Guideline for Pain and Distress in Laboratory Animals: Responsibilities, Recognition, and Intervention

Introduction & Responsibilities:

All research personnel share an ethical and legal obligation to minimize animal pain and distress without compromising study objectives. Pain is a brain-processed sensation, and distress is a complex negative reaction.(1) Reflex responses often measure spinal nociception, not actual pain, but remain the best assessment tool for non-verbal subjects.(2)

The Institute/Center Animal Care and Use Committee (IC ACUC) and Principal Investigators (PIs) must jointly ensure compliance with federal regulations and relevant policies by:(3-6)

- Knowing legal requirements.
- Knowing requirements for institutional accrediting organizations.
- Knowing requirements as set forth by ARAC guidelines and NIH Policies.
- Recognizing species-specific signs of pain/distress.
- Implementing mitigation strategies and defining clear humane endpoints.

Regulatory Requirements & ASP Justification:

The IC ACUC must review all Animal Study Proposals (ASPs), paying particular attention to those with procedures causing more than transient pain or distress, such as procedures that would normally be clinically treated with analgesics or are likely to be painful or distressful for animals, including human subjects.(3, 7)

ASP Requirements for Painful Procedures:

- **Alternatives:** Document that refinements (3Rs) which minimize the severity and duration of pain and distress were explored. In addition, replacement of animals and reduction in animal numbers must be considered and justified.(7, 8)
- **Justification:** If pain/distress cannot be alleviated, a scientific justification must be provided.
- **Management Plan:** Detail experimental procedures, expected clinical outcomes, exclusion criteria, and pharmacological/non-pharmacological interventions.
- **Monitoring:** Define a clear monitoring schedule, parameters, and assigned personnel.
- **Endpoints:** Establish explicit humane endpoints (avoiding severe pain or death whenever possible) and designate decision-making authority.

Ongoing Compliance:

- **Continuous Monitoring:** Animal care staff, researchers, and veterinarians must monitor animals throughout the study. Establish a system to ensure guidelines and procedures are followed.
- **Unanticipated Distress:** If unexpected pain/distress occurs, and is more than an isolated event, the PI must submit an amendment immediately detailing the event and corrective actions, including providing a justification if pain/distress is not mitigated.

Recognition & Monitoring:

- **Training:** Personnel must be trained in species-specific indicators to distinguish normal from subtle abnormal behaviors. Prey species vs predator species display very different behaviors indicating pain or distress.(9)

- **Objective Assessment:** Utilizing validated behavioral metrics and scoring systems ensures highly objective clinical evaluation.(10-13) It should be clarified that procedures expected to cause pain/distress in humans must be assumed to cause similar pain/distress in animals.
- **Frequency:** Animals must be observed at least once daily, with increased frequency for novel procedures or those procedures anticipated/known to elicit pain or distress.(14)
- **Preemptive Action:** Implement early interventions and execute rigorous follow-up monitoring to assess treatment efficacy.(15)
- **Documentation:** All observations, clinical signs, and interventions must be documented.

Intervention Strategies:

Formulate intervention and endpoint strategies in consultation with an IC veterinarian during ASP development to expedite approval.

- **Strategy Selection:** Protocols span euthanasia(16), non-pharmacological measures (e.g., modified housing, husbandry adjustments, dietary changes, acclimation), and pharmacological options[17]. Formularies provide extensive design support.(17, 18)
- **Non-Pharmacological:** Utilize modified housing, husbandry adjustments, dietary changes, and acclimation strategies.
- **Preemptive Care:** Use proficient surgical techniques to minimize tissue trauma. Administer preemptive, analgesics (e.g., ketamine, opioids, or NSAIDs) to prevent pain onset and shorten recovery.(19)
- **Multimodal Analgesia:** Consider combining different drug classes (e.g., local anesthetics, opioids, NSAIDs) or adjuncts (NMDA antagonists (19), alpha_2 adrenergic receptor-agonists (20), tramadol (21), gabapentin (22), or anti-nausea agents [(23)]) to enhance pain relief while lowering individual drug dosages.
- **Advanced Delivery:** For severe pain, consider constant rate infusions (systemic, local, epidural,) or transdermal patches and extended-release formulations under veterinary guidance.(24, 25)

Summary:

Clear responsibilities, appropriate endpoints, careful monitoring, and timely intervention are essential for preventing, minimizing, and relieving pain and distress in laboratory animals. Researchers, veterinarians, and animal care staff can use a variety of established references and formularies to help recognize and manage pain and distress. Experience has shown that a collaborative, team-based approach is most effective for protecting animal welfare while supporting high-quality science.

