Pain assessment in laboratory animals using behaviour & facial expressions

BfR Forum 2011

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Research group

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- Aurelie Thomas
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- Melissa Bateson
- Jon Gledhill
- Claire Richardson
- Amy Miller

To recognise pain, you need to define it…

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" IASP 1979

Although this is a human definition, it can be applied to animals, if we accept they can experience 'emotional' component of pain…

Background

- If we accept animals experience pain then we have an obligation to develop effective methods of:
  - Recognising it and;
  - Assessing it's nature & severity
- If we are unable to do this, then we cannot:
  - Effectively assess pain severity induced by a procedure
  - Be confident that anything we do to reduce pain is effective e.g. type, dose & administration regime of analgesia

Traditional assessments

- Subjective assessments of ‘clinical signs’:
  - Appearance: coat condition, pilo-erection, anorexia etc.
  - Posture/Gait: hunched posture, abnormal gait etc.
  - Demeanour: aggression, hiding etc.
- Objective assessments of:
  - Locomotion / Activity
  - Food & water intake / Bodyweight change
  - Respiratory rate / Heart rate / Blood pressure

Limitations

- Little evidence of how these relate to pain intensity:
  - Changes could be due to other non-pain related causes
  - Changes in measure may not parallel change in pain
- Retrospective: Poor for immediate assessment
- Effectiveness requires detailed species knowledge
  - Indicators are subtle/unfamiliar so difficult to detect
  - Need to know the normal/baseline 'state' of species/ individual
- Problems of between & within observer reliability

Why not just use an analgesic?

- Not a viable alternative to pain assessment…
  - Without this we cannot ensure analgesic appropriateness
- Considerable variation analgesic potency between:
  - Species, strains/breeds, sexes & even individuals…
    - Analgesic sensitivity & stress-induced analgesia etc.
- Universal dose unlikely to control pain in all…
  - Too low: Pain not controlled
  - Too high: Side-effects may occur

} Poor welfare & validity

Behavioural assessment

- Behavioural indicators have improved the effectiveness of pain assessment
- Behaviour provides:
  - Immediate cage/pen side assessment (**not** retrospective)
  - Growing evidence of relationship between pain & behaviour
- So consider ‘better’ than more ‘traditional’ methods
- Schemes developed for a wide range of species:
  - Rodents, rabbits, dogs, cats, lambs and calves etc.

Behavioural assessment

- Underlying principle of behavioural assessments:
  - Pain causes certain behaviours to occur more or less frequently
  - Analgesics should then reduce those that increase (if pain specific) & increase those that decrease (e.g. Activity)
- Appropriate controls are necessary to ensure:
  - Cause-effect relationship & changes are direct response to pain
- Composite scoring necessary for rodents & rabbits

Rat pain assessment

Rat pain behaviours can be viewed at:

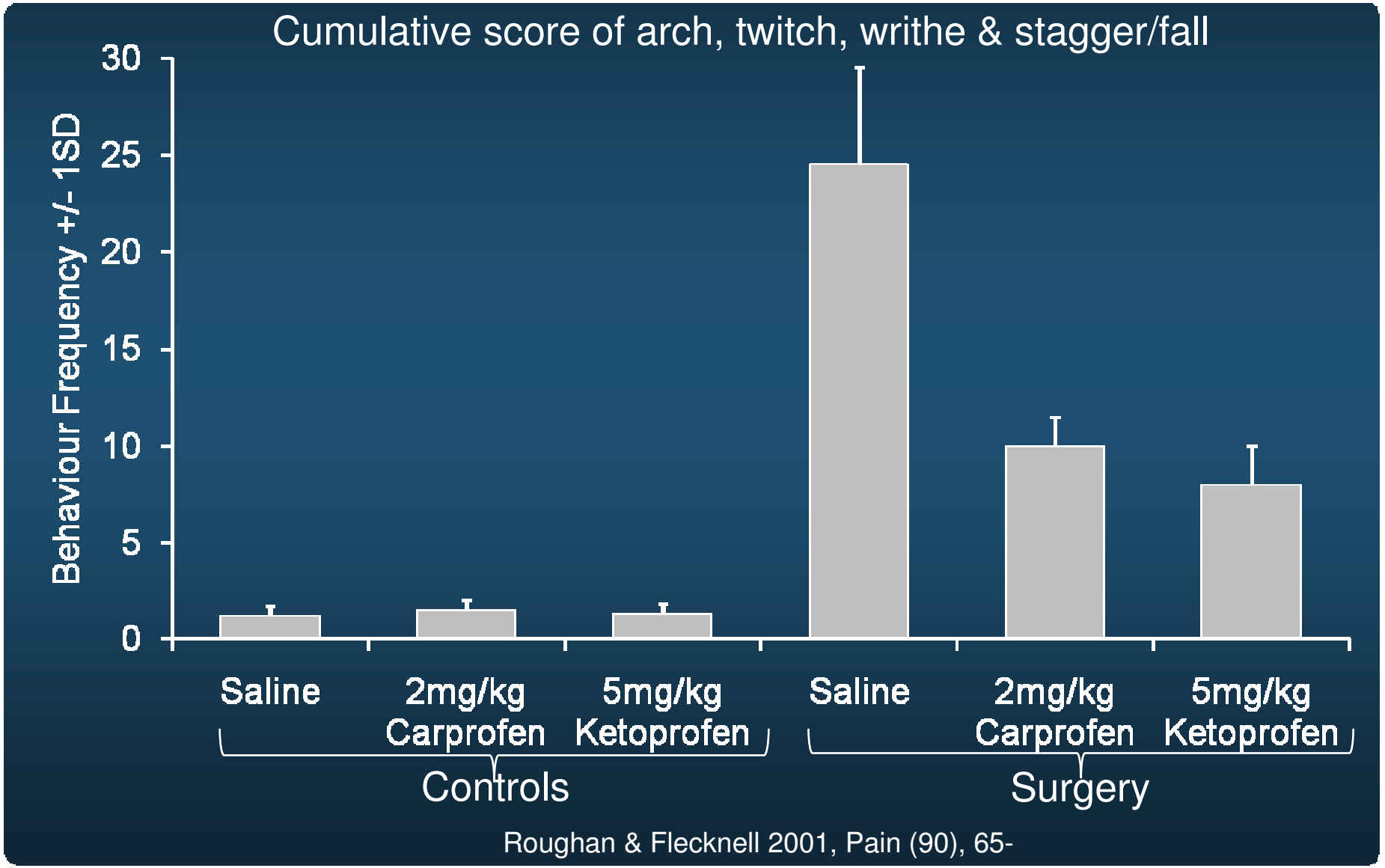
Digires:

