Use of behavior analysis to recognize pain in small mammals

Jörg Mayer, Dr.Med.Vet, MSc

The minimization of pain in laboratory animals is a gold standard with implications for improvements in both animal welfare and research quality. Changes in behavioral parameters may indicate that an animal is in pain, but in order to effectively use behavioral change to assess pain, the observer must be familiar with normal behaviors. The author discusses normal and pain-related behaviors exhibited by rodents, rabbits, and ferrets.

Parameters for measuring animal responses to painful stimuli fall into three general categories: physiological, biochemical, and behavioral. In animal research, physiological parameters, such as changes in body temperature, heart rate, respiration, or body weight, are the most commonly used indicators of pain. Biochemical parameters—including levels of corticosteroids, catecholamines, and various hormones—are also frequently employed to assess pain or distress in laboratory animals. The third category, however—behavioral parameters (see **Box 1**)—is often overlooked or underestimated.

Behavioral changes are often the earliest signs of pain for animal care staff [1]. Not only can behavioral parameters be effective tools for detecting or grading pain, use of these parameters avoids the induction of pain or stress sometimes inherent in collecting biochemical or even physiological data. Behavioral data is especially important in small animals, such as laboratory rodents, because gathering physiological and biochemical data is often not practical in these animals. In many cases, researchers and animal care personnel must make an adequate assessment of pain and discomfort based upon behavioral observations alone.

Although investigators who are primarily focused on analgesic research and pain physiology are constrained by their research parameters to develop a good understanding of how to quantify and qualify pain, other researchers and clinicians often misinterpret the common clinical signs of pain or even completely fail to recognize them, especially in species that do not react to pain in an intuitively 'human way'. The innate 'conservation-withdrawal' reflex of prey animal species is much more pervasive and difficult to interpret than the familiar 'fight or flight' reaction to stress or pain that is frequently exhibited by domesticated animals and predator species. Many times 'conservation-withdrawal' reactions to pain, in which the animal calmly crouches down, are misinterpreted as manifestations of relaxation or sleepiness.

An understanding of normal species behavior is essential to correctly assessing painful behavior. Often non-specific indications, such as a decrease in food consumption or a change in posture, vocalization, locomotion, or temperament, are used to judge pain. These behaviors, however, may not be uniformly manifest among different species and are often so subtle as to appear absent to the eyes of the untrained observer. Without knowing the range of normal behavior, an observer will find it extremely difficult to detect abnormal behavior, especially in prey species, which often only demonstrate cryptic and subtle changes.

Many of the published standards for evaluating behavioral responses to external stimuli in animals highlight the need for understanding normal behavior by implicitly invoking such behavior as the baseline for comparison. For example, Morton and Griffiths's pain-scoring system defines 'category 0' as any "behavioral response normal for the expected condition" and 'category 1' as a minor depression or minor exaggeration of the 'category 0' response[2]. These coarse differentiations would be difficult to implement without knowledge of normal behavior.

Clinical Assistant Professor, Head of Exotics Service, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA. Email: joerg.mayer@tufts.edu.

BOX 1. COMMON CLINICAL SIGNS ASSOCIATED WITH PAIN IN SMALL MAMMALS[a]

Production of fewer, smaller, or no fecal pellets
Anorexia
Half-closed, unfocused eyes
Aggression
Pushing abdomen on the floor
Stiff movements
Immobility/lethargy/isolation
Overgrooming/lack of grooming
Vocalization (squeal usually fear in rabbits)
Stretching with back arched
Stinting on palpation
Hunched posture
Teeth grinding (bruxism)
Reluctance to curl when sleeping (ferrets)
'Tucked' into abdomen
Strained facial expression, bulging eyes
Increased frequency and depth of respirations or rapid shallow breathing
Lameness/ataxia
Polyuria/polydipsia (especially with GI pain)
Head extended and elevated
Piloerection
Porphyrin secretion (especially in rats)
Self-mutilation
Squinting (especially ferrets)
Absence of normal behavior

[a]Adapted from ref. 15.

