Detection and alleviation of pain in fish

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Detecting and alleviating pain in fish

1. Legislation and guidelines on welfare
2. Current definitions of nociception and pain
3. Key principles
4. Examples
5. Automated pain detection
6. Alleviation of pain

UK – Farm Animal Welfare Committee

- **Fish** are able to detect and respond to noxious stimuli, and FAWC supports the increasing scientific consensus that they **experience pain**.
- We therefore recommend that deliberations on management and other processes should be made on this basis.

FAWC

Farm Animal Welfare Committee

Opinion on the Welfare of Farmed Fish

February 2014

Europe

- European Council Directive 98/58/EC concerning the protection of animals kept for farming purposes (including fish), requires that *"owners or keepers take all reasonable steps to ensure the welfare of animals under their care ….. not caused any unnecessary pain…."*.
- However, the Directive excludes fish from the detailed provisions set out in its Annexes.

- Directive 2010/63/EU concerning the protection of animals used for scientific purposes.
- Fish included – *"assessment of pain, suffering distress and lasting harm caused to the animals"*.

- **Need to define pain for its assessment and further decide when alleviation of pain is required.**

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Review

Defining and assessing animal pain

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A R T I C L E I N F O

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The detection and assessment of pain in animals is crucial to improving their welfare in a variety of contexts in which humans are ethically or legally bound to do so. Thus clear standards to judge whether pain is likely to occur in any animal species is vital to inform whether to alleviate pain or to drive the refinement of procedures to reduce invasiveness, thereby minimizing pain. We define two key concepts that can be used to evaluate the potential for pain in both invertebrate and vertebrate taxa. First, responses to noxious, potentially painful events should affect neurobiology, physiology and behaviour in a different manner to innocuous stimuli and subsequent behaviour should be modified including avoid-

IASP Definition

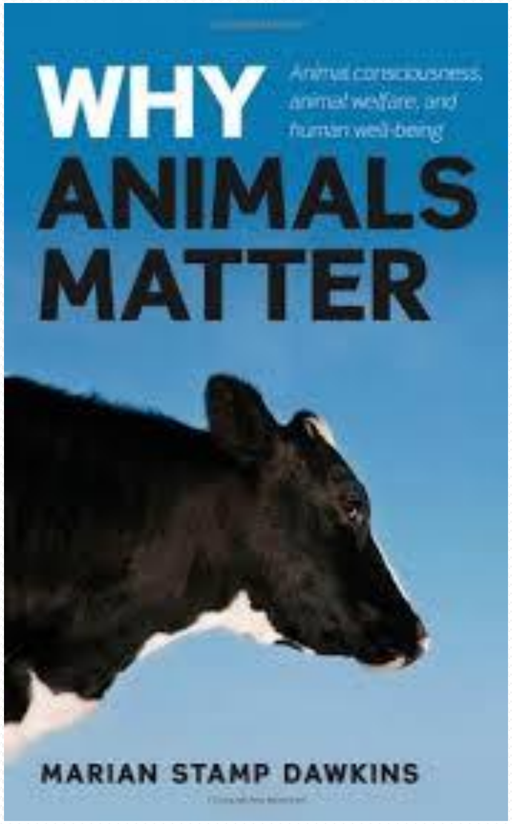
- An unpleasant and emotional experience associated with actual or potential tissue damage.
- Note: The inability to communicate verbally does not negate the possibility that an individual is experiencing pain.

Definition of Nociception and Pain

- Nociception is the simple detection and reflex response to a noxious stimulus
- Pain is a sensory and a psychological experience

Sceptics of animal pain

- Neocortex
- Generates consciousness
- Only primates/humans
- Little known
- Hampers true progression
- Consciousness and perception
- Evolutionary/ecological differences



Function of pain

- Alarm system
- Perceive/avoid damage
- Aversive motivational state
- Results in learning
- Require a definition for assessment
- Leads to pain relief

Defining pain

1. **Whole animal responses to potentially painful events differ from innocuous stimulation**
2. **Change in motivational behaviours after a potentially painful event**

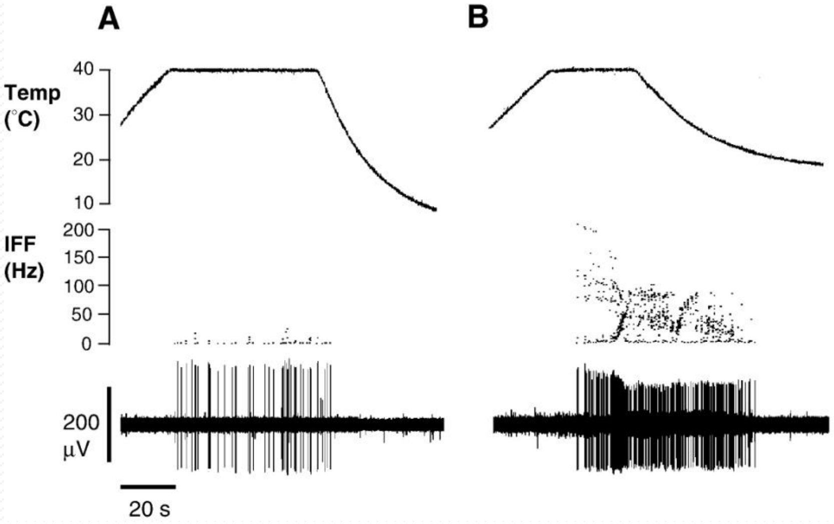
All animals appear to have nociceptors, pathways to the central nervous system (CNS) and altered CNS activity specific to noxious stimuli (where known i.e. invertebrates)