http://www.digires.co.uk

Lab animal welfare website:

http://www.ahwla.org.uk

Laporatomy in Wistar rats



Roughan & Flecknell 2001, Pain (90), 65-

Mouse pain assessment

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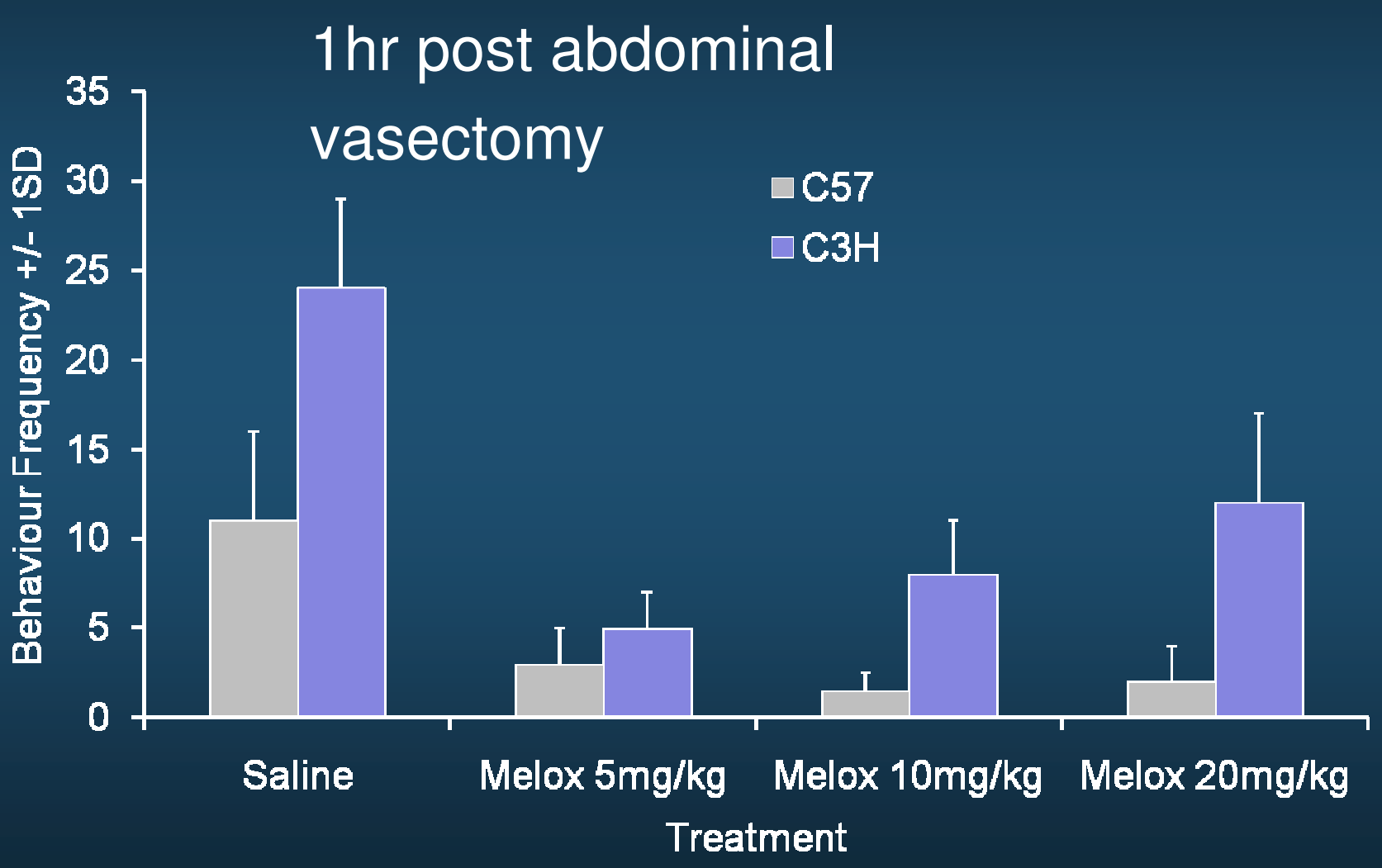
Vasectomy in CD1 & C3H mice



Wright-Williams et al (2007) Pain, 130, 108-118

Rabbit pain assessment

Rabbit pain behaviours can be viewed at:

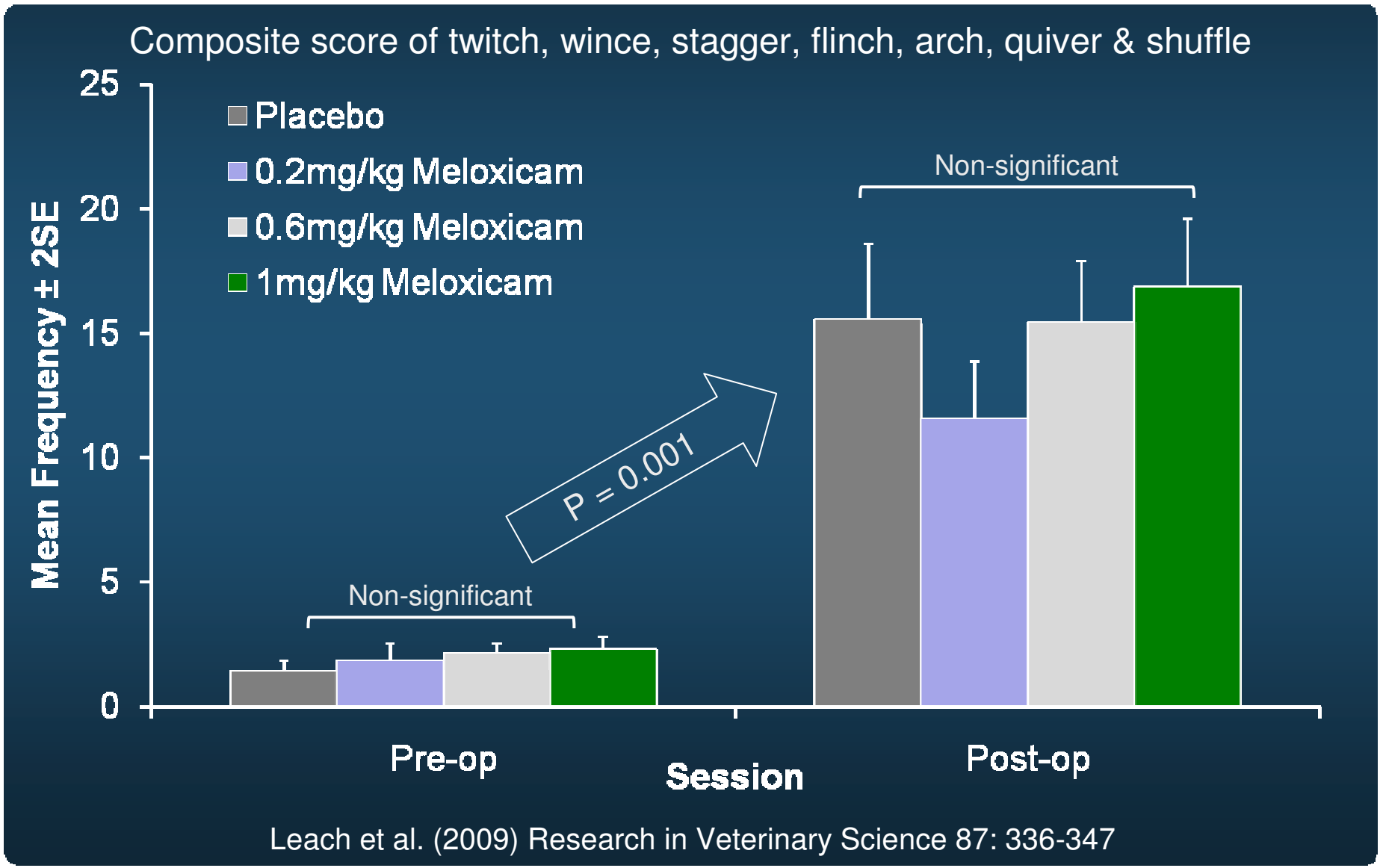
Digires:

http://www.digires.co.uk

Lab animal welfare website:

http://www.ahwla.org.uk

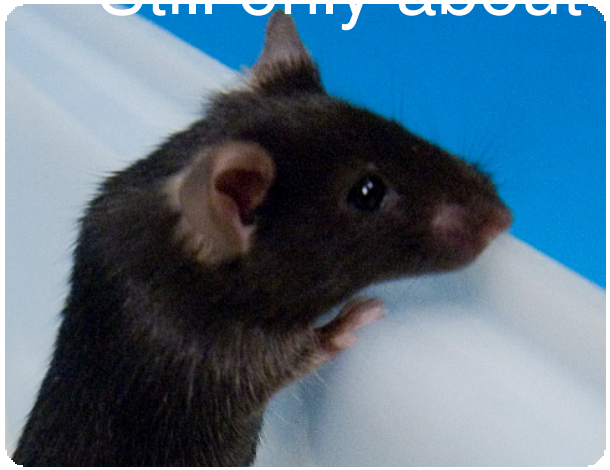
Ovariohysterectomy in NZW rabbits



Leach et al. (2009) Research in Veterinary Science 87: 336-347

Problems with behavioural indicators

- Very few procedures assessed in small no. of species
- Whole process is time-consuming:
  - Establish which behaviours indicate pain
  - Applying a scoring system
- Still only about 80% accurate!

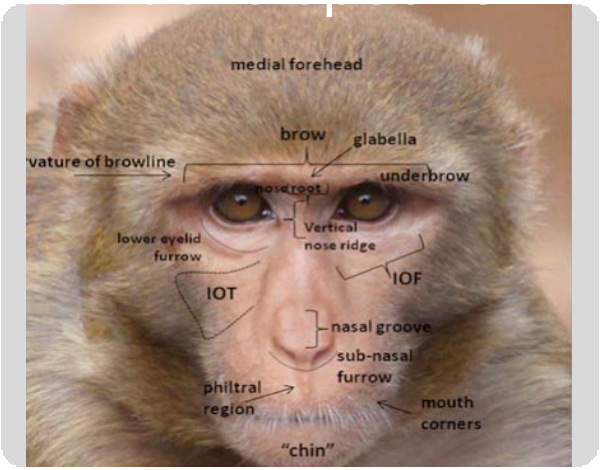


Alternative method of assessing pain

- In humans, pain is assessed using facial expressions
  - Particularly in non-verbal patients (e.g. the elderly & neonates)
- Considered by some as ‘Gold Standard’ assessment:
  - Effective assessment using a limited no. of indicators
  - Rapid & easy to carry out
  - Requires minimal training
- Facial Action Coding Systems (FACS)

What about in animals?