One recent article[3] set down four general guidelines for researchers, animal technicians, and veterinarians assessing behavioral pain parameters:

- Be able to recognize 'normal' animals, at a species, strain, and (ideally) individual animal level.
- Be familiar with clinical signs that indicate both general suffering and that which is specific to each project, species, and (where possible) individual.
- Make behavioral assessment observations at appropriate times for each species (observe nocturnal species at night, for instance).
- Be open to the use of a broad range of techniques for assessing and monitoring animals, and always be prepared to try new methods.

Ideally, researchers and caretakers should not only be able to detect pain in an animal, but should be qualified to differentiate between different levels of pain, in terms such as 'mild' versus 'severe'. For those in the laboratory animal research field, this requires proper education and training on how non-domesticated species manifest pain. (See **Box 2** for suggested articles related to small mammal pain behavior.) A recent article in this publication raised questions about the current practices of pain recognition in laboratory animals[4]. According to Karas, one of the key barriers to successful pain treatment in laboratory animals is "the lack of training of veterinary students and technicians in schools, including training for a culture of regard for pain[4]."

The following segments, adapted from a book dedicated to the recognition of normal and abnormal behavior in exotic pets co-edited by the present author, describe manifestations of pain commonly seen in rodents, rabbits, and ferrets[5].

PAIN BEHAVIORS IN SMALL MAMMALS

Rodents

Small rodents are prey species, so they often do not exhibit the overt signs of pain that may be seen in other species[6]. Their quickness, small body size, nocturnal activity, and burrowing habits compound the difficulty in determining if a rodent is sick or in pain. Subtle changes in behavior that might indicate pain or an underlying medical condition are key in these animals. Normal guinea pigs, for example, will usually try to escape (stampede) or vocalize (squeal) when afraid, whereas guinea pigs in pain will often remain quiet[1].

The common first signs of sickness or pain in rodents include a rough hair coat, a starey coat, or piloerection[7]. Essentially, instead of appearing sleek and shiny, the hair coat sticks out and looks spiky. The careful observer may first notice this difference in appearance without being able to identify the specific change. Animal caretakers may simply describe the animal by noting that it 'just doesn't look right'. Such subtle changes should not be overlooked.

Another common nonspecific sign of pain or illness in rodents is a hunched posture, the clinical presentation of which is a slight increase in the arch of the back in the area of the caudal thoracic and cranial lumbar vertebrae. This can occur with either abdominal or thoracic pain and may be difficult to identify without considerable experience observing normal rodents. The hunched posture also manifests itself in the speed of ambulation; mice, in particular, often slow down and exhibit a 'waddle' or 'drag-car gait' (Morton, D.B., personal communication).

If the pain progresses, the hunched back may become more prominent and affect movement to a greater degree, causing the animal to take shorter steps. Often the hunched posture is associated with pacing or restlessness, especially during the day when the nocturnal animal should be sleeping. If the animal can sleep, it usually stretches out on one side, rather than in the normal tucked position. In a social group setting, the individual in pain frequently removes itself from the others and may even sleep alone on the floor of the cage, apart from the rest of the cagemates in the pile or nest.

Another sign of pain in rodents is anorexia, especially with conditions of the head, the oral cavity, or the abdomen[8]. Small rodents do not generally produce copious amounts of saliva, but the chin of a pained rodent may be wet ('slobbers'), particularly when there are lesions in the mouth, such as those seen with malocclusion of the teeth (in guinea pigs, for instance). Animals attempting to either groom themselves or rub the lesions in the mouth will often have saliva-matted fur on the inside surface of the front feet, another indication of an underlying problem.

