



# ANIMAL TRACKS

A newsletter for the Duke research community



April 2009

<http://vetmed.duhs.duke.edu>

## Duke Lemur Center Receives a “Thumbs Up” from the Association of Zoos and Aquariums!

On March 23, 2009, The Duke Lemur Center received notification from the Association of Zoos and Aquariums (AZA) that the DLC is officially approved as a participant in AZA Species Survival Plans for all prosimian species! The Species Survival Plan (SSP) program began in 1981 as a cooperative population management and conservation program for selected species



in zoos and aquariums in North America. Each SSP manages the breeding of a species in order to maintain a healthy and self-sustaining population that is both genetically diverse and demographically stable.

Beyond this, SSPs participate in a variety of other cooperative conservation activities, such as research, public education, and reintroduction and field projects. Currently, 113 SSPs covering 181 individual species are administered by the Association of Zoos and Aquarium, whose membership includes accredited zoos and aquariums throughout North America.

For the Lemur Center, this approval is the culmination of a process that began last fall with a lengthy and comprehensive application that described the Lemur Center's governance, staff, finances, physical facilities, safety and security, animal collection and care, veterinary services, research procedures and so on. This was followed by a thorough two-day site inspection in February which included meetings with nearly all staff members.



The results were an enthusiastic thumbs up to the Duke Lemur Center for participation in prosimian Species Survival Plans. The Visiting Committee shared particular kudos in the following areas:

1. The entire staff were dedicated, enthusiastic and hard-working.
2. There was a very good staff training program (Both from Duke University and the Duke Lemur Center).
3. There were excellent animal enrichment and animal training programs.
4. The Duke Lemur Center has had no non-compliance items in the last five years from USDA!
5. The DLC has started a \$9 million capital improvement project that will greatly improve indoor and winter holding for the lemurs.
6. The relatively new Director (Dr. Anne Yoder) has reorganized the staff and added several new positions (Operations Director, Research Coordinator and Advancement Officer) to better achieve the Center's three major goals of research, conservation and education. Her enthusiasm for the facility is evident and her vision is clearly articulated.



### Upcoming Dates & Deadlines

*Deadlines are 5 PM on the date listed!*

April 17	Seminar: Rodent Caging Systems
April 20	Amendment deadline
April 27	Seminar: Protocol Writing (Alt. Search)
April 23	New protocol meeting
May 4	New protocol deadline
May 4	Amendment deadline
May 7	Amendment meeting

## A CASE FOR PRE-EMPTIVE ANALGESIA

Pain and distress modifies research outcomes. A simple statement, a true statement, but more thorny when assessing *impactful pain or distress and the extent that such conditions modify research outcomes*. Balanced against a core axiom of veterinary medicine 'to prevent or alleviate pain in the animal patient,' the researcher must consider paradoxical, complicated, and potentially restrictive options.

How can one perform quality research that is free of outcome modifiers such as animal pain or distress? While it may be challenging to do so, an effective engagement of pre-emptive pain management will serve the strongest option for securing a stable biologic platform from which quality animal facilitated investigation may occur!

The Duke animal care & use program requires the use of pre-emptive analgesia for animal based activity as an ethical and scientifically sound method of preventing animal pain and maximizing the integrity of research data outcomes. Some of the evidence to support this institutional position is:

- In a clinical study, titled: Pre-Emptive Analgesia for Post-Operative Pain Relief in Lumbosacral Spine Surgeries: A Randomized Controlled Trial, human patients were evaluated on the effectiveness (or lack thereof) of epidural administration containing bupivacaine and tramadol. The authors reported blinded patient assessments confirmed that pre-emptive analgesia was effective in providing a more comfortable and successful patient procedure. An anthropomorphic application would engender a similar outcome in animal patients; a pain-free animal equals humane use and improved data quality.
- A report titled: A Qualitative and Quantitative Systematic Review of Preemptive Analgesia for Postoperative Pain Relief: The Role of Timing of Analgesia, notes: "In the perioperative setting, preemptive analgesia can be achieved with NSAIDs, COX-2-selective inhibitors, acetaminophen, and longer-acting opioids such as codeine and propoxyphene." The author concludes: "Understanding of pain mechanisms has revealed the importance of proactive interventions in analgesia that aim to prevent initiation of hyperalgesia and central sensitization through preemptive analgesia. An appreciation of balanced approaches to analgesia has allowed for safer pharmacologic strategies for analgesia." While these results were specific for human clinical assessment, the anthropomorphic paradigm remains a rational application for animal based research—that prevention of pain is a good thing for both animal welfare and animal research!
- John C. Schofield BVSc, Dip ACLAM, MRCVS and Virginia M. Williams BVSc, MACVSc, Dip Prof Ethics reported to the New Zealand Ministry of Agriculture in their report Analgesic Best Practice for the Use of

