

AUSTIN HEALTH AEC (2018) GUIDELINES FOR PAIN AND DISTRESS MANAGEMENT IN RODENTS

1. Purpose

The Austin Health AEC requires that all animals undergoing any procedure that is painful or anticipated to be painful or causing distress must have a pain management plan developed as part of the AEC application process. There should also be a plan incorporated into any project if there is the potential distress or stress to any animals, not just pain-this includes such issues as solitary housing of animals, prolonged aging of animals, being housed with other animals that may be in pain. This is in keeping with *The Australian code for the care and use of animals for scientific purposes 8th edition 2013, "The Code"* and serves to ensure respect for the animals underpins all that is done. The approved plan must then be implemented and adapted as needed throughout the project duration to ensure pain and distress management is appropriate for each animal.

This guideline aims to assist investigators in the development of these pain and distress management plans. This guideline is designed for general information and should be utilised to formulate and tailor a pain and/or distress management plan for each individual project. This guideline will address pain and distress management in mice and rats only.

2. Principles

Pain is defined by the *Australian Code for the Care and Use of Animals for Scientific Purposes (the Code)*, as:

"an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It may elicit protective actions, result in learned avoidance and distress, and modify species-specific traits of behaviour, including social behaviour."

The Code's governing principle is respect for animals and this is demonstrated *by avoiding or minimising harm, including pain and distress, to those animals.*

The Code is very specific regarding reduction of pain and distress at all times and states, amongst other things, the following:

Avoid or minimise harm, including pain and distress, to animals

*1.10 Animals have a capacity to experience pain and distress, even though they may perceive and respond to circumstances differently from humans. Pain and distress may be difficult to evaluate in animals. **Unless there is evidence to the contrary, it must be assumed that procedures and conditions that would cause pain and distress in humans cause pain and distress in animals.***

Decisions regarding the possible impact of procedures or conditions on an animal's wellbeing must be made in consideration of an animal's capacity to experience pain and distress.

1.11 Steps must be taken at all times to safeguard the wellbeing of animals by avoiding or minimising harm, including pain and distress to the animals.

1.12 Where the aim(s) of the project involves the animals experiencing pain and distress that will not be alleviated, the planned endpoint of the project must be as early as feasible to avoid or minimise pain and distress in the animals.

1.13 'Death as an endpoint' must be avoided unless it is essential for the aim(s) of the project. In these circumstances, the means to prevent or minimise harm, including pain and distress, must be considered, implemented and reviewed at all stages of the project.

1.14 Prompt action must be taken to alleviate pain and distress that were not anticipated in an approved project or activity, or occur as the result of an emergency. Such action must take precedence over an individual animal reaching the planned endpoint of the project or activity, or the continuation or completion of the project or activity.

Each AEC approved project must have a pain management plan which is tailored to that project and is appropriate for the species, strain and age of animals within it and the research outcome requirements. This plan should also include actions to be undertaken if the need for emergency euthanasia to reduce pain and end suffering arises.

Most laboratory animals are prey species and as such will hide pain and distress until it is significant in amplitude. This means that the early signs of pain and/or distress may be missed by investigators unfamiliar with such species. **As stated above, because of this, if a procedure is likely to cause pain or distress in a human it is assumed to do so in an animal.** This **must** be the underlying assumption for any pain management plan.

Pain and distress result in many poor welfare and experimental outcomes such as reduced movement, aversion to handling, social isolation, excessive vocalisation, cannibalism of affected animal, self-trauma, increased cortisol levels, reduced wound healing, increase blood pressure and heart rate and reduced caloric intake. These are significant welfare concerns and experimental results. Pain and/or distress can reduce consistency in results, introducing potential experimental variation and reduce repeatability in work.

