach customizes the treatment for each animal and avoids over-hydration.

Normal maintenance volumes of Lactated Ringers Solution, or 0.9% saline, or glucose-saline can be injected in boluses of about 3 ml/25 g mouse and about 15 ml/250 g rat per day. The subcutaneous administration of these volumes may begin prior to a study and continue once daily (or split in two doses a day) through the period of expected morbidity.

Therapeutic fluids should be warmed prior to injection because fluids administered at room temperature will chill the animal. Fluids can be loaded into syringes and kept warm in rodent support areas. Analgesic treatments may be combined with daily fluid administrations (for hydration therapy). For convenience in treating multiple animals, you can figure the total fluid volume needed for the study and add the appropriate amount of analgesic to a concentration that will deliver the desired dose in each aliquot administered. For more information on medications, refer to the lesson *Alleviation of Pain and Distress: Pharmacological Treatment*. 
Due to their large ratio of body surface area to mass and high metabolic rate, rodents lose body warmth at a faster rate than do larger animals. Conventional thermometers are not practical for use in rodents and can cause stress if used in unanesthetized rodents.

In studies of toxicology, sepsis, diabetes, or whenever morbidity is expected to be high, investigators may consider the use of implantable microchips to track body temperature (as well as identify an animal) without the need for animal manipulation. Microchips can be injected under the skin using conventional restraint or light inhalation anesthesia. Check with the veterinary staff for information on purchasing a microchip system. (The noise of the microchip reader can frighten a rodent. Consider placing the chip in the animal's rump as opposed to the neck.)

Body temperature is also a useful adjunct in the monitoring of humane endpoints in rodents because a reduction in temperature of sufficient magnitude can be a reliable predictor of death. Body temperature measurements may guide the decision of when to euthanatize an animal, which will end or prevent unnecessary pain/distress and allow for the antemortem harvest of fresh body tissues for histopathologic or other analysis.

**Treatment of Hypothermia**
When under general anesthesia, rodents lose heat very rapidly. A mouse can lose 1 degree of body temperature per 5 minutes. A best practice is to use methods for conserving body heat during a procedure that will induce hypothermia, such as anesthesia and surgery. These methods are the provision of a heat source, thermal insulation, or a combination.

**Caution!** Warming devices should provide gentle heat only (maximum of 40°C or 104°F). Having a high ratio of body surface area to mass, rodents on a heat source heat up as quickly as they lose body heat when chilled. They can readily overheat when high temperature heating systems are used, causing animal injury or death.

The image below shows a rat with burns of the ears from over-utilization of a heat lamp. Burns can occur when a heat lamp is positioned too close to the animal.

There are many practical ways to provide temperature support to rodents, either individually or in cages:

- **Insulated pouch or wrap** Rodents may be placed within an insulated pouch or a wrapping to conserve body heat. The insulation used can be such materials as bubble wrap, drapes, polar-fleece, space foil, etc. Bubble wrap can be taped into a tube through which the animal is inserted. A fenestration may be cut in the bubble wrap for access to a surgical site. Rodents may be wrapped in paper drape while they are recovering from anesthesia. As the animal begins regaining
consciousness, the wrapping offers an additional benefit of a sense of security in hiding. A simple double pouch of polar-fleece can be easily constructed (Lab Animal 29:5, 40-45, May 2000). One pocket holds the mouse or rat and the second pocket may hold a pocket-warmer for heating. Mice wake and move out of the pouch when warmed up. Nondisposable items like a polar-fleece pouch should be washed (and sterilized if necessary) between uses.

- **Insulated pads** Anesthetized rodents should always be placed on a thermally insulated surface (e.g. thick or folded drapes). Counter tops and workstation surfaces (often of stainless steel) are highly heat-conductive and will rapidly chill an animal. Remember, hairless body parts such as the feet, tail, and ears, allow a rapid loss of body heat through these areas. Also, if warming devices are too hot to lay a rodent directly on top, then use insulating pads to protect the animal from excessive heat.

