

Scientific advances in the study of animal welfare

How we can more effectively
Why Pain?
assess pain...

To recognise it, you need to define it...

‘Pain is an unpleasant sensory & emotional experience associated with actual or potential tissue damage’ IASP 1979



As it is the emotional component that is critical for our welfare,
the same will be true for animals

Therefore we need indices that reflect this component!

Q. How do we assess experience?

- As it is subjective, direct assessment is difficult..
- Unlike in humans we do not have a **gold standard**
 - i.e. Self-report
 - Animals cannot meaningfully communicate with us...
- So we traditionally use proxy indices



Derived from inferential reasoning

Infer presence of pain in animals from behavioural, anatomical, physiological & biochemical similarity to humans



In humans, if pain induces a change & that change is prevented by pain relief, then it is used to assess pain



If the same occurs in animals, then we assume that they can be used to assess pain

Quantitative sensory testing

- Application of standardised noxious stimuli to induce a reflex response
 - Mechanical, thermal or electrical...
 - Used to measure nociceptive (i.e. sensory) thresholds
- Wide range of methods used
 - Choice depends on type of pain (acute / chronic) modeled
- Elicits specific behavioural response (e.g. withdrawal)
 - Latency & frequency of response routinely measured
 - Intensity of stimulus required to elicit a response
- Easy to use, **but** difficult to master...

Value?

- What do these tests tell us:
 - Fundamental nociceptive mechanisms & central processing
 - It measures evoked pain, not spontaneous pain
 - Tests of hypersensitivity not pain per se (Different mechanisms)
- What don't these tests tell us:
 - Much about the emotional component of pain
 - Measures nociceptive (sensory) thresholds based on autonomic responses (e.g. reflex) directly relating to sensory component
- Not practical for assessing pain outside laboratory...

Clinical signs

- Subjective assessments of:

- Appearance: 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
- Physiology: Respiration/ Heart rate/ Blood pressure/ Temp

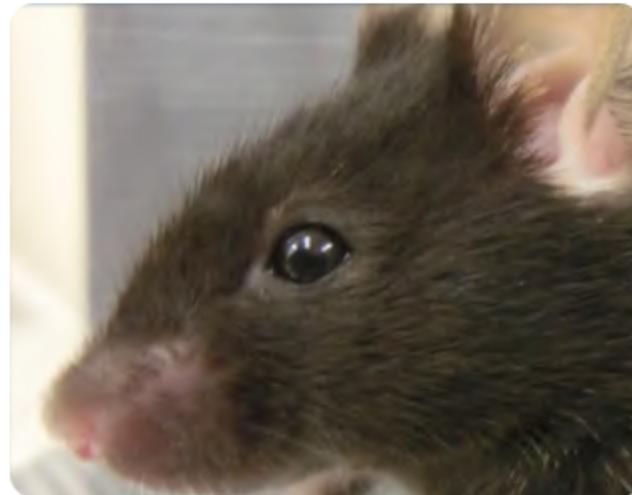


Value?

- Little evidence of how these relate to pain experienced:
 - Changes could be due to other non-pain related causes
 - Changes in measure may not parallel change in pain
- Insensitive to individuals' pain sensitivity or to changes in pain state
- Poor dose-effect relationship with pain relief methods
- Retrospective: Poor for immediate assessment
- Problems of reliability between & within observers

Specific pain behaviours

- Behaviour-based indices are increasingly being used
 - Rodents, rabbits, dogs, cats, lambs and calves etc.
- Considered more effective than traditional methods
 - Immediate cage/pen side assessment (**not** retrospective)
 - Growing evidence of relationship between pain & behaviour



Rat pain behaviours



Stagger



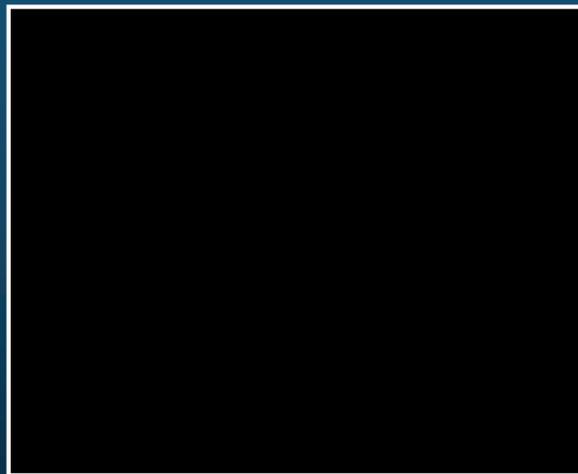
Twitch



Writhe



Flinch



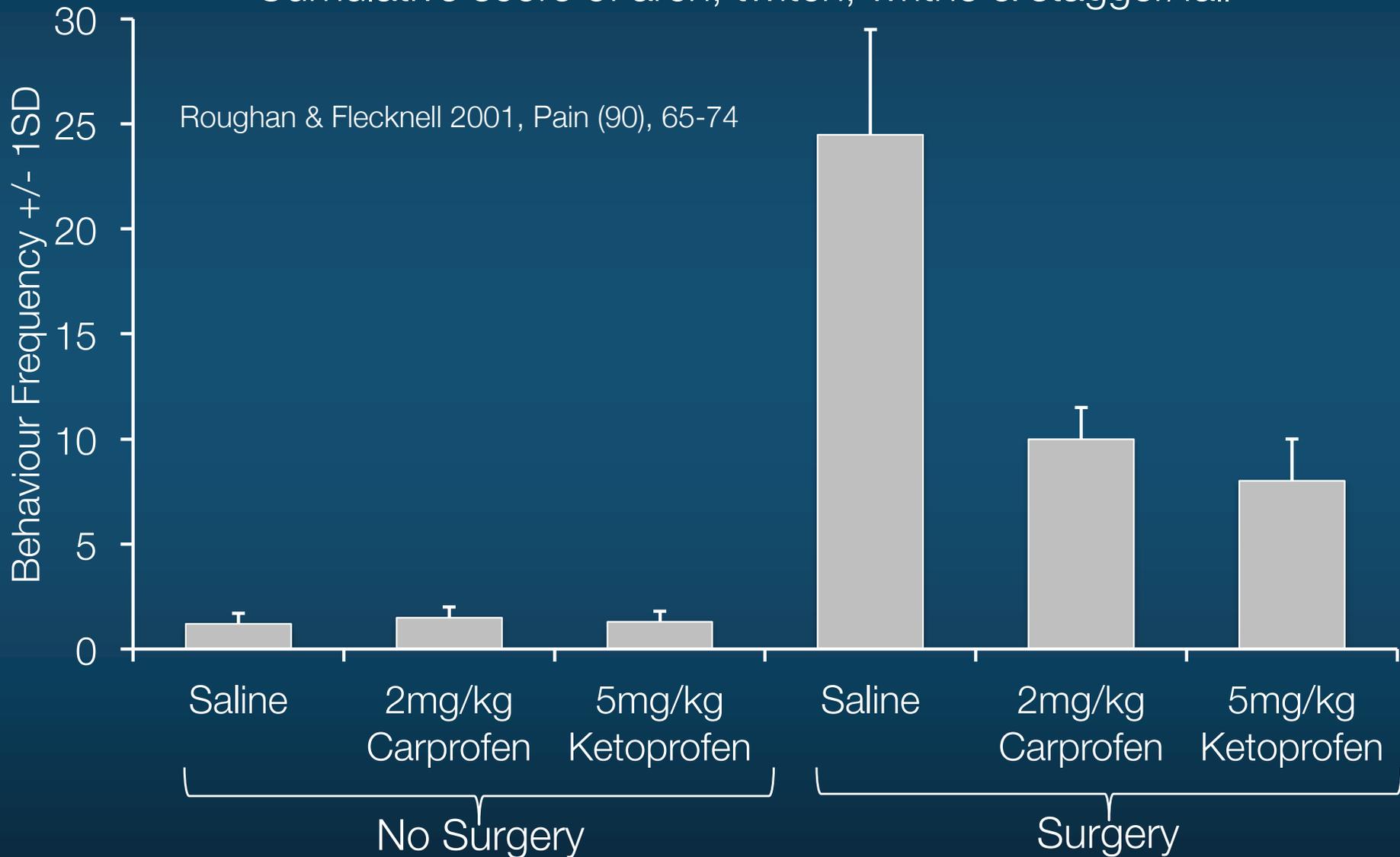
Press



Arch

1 hr post-laparotomy

Cumulative score of arch, twitch, writhe & stagger/fall



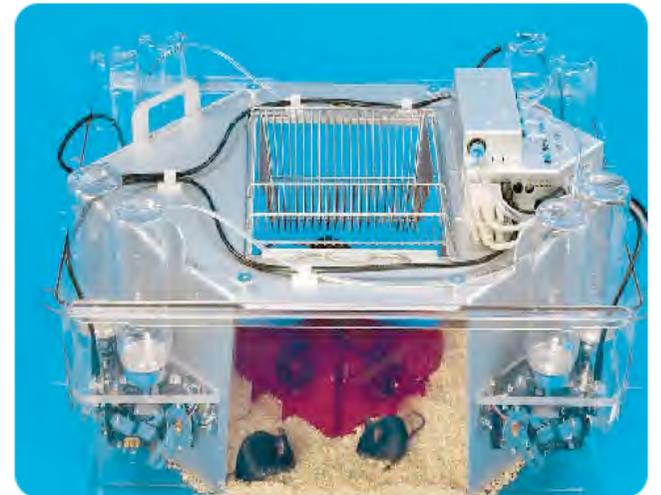
Potential limitations using behaviour

- Time-consuming to develop & implement:
 - Establish which behaviours indicate pain following a procedure
 - Often requires >30mins of training¹
- Pain behaviours vary markedly between procedures
- So behaviour-based schemes only developed for a few species undergoing a limited no. of procedures
 - Mostly laboratory rodents & rabbits undergoing castration & lambs undergoing castration & tail docking...

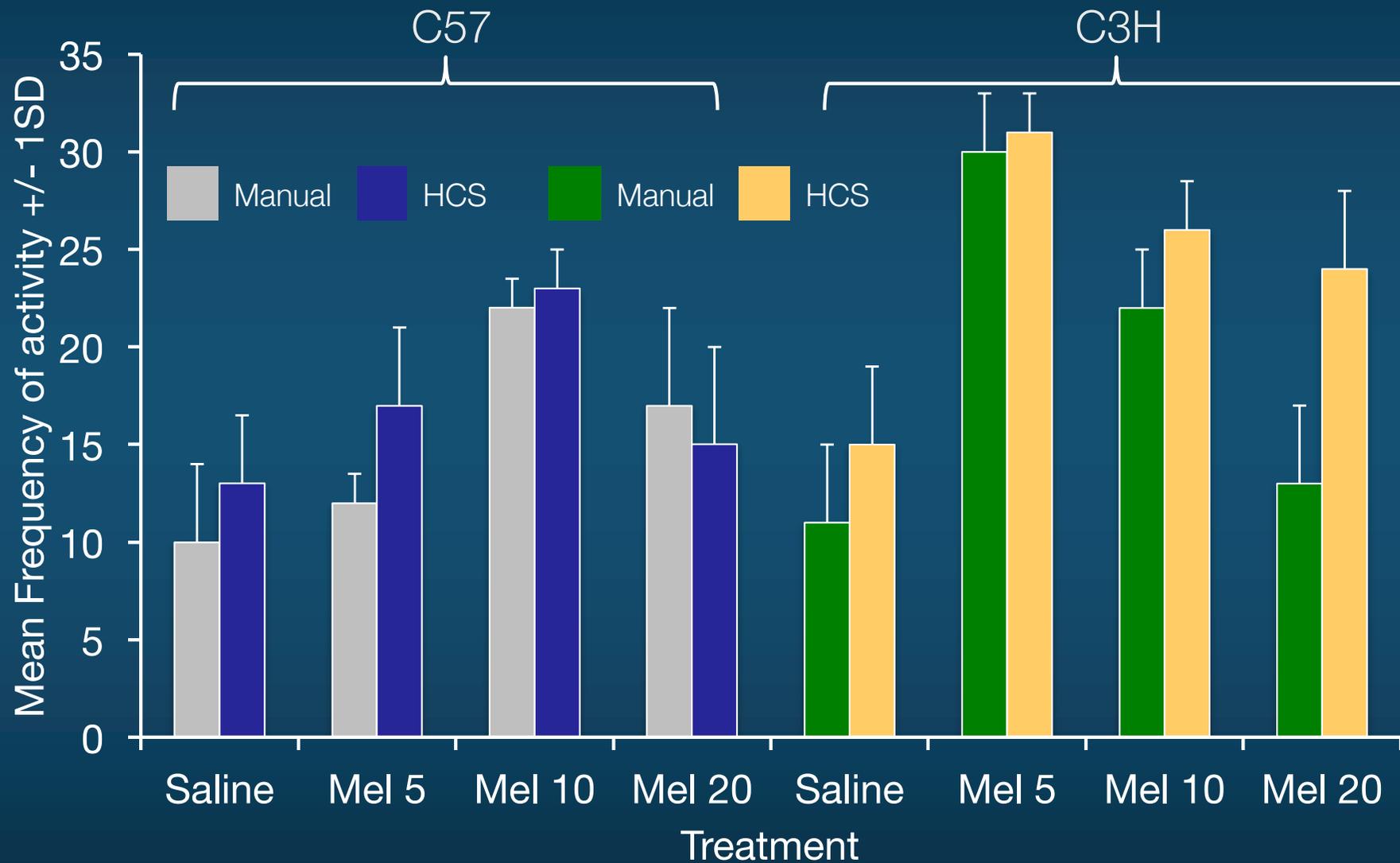
¹Roughan & Flecknell (2006) *App. Ani. Behav. Sci.* 96: 327–342

Potential solution...