3. Application

There are a number of considerations when formulating a pain relief plan for an animal within a research project. Each plan should be tailored for the particular project and there is no "one size fits all" for pain relief. Pre-emptive and multi modal pain relief is the gold standard and should be attempted where at all possible. Pain relief should also be tailored throughout the project for the animals within it. Distress to animals can also be produced in many ways and is species

dependent. What constitutes distress in some species and strains (e.g solitary housing) may not do so for others. Considerations when formulating a pain and distress relief plan include:

- The species, strain and age of the animal
- The research outcomes and data that requires collection
- The cumulative impact of procedures on an animal
- All factors that may cause pain or suffering (e.g. reduced food intake, nausea, hypothermia)
- How pain relief is to be administered and practicality of this given the species
- The pharmacology of the pain relief to be administered and how this may impact physiology of animals (e.g. non-steroidal anti-inflammatories and nephrotoxicity)
- How frequently pain relief may be able to be given, particularly considering limitations with staffing in a facility and project
- How distress may be minimised by such things as enrichment, co-housing, reduction of exposure to other animals undergoing recovery from anaesthetic etc.

4. Procedure

Pre-emptive analgesia should be provided whenever possible. Analgesic drugs are most effective when given prior to the onset of a painful stimulus, allowing for enhanced peri-operative pain management.

Multi-modal analgesia should be utilised whenever possible to provide optimal levels of pain relief. Analgesic drugs with different mechanisms of action provide a complimentary and increased level of pain control when given in combination, and often lower doses of an individual agent may be used. Lower doses of individual drugs may lessen the risk of undesirable side effects.

4.1 Monitoring

Analgesic drugs are only one component of a multi-modal approach to pain control, which begins with prevention and efforts to reduce the intensity of the painful stimulus. Anyone working with animals must be competently trained in correct handling techniques to ensure minimal stress to the animal.

Restraint for procedures should not be performed for any longer than is required to achieve the desired task. As with all work involving animals, it is absolutely essential that their welfare be maintained throughout all aspects of a project.

Activities that decrease anxiety and stress will enhance the effect of concurrently administered analgesic agents. Nonpharmacological methods for reducing stress and therefore pain can include acclimatisation of the animal before the procedure, good husbandry, offering

enrichment, reducing access to bullying/fighting behaviour, nutritional support, and access to conspecifics for social animals.

Some individual variation in how an animal responds to analgesic agents may occur, so animals must be carefully monitored after a procedure to assess this. The dose or frequency of drug administration should be modified according to the effectiveness of pain control in each animal.

4.1.1 Recognising pain and distress in animals

Pain has the ability to cause physiologic changes. An animal may experience fluctuations in body temperature, respiration rate, heart rate and blood pressure. These things are often not practical to measure directly in laboratory animals (if possible at all) and so observations of behaviour, body position and feeding habits are relied upon for monitoring instead.

It is essential that researchers and animal carers familiarise themselves with the normal behaviour and physiology of the species under observation prior to any procedures in order to recognise any abnormalities resulting from pain or distress. The AWO, a veterinarian familiar with the species or an experienced investigator should be consulted if unsure what to look out for. This should occur, if possible, prior to submitting ethics applications, as species-specific knowledge will be required to prepare monitoring and intervention sheets for the project. This can also be achieved in the acclimatisation period- researchers can gently handle and observe their animals in this time to familiarise themselves with the animals in a normal health and low stress state.

General signs associated with pain and distress in animals may include:

- Decreased appetite
- Weight loss
- Alteration from usual demeanour (e.g. calm animals may become aggressive when handled, active animals become unusually lethargic)
- Abnormal vocalisation (e.g. urgent, repetitive sounds)
- Fluctuations in heart and breathing rates
- Reduced interaction with cage mates in social species
- Further species specific signs of pain may also be found within the NHMRC guidelines, available online at <http://www.nhmrc.gov.au/>
- The grimace scale for rodents (indicative of pain response) are an excellent resource and are found at the website for NC3Rs at <https://www.nc3rs.org.uk/grimacescales>.
- These scales are attached to this document as appendices and are available in the BRF holding rooms.

4.1.2 Intervention Criteria

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Evidence of any of the above signs or other concerns warrants further examination and assessment for pain, distress and discomfort. Actions taken may include provision of additional analgesic drugs, supportive care such as warmth and rehydration or nutritional support where appropriate.

Monitoring criteria approved with the AEC project must be adhered to and if there is any doubt regarding an animal's welfare state then advice should be sought immediately from BRF staff and the AWO/veterinarian.