- **Using warming pads** Heating devices can generate heat by electrical resistance (electric heating pads), or circulating warm water, or by chemical reaction. Because rodents absorb warmth readily (due to the large ratio of body surface area to mass), heating devices should be set up to heat the area around the animal to only several degrees higher than body temperature (e.g. 102°F). The range of normal body temperature is:
  
  - For mice – 97.5-100.4°F / 36.5-39.0°C
  - For rats – 96.6-99.5°F / 35.9-37.5°C

- **Chemical warming pads (often too hot)** Chemical warming pads (generally an activated sodium acetate in a gel) may get too hot depending on the sodium acetate concentration, which relates to the intended commercial use of the warming pad. For example, pocket warmers typically reach higher temperatures than infant heel warmers. Like the heating temperature, duration of heating is affected by sodium acetate concentration. Lower temperature pads cool down more rapidly and so have a shorter use time. Rodents should never be placed in direct contact with high temperature pads. Instead there should be insulating material between the animal and the heat source.

- **Circulating water warming pads** Circulating warm water devices often offer greater thermostatic control than electrical heating pads. These devices are generally safe for use with rodents at appropriate settings.

- **Electrical heating pads (use discouraged)** Electrical heating pads may be too hot to be in direct contact with a rodent, and could thus cause thermal injury or death. Electric heating pads are very hazardous because they often lack a fine control of temperature. Their use is generally discouraged. But when they must be used, insulated padding should be placed between the animal and the heat source to protect the animal from overheating. Beware of electrical heating pad units that have a feedback according to the temperature of the animal. They should have a thermostat that limits the heating temperature that can be reached.

- **Warming racks** Some caging vendors provide customized warming racks so that whole rodent isolator units can be placed in a thermally-controlled rack. See: Lab Animal 29:5, May 2000 Page 40-45.
Post-procedure Rats and Mice

- **Heat lamps (use discouraged)** The use of heat lamps, or large animal warming lamps, is discouraged because these devices are too hot for rodents. Animals that overheat may be injured or killed. Also, heat lamps are fire hazards when they come into contact with flammable materials (e.g., drape, bedding).

- **Monitoring area temperature** Rodents absorb warmth readily from heating sources (due to the large ratio of body surface area to mass), so heating devices should be set up to warm the area around the animal to only several degrees higher than body temperature (102°F). The range of normal body temperature is:
  - For mice – 97.5-100.4°F / 36.5-39.0°C
  - For rats – 96.6-99.5°F / 35.9-37.5°C

If a heat source is too hot (or unregulatable), then insert some insulation material (e.g., folded drape) between the animal and the heating device. An inexpensive way to assess the heat provided to the animal is to place a simple glass rectal thermometer beside the animals. Adjust the heating or the insulation to obtain an area temperature of about 102°F. For animals recovering from anesthesia, body temperature may remain low beyond the time the animals begin to ambulate. Therefore, it is best to keep them warm until their activity has returned to normal. In addition, if recovering animals are warmed within a cage, offer an area for escape from the heating device. If recovering animals become too hot, they can leave the heated area for a cooler part of the cage.
The growth of solid or ascitic tumors produces pain and distress in rodents just as in humans and other animals. As some examples:

- Pain is associated with distension of overlaying tissues and ulceration of involved skin.
- Tumors that impinge on joints can impair body movement and locomotion and can restrict the animal's access to food and water.
- Growth of a tumor (any type) may cause the animal not to eat and lose body condition.

**Assessment**

Develop an approach that evaluates both the general effects of cancer, e.g., inappetance, and the specific problems related to the type and placement of the tumor. Assessment of the clinical condition of a tumor-bearing rodent largely depends on characteristics of the tumor's biology, such as tumor growth rate, invasion, distension, ulceration, metastasis, and production of cachectic factors. The body systems most likely affected by the tumor should be identified and examined for clinical signs of illness. Therefore, the tumor model will determine the clinical signs to be monitored. Examples:

- Superficial tumors - ulceration, swellings.
- Intracranial tumors - neurological signs.
- Ascitic tumors - abdominal distension, dyspnea.

Although clinical signs may be anticipated, as related to the tumor biology and location, be mindful that unexpected signs may also occur.