- Automated behavioural analysis...
- Several commercially available systems
 - Development driven by phenotyping of GM mice
- Advantages:
 - Continuous & rapid monitoring of large number of animals



HomeCageScan vs. Manual scoring



1h post-op

Miller et al (2013) Applied Animal Behaviour Science 131: 138-144

Limitations of automated scoring

- So far only scores general behaviour, which may not be pain-specific
 - e.g. Activity etc..
- Not able to be ‘trained’ to score more pain specific behaviours...
- But these automated systems may offer a ‘triage’ method at least for new procedures
 - Indicate when more specific analysis is needed...

Behaviour limitations: species differences

Species differences are important!



Rodents primary response:

- Explore & move around their cage
- Seems to occur when observer present



Rabbits primary response:

- Become inactive
- Rabbits freeze & are motionless when observer present

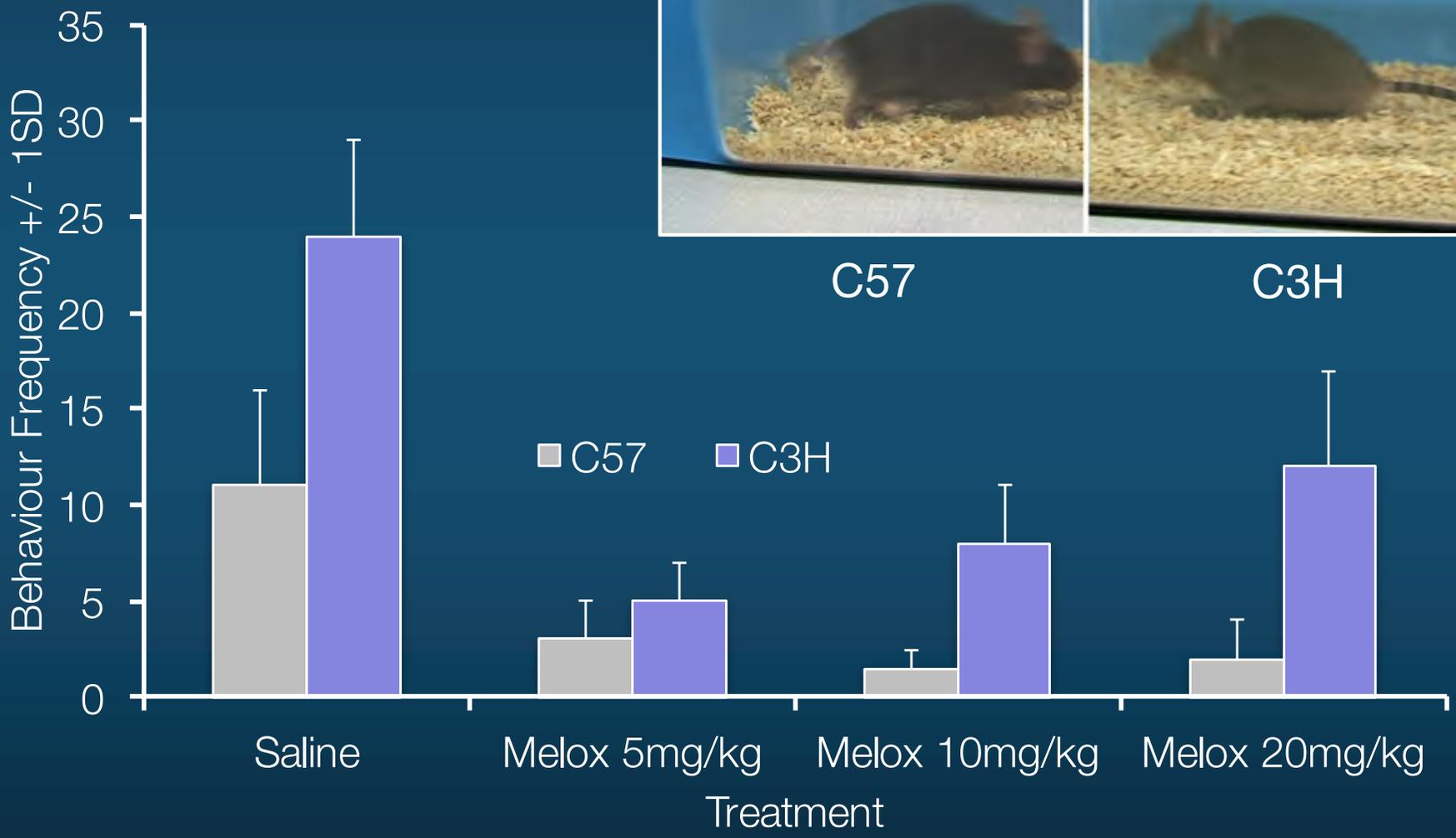
Behaviour limitations: strain differences

1 hr post abdominal vasectomy



C57

C3H



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

Indices of pain 'experience'?

- So far the indices covered at best can be used as an indirect measure of the emotional component
 - Could just measure responses to sensory component
 - Some authors (e.g. Rose et al. 2012) argue that these indices simply represent 'complex' reflex responses...
 - No higher processing, so no emotional reaction...
- We would argue this is an extreme view...
 - Principle of triangulation (Bateson 1991)

So how can we assess 'experience'?



Use approaches utilised with non verbal humans can provide a framework for animal pain assessment

Complex behaviour

- In humans, pain effects complex behavioural patterns
 - Locomotion, getting dressed, washing, keeping house clean
- As behavioural complexity of responses increase
 - Responses go beyond a “stimulus-response” reflex...
 - Likelihood of responses requiring higher processing increases..
 - So more likely to be direct indices of pain experience



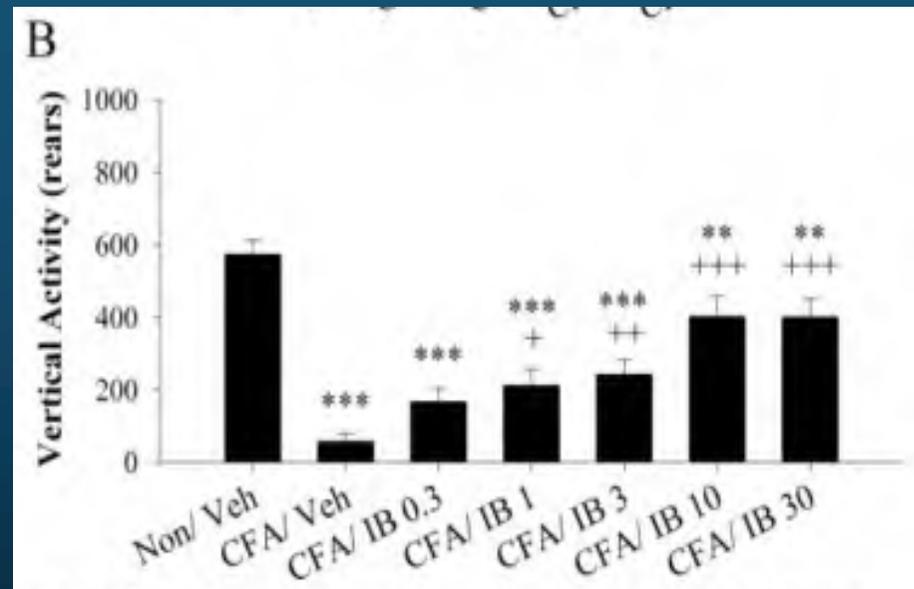
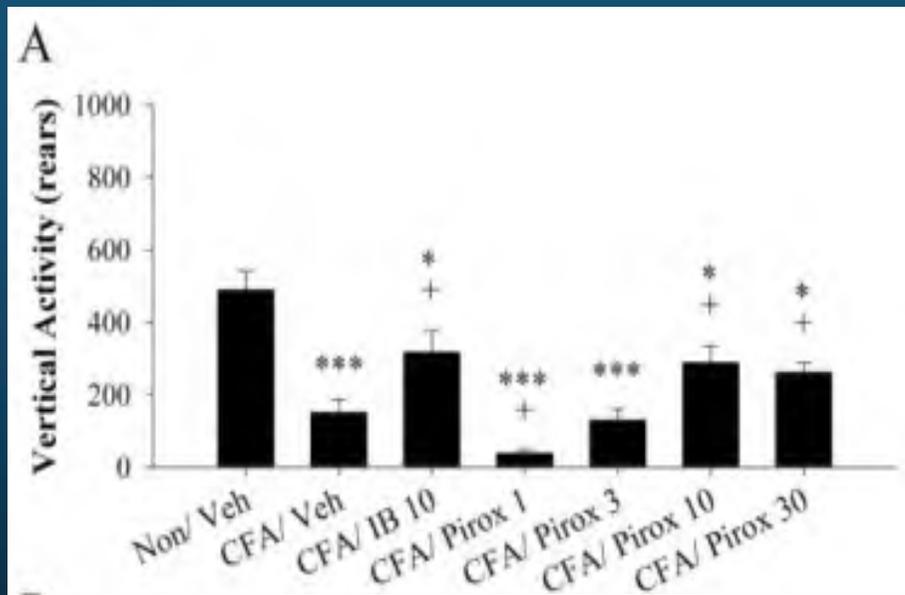
Complex behaviour

- Pain also influences complex behaviour in animals:
 - Locomotion, explorative behaviour & rearing (most species)
 - Nest building (Mice)
 - Grooming & Burrowing (rodents)
- Represent highly motivated behavioural needs
 - Alterations likely indicate important impacts on welfare



Rearing

- Rats placed into novel environment explore & rear
- Pain induced by inflammation of the knee joints (CFA)
 - + /- pain relief (i.e. NSAIDs)
- Horizontal locomotion & vertical rearing measured



Naturalistic behaviours

- Burrowing & Nest Building
- Highly motivated behavioural needs
 - Alterations likely indicate important impacts on welfare
- Wild mice build nests for warmth
 - Lab mice prefer near 30°C (1) , not 20-24°C
 - Lab usually 20-24°C
 - Nests create microclimates within the cage
- Burrowing expression = global wellbeing¹



Nest building

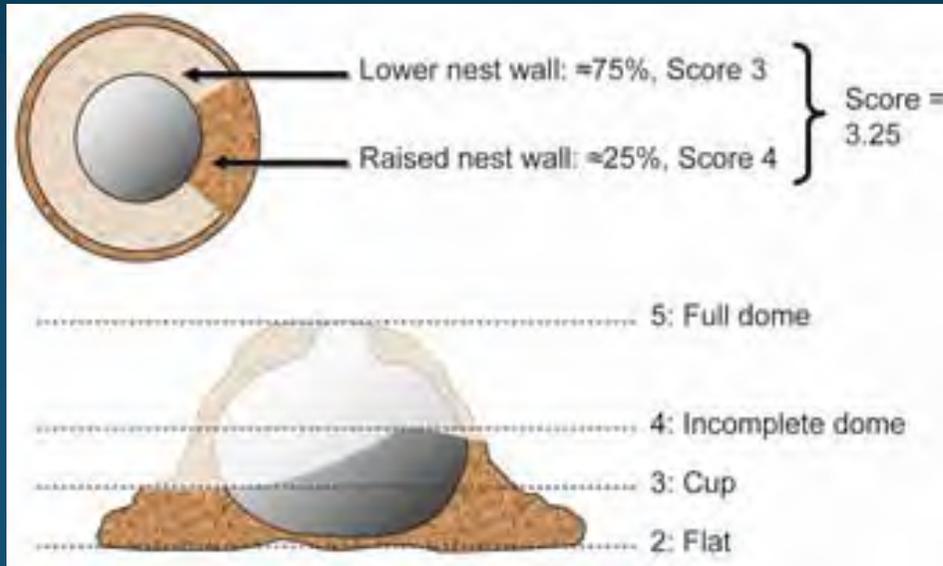


Figure 1. The naturalistic nest score system. Scores are based on the shape of the nest as well as on how much the walls are built up around the nest cavity in order to form a dome. Both a top view and a side view are shown.

Score	View from above	View from side
1		
2		
3		
4		
5		

Hess et al. (2008) JAALAS 47(6) 25-31, Deacon (2006)
 Nature Protocols 1(3) 1117-1119

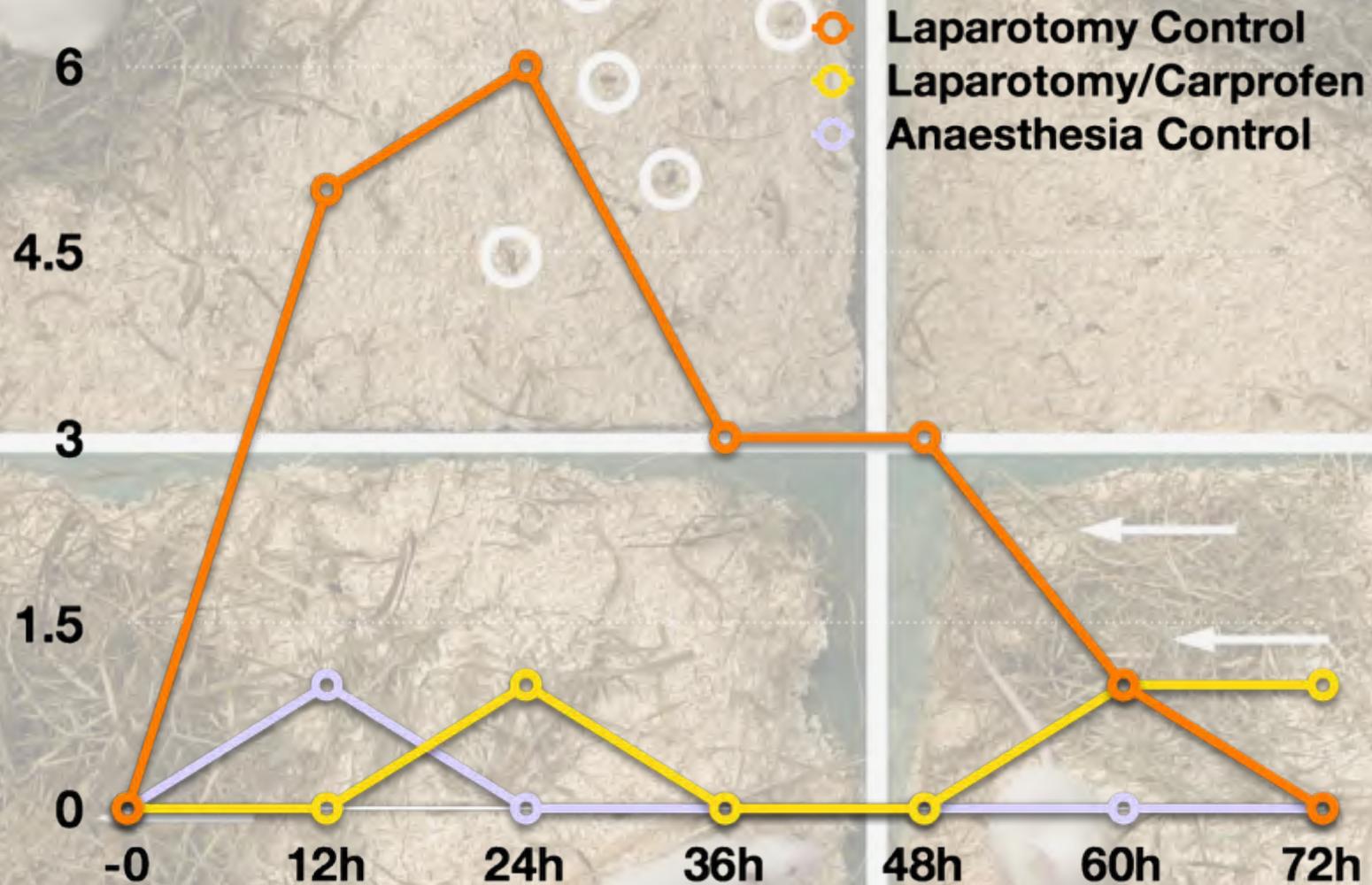


Well-made nests



Poorly-made nests

No. of cages showing poor nest building (/8)