4.2 AEC Requirements

- Adherence to AEC approved project with regards to monitoring and analgesia
- Monitoring performed by person competent to do so and with a good understanding of animal welfare and response to pain and distress
- Weighing of individual animals prior dosing with drugs and for calculation of dosages
- Observation immediately after injections for adverse events.
- Continuous monitoring post surgery or anaesthesia until animals completely recovered and ambulatory and returned to home cage. Further monitoring as per AEC approved protocol
- Consultation with AWO/vet and/or BRF staff immediately any concerns are noted regarding animal condition or welfare
- Adherence to instruction regarding analgesia or humane endpoint from AWO/veterinarian or BRF staff
- Complete, accessible and contemporaneous records of analgesic/anaesthetic administration and procedures performed on animals and by whom.
- Adverse events related to analgesia/pain relief are reported to the AEC as per current AEC approved adverse event guideline.

4.3 Types of Analgesic Agents

4.3.1 Local anaesthetics (e.g. Lignocaine, Bupivacaine)

Provide analgesia by acting directly at nerves near the required site and inhibiting transmission of painful stimuli back to the brain. It may be injected into tissues and allowed to perfuse around nerves or applied topically to skin and given time to permeate through. The time required for lignocaine based preparations to be effective at numbing skin will vary according to the drug preparation, species and their skin type.

Compared to lignocaine, bupivacaine has a significantly slower onset of action, a prolonged duration of action and cannot be safely given intravenously. There may be transient pain associated with injection of local anaesthetic agents; for this reason, they should preferably be used in anaesthetised animals. The use of these agents in the conscious animal should be

considered in the context of the species and the procedure, with the method that causes the least distress or discomfort selected.

4.3.2 Non--Steroidal Anti--Inflammatory Drugs-- NSAIDs (e.g. Meloxicam, Carprofen)

This class of drugs act as non--selective inhibitors of cyclooxygenase (COX) enzyme, and are known to have analgesic and anti--inflammatory effects.

4.3.3 Paracetamol

Generally classed on its own, this is a COX--2 selective drug that can be safely combined with NSAIDs or opioids for multi--modal analgesia. Paracetamol has a narrow safety margin in many animals.

4.3.4 Opioids (e.g. buprenorphine)

Act at opioid receptors (primarily μ or κ) to provide analgesia, though common side effects may also include sedation, respiratory depression, euphoria or constipation. As each species group has varying types and ratios of these receptors, the effects of the agent may vary in different species.

Investigators need to be aware of any species differences before selecting opiates for procedures and should consult with the AWO or a veterinarian where required.

4.4 Minimum Analgesia Requirements

Small skin and subcutaneous tissue injury (such as implantations of chips or pellets) that procedures that leave the underlying muscle wall intact

- Long acting local anaesthetic +/- NSAIDs
- Minimum of 4-6 hours post procedural

Small skin incisions and sub cutaneous injury that leave the muscle intact

- NSAID +/- long acting local anaesthetic
- Minimum 24 hours post procedural analgesia

Incisions through the muscle wall or entering the abdominal cavity, retro--peritoneal space or thoracic cavity

- NSAIDs for 48hrs and opioids for first 24hrs, consider long acting local anaesthetic at skin wound site
- Minimum of 48hrs post--operative analgesia

Orthopaedic procedures

- NSAIDs and opioids, consider long acting local anaesthetic at skin wound site
- Minimum of 48hrs post--operative analgesia

Craniotomy

- Opioids, consider long acting local anaesthetic at wound site
- Minimum of 48hrs post--operative analgesia

- 4.4.1** Analgesia is mandatory where surgical procedures are carried out on rodents. Procedures involving anaesthesia and surgery, opening of body cavities or orthopaedics must receive a minimum of opiate level and NSAID analgesia, unless a demonstrable medical reason exists to omit the NSAID and is approved by the AWO and AEC.
- 4.4.2** Intravenous injections may require topical local anaesthetic prior to being given, however the pain associated with this procedure is of short duration compared to most surgical procedures.
- 4.4.3** Where a novel procedure is conducted that has the potential to be painful, analgesia must be provided as the default standard to maintain animal welfare. The AWO must be invited to observe and provide analgesic advice for procedures where uncertainty exists about the required level of analgesia.

