CCAC TRAINING MODULE ON:
PAIN, DISTRESS AND ENDPOINTS

www.ccac.ca
This training module is relevant to all animal users working with animals housed in vivaria which are enclosed areas such as laboratories where animals are kept for research, teaching or testing.

This training module covers the following animals housed in vivaria:

- rodents
- rabbits
- birds
- amphibians
- reptiles
- non-human primates
- other mammals
Training Module Goals

- To provide a framework to identify factors that could have a profound effect on animal welfare and experimental results
- To provide tools to recognize and minimize pain and distress in the animals used in science
- To provide a framework to set and monitor endpoints

See the CCAC training module on: analgesia (2003) and the CCAC training module on: anesthesia (2003) for further information on these topics.
Introduction to pain and distress
Predicting pain and distress
Recognizing pain and distress
Introduction to endpoints
Setting endpoints
Monitoring endpoints
We have an obligation to prevent unnecessary pain and distress in the animals used in research.
Introduction to Pain and Distress

- **Discomfort**: a mild form of distress

- **Stress**: response to a threat to an animal’s homeostasis

- **Pain**: unpleasant experience eliciting protective motor and vegetative reactions, resulting in avoidance behaviour and modifying species-specific behaviour

- **Distress**: state at which homeostasis cannot be achieved and may result in disease or pathological changes
To prevent pain, we must recognize it

Recognizing pain requires:

- knowledge of normal behaviour
- understanding of situational, behavioural and physiological indicators of stress, pain and distress

Photo courtesy of Dr. K. Banks

Could this mouse be in pain or distress?
To minimize pain and distress:

- experiments must be assessed to provide some measure of risk for the animal to experience pain or distress
- consideration should be given to their:
  - anticipated intensity
  - anticipated duration
  - potential sources

See the CCAC policy statement on: categories of invasiveness in animal experiments (1991) for further information on this topic
Potential sources of stress, distress or pain:

- poor husbandry practices
- lack of acclimatization
- poor post-operative care
- poor tissue handling
- lack of investigator experience

All these sources can significantly increase the level of pain and distress an animal experiences as part of the primary experimental intervention.
Recognizing Pain and Distress – Evaluating Behaviour

**Behaviour**
- Behaviour changes in response to pain and distress

**Variation of Expression**
- Prey species such as mice, rats and rabbits will mask signs of pain and distress to avoid predation

**Deviations**
- Difficult to evaluate deviations from normal without knowledge of normal behaviour
Animals may significantly change their behaviour depending on whether it is aware that it is being observed.

Common behaviours when experiencing pain/distress:

- failure to groom
- changes in posture and gait
- decrease in food and water intake
- lethargy or reluctance to move
- vocalization
- failure to interact with conspecifics
- guarding
- avoidance or resentment of handling
- scratching or biting
This rat is displaying porphyrin staining under its eyes, a common sign of stress in the rat. The rat’s eyes are also held nearly closed, a consistent sign of acute pain in many species.

Photo courtesy of Dr. K. Banks
Recognizing Pain and Distress – Evaluating Behaviour

- **Failure to groom**
  - hair coat may be standing up, matted or clumped

- **Changes in posture and gait**
  - hunched posture
  - partial or full closure of the eyes

- **Failure to interact with conspecifics**
  - isolated from the group
  - appears to resent engagement
Response to stimulation

Decrease in food and water intake

- cannot maintain weight
- dehydrated: sunken eyes and abdomen, face looks pinched

Click on the images to start the videos

Hydrated skin has turgor or pressure allowing flexibility and resilience

In dehydration, turgor is diminished

Videos courtesy of Dr. K. Banks
Other considerations in experimental intervention:

- animals should be habituated to handling, restraints and commonly applied procedures
- use of tranquillizers and anti-anxiety agents

Chronic or long-term pain:

- previous non-painful stimuli may become painful
- trauma to the painful area

Inappropriate or inadequate analgesia following a painful procedure can lead to over grooming and scratching at the painful area.
Behavioural changes diminished or terminated by analgesics are a good indication that pain was the basis for the observed changes.

New technologies allow recognition of pain and distress.
Environment that does not allow expression of normal behaviour can cause stress

Environmental enrichment is requisite for the expression of behavioural repertoire of all laboratory species

Hammocks can be used in cages to make them more interesting and stimulating to ferrets

Toys and chewable objects allow the expression of species-typical postures and activities

See the CCAC training module on: environmental enrichment (2003) and the CCAC Three Rs microsite at: www.ccac.ca/en/alternatives for further information on this topic
There are many excellent resources for an introduction to normal behaviour in many laboratory species that may assist the investigator new to the subject.
Pain, stress and distress produce changes in physiology

Physiological changes
- can be mediated by:
  - hypothalamic-pituitary-adrenal axis
  - sympathetic nervous system
- help to cope with threats to homeostasis