Burrowing



Burrowing

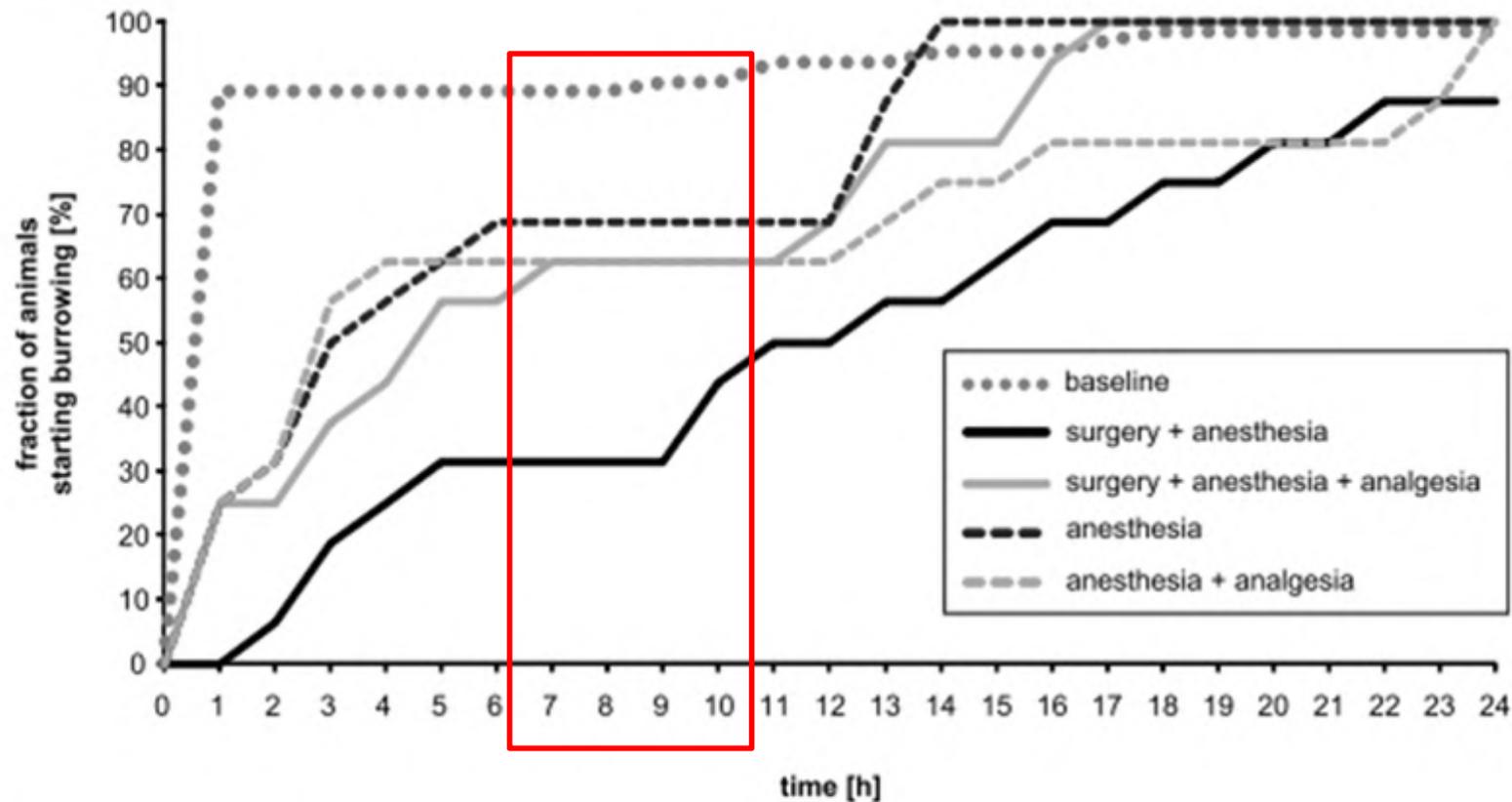
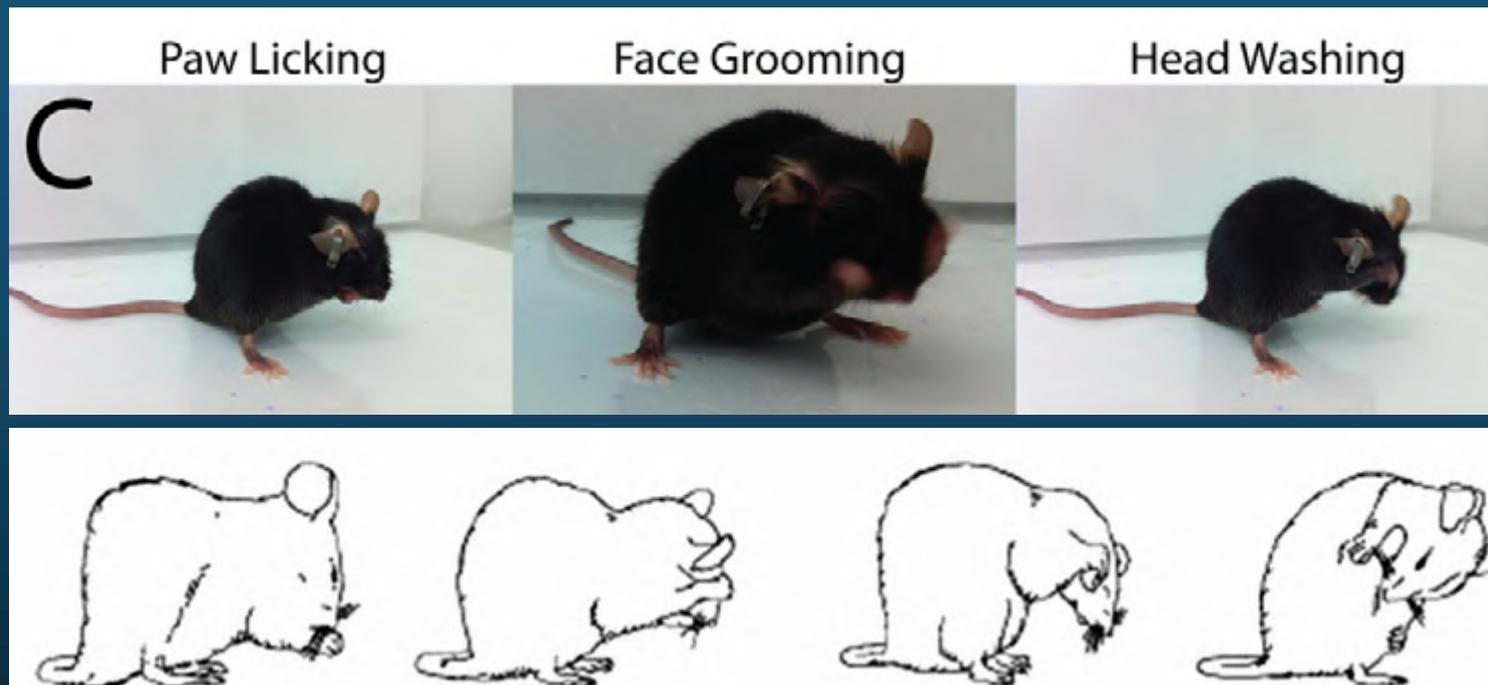


FIGURE 3 | Percentage of mice/hour that started burrowing at baseline vs. experimental conditions. Healthy mice started burrowing immediately, mostly within 1 h (baseline). Between-treatment differences were greatest between hours 7 and 9.

Self-grooming (?)

- Complex & evolutionary conserved behaviour in rodents
 - 15-50% of awake time spent grooming
- Stereotyped sequence:
 - Nose > Eyes > Ears > Body > Tail/genitals



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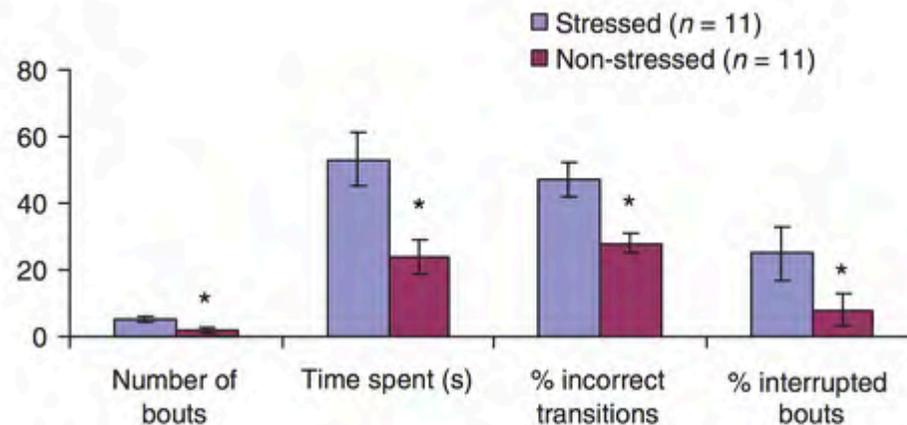


Figure 2 | Example of stress-evoked alterations in grooming sequencing in rats detected using the GAA (rats have been stressed by exposure to a brightly illuminated novel environment for 5 min; * $P < 0.05$, U -test).

Self-grooming (?)

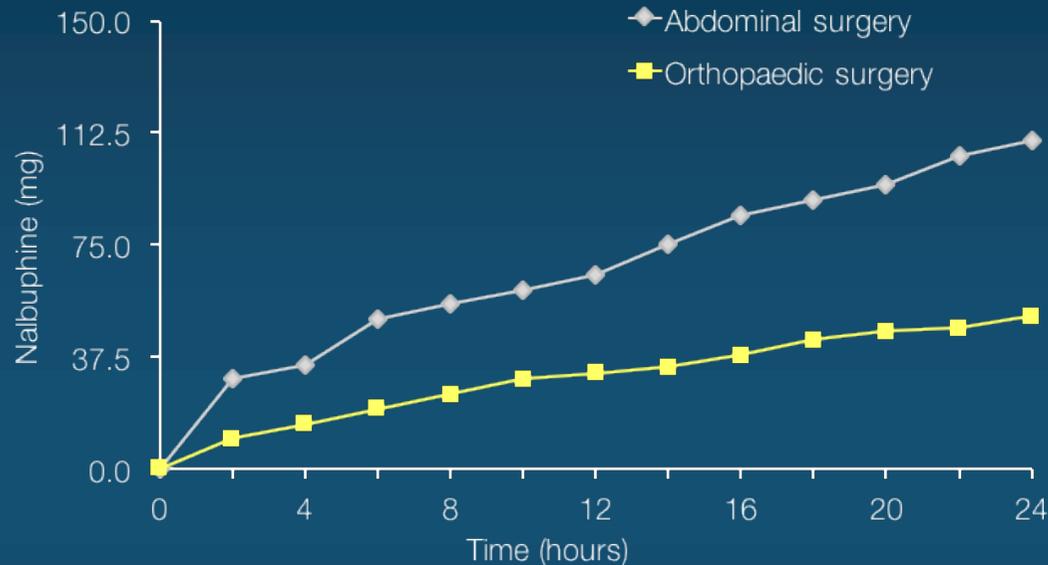
- Complex & evolutionary conserved behaviour in rodents
 - 15-50% of awake time spent grooming
- Stereotyped sequence:
 - Nose ➤ Eyes ➤ Ears ➤ Body ➤ Tail/genitals
- Sensitive to various experimental manipulations
 - Complex neurobehavioral disorders, e.g. basal ganglia disorders, autism, OCD & attention-deficit/hyperactivity disorder
 - Stress
- Could it be used to assess pain?