Animals in Research and Teaching - An Interpretative International Literature Review: "The notion that post-operative pain can be forestalled or even prevented derived from the simple observation of human patients who underwent orthopedic surgery. Patients who received opiate premedication prior to general anesthesia took four times longer to request pain relief after surgery than those who received a general anesthetic without any premedication; these unpremedicated patients all requested pain relief within two hrs." Animal observations are consistent with these reports in people.

- Other authors note that elderly patients receiving pre-emptive analgesia prior to surgical leg amputations for ischemia and diabetes reported no pain at 6 months, whereas 50% of patients who received no premedication reported pain at 6 months. Similar results occurred at 7 days post surgery and 12 months later. Such evidence provides strong motivation to alleviate animal discomfort—whether you can see signs of pain or not in your animal patient!

Many other arguments supporting pre-emptive analgesia could be considered, but it is clear there exists adequate scientific based human clinical evidence to support the use of pre-emptive analgesia in the animal patient.

How can this information be used by the Duke research community? When designing your animal use protocol:

- Evaluate an anticipated level of pain or distress for the procedures being proposed. Lacking any clear basis for expectation, asking oneself if the same procedure was performed in a human (and realizing that many research procedures would not be performed in people due to the unethical nature of such), what would be the anticipated pain management plan?
- Develop a pain management plan for all animals used in research. Call on a Duke veterinarian for advice on which medications/techniques might be preferred.
- Employ pre-emptive and multimodal methodology, maximizing the humane aspects of research and insuring the integrity of the research data.
- Consider agent, route of delivery, and dosage. Choose the best option for the patient & the research.
- Determine the MAXIMAL duration of anticipated pain and assure there is sufficient duration of coverage to address potentially painful periods.
- Define the signs of distress, specific to the species being used, which will be used as indicators of failed analgesia (observation of these signs is an indication of insufficient frequency of provision of analgesia).
- Establish endpoints to prevent unnecessary pain or distress, and be ready to remove the animal from the study.

*Wishing you a productive research month,*



# HANDLING & TRANSPORT OF AGRICULTURAL ANIMALS USED IN RESEARCH

By Temple Grandin, PhD  
Department of Animal Sciences  
Colorado State University  
Part 1

Researchers and technicians who work with agricultural animals should understand the basic principles of livestock behavior during handling. An understanding of behavior will improve animal welfare, reduce stress, which could confound research results, and prevent injuries to both people and animals. Calm animals are easier to handle than agitated fearful animals. If an animal becomes agitated, it takes 20 to 30 minutes for it to calm down. Waiting for it to calm down will make handling and restraint easier. Stress associated with transportation, restraint, or handling diminishes immune function in cattle, pigs, and sheep (Kelley et al. 1981; Mertsching and Kelley 1983; Blecha et al. 1984; Coppinger et al. 1990). Detrimental effects of handling and transit stress on rumen and reproductive function in cattle and sheep were shown by Doney et al. (1976), Galyean et al. (1981), Hixon et al. (1981), and Stoebel and Moberg (1982; Grandin, 1997a). Good handling and transport procedures will reduce detrimental physiological changes.

**Hearing and Handling:** Cattle and sheep are more sensitive than people to high-frequency noises (Ames and Arehart 1972; Kilgour 1983; Heffner and Heffner, 1998). The auditory sensitivity of cattle is greatest at 8,000 Hz and that of sheep at 7,000 Hz (Ames 1974a). The human ear is most sensitive at 1,000 to 3,000 Hz. Loud or novel noises can be highly stressful to livestock. Sheep exposed to exploding firecrackers or noises in a slaughter plant have increased thyroid hormone and cortisol levels (Falconer and Hetzel 1964; Pearson et al. 1977). Dairy cows exposed to exploding paper bags produce only 70% of the normal amount of milk (Ely and Petersen 1941). A loud ringing bell from an outdoor telephone will raise a calf's heart rate by fifty to seventy beats (T. Camp, pers. com.). Yelling at cattle is highly stressful and should be avoided (Waynert et al., 1999; Pajor et al., 2002). Physiological changes induced by sudden noises could alter the results of experiments.