Severe stress activates the sympathetic nervous system: the fight-or-flight response
The fight-or-flight response

<table>
<thead>
<tr>
<th>Effector</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iris</td>
<td>Pupil dilation</td>
</tr>
<tr>
<td>Heart</td>
<td>Increased force of contraction, increased rate</td>
</tr>
<tr>
<td>Blood vessels/capillaries (blood supply to periphery, GI tract, urinary tract)</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>Blood vessels/capillaries (blood supply to skeletal muscle)</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>Brochioles, bronchi</td>
<td>Dilation</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Peristalsis slows, sphincter constriction</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>Bladder relaxes, sphincter constricts</td>
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<tr>
<td>Adrenal medulla</td>
<td>Catecholamine secretion</td>
</tr>
<tr>
<td>Liver</td>
<td>Glycogen converted to glucose</td>
</tr>
</tbody>
</table>
Changes in physiology consistent with pain, stress or distress may be:

- directly observed (e.g. pupil dilation, elevated respiration)
- measured with relatively simple equipment (e.g. blood pressure by tail cuff)
- measured by sampling blood, other fluids
- measured non-invasively (e.g. telemetry)
"Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard, whether in research, in teaching, or in testing procedures (...)

(CCAC policy statement on: ethics of animal investigation (1989))
It is our responsibility to:

- accomplish scientific goals while keeping levels of pain and distress to a minimum
- decide at which point an experiment may be stopped while meeting the objectives of the research

See the CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998) for further information on this topic.
Endpoint is a point at which an animal’s pain and/or distress is terminated, minimized or reduced by:

- euthanizing the animal
- terminating a painful procedure
- giving treatment to relieve pain and/or distress
- restoring a basic requirement

See the CCAC training module on: analgesia (2003) and the CCAC training module on: anesthesia (2003) for further information on these topics.
Selecting Endpoints

- Earliest endpoint that is compatible with the scientific objectives of the research

- Determining endpoints involves:
  - principal investigator
  - laboratory animal veterinarian
  - animal care committee (ACC)
Limiting clinical signs:

- point at which it becomes obvious that unless action is terminated, animal will go on to die

Example - regulatory safety testing of rabies vaccine in mice where those scoring 2 went on the die:

- Score 1: ruffled fur, hunched back
- Score 2: slow movements, circling plus >15% weight loss
- Score 3: trembling, shaky, convulsions
- Score 4: lameness, paralysis, permanent recumbency

Endpoint could be set at a score of 2 without affecting the outcome of the test
Three areas of observations:
- behaviour and physical appearance
- body weight and/or body condition
- physiology

Two types of observations:
- parametric signs: body weight and temperature, blood pressure, respiratory rate etc.
- non-parametric signs: ruffled coat, closed eyelids, nasal discharge, lameness, self-trauma etc.

Video and digital cameras, transmitters, monitors and the development of checklists can assist in the recording of these observations
Checklists for the Determination of Endpoints

Checklists should:

- be specific for each experimental protocol
- capture as many signs as possible that reliably predict increasing severity of the clinical course of the condition or disease

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998) provides additional information on the development of checklists
Body weight:
- total body weight loss of 20%

Body temperature:
- a 6°C drop in temperature in rodent models can be used as an endpoint

Activity level:
- lethargy
- depression
- decreased activity

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998) recommend a minimum of two or three observations each day during critical periods.
Challenges in Setting and Monitoring Endpoints

- **Principal investigators:**
  - setting the earliest endpoint possible
  - defining limiting clinical signs
  - using best technologies for observation

- **Animal care committees (ACCs):**
  - balancing high quality science while minimizing pain/distress

- **Veterinary, animal care and research staff:**
  - ensuring careful, objective monitoring
  - documenting observations
  - identifying animals nearing pre-determined endpoints
To monitor endpoints, one must consider the following questions:

- Based on previous information, what is expected time, from the initial treatment to first signs of pain/distress to the death of the animal?
- When are the effects expected to be most severe?
- If the course of the disease and expected signs of the adverse effects are unknown, could an initial study answer these questions?
- Has a checklist of observations been established?
- Who will monitor the animals and keep the records?
- Has a clear chain for reporting observations been established?
- What will be the frequency of animal observations?
- Do investigator(s), animal care and technical staff have the training and expertise to monitor the animals adequately?
- Has existing toxicological data been evaluated?
Investigators should ensure:

- to work with their ACC, laboratory animal veterinarians and technical staff to continue to refine endpoints where possible and to assure ongoing compliance with approved protocols.

- laboratory animal veterinary staff can provide expertise with regard to clinical signs of pain and/or distress.
Summary

- Determine risk for pain and distress
- Evaluate changes in physical appearance, behaviour and physiology
- Minimize pain and distress by choosing the earliest endpoint
- Achieve scientific goals in line with the best possible animal welfare standards

Quality Animal Care = Quality Science