Pain & Distress: Measurement, Avoidance, and Relief

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Why Should Scientists be Concerned About Pain & Distress?

- Ethical Responsibility
- Higher Standard
- Better Data
  - Less Variability
  - More Attention to Detail
Definitions: Distress

An aversive state in which an animal is unable to cope resulting in maladaptive behaviors and/or abnormal physiological responses that if continued for prolonged periods will be detrimental to the animal’s health.

Should be differentiated from stress which is an aversive state that elicits an adaptive response that ultimately enriches or primes the animal to become better adapted to its environment condition.
Potential or actual tissue damage that is perceived and results in a CNS response characterized by
– avoidance or
– a need for avoidance
Management of Pain and Distress

Anesthetics

Observation

Analgesics

Defined Endpoints
Anesthesia

Total loss of sensation in a part or in the entire body as the result of drug induced depression of nervous tissue either locally (peripheral) or centrally.
Anesthesia Considerations

- Individual Animal
  - Species/Strain
  - Sex
  - Age
  - Mutation
- Duration of Procedure
- Additional Support
- Experimental Procedure
Potential for Pain is Predicted
Pre-Emptive Analgesia

Enhancing Humane Science Improving Animal Research
Methods of Anesthetic Delivery in Rodents

- **Intraperitoneal** – very commonly used
- **Intravenous**
- **Intramuscular** – not encouraged in rodents due to limited muscle mass
- **Inhalation**
Commonly Used Injectable Anesthetics in Mice

Ketamine / Xylazine
200mg/kg  10mg/kg

Ketamine / Xylazine / Acepromazine
200mg/kg  10mg/kg  3mg/kg

Tribromoethanol (Avertin)
2.5% (0.01ml/g)
Ketamine/Xylazine

- Most commonly used combination
- Ketamine alone has little analgesic effect in rodents
- Potential side effects include:
  - Transient hyperglycemia
  - Increased intra-ocular fluid glucose
  - Polyuria
  - Bradycardia
- Warmth is Important

25g Mouse Preparation
Add 0.5 mls Ketamine and 0.125mls Xylazine to 4.875 mls PBS
Inject 0.3 mls IP
Tribromoethanol (Avertin)

• Toxic byproducts due to light exposure and storage at incorrect temperature
Tribromoethanol (Avertin)

- Pass through a 0.2µ filter.
- Store at 4°C in a foil-wrapped bottle in dark
- Label date on each bottle
- Discard if:
  - One month old
  - pH >5
  - crystals
  - discolored

Avertin (10/ 02)
Inhalant Anesthetics

Isoflurane

• Very rapid induction and recovery
• Little metabolized
• Minimal effect on cardiovascular system
• May alter immune cell functions
  (Halothane)
Monitoring
Depth of Anesthesia

- Pedal withdraw reflex is the standard measurement for surgical tolerance
- Start surgical procedures slowly with close monitoring of response (twitch)
- Response to anesthesia may be variable between species, stains, and sex.
Monitoring Depth of Anesthesia

- The following should be monitored throughout every 5 minutes during the procedure.
  - Mucous membrane color
  - Respiration
  - Skin twitching
  - Heart rate
What is Normal?
Observation Teams

Laboratory Team:  
- Principal Investigator  
- Post Docs / Grad Students  
- Technicians

Animal Care Staff:  
- Animal Care Technicians  
- Veterinary Technician  
- Veterinarians
Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice

Mollie H. Ullman-Cullere and Charmaine J. Perez

BC 1
Mouse is emaciated.
- Skeletal structure extremely prominent; little or no flesh cover.
- Vertebrae distinctly segmented.

BC 2
Mouse is underconditioned.
- Segmentation of vertebral column evident.
- Dorsal pelvic bones are readily palpable.

BC 3
Mouse is well-conditioned.
- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.

BC 4
Mouse is overconditioned.
- Spine is a continuous column.
- Vertebrae palpable only with firm pressure.

BC 5
Mouse is obese.
- Mouse is smooth and bulky.
- Bone structure disappears under flesh and subcutaneous fat.

A "+" or a "−" can be added to the body condition score if additional increments are necessary (i.e., ...1+, 2−, ...).
Behavioral Assessment

Movement

Exploration

Escape
Pain Assessment Scoring

- Complimentary aid
- Educational tool
- Endpoints

### Numerical score sheet for Inflammatory Bowel Disease

**DISTRESS SCORING SHEET: INFLAMMATORY BOWEL DISEASE RATS**

<table>
<thead>
<tr>
<th>ANIMAL IDENTIFICATION</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPEARANCE</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>General lack of grooming</td>
<td>1</td>
</tr>
<tr>
<td>Coat staining, ocular/nasal discharge</td>
<td>2</td>
</tr>
<tr>
<td>Pinched features, ridge lines</td>
<td>4</td>
</tr>
<tr>
<td>BODYWEIGHT</td>
<td></td>
</tr>
<tr>
<td>Normal - 7 &lt; 5%</td>
<td>0</td>
</tr>
<tr>
<td>Body wt. 5-10%</td>
<td>1</td>
</tr>
<tr>
<td>Body wt. 5-15%</td>
<td>2</td>
</tr>
<tr>
<td>Body wt.15-22%</td>
<td>4</td>
</tr>
<tr>
<td>CLINICAL SIGNS</td>
<td></td>
</tr>
<tr>
<td>Faeces normal - slightly soft</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1</td>
</tr>
<tr>
<td>Soft distended gut + no faeces</td>
<td>2</td>
</tr>
<tr>
<td>Hard and hot distended gut</td>
<td>4</td>
</tr>
<tr>
<td>PROVOKED BEHAVIOUR</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Minor depression or exaggerated</td>
<td>1</td>
</tr>
<tr>
<td>Moderate change</td>
<td>2</td>
</tr>
<tr>
<td>Reacts violently/vocalisation</td>
<td>4</td>
</tr>
</tbody>
</table>

**START BODY** | **MIN**

If scored 4 more than once score 1

**TOTAL**

**JUDGEMENT**

9 – 4 Normal
5 – 9 Monitor carefully, consider analgesics.
10-14 Suffering, provide relief, observe regularly. Seek second opinion from NACWO and/or NVS. Consider termination.
15-20 Severe pain or distress; does this procedure need refining?

Appearance

- Piloerection
- Oily Coat
- Hunched Posture
- Abdominal Breathing
- Dull Sunken Eyes
- Poor Body Condition
Management of Pain and Distress

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Defined Endpoints
Analgesia

A state in which the perception of a painful stimulus is either not perceived, decreased, or altered such that it is no longer aversive or more tolerable.

Response to analgesic may be variable between species, strains, and sex.
Buprenorphine

- Dose (0.005 – 0.1 mg/ml) Intraperitoneal/Subcutaneous
- Most commonly used analgesic
- 6-8 hours of analgesic effect
- Decreased weight gain
- Pica behavior
- Rebound sensitivity
Fentanyl

- 0.01 mg/kg IP
- 80X more potent than morphine
- Patch (used in swine and dogs)
Carprofen

- Nonsteroidal Anti-Inflammatory (NSAID)
- 5mg/kg SC every 24hrs
- Preemptive (before or during surgery)
- Less appetite suppression (mice)
- Return to normal behavior sooner
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Defined Endpoints
Avoidance of Distress

- Accommodate Social Interaction
- Provide Enrichment
- Habituation
- Anesthetize or Sedate
- Appropriate Training of Technician
Interference with Research

• Pilot Study

• Consider effects of uncontrolled pain/distress upon research results
No Pain...

Lots of Gain