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Refinement Issues in Animal Research

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Section A

Introduction, Concepts, and Definitions
It has been proposed that the following three issues should be considered for animal welfare:

1. Ability of the animal to live under conditions natural to its species
2. Health of the animal
3. Avoidance of affective (uncomfortable) states
Refinement Alternatives

- Methods that...
  1. Alleviate or minimize potential pain and distress
  2. Enhance animal well-being
When polled, most members of the public are favorable toward the use of animals in biomedical research, but not when the animals are subjected to pain.
“... by now it is widely recognized that [the most humane] possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments.”

Refinement Considerations

- Pain—recognition and alleviation
- Distress—minimization (prevention), recognition, and alleviation
- Humane endpoints
- Environmental enrichment
“Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”

— International Association for the Study of Pain
Some Potential Causes of Pain During Experiments

- Improper or prolonged restraint
- Experimental infections
- Chemical-induced toxic effects
- Surgical and experimental procedures
- Post-operative complications
- Chronic inflammation
- Tumor-induced pain
- Improper euthanasia techniques
- Pain research
Assessment of Pain in Animals

- Pain can be assessed by evaluating behavioral measures such as eating, socializing, and withdrawal reflexes, and physiological measures such as blood pressure, heart and/or respiration rate.

- Behaviors will be specific to each species and to individuals within a species.

- Some animals may hide signs of pain.
Challenges to Assessing Pain

- Recognizing a departure from the normal
- Subjectivity of pain scales
- Biases of observer
- Chronic vs. acute pain
## Rodents and Rabbits

### Indicators of pain in several common laboratory animals

<table>
<thead>
<tr>
<th>Species</th>
<th>General behavior</th>
<th>Appearance</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodents</td>
<td>Decreased activity; excessive licking and scratching; self-mutilation; may be unusually aggressive; abnormal locomotion (stumbling, falling); writhing; does not make nest; hiding</td>
<td>Piloerection; rough/stained haircoat; abnormal stance or arched back; porphyrin staining (rats)</td>
<td>Rapid, shallow respiration; decreased food/water consumption; tremors</td>
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<tr>
<td>Rabbit</td>
<td>Head pressing; teeth grinding; may become more aggressive; increased vocalizations; excessive licking and scratching; reluctant to locomote</td>
<td>Excessive salivation; hunched posture</td>
<td>Rapid, shallow respiration; decreased food/water consumption</td>
</tr>
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### Indicators of pain in several common laboratory animals

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<td>Dog</td>
<td>Excessive licking; increased aggression; increased vocalizations, inclusive of whimpering, howling, and growling; excessive scratching; self-mutilation</td>
<td>Stiff body movements; reluctant to move; trembling; guarding</td>
<td>Decreased food/water consumption; increased respiration rate/panting</td>
</tr>
<tr>
<td>Cat</td>
<td>Hiding; increased vocalizations, inclusive of growling and hissing; excessive licking; increased aggression</td>
<td>Stiff body movements; reluctant to move; haircoat appearing rough, ungroomed; hunched posture; irritable tail twitching; flattened ears</td>
<td>Decreased food/water consumption</td>
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Nonhuman Primates

- Indicators of pain in several common laboratory animals

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<td>Nonhuman primate</td>
<td>Increased aggression or depression; self-mutilation; often a dramatic change in routine behavior (e.g., decreased locomotion); rubbing or picking at painful location</td>
<td>Stiff body movements; reluctant to move; huddled body posture</td>
<td>Decreased food/water consumption</td>
</tr>
</tbody>
</table>
What Is Unacceptable Pain?

- As a stimulation approaches an animal’s pain tolerance level, the animal’s behavior will be dominated by attempts to avoid or escape the stimulus.

- This degree of pain must be alleviated, unless scientifically justified.
Alleviation of Pain

- General anesthesia
- Local anesthesia and/or analgesia
- Preemptive analgesia and balanced anesthetic regime
- Post-surgical analgesics
- Training of animals to avoid situations that produce pain (behavioral studies)
- Controlling the intensity of the stimulus
What Is Distress?

- Distress is an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behaviors (1992 definition)

- New report—no definition (we can’t define it, but we know it when we see it)
Recognition and Alleviation of Distress in Laboratory Animals (National Research Council, 2008)
Model of Stress-to-Distress (Moberg)

Strategies for Dealing with (Potential) Distress

- Prevention (by appropriate housing and husbandry) or minimization (incorporation of humane endpoints)
- Recognition when it occurs (knowledge of species)
- Alleviation
Some Potential Causes of Distress

- These potential causes of distress may or may not be anticipated
  - Prolonged, unrelieved pain
  - Large tumor burden
  - Social deprivation
  - Environmental status, e.g., transportation, boredom, inappropriate housing/husbandry
Challenges

- Determining when stress becomes distress
- Animal’s mental state can only be inferred
- Evaluating the stress of animal husbandry and residence in a research laboratory
- Assessing the types of stress in the research setting
- Difficulty in measuring techniques which themselves may cause stress
Some Potential Signs of Distress

- Spontaneous self-injurious behavior
- Severe weight loss
- Dehydration
- Hunched posture
- Stereotypies
- Labored breathing
Recognizing Distress

- In general, it is necessary to know the normal behavior of the animal to be able to recognize abnormal behavior (species and strain dependent)

- Use of ethograms may help
Team Approach to Alleviate Distress

- To ensure the best science and the best animal welfare, decisions regarding the fate of an animal in a study must be made as a team
  - Investigator
  - Veterinarian
  - Animal care staff
Team Approach to Alleviate Distress

- New protocol
- Training animal
- Breeding colony

Subject Animal

Observed Distress

Team Dialogue
- Principal investigator
- Veterinarian
- Other staff

Recovery

Yes
- VET
- Euthanize

No Recovery
- VET

Treat

Yes
- Pull

No

Stay on protocol with veterinarian approval

Recycle back to protocol