4.5 Common Analgesic Agents

- 4.5.1** *Appendix I* contains a list of some commonly used analgesic drugs and outlines dosing information from the NHMRC guidelines (where available) and current published literature. It is not intended to provide an exhaustive list, and the AWO or a registered veterinarian should be consulted for additional information where required.
- 4.5.2** Investigators should ensure they are aware of any potential side effects. They should also be aware that not all analgesic drugs are registered for use in all species. Use of a drug in a species for which it is not registered is considered “off-label” use.

4.5.3 Methods of delivery for analgesic drugs

4.5.3.1 Subcutaneous (SC)

An injection under the skin, typically over the scruff or flanks, is the most common and straightforward method of analgesic delivery. Ideally, solutions should be non-irritant to the animal to reduce pain or discomfort associated with the procedure.

4.5.3.2 Intramuscular (IM)

The substance is injected into the belly of a muscle or group of muscles. Some agents may cause muscle necrosis or pain if given repeatedly by this method, so sites should be rotated and the minimum volume size used to limit this.

4.5.3.3 Intravenous (IV)

The substance is injected into a vein. Where repeated administration is required, investigators are encouraged to assess if indwelling intravenous catheters (permanent or temporary) may be suitable. This will reduce the discomfort experienced by the animal,

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ensure consistent delivery of the substance into the vein and minimise trauma to the vessel and perivascular area.

4.5.3.4 Intraperitoneal (IP)

An injection is made into the lower abdominal cavity (known as the ‘peritoneal cavity’ or ‘peritoneum’). This is most commonly used in rodents as it can be done without the need for anaesthesia by trained technicians/researchers. Caution should be exercised to avoid inserting the needle too far into the abdomen and causing damage to the bowel and other organs.

4.5.3.5 Orally (PO)

A substance can be delivered directed into the mouth by oral gavage or syringe or in food or water. Care should be used to mask the presence of any unpleasant tasting substances in order to promote a positive experience for the animal.

4.5.3.6 Oral transmucosal (OTM)

The substance needs to come into contact with a mucosal surface, such as the inner cheeks, under the tongue or rectum. No needle is generally used to deliver the agent topically over a mucosal surface where it is absorbed into the body.

4.5.3.7 Local infiltration

This requires the anaesthetic substance (usually a local anaesthetic) to be drawn up into needle and syringe. The needle with attached, filled syringe is then inserted into the middle of the tissue region where numbing is required, and the drug is slowly injected into the tissue as the needle is withdrawn. This allows the anaesthetic agent to diffuse into the nearby tissue. The ideal time for injection of local anaesthetic agents for this purpose is after the animal is under general anaesthesia, but before any tissue damage (e.g. surgery) has occurred.

4.6 Use of topical analgesic creams

The use of topical gels or creams containing lignocaine, prilocaine or combinations of these drugs should be considered where the pain associated with conducting a procedure is greater than the stress induced by restraint to apply the gel/cream in the first instance. Topical creams must be applied and given time to take effect before the painful stimulus occurs in order to prevent the start of the painful wind up. Analgesic creams must be applied to the designated area of skin following removal of hair, for sufficient time to cause numbing and loss of nerve sensation in the dermis and epidermis of the skin. The onset and duration of analgesia and the depth of activity is dependent on the duration of application and will vary with each species.

4.6.1 EMLA cream

The most widely available preparation is EMLA[®] cream (2.5% lignocaine and 2.5% prilocaine) which has been extensively studied in humans, however limited published data is available for animal species. The most common undesirable side effect noted across most species is a blanching or mild erythema of the skin in contact with the cream.

4.6.1.1 Ingredients

Each 1 gram of cream contains 25 mg of lignocaine, 25 mg of prilocaine, polyoxyethylene fatty acid esters (as emulsifiers), carboxypolymethylene (as a thickening agent), sodium hydroxide to achieve a pH of 9.4, and purified water.

4.6.2 Use in humans

The cream is applied to skin as a 2 mm layer then covered with an adhesive bandage for 60 minutes for standard application in humans. At the end of this time the bandage is removed and the cream wiped away. The local anaesthetic agents have been shown to penetrate 2.9 mm deep in human skin after 60 minutes and achieve up to 180 minutes of analgesia due to the reservoir effects of the drug in the dermis and epidermis.