Some rodents are also notorious for exhibiting avoidance behaviors when they are in pain. Mice and other prey species tend to mask their clinical signs from human observers, which presents a challenge to veterinary personnel assessing their level of discomfort[8]. For this reason, animals that do not move around the cage in a normal fashion should be closely examined. Handlers may also notice animals that previously seemed to enjoy interaction avoid being touched or shield specific areas of the body (such as the tail, foot, or mouth) from physical contact[5]. Animals exhibiting such behavior should be examined, at times with anesthesia to allow for adequate scrutiny.

Researchers and veterinarians who work with rodents must be especially aware of the need for pain management in the initial treatment of these animals. Small mammals have the same or similar neurologic components for perceiving pain as those found in other domestic species, as well as in humans, including antinociceptive mechanisms to modulate pain[9]. This means that rodents respond to stimuli known to be painful in other species. In the clinical setting, pain management should be addressed immediately, before performing diagnostic procedures and other treatments.

BOX 2. SUGGESTED LITERATURE[a]

Bradley Bays, T., Lightfoot, T. & Mayer, J. *Exotic Pet Behavior: Birds, Reptiles, and Small Mammals* (Elsevier, St. Louis, MO, 2006).

Baker, R.M., Jenkin, G. & Mellor, D.J. (eds.) *Improving the Well-being of Animals in the Research Environment* (Glen Osmond: Australian and New Zealand Council for the Care of Animals in Research and Teaching, Wellington, New Zealand, 1994).

FELASA Working Group on Pain and Distress. Pain and distress in laboratory rodents and lagomorphs. *Lab. Anim.* **28(1)**, 97–112 (1994).

ILAR Committee on Regulatory Issues in Animal Care and Use. *Definition of Pain and Distress and Reporting Requirements for Laboratory Animals* (National Academy Press, Washington, DC, 2000). http://books.nap.edu/books/0309072913/html.

ILAR Committee on Pain and Distress in Laboratory Animals. *Recognition and Alleviation of Pain and Distress in Laboratory Animals* (National Academy Press, Washington, DC, 1992). http://www.nap.edu/catalog/1542.html.

Wallace, J. *et al.* The assessment and control of the severity of scientific procedures on laboratory animals. *Lab. Anim.* **24(2)**, 97–130 (1990).

Flecknell, P.A. & Waterman-Pearson, A. (eds.) *Pain Management in Animals* (WB Saunders, London, 2000).

[a]List adapted from ref. 15.

Small rodents in pain rarely vocalize unless they are physically handled or restrained. When they do vocalize, it may be accompanied by some degree of aggression toward their cagemates or humans. Vocalization will also occur at frequencies inaudible to the human ear[10]. Ultrasonic vocalizations in response to both acute and chronic pain have been widely studied in the laboratory rat[11], however, detecting these responses is often impractical because it requires electronic listening devices and the proper training to operate them, which are not readily available in most research settings.

Biting can also be a sign of pain. Mice and hamsters will almost always attempt to bite if they are in pain. Rats and gerbils are more likely to bite when in pain than when they are healthy, but not all rats and gerbils will bite even when in extreme pain. When housed in groups, typical group behavior—like mutual grooming—is often altered when animals are in pain.

It is critical to respond when small rodents exhibit a behavior change for reasons that are not immediately obvious. Even the most subtle change in behavior or level of aggression may be an indication of an underlying medical condition, even if no lesions are obvious. When animal caretakers can accurately describe behavioral changes, it is much easier to get a complete picture of the pain situation and make an appropriate diagnosis.

Rabbits

Rabbits in pain can appear apprehensive, anxious, dull, or inactive; may assume a hunched appearance or attempt to hide; and, with severe pain—or in panic—may vocalize with a squeal or cry[1]. The clinical manifestation of these behaviors varies with the kind of pain they experience (chronic versus acute, for instance). Sometimes rabbits show aggressive behavior with increased activity and excessive scratching and licking[1]. With abdominal pain, they sometimes grind their teeth, which needs to be differentiated from 'teeth purring', a sign of comfort. When documenting the behavioral responses of the rabbit, the nocturnal-to-diurnal behavior pattern[12] should be considered when recording activity levels, such as wheel running.