Using complex behavioural tests

- Pain experience assessed using operant testing
- Animals use their pain to inform their decisions
 - Requires animals to use their underlying ‘experience’
 - This goes beyond even complex reflex response & requires cerebral processing

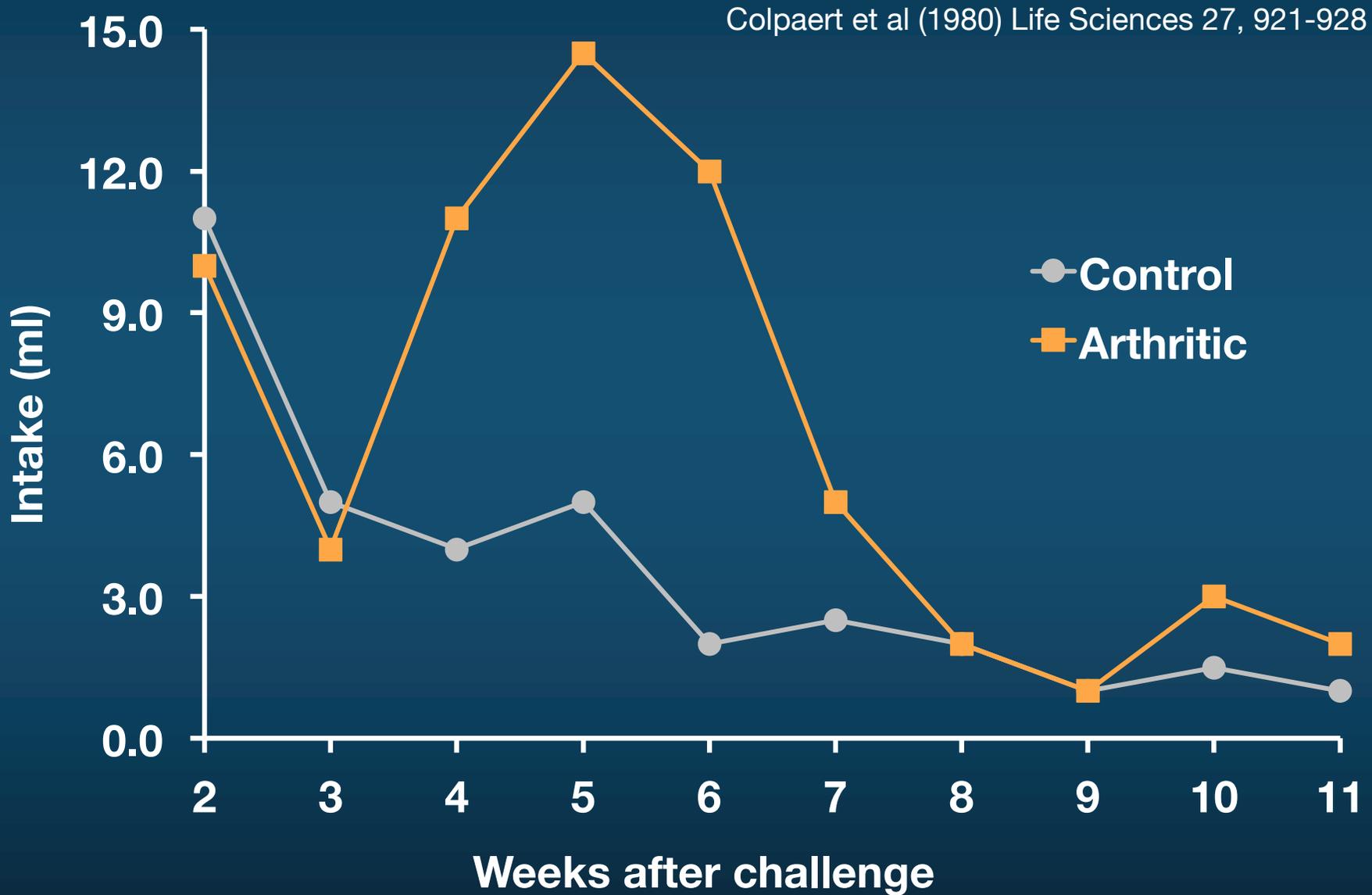


Self-administration



- People in pain can self-medicate to reduce their pain
 - Animals have similar receptors for pain relief
 - Pain relief alters behaviour of animals in pain
- Will animals in pain give themselves pain relief?

Suprofen intake in rats with arthritis



Conditioned discriminations

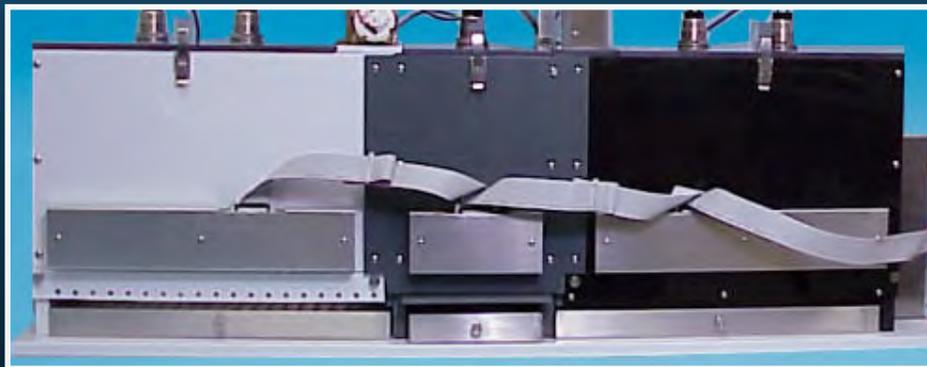
- Methods borrowed from drug abuse literature
 - Used to establish affective value of drugs
- Conditioned place aversion:
 - Animals choose to spend less time in a location previously associated with a negative experience (e.g. pain)
 - e.g. Being in pain is a negative experience, so pain should induce a place aversion
- Conditioned place preference:
 - Animals choose to spend more time in a location previously associated with a rewarding experience (e.g. analgesia)
 - e.g. Relief from pain is a rewarding experience, so pain relief should induce a place preference

Conditioned discriminations

- Conditioned drug discrimination:
 - Animals use the feeling evoked by a drug to solve a conditional discrimination
 - If pain is a feeling that animals can access, they should be able to use the presence/absence of the feeling to solve a discrimination
 - Animals in pain discriminate the difference in the way they feel when given pain relief?

Conditioned place aversion

- Animals confined in a chamber & given a noxious stimulus (CPA), or no stimulus
- When animals given a free choice of chamber, they should choose the one where they did not receive noxious stimulus
- May be combined by making the “no pain chamber” environment one that is mildly aversive (eg light rather than dark)



Conditioned place aversion

Training (?)

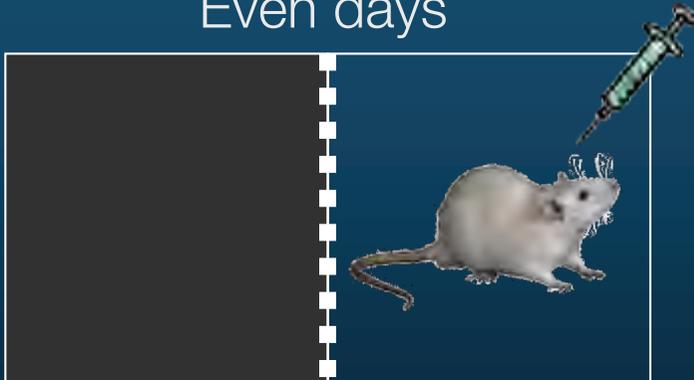
Formalin (S+)

Odd days



Even days

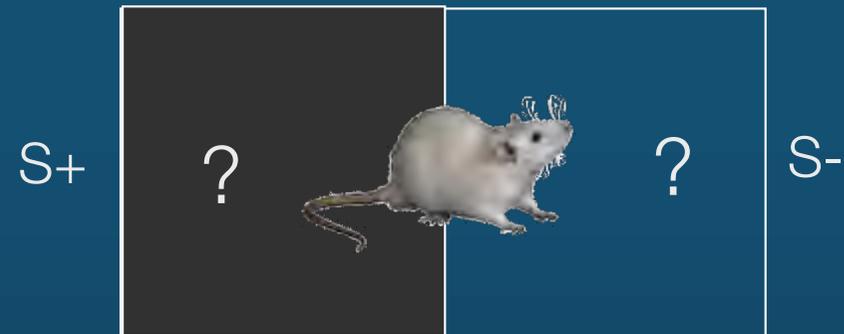
Vehicle (S-)



Tests

Measures:

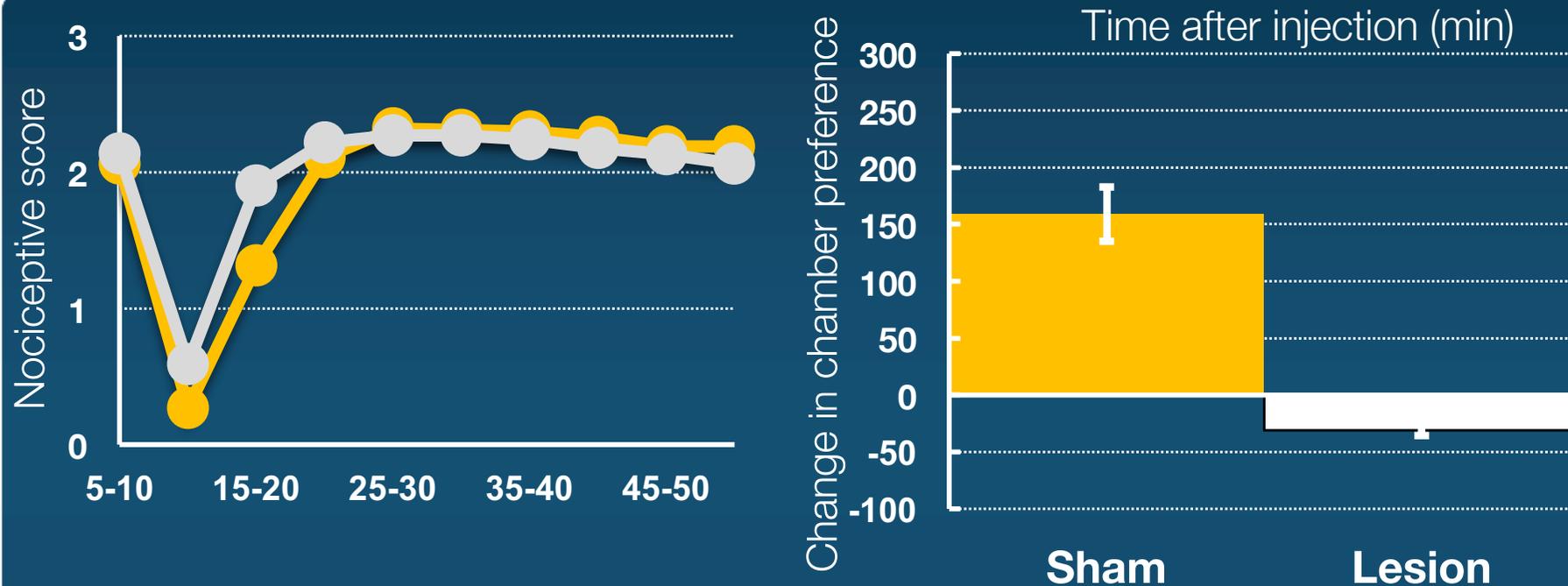
- Time preference
- Choice preference



Compare:

- Un-lesioned rats
- Lesioned rats

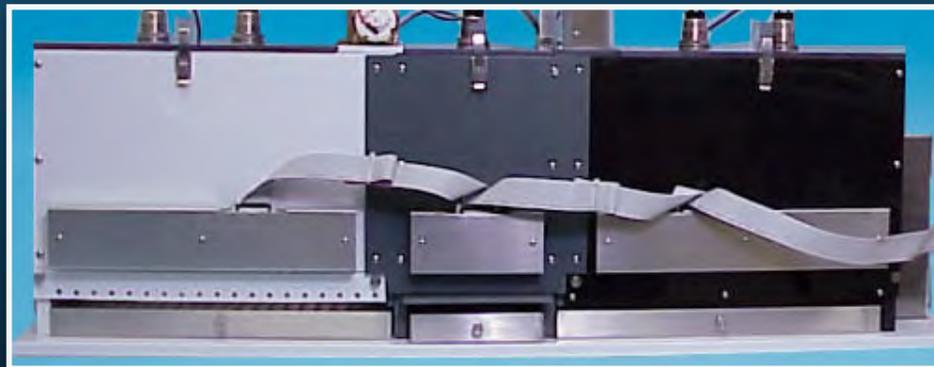
Conditioned place aversion



- Spontaneous nociceptive behaviours (licking foot) unchanged in rats with anterior cingulate cortex lesions
- Conditioned place aversion blocked by lesioning