4.6.3 Use in animals

A handful of studies are available looking at the effects of EMLA cream on rabbit, rat and feline skin. EMLA cream to already furless areas of skin on the tail of rats was found to have no appreciable benefit prior to venipuncture. It is important to note that in many laboratory species the stress of restraint, hair removal, cream application and bandage placement/ removal may outweigh any potential benefit obtained by numbing. In these instances, the stress involved with the use of analgesic creams may pose a greater welfare risk than the brief venipuncture alone. Consultation with the AWO is advised when considering the use of a topical analgesic cream.

4.6.3.1 Method of application

Fur should be removed by clipping or shaving the area (depilatory creams are not suitable) prior to applying a 1--2 mm layer of EMLA cream and covering the area with a non--absorbent dressing (e.g. Cling wrap and /or Vetwrap). The amount used should be the smallest possible volume that achieves analgesia for the animal but avoids passing a toxic threshold. It can be difficult with topical drug application to determine how much active ingredient is being absorbed systemically, so a maximum dose should be calculated based on lignocaine concentration prior to use.

4.6.3.2 Duration of application

The duration and depth of analgesia in human skin is a useful starting point, however it should be noted that the structure, thickness and permeability of animal skin may be vastly different. This information may be applicable to other species depending on the thickness of the tissue and contact time, but as animal skin is often more permeable (e.g. Rabbits) the duration of onset may be much shorter. Unpublished observations from experienced investigators and veterinary staff should be considered in the absence of clear scientific data.

Adapted from University of Melbourne Animal Care and Use Standard-Analgesia

APPENDIX 1

Suggested Dose Rates and “Recipes” of Commonly Used Analgesics

1. MICE

a. Local Anaesthetic

- Lignocaine (2%)**
- Dilute to 0.5% and do not exceed 7mg/kg total dose
 - Use as local block once animal anaesthetised
 - Approx. 1 hour duration but rapid onset
- Bupivacaine (0.5%) (Marcain™)**
- Dilute to 0.25% and do not exceed 8mg/kg total dose.
 - Use as local block once animal anaesthetised
 - Duration of action 4-8 hours but slower onset of action

Best to use in combination to gain quick onset of action plus length of duration

Combination: Recipe for 25gm mouse to receive 0.03ml subcutaneous injection:

Combine all in the same syringe:

- 1ml of 20mg/ml lignocaine
- 1ml of 5mg/ml bupivacaine
- 2ml of injectable saline

For straight lignocaine:

Combine in same syringe:

- 1 ml 20mg/ml lignocaine
- 3 ml saline

Mix the drugs and saline together-makes up 4 ml of solution .Can keep in the fridge for up to 10 days once made up. If require less then use same ratios.

Maximum doses by weight:

Weight of Mouse (gm)	Max volume of diluted Lignocaine (0.5%) Do not exceed the following	Max volume of diluted Bupivacaine (0.25%). Do not exceed the following
25	0.03 ml	0.08 ml
35	0.05ml	0.11 ml
45	0.06ml	0.14ml

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55	0.07ml	0.17ml
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b. Non-Steroidal Anti-Inflammatories

Use with care with compromised renal, hepatic or GIT systems and if measurement of inflammatory mediators is required

Carprofen: 5mg/kg sc, ip every 24 hours
(50mg/ml)

Always dilute prior injection as undiluted solution causes scabbing and significant irritation

Recipe for Dilution:

0.1 ml of stock solution (50mg/ml) into 9.9 ml of saline/water for injection (use 22g needle fort this as stock very thick).

This yields a solution of 5mg/10ml or 0.5mg/ml

Dose is 5mg/kg (or 10ml/kg)
(→ 10ml/kg=1ml/100g →0.1ml/10gm mouse)

So mouse requires
0.1ml/10gm of mouse

Meloxicam: 2mg/kg sc every 24 hours
(5mg/ml)

Recipe for Dilution:

0.1 ml stock solution (5mg/ml) into 1.9 ml saline

This yields a solution of 0.25mg/ml

Dose is 2mg/kg (or 8ml/kg)
(→8ml/kg=0.8ml/100gm→0.08ml/10gm mouse)

So mouse requires

0.08 ml/10gm mouse

c. Opiates

The supply of these drugs is strictly regulated and as such the supply of them will be in pre diluted small aliquots and dispensed by the BRF veterinarian.