- Facial action coding systems developed for:
  - Chimpanzee's (Ekman & Friesen 1993) & Macaques (Parr et al. 2010)
- But none specifically assess pain in these animals
- However, since 2010!
  - Systems have been developed for rodent, rabbit &



The Mouse Grimace Scale…

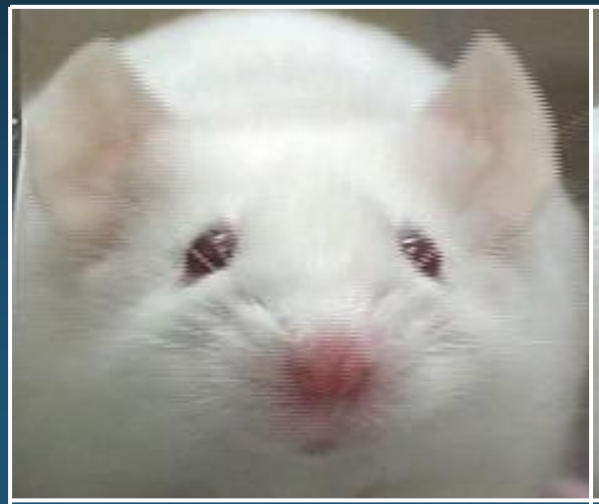
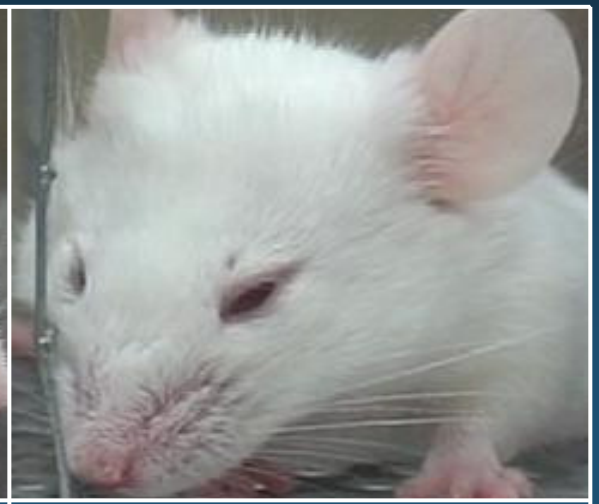
Mouse Grimace Scale (MGS)

- Developed & validated using routine nociceptive tests
  - e.g. acetic acid writhing, formalin, paw incision etc.
- Images of mouse faces compared:
  - Pre vs. post procedure (Scored by a blind observer)
- MGS comprises 5 facial action units (FAUs)

Langford et al. (2010) Nature Methods 7: 447-449



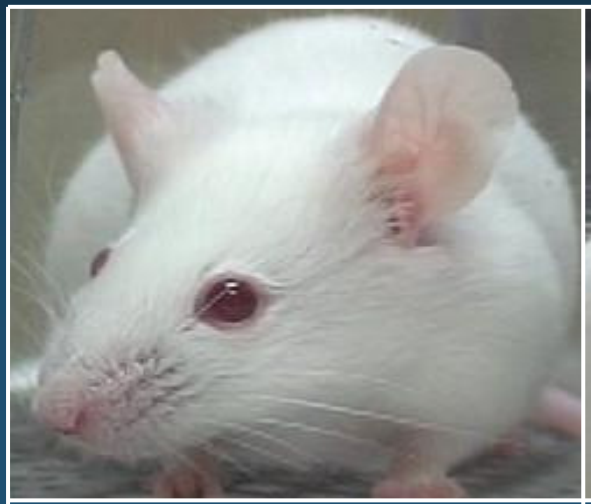
Orbital tightening

		
0	1	2
Not present	Moderate	Obvious

A narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. An eye squeeze is defined as the orbital muscles around the eyes being contracted. A wrinkle may be visible around the eye.

Images & description reproduced from **The Mouse Grimace Scale Manual** produced by Dr. Jeffery Mogil.

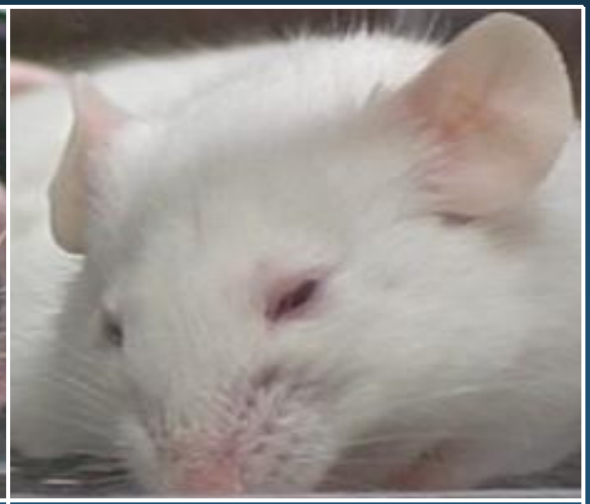
Nose bulge

		
0	1	2
Not present	Moderate	Obvious

A bulge on top of the nose. Skin & muscles around the nose are contracted creating a rounded extension of skin visible on the bridge of the nose. Vertical wrinkles down the side of the nose from the bridge may also be seen.

Images & description reproduced from **The Mouse Grimace Scale Manual** produced by Dr. Jeffery Mogil.

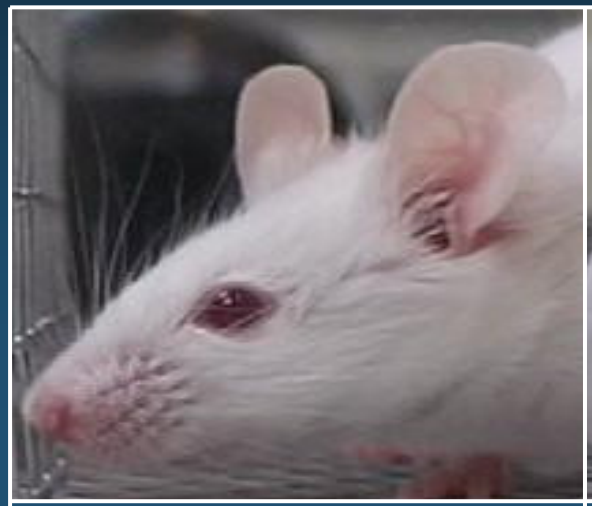
Cheek bulge

		
0	1	2
Not present	Moderate	Obvious

The cheek muscle is contracted & extended relative to the baseline condition, appearing convex from its neutral position. The cheek is directly below the eye & extends to the start of the whiskers.

Images & description reproduced from **The Mouse Grimace Scale Manual** produced by Dr. Jeffery Mogil.

Ear position



0	1	2
Not present	Moderate	Obvious

Ears pulled back from baseline position, or laid flat against the head. Baseline ears are roughly perpendicular to the head & directed forward. In pain, ears rotate outwards and/or back, away from the face. Space between the ears increases.