As in all species, the response to pain in rabbits can include immune suppression, impaired wound healing, decreased food and water intake[13], and secondary medical problems such as gastric ulcers, gastrointestinal stasis, decreased peripheral circulation and body temperature, shock, and even death. In general, a higher rate of anesthetic mortality is associated with surgery in exotic species, and this might be related to the inadequate use of preoperative and postoperative analgesics[13].

Physiologic changes that accompany pain can create secondary medical problems, may inhibit response to treatment, and may even precipitate death in the animal. These detrimental responses make it necessary for practitioners to reevaluate the need for analgesia

BOX 3. COMMON RABBIT POSTURES, BEHAVIORS, AND VOCALIZATIONS[a]

Purring or teeth purring—A sound made by lightly and quickly grinding/vibrating the teeth as the whiskers quiver; a sign of contentment.

Oinking or honking—A sound made to gain food or attention or during courtship.

Clicking—A happy sound often made after a welcomed treat is given.

Wheezing or sniffing—Nasal sounds made by 'talkative' rabbits; can be distinguished from abnormal respiratory sounds because they are intermittent and stimulated by interaction with the rabbit.

Whimpering or low squealing—A fretting noise that is made when one picks up a rabbit that is reluctant to be handled; made more often by pregnant and pseudopregnant does.

Chinning—Rubbing the secretions from the scent glands under the chin on inanimate objects and people to mark possession. Glands are more developed in males than females.

Nudging or nuzzling—The nose is used to nudge a person's hand or foot, or the rabbit may pull on a pant leg to signal a desire for attention. When enough petting has been done the rabbit may push the hand away.

Head shaking, ear shaking, body shudder—A shake of the head or body in response to an annoying smell or unwanted handling; often occurs as a rabbit settles down and becomes relaxed enough to begin eating and grooming.

Courting or circling—A sexual or social behavior whereby a rabbit circles another rabbit or the feet of a human while softly honking.

Scratching at the floor—A rabbit may scratch at the floor with its forepaws in order to get a person's attention or to be picked up.

Nipping—Not always done in anger, this can mean 'move over' or 'put me down'.

Presentation—The head is extended forward with the feet tucked under the body and the chin placed on the floor in order for the rabbit to present itself as subordinate for petting from humans or to be groomed by another rabbit.

Flattening—A fear response wherein the rabbit flattens its abdomen onto the floor with ears laid back against the head; the eyes may be bulging.

Thumping—A sharp drumming of the hind feet as a warning or an alert to other rabbits of danger; often accompanied by dilation of the pupils and seeking of refuge.

Teeth grinding—A slower, louder teeth crunching, sometimes seen with bulging of the eyes and usually indicating discomfort, pain, or illness.

Snorting or growling—A warning sound, either hissing or a short barking growl, that occurs with aggression or fear and is often seen with the ears flattened against the head and the tail up and in the grunt-lunge-bite sequence.

Isolation—When a rabbit that normally seeks attention from its mates and human companions isolates itself and is less active. Such a rabbit should be checked for illness.

Kicking—If a rabbit feels insecure when being picked up it will kick violently in an effort to escape. The hindquarters *must* be supported to prevent trauma to the spine or legs. A rabbit should be placed hind-end first into a cage in order to help prevent injuries caused by kicking.

Aggression—Strained, upright stance with tail stretched out and ears laid back in defensive posture; the rabbit may also kick high and backwards.

Loud, piercing scream—Similar to a human baby crying; signaling pain and fear, as when the rabbit is caught by a predator.

Scanning—A rabbit with impaired vision may move its head from side to side to scan the area around it.

[a]Reprinted with permission from ref. 5.

in rabbits that are experiencing trauma, disease, surgery, or other invasive procedures in which pain is a known component. As with all species, a return to normal behavior (see **Box 3**) indicates a positive response to analgesic treatment (**Fig. 1**). Specifically for rabbits, this includes eating and drinking, defecating, sleeping, playing, stretching, and grooming. Any disease, trauma, or process that causes pain in other species, including humans, should be considered painful in rabbit species and treated accordingly.