Buprenorphine: 0.05mg/kg -0.1 mg/kg (approx. 0.001-0.002 mg/mouse) sc, ip or iv every 4-12 hours
(300ug/ml)

The premix used within the BRF is 0.015mg/ml so designed to give **0.1ml per 20gm mouse**.

Butorphanol: 1-2mg/kg sc, ip every 2-4 hours

This drug is currently not used within the BRF but may be attained if required.

2. RATS

a. Local Anaesthetics

Lignocaine and Bupivacaine dose as per mice
Dilution recipe as per mice

Maximum doses by Weight:

Weight of Rat	Maximum Volume Diluted Lidocaine (0.5%) Do not exceed:	Maximum Volume Diluted Bupivacaine (0.25%) Do not exceed:
250g	0.35ml	0.8ml
350g	0.49ml	1.12ml
250g	0.63ml	1.44ml
550g	0.77ml	1.76ml

b. Non-Steroidal Anti-Inflammatories

As for mice- care with use in renal or hepatic injury or with GIT insults or dehydration.

Carprofen: Dose is **5mg/kg** ip, sc once a day-must dilute pre injection to prevent sores and injection site irritation.
(50mg/ml stock solution)

Dilution recipe: Add 0.2 ml of carprofen to 3.8 ml of sterile saline/water to produce 2.5mg/ml

Average rat weighs 250gm –dose is 5mg/kg= 1.25 mg/250gm once a day

Therefore 250gm rat needs 0.5ml of diluted carprofen

Meloxicam: Dose is **2mg/kg** sc,ip once a day
(5mg/ml stock solution injectable)

Dilution recipe: Dilute 0.4 ml meloxicam with 0.6 ml saline/water for injection to give a solution of 2 mg/ml.

Dose for rats is 2mg/kg or 1ml/kg of diluted solution.

Give 0.25 ml of diluted solution to 250gm rat once a day

c. Opiates

Buprenorphine: Dose is 0.01-0.05mg/kg sc, ip 8-12 hourly
(300ug/ml stock solution)

Diluted solution in BRF is 0.06mg/ml-so give 0.1 ml per 200gm rat of diluted solution

Minimum Analgesia Requirements

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- Long acting local anaesthetic +/- NSAIDs
- Minimum of 4-6 hours post procedural

Small skin incisions and sub cutaneous injury that leave the muscle intact

- NSAID +/- long acting local anaesthetic
- Minimum 24 hours post procedural analgesia

Incisions through the muscle wall or entering the abdominal cavity, retro--peritoneal space or thoracic cavity

- NSAIDs for 48hrs and opioids for first 24hrs, consider long acting local anaesthetic at skin wound site
- Minimum of 48hrs post--operative analgesia

Orthopaedic procedures

- NSAIDs and opioids, consider long acting local anaesthetic at skin wound site
- Minimum of 48hrs post--operative analgesia

Craniotomy

- Opioids, consider long acting local anaesthetic at wound site
- Minimum of 48hrs post--operative analgesia

APPENDIX 2 GRIMACE SCALE MOUSE



**NC
3R^s**

**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 pain-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"
Orbital tightening - Closing of the eyelid (narrowing of orbital area) - A wrinkle may be visible around the eye			
Nose/cheek flattening - Flattening and elongation of the bridge of the nose - Flattening of the cheeks (potentially sunken look)			
Ear changes - Ears rust (inwards) and are angled forward to form a 'pointed' shape - Space between the ears increases			
Whisker change - Whiskers stiffen and angle along the face - Whiskers may 'cling' together - Whiskers lose their natural 'downward' curve			

Read the original paper:
 Brocchieri M, Sorge RE, Zakaria A, Tittle AK, Mertz LJ, Wesskopf JS, Maplebeck JCB, Wei P, Chen S, Zhang S, McDougall JJ, King CG, Mogil JS. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Neuroscience* 177:35. doi:10.1016/j.neuroscience.2011.04.026-7-26

For guidance on using the Rat Grimace Scale, research papers that describe the techniques and for grimace scales in other species, visit www.nc3rs.org.uk/guide-to-grimace-scales. To request copies of this paper, please email enquiries@nc3rs.org.uk. The NC3Rs provides a range of 3Rs resources at www.nc3rs.org.uk/resources. Images kindly provided by Dr Jeffrey Mogil, McGill University.