Animals will readily adapt to reasonable levels of continuous sound, such as white noise, instrumental music, and miscellaneous noises. Continuous exposure to loud sounds over 100 dB has been reported to reduce daily weight gain in sheep (Ames 1974a). However, continuous background sound at lower levels can actually improve weight gain in some cases. Ames found that sheep exposed to 75 dB of miscellaneous noises (roller coasters, trains, horns, etc.), white noise, or instrumental music gained weight faster than controls without continuous background sound.

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Livestock producers and researchers have learned from experience that continuous playing of a radio with a variety of talk and music will reduce the reaction of pigs to sudden noises. Providing controlled amounts of continuous but varying background sound may help prevent experiments from being confounded by extraneous noises.

In facilities where livestock are handled for experimental or veterinary procedures, sudden or novel noises should be avoided. It may be advisable to have the same radio station or background sound that is provided in the living quarters. Research is needed to determine if exposing animals to sounds they may hear while being handled and tested in their living quarters will reduce stress.

The sound of banging metal can cause balking and agitation. Rubber stops on gates and squeeze chutes will help reduce noise. The pump and motor on a hydraulic squeeze chute should be located away from the squeeze. If the pump is noisy, replace it with a quieter vane pump. The loud sound of a noisy pump is also stressful to people. Equipment that produces a high-pitched whine will cause livestock to become agitated and difficult to handle.

## Part 2 in the May Issue of Animal Tracks



## IACUC A ADVANCED HELD IN RTP (June 2009)

Scientist Center for Animal Welfare (SCAW) is hosting an IACUC Advanced for June 15, 2009 at the Glaxo-Smithkline campus in RTP. Secure registration can be completed at the SCAW webs site [info@scaw.com](mailto:info@scaw.com).

Topics presented at these educational workshops will help to fulfill the training requirements in USDA.APHIS/AC Policy 15 that states "IACUC members should be trained in understanding the Animal Welfare Act, protocol review and facility inspections." The program format will let small groups discuss specific, complex topics that are relevant to IACUC functions. The focus for this meeting will be 'Distress.' How to recognize and evaluate the level of pain and distress; appropriate end points stated in the protocol; special environmental conditions required because of potential pain and/or distress.

SCAW conferences and workshops will be reviewed and approved by the American Association of Veterinary State Boards (AAVSB) RACE program and will meet the requirements for continuing education credit for Veterinarians and Veterinary Technicians. SCAW's AAVSB/RACE provider number is #293.

# EFFECTIVE MONITORING AND RECOVERY OF THE ANESTHETIZED ANIMAL PATIENT

Anesthesia is the act of rendering the patient senseless to pain or discomfort. Anesthesia is used for surgical procedures as well as non-surgical procedures. Assuring a safe and effective level of anesthesia is necessary for quality research and humane use of animals. Assuring a safe and effective level of anesthesia requires monitoring.

According to Duke animal program policy, ALL anesthetized animals must be observed and monitored to assess adequate level of anesthesia, and assure the animal is anesthetized. Failure to monitor properly, especially if the animal experiences adverse outcomes from anesthesia, could be a non-compliance reportable to the federal regulatory agencies (the NIH, the USDA, and AAALAC).

There are as many acceptable methods to monitor anesthesia as there are species of animals, but select monitoring processes are fairly common between species. These include:

- **Toe pinch:** Effective if the animal has a toe large enough to pinch. A gentle pinch at or near the nail bed, a pinch which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal has inadequate anesthesia. Any observed movement (e.g. withdrawing the paw) indicates that the animal is not sufficiently anesthetized to perform a painful activity (e.g. surgery).
- **Skin pinch:** Similar to the toe pinch but using any skin on the body. More sensitive areas of skin work best. A gentle pinch of a small fold of skin, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal is too light. Any observed movement (twitching of the skin) indicates that the animal is not sufficiently anesthetized to do surgery.
- **Jaw "tone":** Generally this can be a good indicator of muscle relaxation. The lower jaw is gently opened to its maximum extent. Any observed resistance to opening, or closing of the mouth, is an indicator that the animal is too light to do a painful procedure.
- **Respiratory rate:** this can be used as a good indicator of the depth (or level) of anesthesia. Rapid, shallow respirations usually indicate the animal is too "light," not sufficiently anesthetized to perform painful procedures. A very slow relaxed respiration

may be an indication the animal is very 'deep,' even approaching euthanasia. Animals in deep anesthesia often take a very long time to recover, and will require additional supportive care during recovery. The best respiratory rate is one that is just barely below normal. Since normal respiration rate varies among animals, it is always important to observe your patient for a few minutes while they are resting to determine their normal respiration rate. Veterinary textbooks can provide a range of normal respirations, but even these vary from one animal to another.