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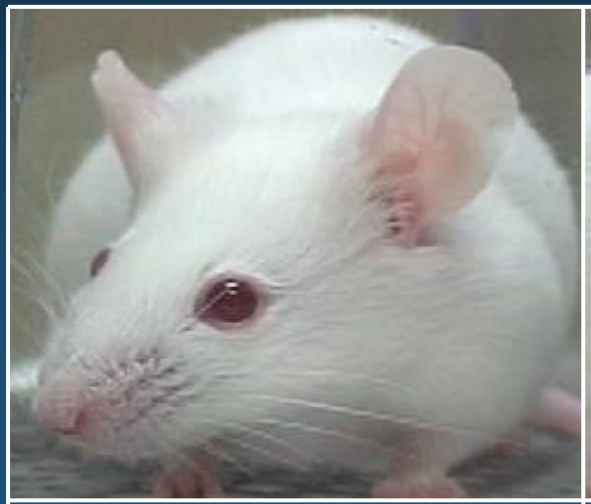
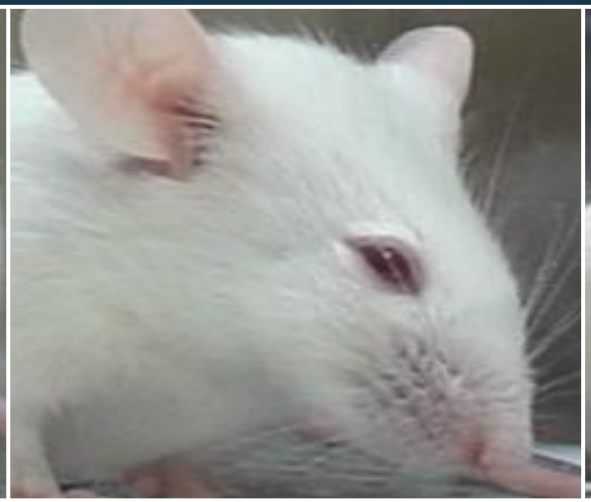
Whisker change

		
0	1	2
Not present	Moderate	Obvious

Whiskers moved from the baseline position by either being pulled back to lay flat against the cheek or pulled forward as if to be “standing on end”. They can also clump together compared to baseline when they are fairly evenly spaced.

Images & description reproduced from **The Mouse Grimace Scale Manual** produced by Dr. Jeffery Mogil.

Mouse Grimace Scale (MGS)

- Demonstrated a significant increase in MGS score from pre to post procedure…
- High reliability & accuracy
  - Accuracy: 72-97% (depends on experience & video quality)
  - Highly consistency & reliability (ICC: 0.9)

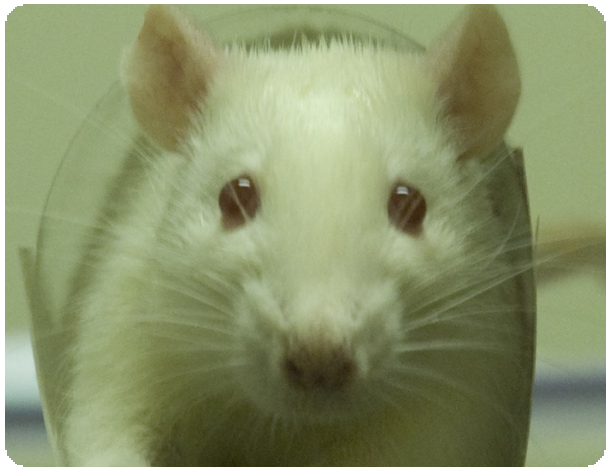
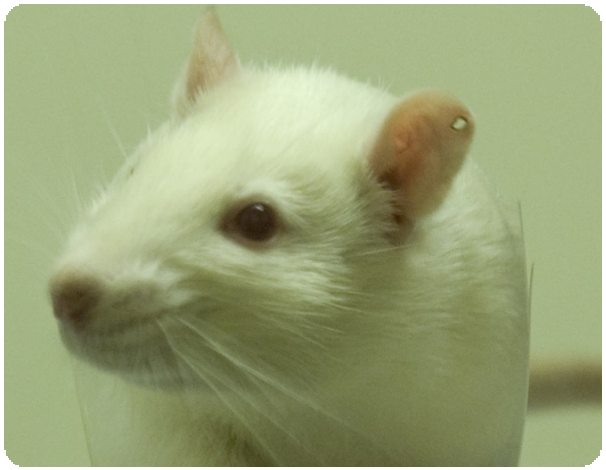
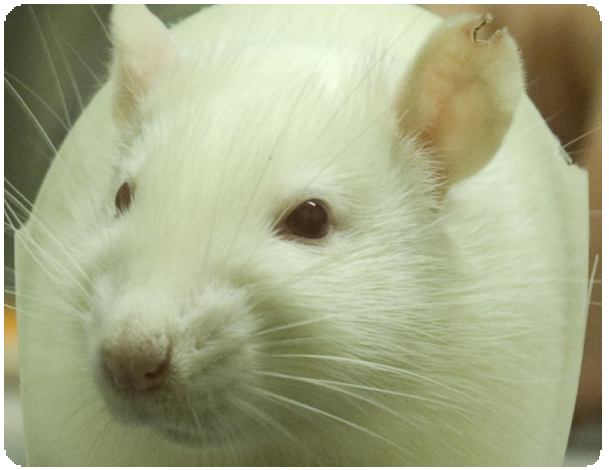
Langford et al. (2010) Nature Methods 7: 447-449

The Rat Grimace Scale…

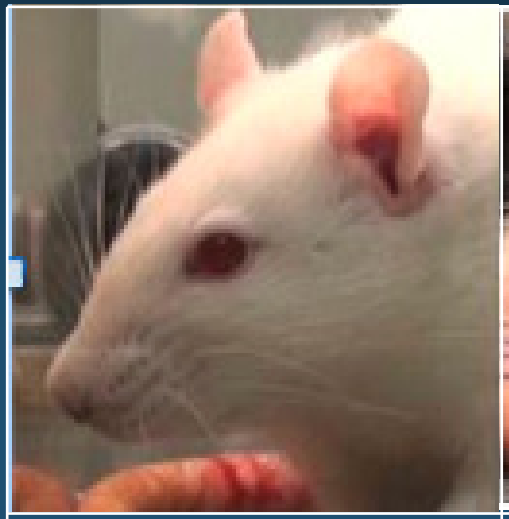
Rat Grimace Scale (RGS)