Becoming familiar with the normal behaviors of each species that a researcher or veterinary practitioner treats is the first step needed in order to recognize pain behaviors as early as possible. Identification of pain behaviors in rabbits and other prey species, however, is made more complicated because they exhibit less overt pain-associated behaviors in order to decrease their chances of being caught by predators[14]. Immobility (**Fig. 2**) is a common behavior displayed by all prey species that are presented for examination, making it even more difficult to determine if pain is present[15]. This is especially true of rabbits that are excessively nervous or not well socialized. Anxiety has also been associated with a lowered threshold of pain perception in rabbits as well as in other species[15].

Ferrets

Recognizing changes in ferret behavior associated with pain requires careful observation of the undisturbed ferret. An uncomfortable ferret is reluctant to curl into

FIGURE 1 | A relaxed and comfortable rabbit. Note how the whole body is stretched out and the animal is lying down, without any sign of a stressed demeanor.

its normal, relaxed sleeping position and may have a strained facial expression and the appearance of being 'tucked' into its abdomen. Pained ferrets may also have more frequent and deeper respirations. The gait may be stiff, with the head elevated and extended forward. Most ferrets in pain are lethargic and anorexic.

A painful abdomen is a common sequela to ferret gastrointestinal diseases, including gastric ulcers, gastrointestinal foreign bodies or trichobezoars, Epizootic Catarrhal Enteritis (ECE), and *Helicobacter* infections. Caretakers often report that the ferret is hunched with an arched back, immobile, walking with a stilted gait, or grinding its teeth—all common signs of abdominal pain. A less-astute caretaker may not recognize spasmodic teeth-grinding behavior in a ferret that holds its head down and rhythmically moves its facial muscles back and forth, wiggling its ears in response to painful stimuli.

Postoperative and traumatic pain are usually manifested as a reluctance to move and a facial expression demonstrating dull, half-open, non-inquisitive eyes, which are overall expressions of tension. When pain medication is administered to a pained ferret, there is a marked, easily discernable change in behavioral attitude and facial relaxation. If possible, analgesics should be provided before painful stimulus occurs. Administering preemptive analgesia as part of the preanesthetic protocol or administering analgesics intraoperatively before discontinuing general anesthesia diminishes the wind-up effect of pain and decreases the postoperative pain caused by neuropathic and inflammatory pain[16].

Each ferret must be evaluated individually when analgesic protocols are chosen, with frequency, duration, and type of analgesic used based on clinical judgment, hematologic and biochemical values, and patient response. A return to normal attentive behavior, curling up under a towel to sleep, and a good appetite are all behavioral signs in the ferret that postoperative analgesia is adequate.

CONCLUSIONS

Alleviating pain experienced by laboratory animals has many benefits for the animals and researchers. Often analgesic therapy in research is suboptimal. While there are multiple factors responsible for this, one of the most common problems is the lack of recognition of painful behaviors in the non-domesticated and non-traditional research species.

The decision to give analgesics or to withhold them can be difficult in some situations or might be perceived to interfere with the primary research goal. The author proposes that analgesics should be used every time a procedure is performed on an animal that would warrant the use of analgesics if the same procedure were performed on a human. While the administration of repeated doses of analgesics to animals that are not experiencing pain might be detrimental[6], a far more common problem is the failure to provide analgesics appropriately because behavior-exhibiting pain in the animal is not acknowledged.

Pain 'score sheets' can help maximize the efficacy of pain scoring based on behavioral analysis. Because behavior is difficult to quantify, score-sheet descriptions of behavior must be refined and specific so as to avoid varied scoring by different observers. Once such a system is implemented, scoring can be done by well-trained non-veterinary staff. Morton and

FIGURE 2 | A stressed or painful rabbit. The body is positioned in a corner and all the legs are tucked under the body, which is held in a hunched position. Often these rabbits have a glazed look on their faces and are unresponsive to visual stimuli.