**Endpoints**

Unless otherwise approved by the IACUC, animals should be euthanized before they become moribund or die due to tumor load. Also, animals should be euthanized before the tumor mass becomes excessive, ulcerates, or impairs the animal's bodily functions or behavior.

The criteria for endpoints in tumor development should be established in the animal protocol. These are generally a combination of:

- Tumor mass or burden
- Body condition, e.g. cachexia
- Impairment of body functions, e.g. gait.
- Ulceration

The image to the right shows a nude mouse with an implanted tumor. The mouse has reached a humane endpoint in the experiment because of the tumor's size and because the tumor has become necrotic and ulcerated. This mouse was euthanized.
Lesson 9. Alleviation of Pain and Distress

General Approach
The detection and alleviation of pain or discomfort in rats and mice have been discussed in this course. The effective recognition of pain and distress should not rely on a single clinical observation but rather on a composite of signs and measurements that together reflect animal well-being in terms of pain or distress.

In the image to the right, a rat is shown 36 hours after a neurosurgical procedure. He has porphyrin staining or “red tears” around his eyes, nose, and medial forepaws. His incision appears swollen and painful. He has not been grooming. This animal should receive treatment to alleviate his pain and distress.

The results of the systematic clinical exam described in this course should be documented in a study record for animal health. (See the next lesson.)

When animals are found to be in pain or distress, appropriate individuals should be contacted (i.e., veterinary staff and investigators). Determining the appropriate response involves a team approach with both scientific and veterinary input. A strategy to manage the adverse effects of the experimental procedures should be addressed in the protocol. Possible treatments may include the administration of analgesics, antibiotics, warmth, fluid therapy, nutritional supplements, etc.

Pharmacological Treatment
A number of analgesic options are available. Refer to your institution's veterinary staff for treatment recommendations. Generally you should consider the use of local anesthetics, opioids, and non-steroidal anti-inflammatory drugs (NSAIDs). The opioids are controlled drugs and may be dispensed from an animal facility pharmacy.

Commonly used analgesics are:

- Opioid:
  - Buprenorphine hydrochloride

- NSAIDs:
  - Ketoprofen
  - Carprofen (lasts 24 hours)
  - Banamine
  - Tylenol derivatives

A practical approach to using analgesics in rodents is to prepare a batch of doses for a population of animals over the period of a study.

1. First calculate the total fluid volume required to dose all animals.
2. Then make a solution of the analgesic at a concentration that will deliver the desired dose per aliquot administered.

This approach can be used to medicate the animals with analgesic only or it can be used as a combination with hydration therapy. Remember to adjust the analgesic concentration according to whether the fluid aliquots will provide for hydration therapy or not.

If injections are not necessary (i.e. for hydration therapy), you may consider offering analgesic orally. A common approach is to add the analgesic (usually an opioid) to a gelatin treat, such as grape jelly, jello, and various commercial doughs and gels. (Rodents may prefer berry flavors and may avoid artificial citrus flavors.) Your veterinary staff will be familiar with these techniques.

When administering a medicated treat, it is important to be sure that the intended animal (and not cage mates) eats the whole dose.

If there is concern whether an analgesic may interfere with the experiment, conduct a pilot study to determine whether the analgesic may affect the study or not.

An important consideration in the use of analgesics is to reassess the animal for pain as the analgesic effect wanes. Perform a clinical exam for signs of pain to determine if another dose is needed. For information on a record-keeping system, refer to the next lesson *Documentation of Post-Procedure Care: Monitoring and Treatment.*
Lesson 10. Monitoring and Treatment  Top of Page

Previous lessons have discussed practical methods for conducting a clinical exam on rodents to assess morbidity, pain, and distress. This lesson addresses the documentation of exam findings and treatments. A records management system aids in documenting the status of your animals over time. And in cases when multiple staff take turns monitoring the animals, a record system facilitates good communication among all persons involved in the care and use of these animals.

The image below shows cages of rats that recently underwent surgery. The cages have been affixed with a "watch" card so that it is easy to find these cages on the rack when a person enters the room. Each cage card also corresponds to a 5”x8” medical record in a desk just outside the room. (There is a fresh orange for added nutrition - just be careful not to leave fruit in the cage more than 12 hours so it does not spoil.)