- Developed & validated using routine nociceptive tests
  - e.g. acetic acid writhing, inter-planter Freund's etc.
- Images of rat faces compared:
  - Pre vs. post procedure (Scored by a blind observer)
- RGS comprises 4 facial action units (FAUs)

Sotocinal et al. (2011) Molecular Pain 7:55

Orbital tightening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Not present	Moderate	Obvious

A narrowing of the orbital area, a tightly closed eyelid, or an eye squeeze. An eye squeeze is defined as the orbital muscles around the eyes being contracted. A wrinkle may be visible around the eye.

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Cheek/Nose Flattening

		
0	1	2
Not present	Moderate	Obvious

A lack of bulge on top of the nose (i.e., a flattening of the nose). The bridge of the nose flattens and elongates, causing the whisker pads to flatten. At this time the crease between the pads and the cheek is no longer present.

Images & description reproduced from **The Rat Grimace Scale Manual** produced by Dr. Jeffery Mogil.

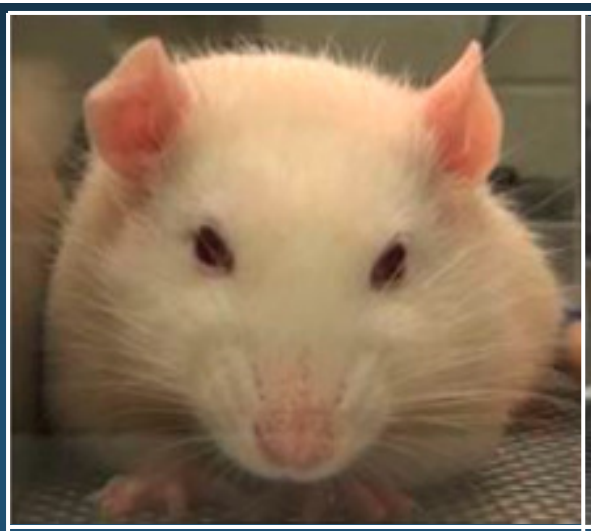
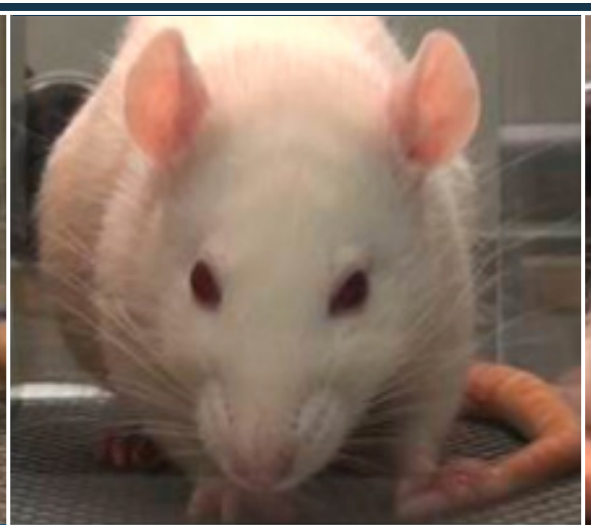
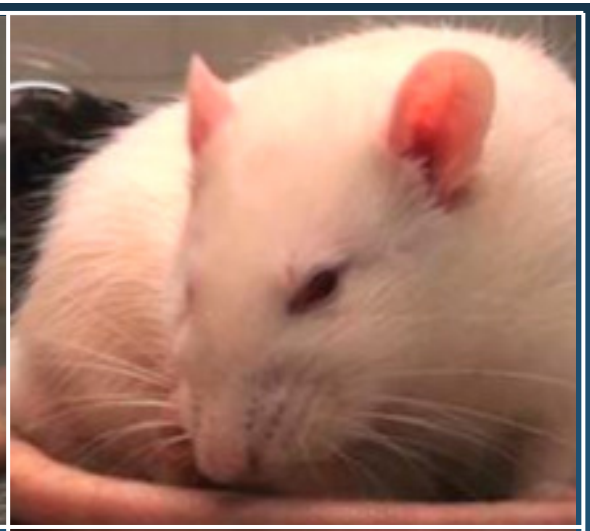
Ear position

0	1	2
Not present	Moderate	Obvious

Normal ears are perpendicular to the head, face forward, angled slightly backward & are rounded. In pain, the ears tend to fold, curl inwards & angled forward resulting in a “pointed” shape. The space between the ears appears wider relative to baseline.

Images & description reproduced from **The Rat Grimace Scale Manual** produced by Dr. Jeffery Mogil.