- **Heart rate:** An increase in heart rate and/or blood pressure usually indicates a decrease in anesthetic depth. Normal heart rates vary greatly among species, consult veterinary text for normal values, or Email our clinical veterinarian.
- **Palpebral:** The blink reflex is quite variable, depending on the anesthetic agent used, and difficult to assess in small animals (i.e. mice and rats). DO NOT USE FOR RODENT ANESTHESIA!
- **Corneal:** The cornea can be damaged, if not protected, but when used carefully, it is a good reflex. Touch the edge of the cornea with a gauge sponge or cotton q-tip. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.
- **Body Temp:** Most anesthetic agents depress body temperature to a significant degree. Therefore, it is important that anesthetized animals be maintained on some type of material which shields them from contact with cold surfaces and reduces heat loss. The use of a supplemental heat source is a good idea, but must be used with caution, since burns can occur from electric blankets or water bottles that are too hot.

**Anesthesia Recovery:** All animals recovering from anesthesia must be constantly attended until they have recovered their swallowing reflexes. As a general statement, animals must be observed and the observation must be recorded at least every 15 minutes. Observing recovering animals less frequently than every 15 minutes requires IACUC approval. Observations (and recording of the observations) must continue until the animal regains motor control. In the case of most animals, this is usually indicated by the animal starting to move around the cage and being able to stand and walk without falling.

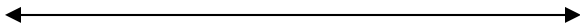
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Observations may be recorded in a research notebook or a medical record notebook, but should be available for IACUC review.

**Support of the Recovering Patient:** Rodents be supported, and the duration of recovery shortened, by keeping the animal warm. A heat lamp, Snuggle Safe or a heating pad may be used, but the animal should not be close to the heat lamp (they will get skin damage from being too close) nor should they be in direct contact with the heating pad (the heating wire will also burn the animal). Usually, the best approach is placing the animal's cage half on and half off a heating pad, or wrapping the animal in a small towel placed in the bottom of the cage. Care must be taken to avoid overheating when a heating lamp or heating pad is used. Whenever a heat source is used, a thermometer should be placed at the animal's level to monitor actual heat. Animals which have had any significant blood/fluid loss during surgery should be provided with fluid or blood replacement during surgery and/or the anesthesia recovery period. In small rodents, this is best accomplished via the intraperitoneal or subcutaneous route and as described in the protocol. If you should have any concerns at any time during the animal's recovery, contact the veterinarian on call by paging 970-9410 for immediate assistance.



## Home Improvement: C57BL/6J Mice Given More Naturalistic Nesting Materials Build Better Nests

In the November 2008 issue of JAALAS, researchers from Purdue University report shredded paper strips allowed the mice to build higher quality nests than those built with any of the other materials. Nests built with tissues were of intermediate quality, and nests built with compressed cotton squares were of poor quality, similar to those built by the control group. These results suggest that C57BL/6J mice given appropriate nesting materials can build nests similar to those built by their wild counterparts.

Hess et al cite the advantages of environmental enrichment for laboratory, while recognizing the debate which arises over the means of enrichment and its ability to be used in a sterile environment. The authors remind us that mice in nature build dome-shaped, complex, multilayered nests. This study focused on the use of naturalistic nesting materials to evaluate nest quality through the use of a 'naturalistic nest score' system.

Their results suggest that C57BL/6J mice, a strain of mice more often associated with cage mate fighting and self induced trauma, given appropriate nesting materials could build nests similar to those built by their wild counterparts. Such enterprises may discourage aggressive tendencies in this strain by provided opportunity for species specific activity.

## AALAS LEARNING LIBRARY AVAILABLE TO DUKE RESEARCHERS

The Duke animal program has an institutional license for the AALAS Learning Library, which can provide essential training for technicians, managers, and investigators working with animals in a research or education setting. The Learning Library emphasizes appropriate handling, care, and use of animals, and meets all federal and local IACUC requirements for qualified training; and its FREE to members of the Duke animal care and use community!

