1. Purpose

To provide guidance on recognizing pain and distress in rodents.

1. Scope

This guideline applies to all personnel who are responsible for observing and monitoring animals following experimental and or surgical procedures. The Principal Investigator is responsible for ensuring that personnel are adequately trained and assigned to monitor animals for pain and distress after experimental and surgical procedures.

1. General Information
2. Unless otherwise indicated in the IACUC protocol, animals should return to normal behavior as soon as possible following experimental or surgical procedures. Animals must be evaluated for signs of pain or distress. The AV must be contacted immediately if signs of pain or distress that are not described in the IACUC protocol are observed.
3. The addition of a small amount of nesting material to the cage can be used as a mechanism to evaluate mouse health and well-being. Mice that are free of pain will utilize the new nesting material within 30 minutes.
4. The following is a list of clinical signs and behaviors that may indicate pain:

| **Sign or Behavior** | **Comments** |
| --- | --- |
| Abnormal posture | Hunched posture (or a tucked abdomen) is a general sign of pain or disease. Writhing and stretching behavior are associated with pain following laparotomy. |
| Behavior changes during handling | May be unusually aggressive or placid. |
| Changes in activity/mobility | Decreased mobility may be localized to the area of pain (e.g. limb) or may be generalized. Rodents in pain generally have decreased activity, but increased activity may also be seen. Other abnormal behaviors include back-arching, belly-pressing, twitching, and staggering. |
| Changes in facial expression | Utilizing a grimace scale may be beneficial in identifying pain. See: <https://nc3rs.org.uk/grimacescales#posters> |
| Decreased body temperature | Animals that feel cold to the touch are likely severely hypothermic and may be moribund. |
| Decreased food and/or water intake | May be associated with weight loss, dehydration, and decreased urine/fecal output.  Prolonged skin tent indicates dehydration.  Body condition scoring may be more useful than body weight in some circumstances (e.g., development of solid tumors). See: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846001/> |
| Decreased grooming, piloerection | Coat appears scruffy, hairs may be raised. |
| Decreased nest-building | Particularly meaningful in mice, which have a strong drive to build nests. Adding a small amount of nesting material to the cage daily allows for best assessment of nest building behavior. May not be as useful if multiple mice are housing per cage. |
| Decreased response to external stimulation | Attempts to escape when handled are normal in rodents unless well-habituated to handling – failure to exhibit this behavior may indicate pain or distress. |
| Licking, scratching at painful site | May result in trauma or exacerbating lesion severity due to self-mutilation. |
| Pale mucous membranes/extremities | Easiest to visualize by examining paws, tails, ears; can also look in mouth. |
| Porphyrin (red pigment) staining around eyes, nose, paws, forelimbs | Generally, pertains to rats. |
| Separation from group | Mice & rats are social and normally rest in close proximity to one another during the day (are nocturnal). |
| Shallow and/or rapid respirations | Normal respiratory rate = 100-230 breaths per minute |
| Squinting of eyes | By itself this sign could indicate pain associated with ocular problem, but if combined with other signs, such as ear position, whisker change, nose bulge, it could also indicate an extra-ocular source of pain. May also indicate dehydration. See: <https://nc3rs.org.uk/grimacescales#posters> |
| Teeth chattering, vocalization |  |
| Weight loss > 20% | Weight loss is associated with generalized pain leading to decreased food consumption. |
| Dehydration | Sunken eyes, prolonged skin tenting, tacky mucus membranes |

References:

Turner PV, Pang DS, Lofgren JL. A Review of Pain Assessment Methods in Laboratory Rodents. Comp Med. 2019 Dec 1;69(6):451-467. doi: 10.30802/AALAS-CM-19-000042. Epub 2019 Dec 20. PMID: 31896391; PMCID: PMC6935698.