Whisker change

		
0	1	2
Not present	Moderate	Obvious

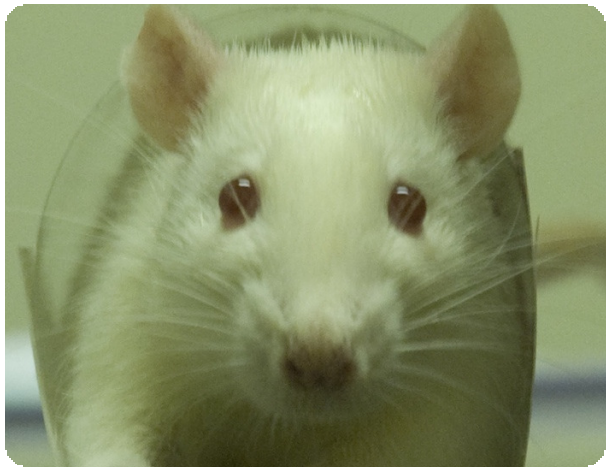
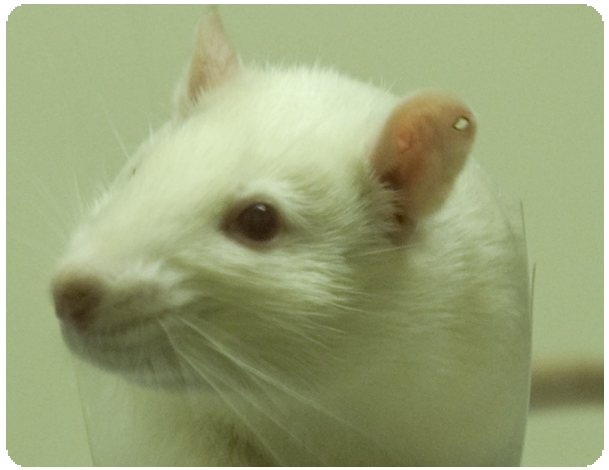
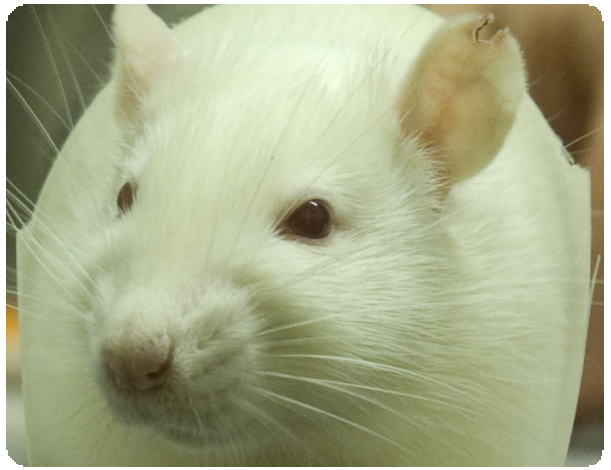
Normal whiskers are relaxed drooping slightly downwards. As pain progresses, tension in the pads increases angling the whiskers back along the head. The whisker pad contracts causing the whiskers to bunch directing away from the face.

Images & description reproduced from **The Rat Grimace Scale Manual** produced by Dr. Jeffery Mogil.

Rat Grimace Scale (RGS)

- Demonstrated a significant increase in RGS score from pre to post procedure…
- Demonstrated dose dependent decrease in RGS with 1-5mg/kg morphine
- RGS is highly reliability (ICC: 0.9) & accurate (>81%)

Sotocinal et al. (2011) Molecular Pain 7: 55

Facial expressions change in response to nociceptive stimuli in mice & rats…

But do these change in response to painful procedures routinely carried out on animals?

Can they be used to effectively assess the pain associated with routine procedures?

Vasectomy in CD1 mice

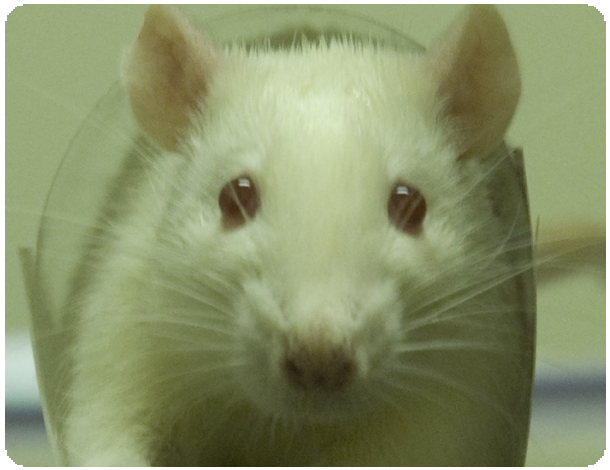
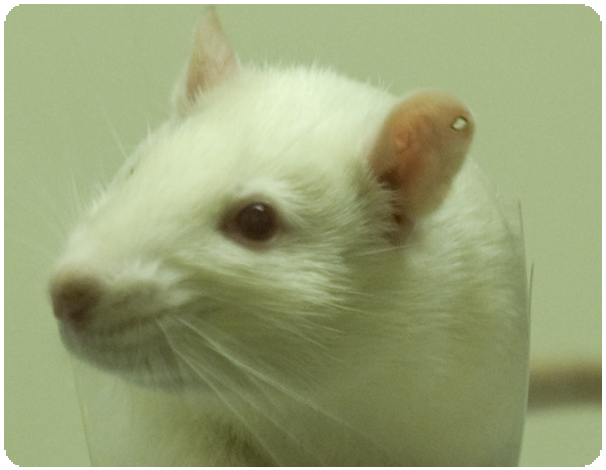
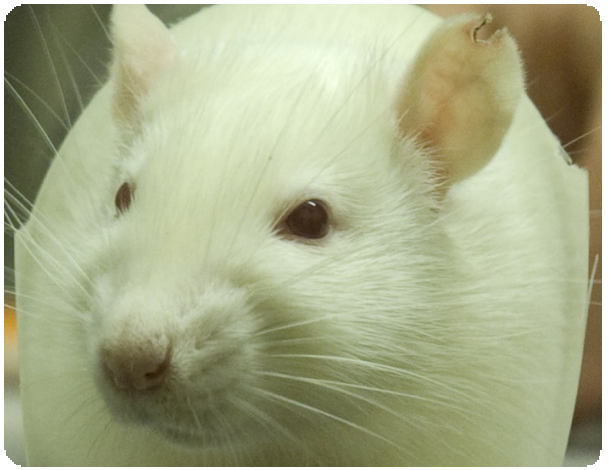
- 3 treatment groups:
  - Saline (2ml/kg *SC*)
  - Meloxicam (20mg/kg *SC*)
  - Bupivacaine (5mg/kg *Local Infiltration*)
- Pain scored using pre & post surgery:
  - MGS (4 FAUs NOT whisker position)
    - Using method of Langford et al. 2010
  - Validated mouse pain behaviours