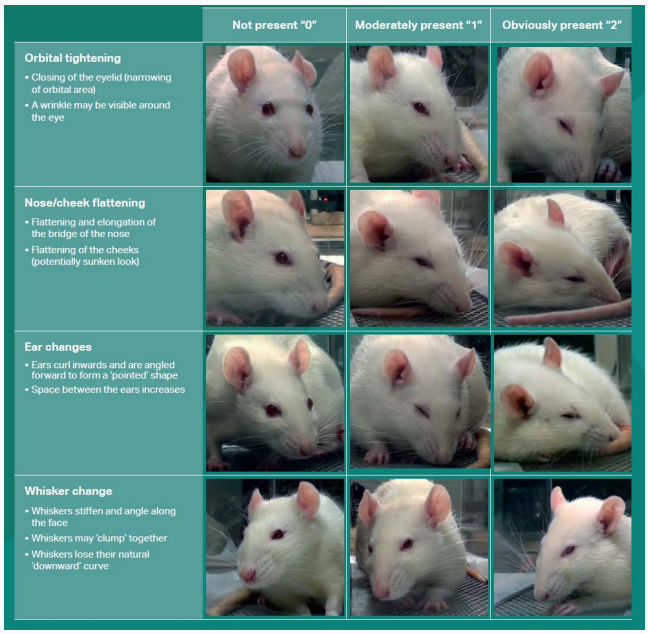
Grimace Scales for Various Animal Species

A Grimace Scale is a standardized behavioral coding system used to assess pain in animals by observing changes in their facial expressions. It is a critical non-invasive tool for researchers and veterinarians to ensure animal welfare. Below is a visual layout divided into four sections for four different species: Mouse, Rat, Rabbit, and Macaque.

1. Mouse Grimace Scale

Source: Mouse Grimace Scale (ref - Langford DJ et al. (2010). Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7(6): 447-449. doi:10.1038/nmeth.1455)
NC3Rs Resource Library. 2021. Grimace scales. https://nc3rs.org.uk/3rs-resources/grimace-scales

2. Rat Grimace Scale



Source: Rat Grimace Scale (ref - Sotocinal SG et al. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Molecular Pain 7: 55. doi:10.1186/1744-8069-7-55)
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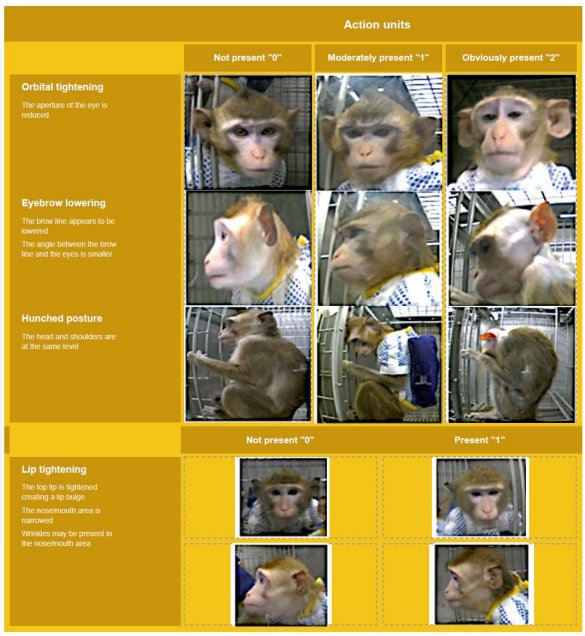
3. Rabbit Grimace Scale

Source: Rabbit Grimace Scale (ref - Keating SCJ et al. (2012) Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. PLOS ONE 7(9): e44437. doi:10.1371/journal.pone.0044437)
NC3Rs Resource Library. 2021. Grimace scales. https://nc3rs.org.uk/3rs-resources/grimace-scales

4. Cynomolgus Macaque Grimace Scale



Source: Paterson, E.A., O'Malley, C.I., Moody, C. et al. Development and validation of a cynomolgus macaque grimace scale for acute pain assessment. Sci Rep 13, 3209 (2023). https://doi.org/10.1038/s41598-023-30380-x

Note: These are not the only species with grimace scales, but can be extrapolated to other species, though specific signs and facial expressions of pain may vary. Always refer to species-specific validated scales when available to ensure accurate pain assessment and optimal animal welfare.

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