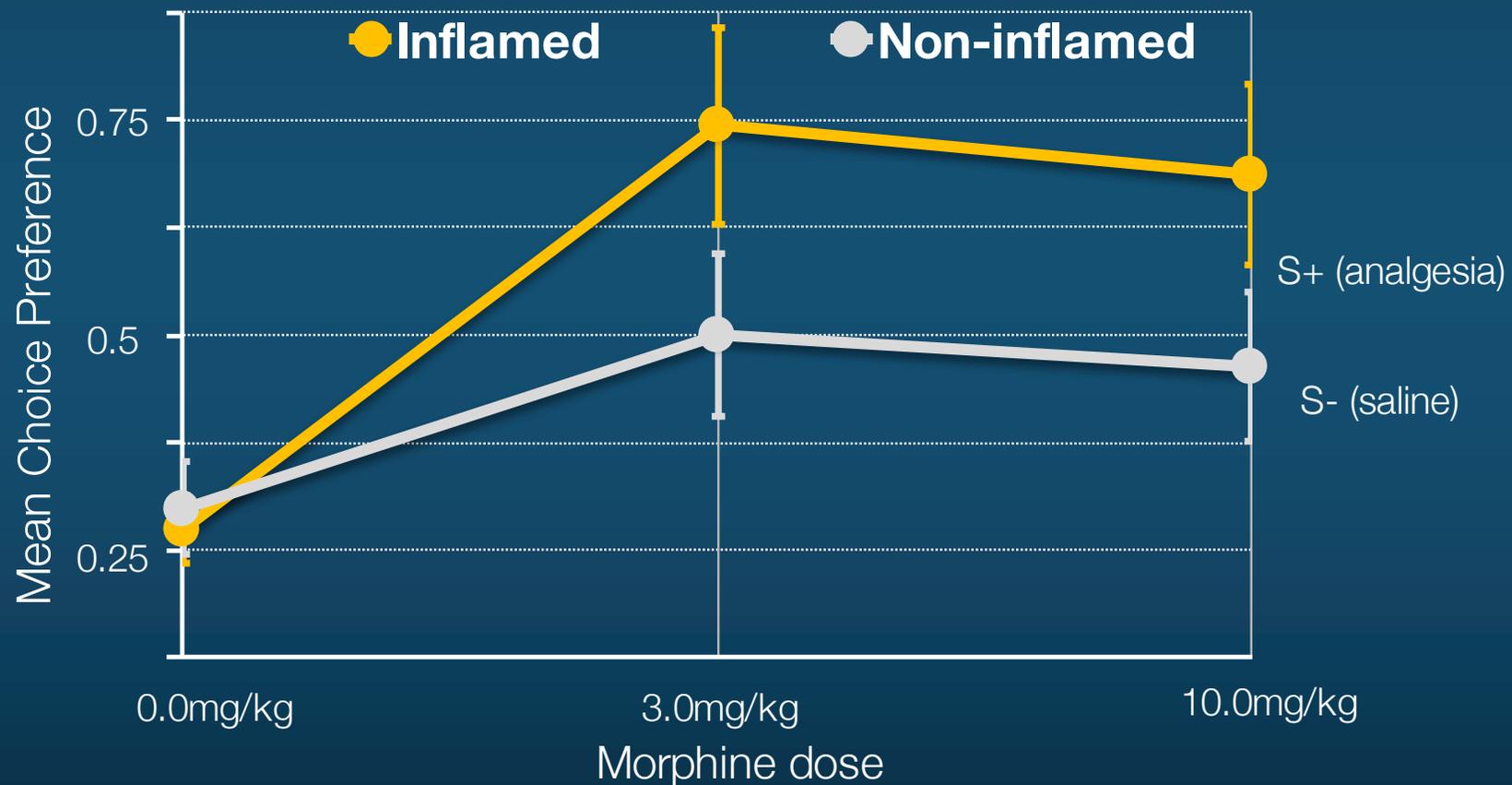
Conditioned place preference

- Animals given analgesic & placed in one chamber, or saline & placed in other chamber
- When animals in pain given free choice of chamber, they should choose the one where they received analgesic
- Animals must be able to use their “feeling” of pain, and its change in intensity, in order to make this choice



Conditioned place preference

Rats in pain show a preference for a context previously associated with analgesic administration



Sufka, K. J. 1994. *Pain* 58, 355-366.

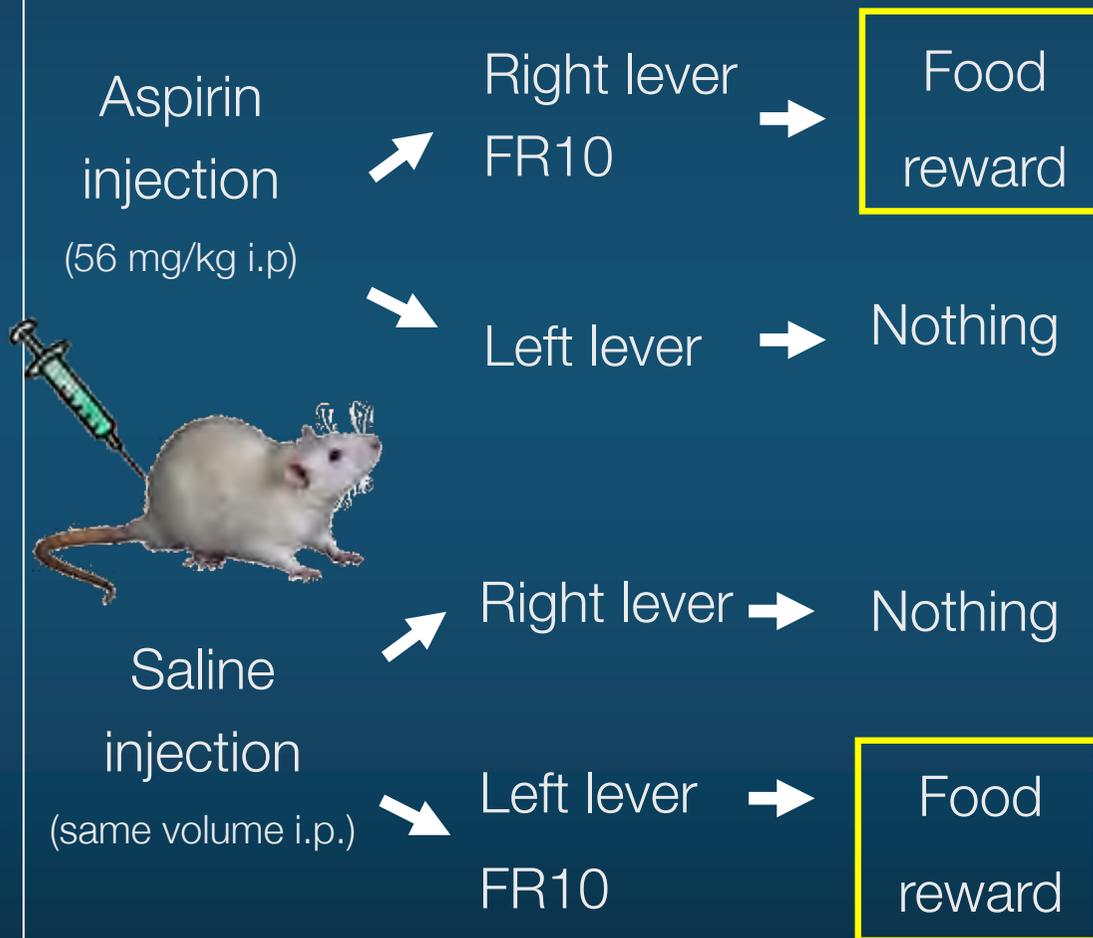
Conditioned drug discrimination

- When given analgesic receive food reward when the press left lever (only)
- When given vehicle receive food reward when the press right lever (only)
- When animals in pain given free choice of levers, they should choose the one associated with analgesic
- Animals must be able to use their “feeling” of pain, and its change in intensity to make the correct choice

Are arthritic (painful) rats better than normal/pain free rats at discriminating between analgesic and placebo?

Conditioned drug discrimination

Train (14 days)



Test

Measures:

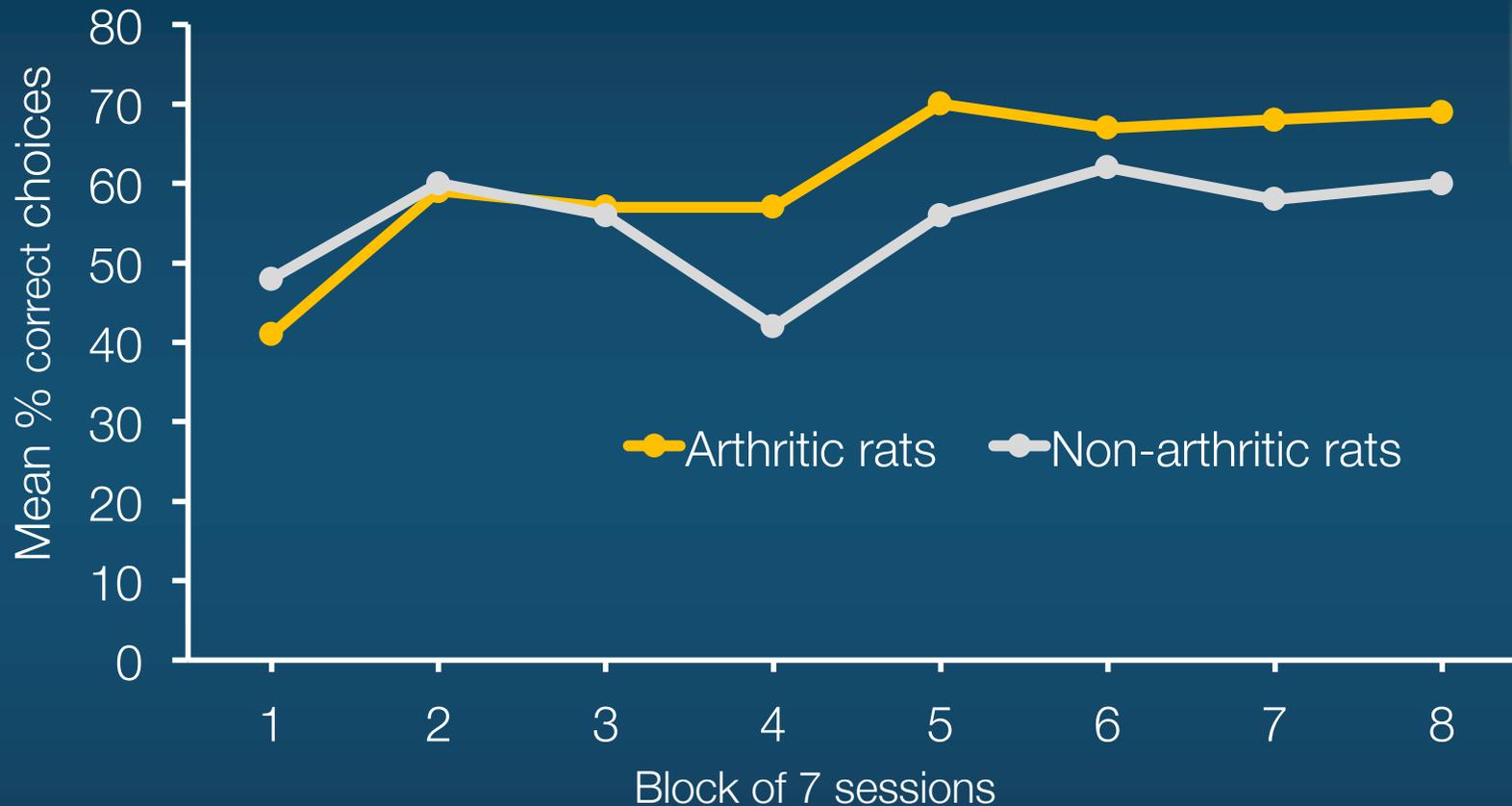
- Lever presses

Group:

- Control: Untreated
- Pain: Arthritis-induced

Compare: Discrimination performance in two groups

Conditioned drug discrimination



Arthritic rats better at learning the discrimination:
Used “how they felt” to make the correct choice

Cognitive bias

- New method to identifying affective states in animals
 - In humans, emotions are associated with adaptive biases in information processing
- Emotional state influences decision making
 - Negative affective states associated with expectations of poor outcomes & makes individuals more risk averse



Put another way...

- Emotional state can influence decision making
- Optimists (or a positive emotional state) sees the glass as half-full
- Pessimists see the glass as half-empty
- Can we assess whether rats are optimists or pessimists?