Results

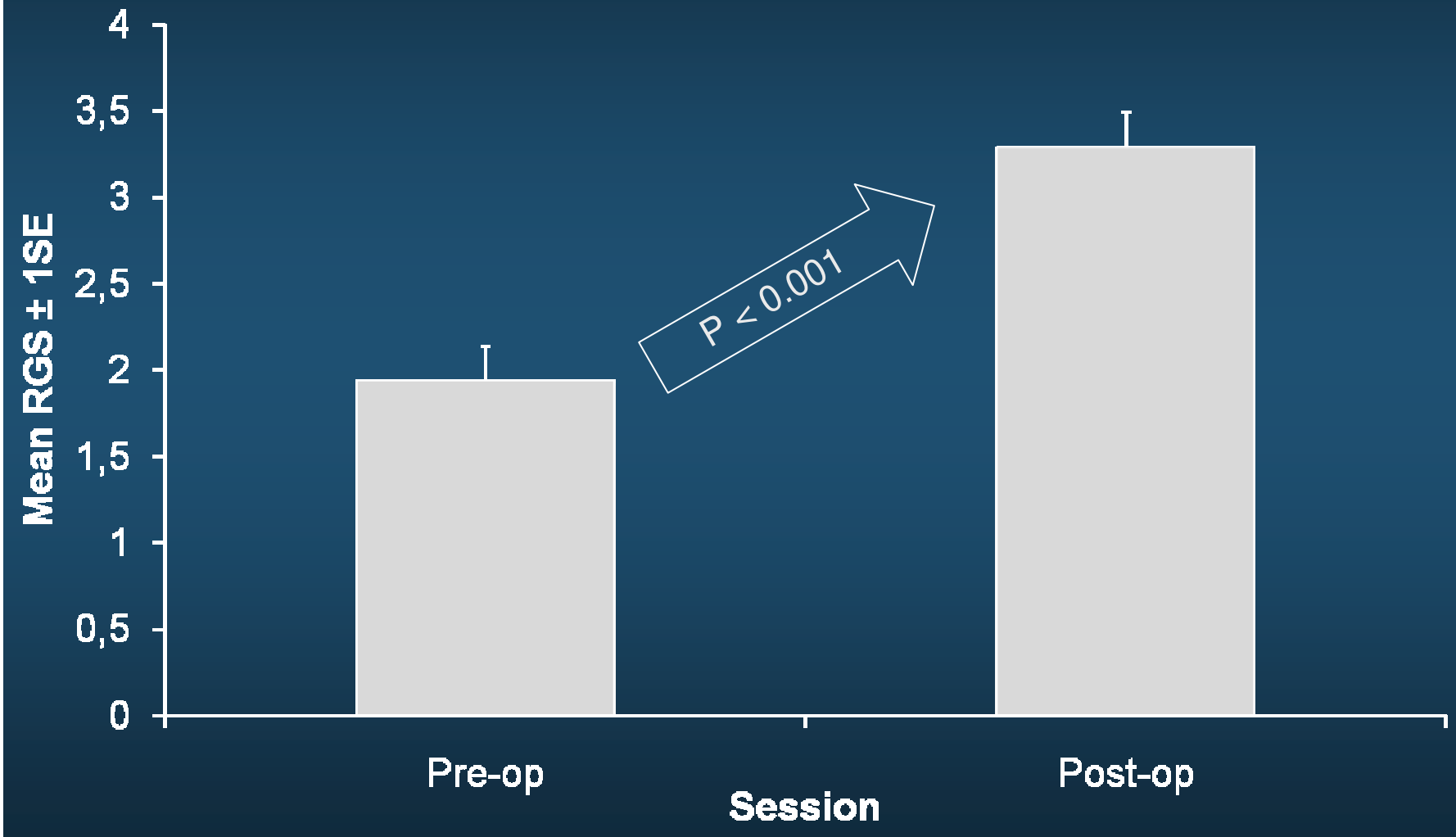
- Demonstrated that the MGS & composite pain behaviour score:
  - Very low pre-surgery with no difference between treatments
  - Significantly increased from pre to post surgery
  - Was significantly greater post-surgery in the saline treated groups compared with the analgesic groups
- MGS & composite pain behaviour score were highly correlated
  - High MGS score associated with high behaviour score

Laporatomy in Wistar rats

- All animals received 1mg/kg Meloxicam analgesia
- Pain scored using pre & post surgery:
  - RGS (4 FAUs)
    - Using method of Sotocinal et al. 2011

Rat Grimace Scale (RGS)



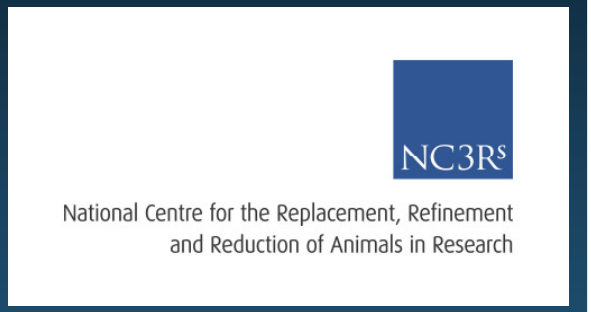
Thomas et al. (2011) Proceeding of the 2011 AVA Congress

Ongoing Grimace Scale Developments

- We are currently developing:
  - Rabbit Grimace Scale (RbtGS)
  - Rhesus Macaque Grimace Scale (MqGS)
- These scales are being developed & validated using:
  - Routine surgical procedures
  - Routine potentially pain husbandry procedures

Acknowledgements

- **National Centre for the 3Rs**
- AMGEN
- BBSRC
- Charles River UK
- Medical Research Council
- Pfizer plc
- Swedish Ministry of Rural Affairs
- UFAW
- Wellcome Trust
- Boehringer Ingelheim GmbH
- VETO



Workshop on Pain Assessment and Alleviation

18th & 19th of June 2012

Newcastle University

This workshop will provide an up-to-date review of pain assessment and pain management in laboratory animals.

Seminars will be extensively illustrated with video material and ample time provided for discussion.

Topics include:

- Anatomy and physiology of animal pain
- Pain assessment in animals
- Analgesics
- Problem solving

Cost: £309, lunch and evening meal included. Accommodation can be booked on your behalf (£74 per night) at a local hotel.

For more information and a registration form, please email

cbc-office@newcastle.ac.uk