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### 1. PURPOSE

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This Standard Operating Procedure (SOP) intends to describe methods of assessing pain in rodents and mitigating pain by administering analgesic medications.

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### 2. RESPONSIBILITY

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Principal investigator (PI) and their research staff.

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### 3. GENERAL CONSIDERATIONS

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- 3.1. A procedure expected to be painful in humans is considered painful in animals.
  - 3.2. When there is a question of whether a procedure is painful, the animal should benefit from analgesia.
  - 3.3. Analgesia should be provided at an appropriate dose and frequency to control pain.
  - 3.4. Any deviation from this procedure must be justified by the investigator and approved by the BGU ethical committee.
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### 4. PAIN RECOGNITION AND ASSESSMENT

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- 4.1. Adapt the observation frequency to the procedure's invasiveness (minimum once a day).
- 4.2. Start by observing the animal from a distance, so the observer's presence does not alter the animal's behavior. Then proceed to observe the animal more closely.
- 4.3. Look for any changes in the behavior. Report animals that are in pain to the vets.
- 4.4. Common clinical signs indicative of pain or distress include (but are not limited to): avoidance, vocalization, aggressiveness, low spontaneous activities, isolation from the social group, altered gait, hunched posture, piloerection, reduced grooming, dark-red stain around the eyes and nostrils, weight loss and poor nesting behavior.

**Note:** *The most reliable signs of pain and distress are changes in behavior and no manipulation of enrichment materials.*

- 4.5. The Mouse and Rat Grimace Scale (Langford et al. 2010, Sotocinal et al. 2011): Posters demonstrating the scale are posted in the hallways and in Appendix 1 and 2.

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## 5. ANALGESIA PLAN

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- 5.1. Specify the analgesia plan in your animal protocol.
- 5.2. Provide analgesia just before the painful stimulus whenever possible, as it is more effective in preventing pain (e.g., give analgesic before surgery).
- 5.3. Use a combination of analgesics, often more effective than a single agent.
- 5.4. Extend analgesia from pre-op to 72 hours post-op for surgical procedures unless specified otherwise in the Animal Use protocol and approved by the BGU Ethical committee.

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## 6. LOCAL ANESTHESIA

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- 6.1. Infiltrate or apply local analgesics to areas where a painful stimulus may be induced. Repeat the application of local agents at specified intervals to maintain analgesia. In some cases, a sedative is recommended when using local analgesia.

Analgesic	Dose	Route	Duration	Note
Lidocaine	< 2 mg/kg	SC, Infiltration of surgical wounds	30–60 min.	Use lidocaine HCl 2% (20mg/ml) injectable solution. Because this drug is acidic, it is recommended to dilute it 3:1 with sodium bicarbonate injectable solution (at 5 or 8.4%).  Dilution must be prepared immediately before use and should not be stored. A diluted solution is as effective, but induction of analgesia is slightly prolonged.  *Dilution with sodium bicarbonate is unnecessary if lidocaine is administered to an anesthetized animal.
EMLA cream	Thick spread	Topical	30–60 min.	Apply only to intact skin. Shave or pluck the fur. Ideally, 10 minutes before the painful procedure
Lidocaine	Thick spread			Use lidocaine HCl 2% (20mg/ml) cream or gel. Apply only to intact skin. Shave or pluck the fur. Ideally, 10 minutes before the painful procedure
Eye drops	1-2 drops	Ocular	30-60min	
Bupivacaine	< 2 mg/kg	SC, Infiltration of surgical wounds	3–4 hrs.	Use bupivacaine HCl 0.50% (5mg/ml) injectable solution.  Same comment as for lidocaine.

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## 7. SYSTEMIC ANALGESIA

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- 1.1. Administration of non-steroidal anti-inflammatory drugs (NSAIDs):
  - 1.1.1. NSAIDs include carprofen, ketoprofen, meloxicam, and dipyrone.

- 1.1.2. Ensure good water intake and monitor hydration status during the treatment period.
- 1.1.3. Suspend water restriction before administration of NSAIDs.
- 1.1.4. Do not administer NSAIDs to neonatal rodents.
- 1.2 Administration of opiates:
  - 1.2.1. Opiates include Buprenorphine and butorphanol.
  - 1.2.2. Ensure good water intake and monitor hydration status during treatment.
  - 1.2.3. Suspend water restriction before administration of opiates.