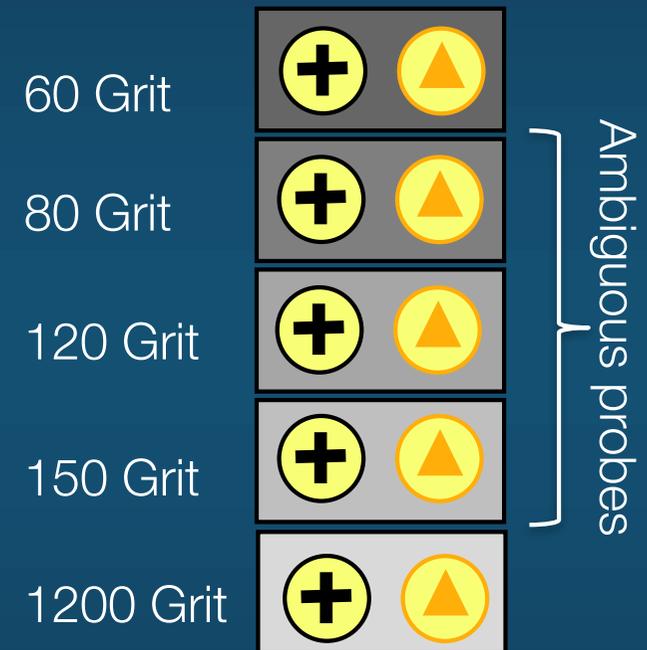


Rat enrichment & cognitive bias

Train

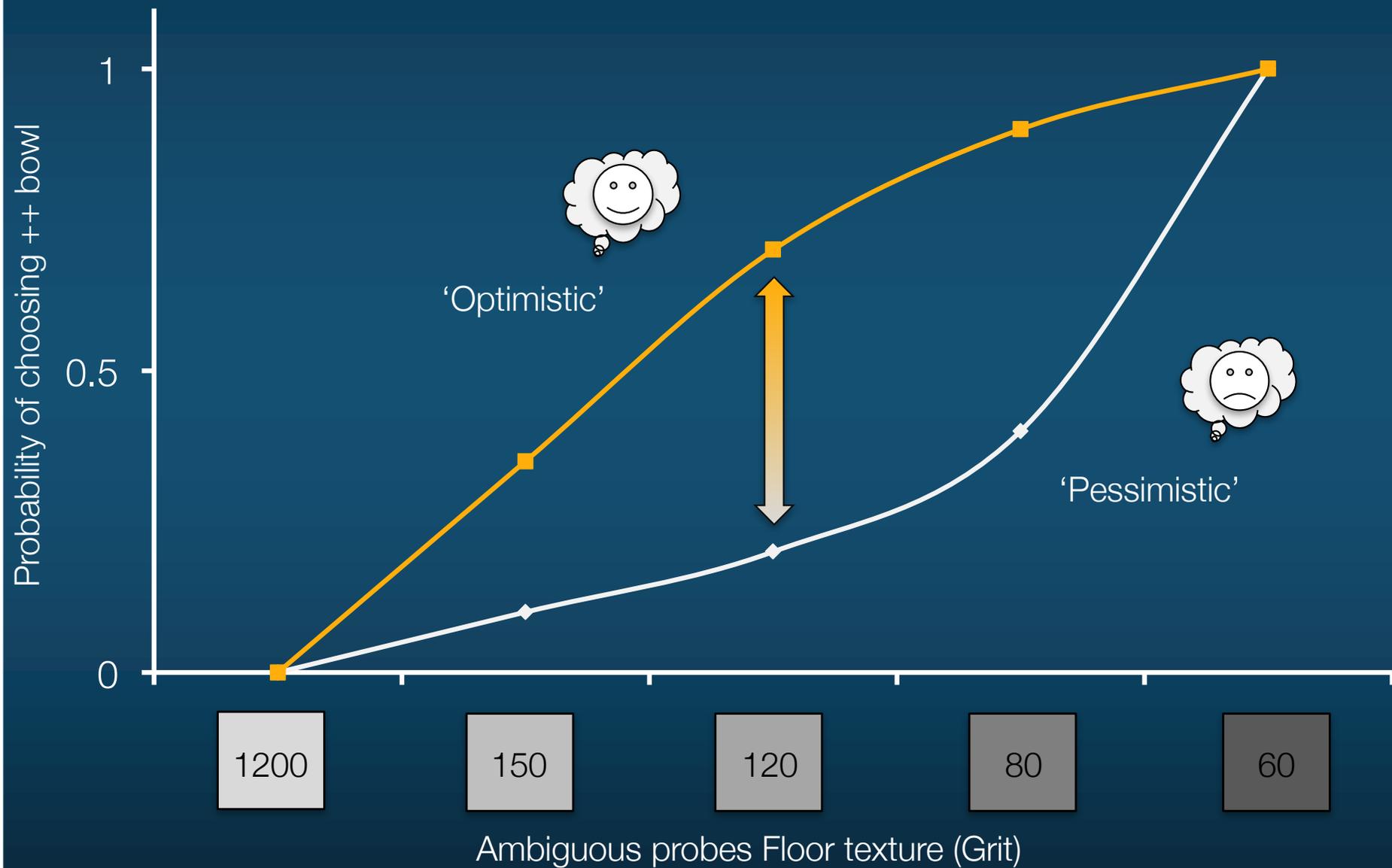


Test (5x)

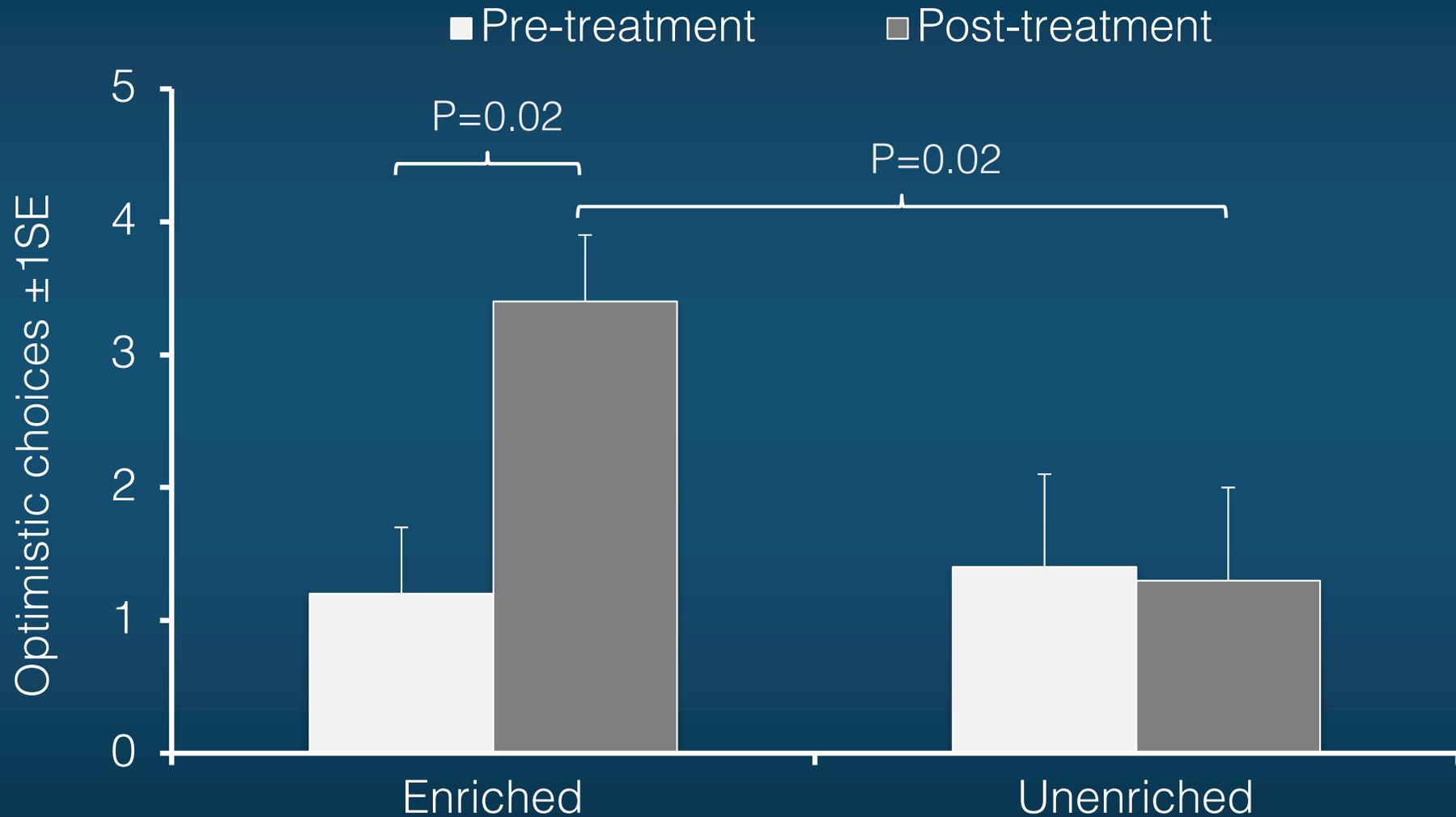


Record which bowl is chosen?

Predictions...



Findings



Has not been used to assess pain yet, but we are working on it...

Cognitive bias & pain?

- Well recognised in humans...
- Pre-operative affective state determines;
 - Perceived pain severity, surgical outcome
- Anxious, depressed or painful humans (–ve emotions)
 - Report more severe pain & require more analgesia
- Is this also true in animals?
- In theory yes...
 - We are working on this...

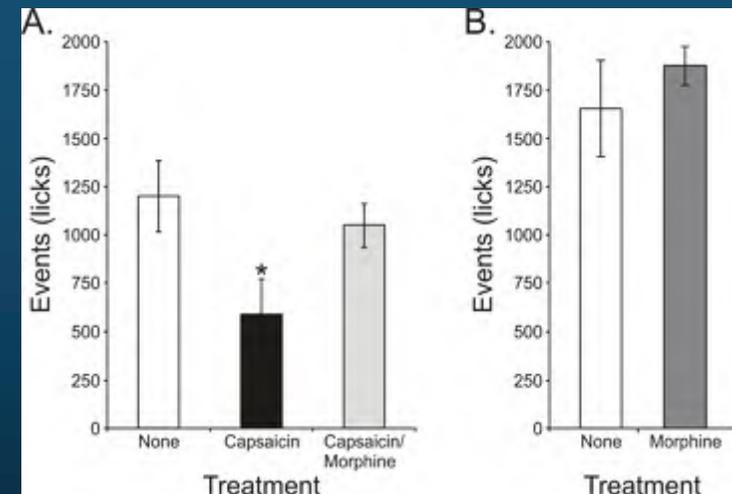


New (ish) operant test

- Orofacial Operant Pain Assay (OOPA)
- Uses a reward-conflict paradigm
 - Ask animal to titrate pain (heat) against its willingness to access a palatable reward (e.g. condensed milk)
- Requires higher-level cognitive processing
 - The animal decides, according to its perceived pain, whether it will complete the task of maintaining contact with the thermode while obtaining a palatable reward
- Minimally invasive using transient pain that the animals terminate themselves

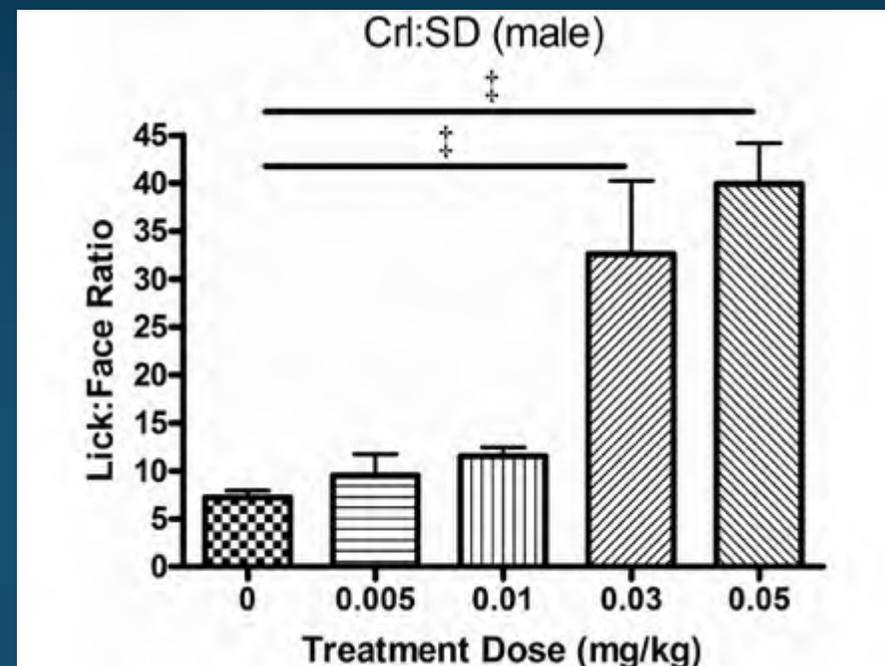
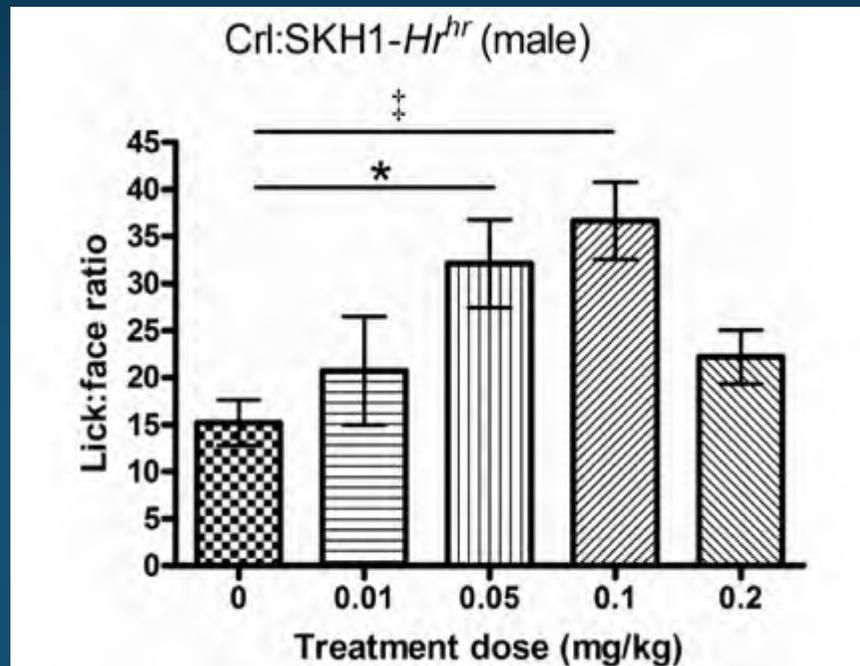
New (ish) operant test

- Mice/rats trained to seek a fluid reward
 - To access sweetened condensed milk they have to keep cheeks in contact with heated bars (e.g. 37°C)
- As temperature increases (41°C) licking decreases
- Animals show less licking when orofacial pain was induced with capsaicin



Photos and data from Neubert et al. (2008) *Molecular Pain*, 4: 1-14

New (ish) operant test



Effect of buprenorphine dose on the lick:face ratio in male mice & rats.

Rodents were more willing to complete the task (higher lick/face ratio) with pain relief

New NC3Rs grant to further validate this technique & to identify more effective analgesia

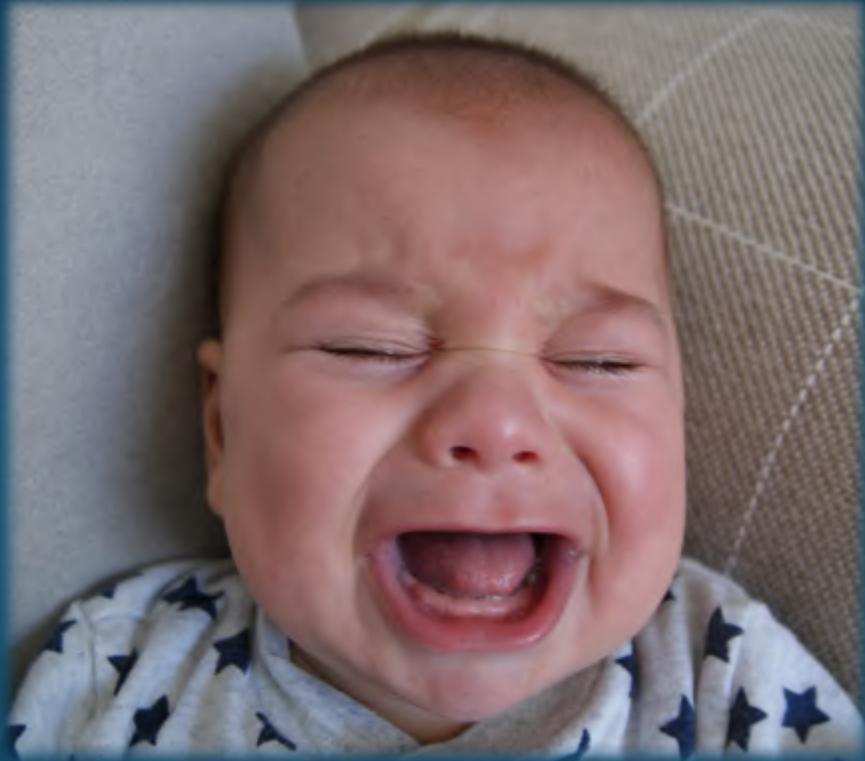
Ramirez et al. (2015) JALALAS 54: 426-432

Limitations of complex behaviour

- Impractical at the cage-side, i.e. a 'clinical' scenario!
- May not be pain-specific, rather negative state specific
 - i.e. change in response to pain, distress, anxiety or combinations of these states!
 - But does this matter!
- But could be used as adjuvants to other cage side methods for the purposes of validation!