Typically, there are three components to a record system.

1. A cage identification system. Cages to be monitored should be flagged to help an observer quickly locate the cages to be checked among all others in the animal room. Once the animals are no longer being treated, a different cage flag can be used to indicate the need for a later recheck of these cages. Colored stickers, hanging tabs, or index cards may be used. Consider a color coded system for distinguishing the type of monitoring.

2. A health record. A health record is used to document the clinical observations and physical exam findings. Records may be maintained for individual animals or a cage of animals. Consider using an index card, which can be kept in the cage card-holder throughout the monitoring period. A scoring system for clinical signs can be incorporated into the health record to provide both an efficient way to track the animals' clinical profiles over time and to compile numerical data useful for scientific purposes. For more information, refer to the next section Scoring Systems for Clinical Exam Data.

3. An accessible record archive. It is helpful to archive animal health records so that they are accessible for routine use, for example in a procedure area adjacent to an animal room. The archive can be organized with a section of current cases. Staff who monitor the animals should routinely check this file before entering the room. This system allows for animals to be checked on a frequency that is appropriate to the condition – daily or weekly, for example.

Scoring Systems for Clinical Exam Data
A defined scoring system of clinical parameters is a valuable aid for monitoring animal morbidity. The clinical parameters and scoring standards should be appropriate for the animal species and the disease model. This system can facilitate the decision to intervene to allay an animal's pain/distress, e.g., to administer treatment or euthanasia.
If appropriate clinical parameters are not known for a particular disease model, you can perform a pilot study on a small number of animals to:

- Characterize the relevant clinical parameters;
- Define the time course of the disease and related critical events;
- Refine the endpoints; and
- Determine the timing and frequency for animal monitoring.

**Scoring Systems: Guidelines**

Some practical guidelines in developing a scoring system are to:

- Identify the clinical sign or signs that can be used to recognize the need for immediate euthanasia.

- Over successive studies, be mindful that the scoring system may need refinement. New and useful assessment methods may become available. Or, the clinical profile may change and require assessment by other parameters.

- Collect data on the numbers of animals that are euthanized vs. die unexpectedly. These data will help you refine the scoring system to minimize the number of animals dying from the experimental procedure without benefit of euthanasia.

- Publish your scoring system so that others may refine their methods based on your work.

A scoring system can be incorporated into the health record. This composite record can track the animals' clinical profile and document the administration of treatments.

**Scoring Systems: Examples**

Two example scoring systems are presented below. These are sample score sheets illustrating scoring standards:

**Example 1.**
For postoperative monitoring of rodents in surgical models. Lab Animal 29:5, 40-45, May 2000. In this example, five parameters are used:

1. Attitude
2. Porphyrin staining
3. Gait and posture
4. Weight
5. Food intake

All parameters are rated on a scale of 0.0, 0.1, and 0.4. Score standards are defined for each parameter. A total score equal to or greater than 1.0 indicates the need for veterinary attention.
Example Score Sheet for Assessing Rodents Postoperatively

National Institutes of Neurological Disorders and Stroke (NINDS)


<table>
<thead>
<tr>
<th>Observation</th>
<th>Individual Score (mark score here)</th>
<th>Score Standards</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude</td>
<td>0</td>
<td>Bright and alert.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Burrowing or hiding, quiet, but rouses when touched.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>Burrowing or hiding, quiet, but rouses when touched. No exploration when lid off, burrows, hides, head presses. Might be aggressive when touched.</td>
<td></td>
</tr>
<tr>
<td>Porphyrin staining</td>
<td>0</td>
<td>None.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Mild around eyes and/or nostrils</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>Obvious on face or paws.</td>
<td></td>
</tr>
<tr>
<td>Gait and postures</td>
<td>0</td>
<td>Normal.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Mild incoordination when stimulated, hunched posture, mild piloerection.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>Obvious ataxia or head tilt, hunching, drags one or both limbs, severe piloerection.</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0</td>
<td>Up to 5% weight loss over preoperative weight.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>5-10% weight loss over preoperative weight.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>10-20% weight loss over preoperative weight.</td>
<td></td>
</tr>
<tr>
<td>Appetite</td>
<td>0</td>
<td>Normal: eats dry food, evidence of urine and feces, food missing from feeder or floor, gelatin gone within 8 hours, evidence that fruit is chewed on.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>No evidence of eating dry food but likes gelatin and fruit, drinks and appears hydrated (skin does not “tent”).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>No interest in food or treats and or appears dehydrated (poor skin turgor).</td>
<td></td>
</tr>
<tr>
<td><strong>Total Score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example 2.