#### Mouse / Gerbil / Rat

Analgesic	Dose	Route	Frequency	Note
Buprenorphine	0.1 mg/kg	SC, IP	4–8 hrs.	Mild to moderate pain. Controlled drug.
Buprenorphine long-acting	Mouse -3.25 mg/kg Rat – 0.65mg/kg	SC, IP	72 hrs.	Mild to moderate pain. Controlled drug. From 1.3mg/ml stock: 0.05ml/20gr mouse 0.1ml/200gr rat
Carprofen	20 mg/kg	SC, PO	12–24 hrs.	Mild to moderate pain. Use carprofen 50mg/ml injectable solution. To prepare a 4mg/ml dilution: add 0.8 ml of carprofen 50mg/ml to 9.2 ml of sterile water for injection. Administer 5µL/g of body weight. Store at room temperature. Discard dilution after one month.
Meloxicam	20 mg/kg	SC, PO	24 hrs.	Mild to moderate pain.
Ketoprofen	15 mg/kg	SC	12-24 hrs.	Mild to moderate pain.
Dipyrene	200 mg/kg	PO	In water bottle	Mild to moderate pain.

#### Mouse Neonates

Analgesic	Dose	Route	Frequency	Note
Buprenorphine	0.05-0.1 mg/kg	SC, IP	4–8 hrs.	Severe to moderate pain. Controlled drug.

#### Guinea pig

Analgesic	Dose	Route	Frequency	Note
Buprenorphine	0.01–0.05 mg/kg	SC, IP	8–12 hrs.	Severe to moderate pain.
Dipyrene	200mg/kg	PO	3 days	Mild to moderate pain. Change water after 3 days.

### Appendix 1: The Mouse Grimace Scale

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals In Research

## The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>			
<b>Nose bulge</b> <ul style="list-style-type: none"> <li>Bulging on the bridge of the nose</li> <li>Vertical wrinkles on the side of the nose</li> </ul>			
<b>Cheek bulge</b> <ul style="list-style-type: none"> <li>Bulging of the cheeks</li> </ul>			
<b>Ear position</b> <ul style="list-style-type: none"> <li>Ears rotate outwards and/or backwards, away from the face</li> <li>Ears may flap to form a 'pointed' shape</li> <li>Space between the ears increases</li> </ul>			
<b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers are either pulled back against the cheek, or pulled forward to stand out and</li> <li>Whiskers may clump together</li> <li>Whiskers lose their natural downward curve</li> </ul>			

Read the original paper: Langley CJ, Bailey JL, Charalab, Crane DJ, Durrumore D, Eklund S, Glick SJ, Grigg J, Dunnington T, Cordero-Rodriguez, Hohenhaus S, et al. (2018) The Mouse Grimace Scale: A partially automated method for quantifying pain in the laboratory rat and mouse. Molecular Pain 7: 55. doi:10.1186/s12908-018-0056-7-55

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique and rat grimace scales (when relevant) are available on request. To request copies of this paper, please email: [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk). The NC3RS provides a range of all resources at [www.nc3rs.org.uk/resources](http://www.nc3rs.org.uk/resources). Images kindly provided by Dr. Jeffrey Mogil, McGill University.

### Appendix 2: The Rat Grimace Scale

**NC 3R<sup>s</sup>** National Centre for the Replacement, Refinement & Reduction of Animals In Research

## The Rat Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in rats.

The specific facial action units shown below have been used to generate the Rat Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
<b>Orbital tightening</b> <ul style="list-style-type: none"> <li>Closing of the eyelid (narrowing of orbital area)</li> <li>A wrinkle may be visible around the eye</li> </ul>			
<b>Nose/cheek flattening</b> <ul style="list-style-type: none"> <li>Flattening and elongation of the bridge of the nose</li> <li>Flattening of the cheeks (potentially sunken look)</li> </ul>			
<b>Ear changes</b> <ul style="list-style-type: none"> <li>Ears curl forwards and are angled forward to form a 'pointed' shape</li> <li>Space between the ears increases</li> </ul>			
<b>Whisker change</b> <ul style="list-style-type: none"> <li>Whiskers stiffen and angle along the face</li> <li>Whiskers may 'clump' together</li> <li>Whiskers lose their natural downward curve</li> </ul>			

Read the original paper: Bokrosi SD, Singh RE, Zakaria A, Tuttle AH, Martin LJ, Wessling JB, Mappelback JCS, Wolf Z, et al. (2018) The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat and mouse. Molecular Pain 7: 55. doi:10.1186/s12908-018-0056-7-55

For guidance on using the Rat Grimace Scale, research papers that underpin this technique and rat grimace scales (when relevant) are available on request. To request copies of this paper, please email: [enquiries@nc3rs.org.uk](mailto:enquiries@nc3rs.org.uk). The NC3RS provides a range of all resources at [www.nc3rs.org.uk/resources](http://www.nc3rs.org.uk/resources). Images kindly provided by Dr. Jeffrey Mogil, McGill University.

**SOP 101 RODENT ANALGESIA**  
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