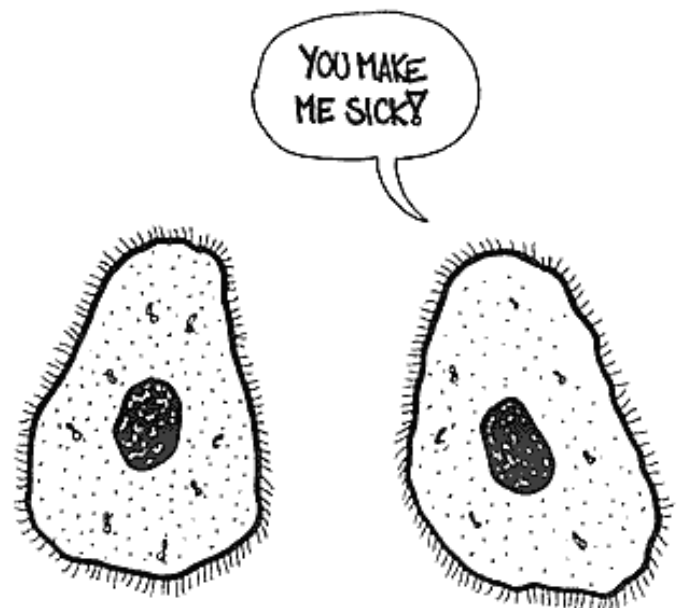
The AALAS Learning Library organizes courses in libraries according to topic area or source of the material. The [Animal Care and Use Library](#) has courses on certification, regulatory mandates, bioethics, biostatistics, biosafety, and management.

While use of the Learning Library is free to the Duke animal care & use community, you **MUST** have a [USERNAME](#) and [PASSWORD](#). Contact Bill Wade at the Office of Animal Welfare Assurance to obtain the necessary information. You can reach me at 919.668.6724 or my email at [w.wade@duke.edu](mailto:w.wade@duke.edu).

If you have thoughts on particular topics that are needed by your laboratory members, please let me know.

Bill

[www.4allfree.com](http://www.4allfree.com)



GASPIRTZ

**Dysfunctional Germ Family**



**Duke Animal Care & Use Program  
Brown Bag Seminar**

**Friday April 17th, 2009  
Noon – 1 p.m.  
Bryan Research Building: Room 103**

**Mr. Scott Hoy  
Mr. Mike Sidelsky  
Allentown Inc.**

**Will be presenting:**

## **Rodent Caging Systems: Components, Operation and Use**

**Rodents are housed in a variety of caging systems here at Duke. This presentation will discuss the more common systems used and their functions, applications and user protocols. This will include specific training on the use of Individually Ventilated Caging (IVC) systems used for both barrier and containment areas.**

The presentation will be on **Friday April 17<sup>th</sup>, 2009 from noon to 1 p.m.**

The session will be held in room **103 of the Bryan Research Building**, located at 421 Research Drive, on Duke University's West Campus.

Attendees are encouraged to bring a lunch. OAWA will provide drinks and desserts.

Please plan on arriving prior to noon in order to get refreshments, sign in, and be seated.

For those who will be coming from off campus, driving directions and parking information can be found at the following link: <http://neuro.duke.edu/Links/map.htm>

**This session will count for 1 CEU of AALAS In-house Training Credit**



## OAWA's Brown Bag Seminar

Monday April 27<sup>th</sup>, 2009

Noon – 1 p.m.

Bryan Research Building: Room 103

Mr. Teddy Gray  
Head, Biological and  
Environmental Sciences Library

Will be presenting:

# Alternatives Searching for the Researcher

**THIS SEMINAR WILL COVER HOW TO EFFECTIVELY SEARCH FOR THE THREE R'S (REFINEMENT, REDUCTION, REPLACEMENT) OF ANIMAL RESEARCH. WE WILL HIGHLIGHT THE BEST LITERATURE DATABASES TO SEARCH AND HOW TO CHOOSE AMONG THEM. WE WILL THEN COVER CREATING EFFECTIVE SEARCH STRATEGIES INCLUDING THE USE OF SUBJECT HEADINGS AND DEMONSTRATE SAMPLE SEARCHES IN PUBMED. FINALLY, WE WILL POINT OUT ADDITIONAL RESOURCES FOR RESEARCHERS USING ANIMAL MODELS.**

The presentation will be on **Monday, April 27<sup>th</sup>, 2009 from Noon to 1 p.m.**

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