Griffiths introduced one of the more popular score sheets, mentioned in the first segment of this article. Several other score sheets exist that aid in monitoring for painful behavior. However, knowledge of the natural history—and therefore behavioral repertoire of the species involved—is necessary in order to correctly evaluate the behaviors associated with pain or stress. Hawkins concluded in a study performed in 2002 that "people are very concerned about the potential for animal suffering and want to be able to detect and alleviate it effectively[17]." By properly educating researchers and veterinary staff on the behavioral aspects of pain in research animals, the specter of animal pain in the laboratory can be banished.

ACKNOWLEDGMENTS

The author thanks Teresa Bradley-Bays (rabbit), Peter Fisher (ferrets), and Elisabeth Evans (rodents) for the inspiration and template provided by their original chapters in ref. 5.

COMPETITNG INTERESTS STATEMENT

The author declares no competing financial interests.

Received 6 November 2006; accepted 9 February 2007. Published online at http://www.labanimal.com

1. Institute of Laboratory Animal Resources, National Research Council. *Recognition and Alleviation of Pain and Distress in Laboratory Animals* (National Academy Press, Washington, DC, 1992).
2. Morton, D.B. & Griffiths, P.H. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Vet. Rec.* **116(16)**, 431–436 (1985).
3. Stasiak, K.L., Maul, D., French, E., Hellyer, P.W. & VandeWoude, S. Species-specific assessment of pain in laboratory animals. *Contemp. Top. Lab. Anim. Sci.* **42(4)**, 13–20 (2003).
4. Karas, A.Z. Barriers to assessment and treatment of pain in laboratory animals. *Lab Anim. (NY)* **35(7)**, 38–45 (2006).
5. Bradley Bays, T., Lightfoot, T. & Mayer, J. (eds.) *Exotic Pet Behavior: Birds, Reptiles, and Small Mammals* (Saunders, Philadelphia, 2006).
6. Schulte, M.S. & Rupley, A.E. Exotic pet management for the technician. *Vet. Clin. North Am. Exot. Anim. Pract.* **7(2)**, xi–xii (2004).
7. Evans, E.I. & Maltby, C.J. (eds.) *Technical Laboratory Animal Management* (MTM Press, Kansas City, 1989).
8. Flecknell, P.A. & Liles, J.H. in *Animal Pain* (eds. Shortland, C.E. & Van Poznak, A.) 482–488 (Churchill Livingstone, New York, 1992).
9. Decosterd, I. & Woolf, C.J. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* **87(2)**, 149–158 (2000).
10. Williams, W.O. Ultrasonic sound measurement as an indicator of pain and distress in laboratory rodents. http://caat.jhsph.edu/programs/AWE/2004/final/williams.htm. Accessed 19 January 2007.
11. Jourdan, D., Ardid, D., Chapuy, E., Le Bars, D. & Eshcalier, A. Audible and ultrasonic vocalization elicited by single electrical nociceptive stimuli to the tail in the rat. *Pain* **63(2)**, 237–249 (1995).
12. Kennedy, G.A., Hudson, R. & Armstrong, S.M. Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiol. Behav.* **55(2)**, 385–389 (1994).
13. Robertson, S.A. Analgesia and analgesic techniques. *Vet. Clin. North Am. Exot. Anim. Pract.* **4(1)**, 1–18, v (2001).
14. Livingston, A. Physiological basis for pain perception in animals. *J. Vet. Anaesth.* **21**, 15–20 (1994).
15. Mathews, K.A. Pain assessment and general approach to management. *Vet. Clin. North Am. Small Anim. Pract.* **30(4)**, 729–755, v (2000).
16. Bradley, T. Recognizing pain in exotic animals. *ExoticDVM* **3(3)**, 21–26 (2001)
17. Hawkins, P. Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Lab. Anim.* **36(4)**, 378–395 (2002).