Assessing pain in non-verbal species?



Q. How do we assess pain in non-verbal humans clinically?

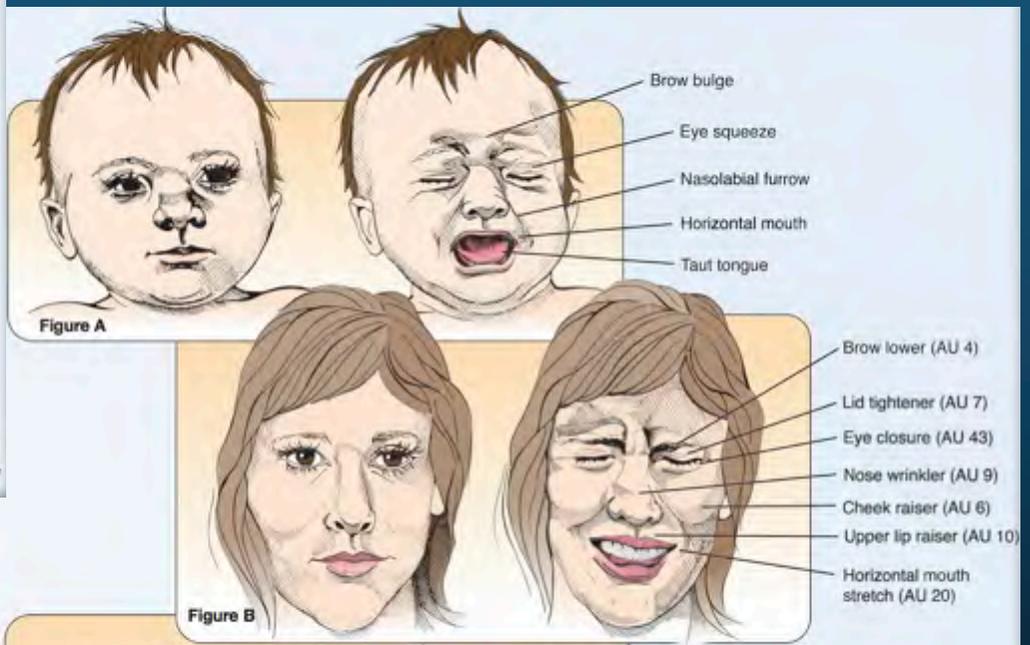
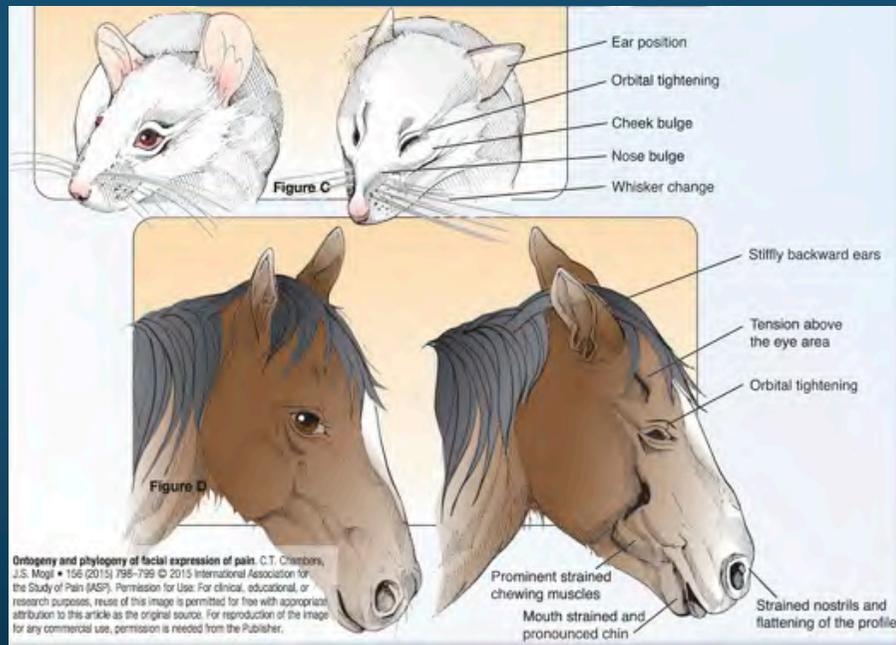
A. We use facial expressions

Q. Why use facial expressions?

- Considered as 'Gold Standard' assessment:
 - Effective using a limited number of indicators
 - Rapid & easy to carry out after minimal training
 - Represents 'generic' response to pain or distress
 - Good accuracy (>80%)
 - Uses the human tendency to fixate on faces
 - e.g. the human face on the moon!

Facial expressions have evolved...

- Share changes in many components of expressions (species generic)
 - Eyes, cheeks, ears, whiskers, mouth, jaw etc.



Current grimace scales

Mouse (MGS)	Langford et al. (2010) Nature Methods 7: 447
Rat (RGS)	Sotocinal et al. (2011) Molecular Pain 7: 55
Rabbit (RbtGS)	Keating et al. (2012) PLoS ONE 7: e44437
Horse (HGS)	Dalla Costa et al. (2013) PLoS ONE 9: e92281
Horse (EPF)	Gleerup et al. (2014) Veterinary Anaesthesia and Analgesia 42, 103–114
Cat	Holden et al. (2014) Journal of Small Animal Practice 55: 615-621
Sheep (SPFS)	McLennan et al. (2016) Applied Animal Behaviour Science 176: 19–26
Cow (CPS)*	Gleerup et al. (2014) Applied Animal Behaviour Science 171: 25–32



Methodology

- Development:
 - Comparison of images (same individual) before vs. after painful event (blinded observer) to identify what changes...
 - Validated method used by human facial coding experts
- Current scoring method:
 - Intensity (presence) of AUs measured on 3-point scale:
 - 0= not present, 1= moderately present, 2= obviously present
 - Overall 'Grimace score' is either:
 - An average of all AUs in the scale
 - The total (composite) of all AUs in the scale

Mouse grimace scale (MGS)



Orbital tightening:

- Closing of the eyelid (narrowing of orbital area)
- A wrinkle may be visible around the eye



Nose bulge:

- Bulging of the bridge of the nose
- Vertical wrinkles extend down the side of the nose



Cheek bulge:

- Bulging of the cheeks



Ear position:

- Ears move back from facing forward to lay on body
- Ears rotate outwards & space between the ears increases



Whisker change:

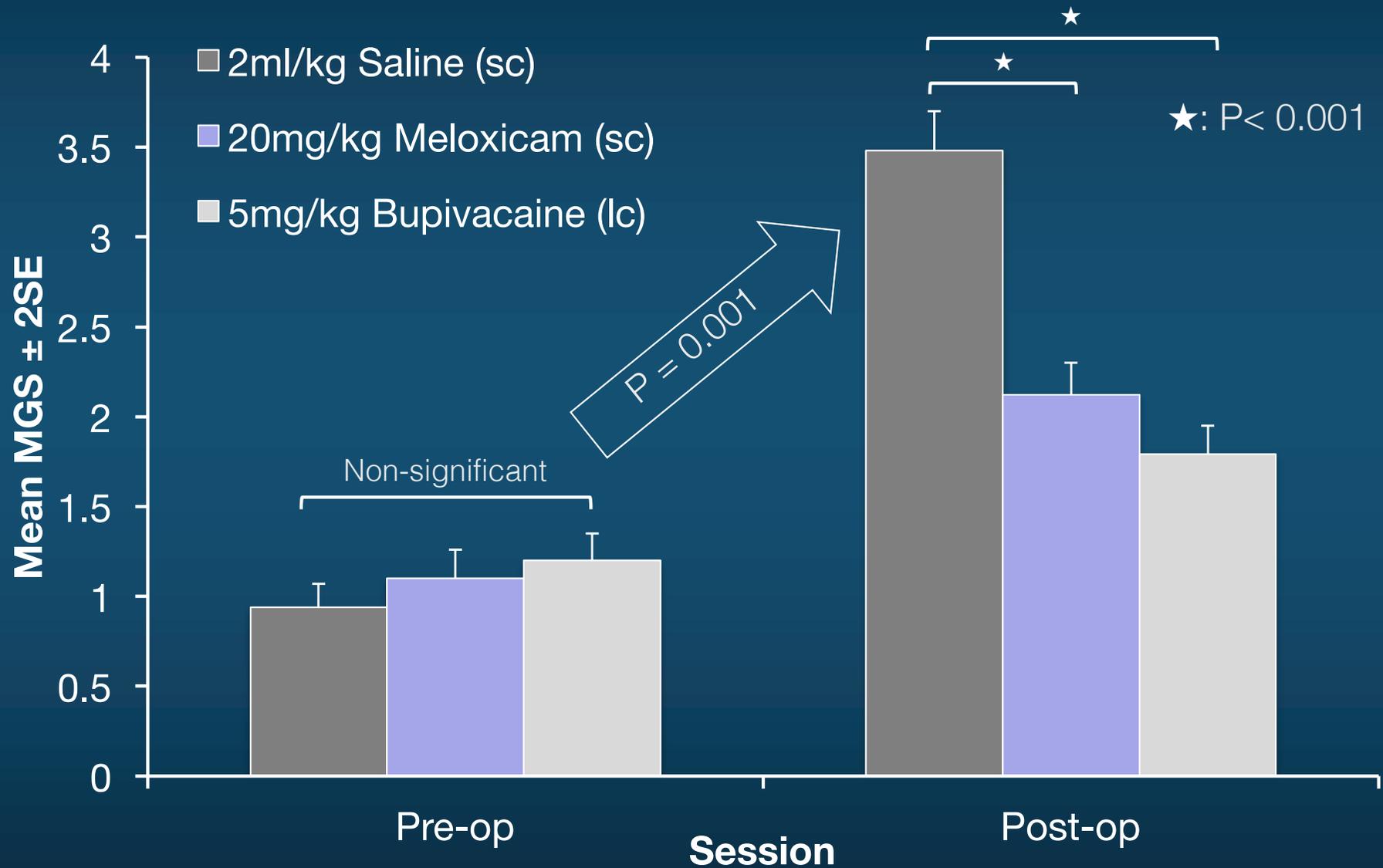
- Whiskers either being pulled back against the cheek or;
- Pulled forward to “stand on end” & clump together
- Whiskers lose their natural ‘downward’ curve

0

1

2

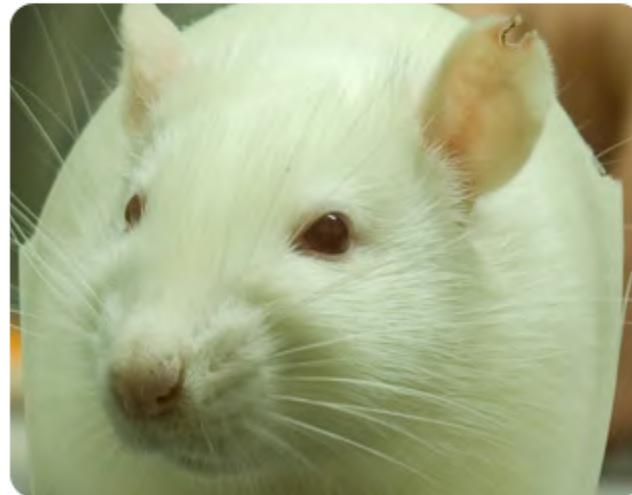
Mouse Grimace Scale (MGS)



Are they reliable & accurate?

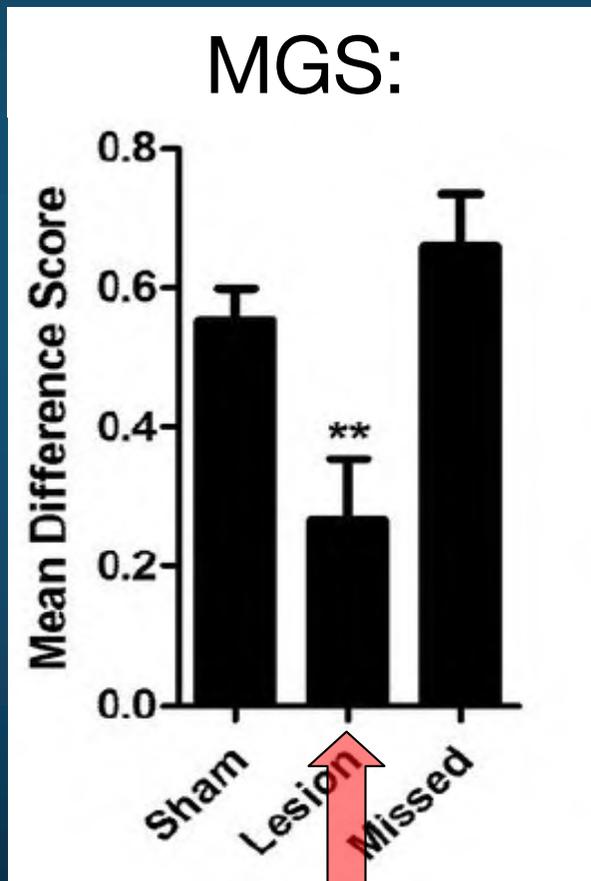
- Inter-rater reliability:
 - Interclass correlation: 0.90 to 0.91 (High)
- Accuracy: 72% to 97%
 - Depends on image quality & species
 - Higher after 5mins of training with grimace scale manual...