Example Score Sheet for Assessing Rodents with Experimental Autoimmune Encephalomyelitis (EAE)
National Institutes of Neurological Disorders and Stroke (NINDS)


<table>
<thead>
<tr>
<th>EAE Grade</th>
<th>Clinical Signs</th>
<th>Intervention Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormality</td>
<td>Baseline weight (average/cage).</td>
</tr>
<tr>
<td>1</td>
<td>Initial signs but no paraparesis: clumsiness, incontinence or atonic bladder, or flaccid tail</td>
<td>If atonic bladder present, express bladder daily and check hydration status twice daily. At this time, fruit, cereal, and other nutrients will be added to the cage bottom. If an animal appears dehydrated, fluids will be given as needed either IP or SQ. Identify individual animals by a mark on the tail with black ink. Initiate a medical record. Initiate an EAE chart and record body weight.</td>
</tr>
<tr>
<td>2</td>
<td>Mild paraparesis: trouble initiating movement but walk well once started, possible atonic bladder.</td>
<td>Same as above; if five mice in a cage and only one affected, consider separating to allow better access to feed. Per veterinary assessment, mice may be fed a high protein liquid diet supplement. Begin weighing animals 3 times a week, recording their weights on the medical record and the EAE chart.</td>
</tr>
<tr>
<td>3</td>
<td>Moderate paraparesis: inability to move one or both hindlegs, noticeable gait disturbance, possible atonic bladder.</td>
<td>Make food and water more accessible (for example, feed mash placed on floor of cage, water bottle w/ long sipper tube, and fruit as fluid supplementation). Express urinary bladder twice daily; give fluids, if necessary. Animals may need supplemental heat (see no. 4 below). Weigh at least 3 times a week. Euthanize if &gt; 20% weight loss.</td>
</tr>
<tr>
<td>4</td>
<td>Moderate quadriparesis/ quadriparalysis</td>
<td>Euthanize, except when investigators develop and implement a plan for at least twice daily gavage and subcutaneous fluid administration, with a medical record kept on each individual animal. Weigh the animal daily and record. Express urinary bladder if needed; wash and dry animal in case of urine staining (incontinent). Animal(s) will also be provided an external means of heat, if needed (heat lamp, heating pads, nestlets with additional bedding, etc.)</td>
</tr>
<tr>
<td>5</td>
<td>Moribund</td>
<td>Euthanize within the day.</td>
</tr>
</tbody>
</table>
Lesson 11. Summary  

Good science requires good animal care. Animals that are in poor condition, discomfort or pain are poor research subjects if such problems are extraneous to the objectives of the research. The impact on the animals' physiology can alter the outcome of the research data. In these cases, animal well-being supports the integrity of the research.

In studies where animal morbidity is an expected outcome of the procedure (i.e., in a disease model when clinical symptoms are manifested), humane experimental endpoints should be established that do not conflict with the scientific objectives. The use of humane endpoints often benefits research by allowing the pre-mortem collection of biological samples. Using pre-established endpoints can avoid spontaneous death that results in loss of tissue due to post-mortem autolysis.

The strategies described here for assessing animal well-being and pain or distress are guidelines that can assist you in developing animal assessment methods that are appropriate for your experimental procedures.

Alleviation of pain and distress in animals is not achieved solely by the use of analgesics. Experimental procedures offer many opportunities for enhancing the animals' well-being by the refinement of procedures to reduce the severity of injury or stress and by the provision of supportive care. Many such refinements were described in this course. Using a system to assess animal well-being will help document the improvements in technical procedures and the benefits from supportive care.
References