*Langford et al. (2010), Sotocinal et al. (2011), Keating et al. (2012)

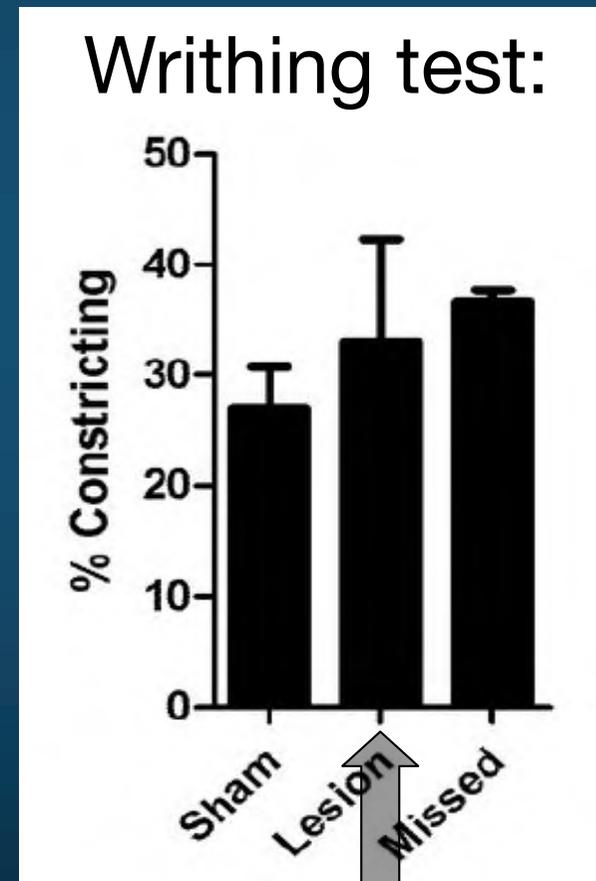


Could assess pain experience!

- Ibotenic lesioning of the rostral anterior insula
 - Activation associated emotional component of pain (humans)



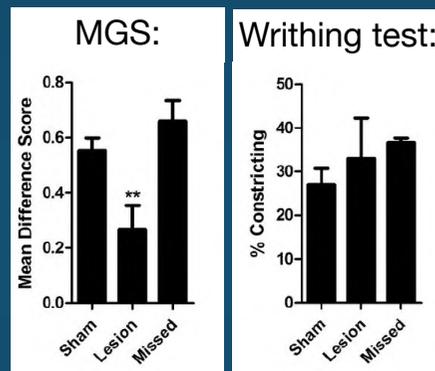
Attenuation of pain face



No effect on writhing

Could assess pain experience!

- Ibotenic lesioning of the rostral anterior insula
 - Activation associated emotional component of pain (humans)



- Lesion could have reduced emotional but not sensory component of pain in mice...
 - Facial expressions are a direct measure of experience...
- But this is one study in only 6 mice...

Things we don't know...

- MGS changes in response to other emotional states¹
 - Current scales only seem sensitive to negative states
 - No change seen in RGS with positive emotions²

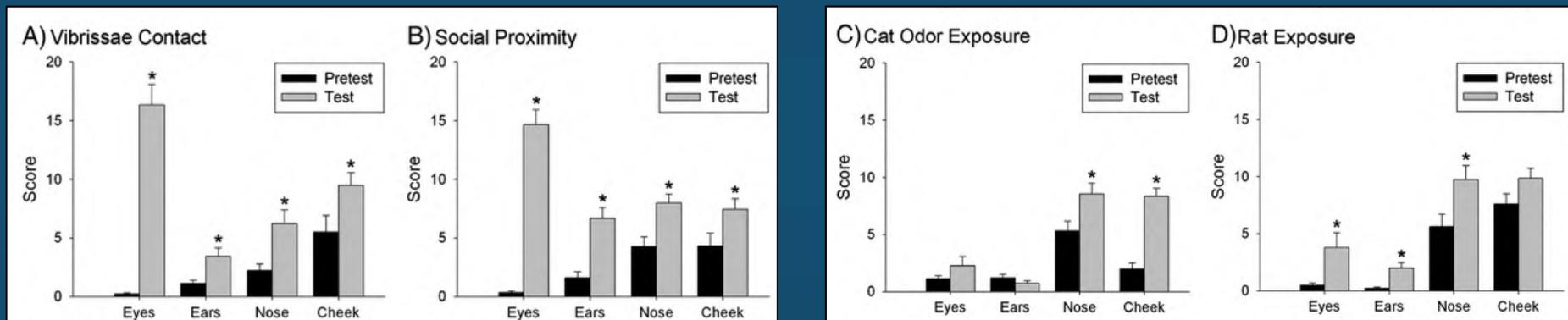
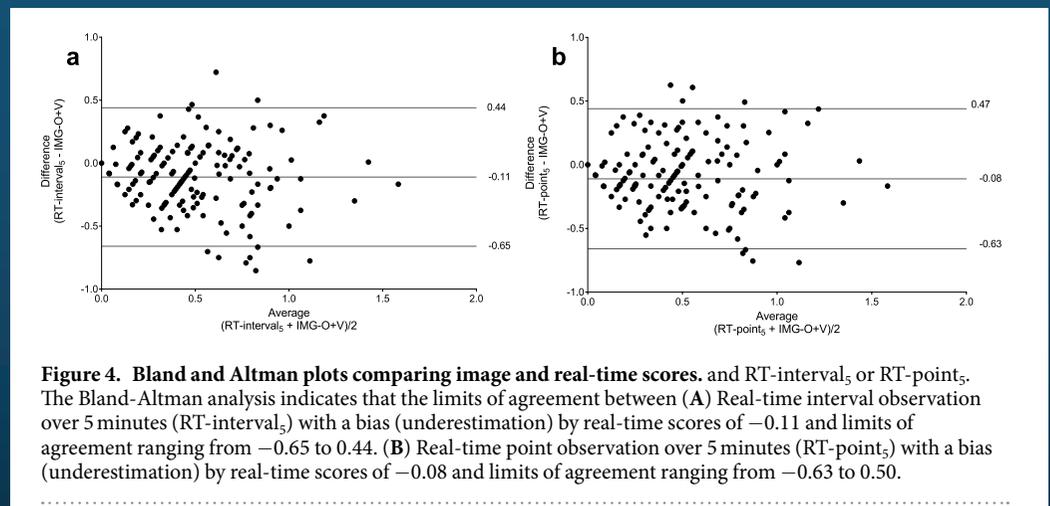
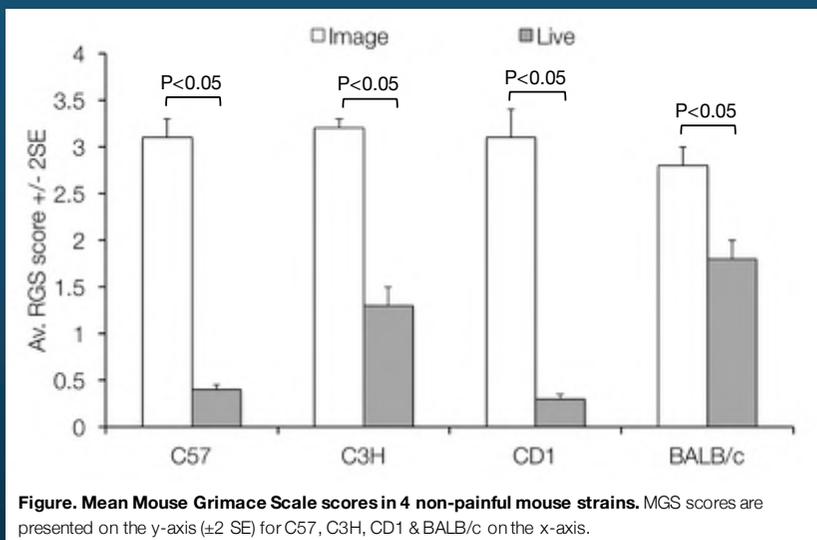


Fig. 2. Facial scores in the (A) vibrissae contact, (B) social proximity, (C) cat odor exposure and (D) rat exposure tests presented as mean \pm SEM. Mice showed changes in the eyes, ears, nose, and cheek when contacted with a brush and when placed in social proximity. Only nose and cheek changes were observed when exposed to a cloth containing cat odor. Changes to the eyes, ears, and nose, but not cheek, were displayed by mice when exposed to a live rat. * $p < 0.05$.

- Current scales more pain related rather specific!
 - At least true for MGS, but for the others we don't know!

Things we don't know...

- Currently it seems practical in experimental context
 - i.e. where we can take images/video & are not time restricted
- Clinical scoring seems to be variable...



Things we don't know...

- Grimace scales may be influenced by other factors?
 - Breed/strain & gender of animals (MGS)^{1,2}
 - Anaesthesia & analgesia (MGS)^{1,2}
 - Presence & gender of observers (MGS, RGS)³

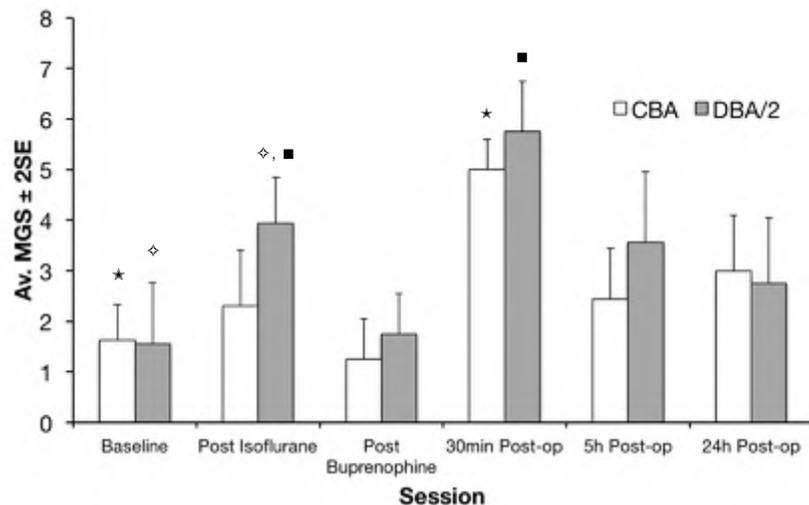


Figure. Mean Mouse Grimace Scale scores in CBA & DBA/2 mouse strains. MGS scores for mice receiving 0.05mg/kg buprenorphine with the post-vasectomy recording sessions on the x-axis (*P<0.001, *P<0.01, ♦P<0.05).

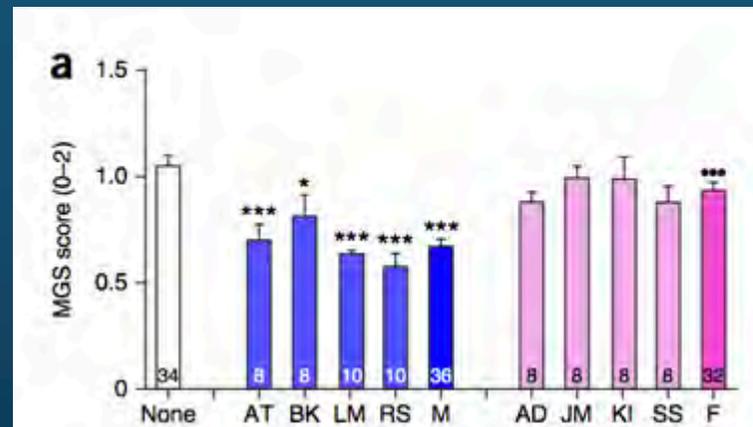
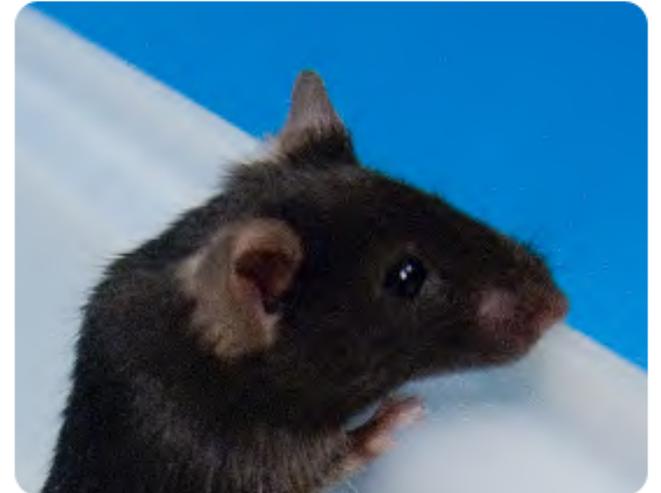


Figure 1 | Male-associated olfactory stimuli produce decreased pain behavior in mice. (a,b) Zymosan-induced facial grimacing in response to four male (M) experimenters and four female (F) experimenters (a; letters on the x axis indicate the initials of the experimenter)

***P < 0.01, ***P < 0.001 compared to analogous male-related stimulus group (T- mice compared to T+ or Str. mice) by ANOVA

Limitations

- Should only be used in awake animals...
 - False positives: falling asleep, asleep or waking up...
 - So sedation & anaesthesia have a confounding effect...
- Most scales developed to assess acute pain (10mins to 24h)¹⁻⁴
 - SPFS developed for chronic pain (lameness & mastitis)
- Currently we still need baseline observations...



Maximising pain assessment

- Tailor assessment to the context/study etc.
- Use a combination of indices
 - As all the methods have their limitations
 - But one index can often compensate for limitation of another!
- Be familiar with:
 - Different methods of assessing pain
 - Species & strain being assessed & how this affects pain assessment



Thank you listening....

If you any questions or wish to discuss anything, then either chat to me or email me at:

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