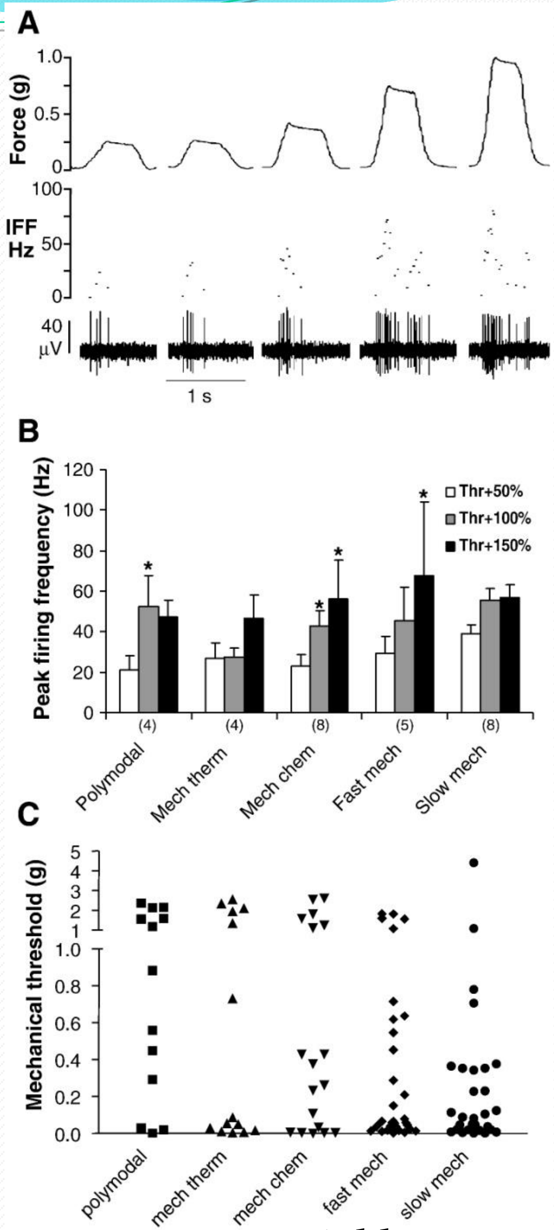
Whole animal responses

- Nociceptors, pathways to CNS, central processing in areas that regulate motivated behaviour (including learning and fear)
- Nociceptive action responsive to endogenous modulators (e.g. Opioids in vertebrates; FMRFamide in *Aplysia*)
- Nociception activates physiological responses linked to stress
- Not just a nociceptive withdrawal reflex
- Alterations in future behaviour
- Protective behaviour such as wound guarding, limping, rubbing, licking or excessive grooming
- All of the above reduced by analgesia or local anaesthetics

Fish: Electrophysiological Properties

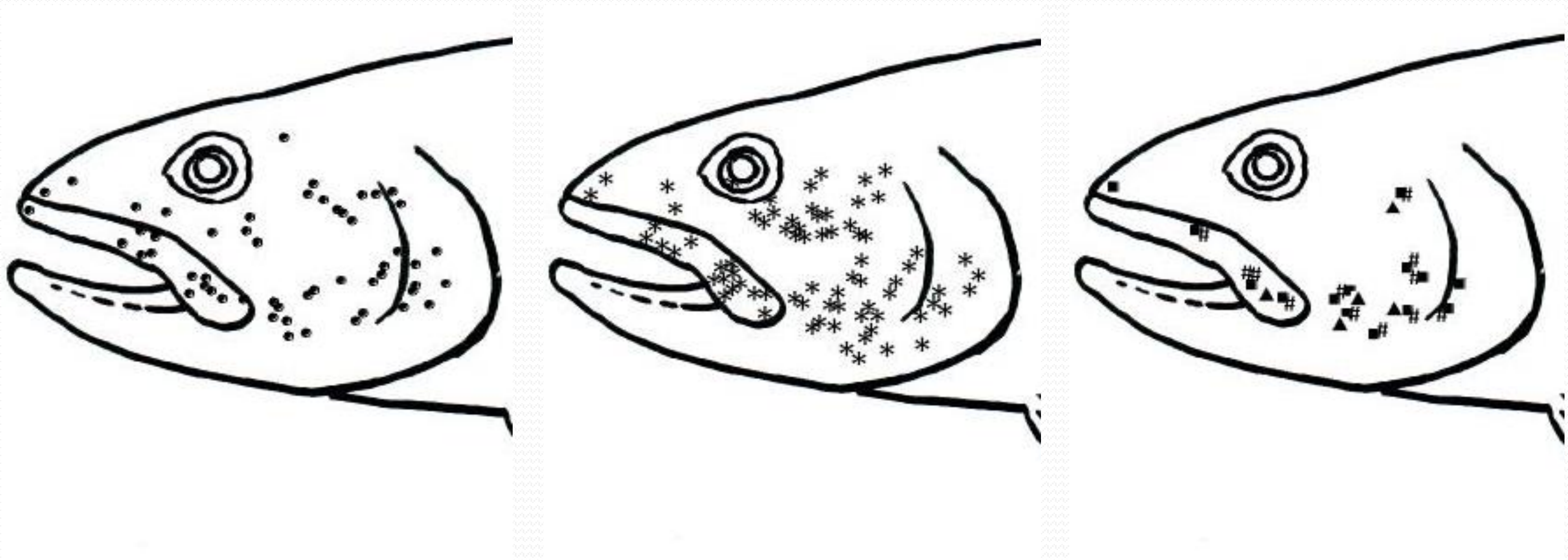
Similar to mammals





Sneddon 2003 Brain Res. 972, 44-52; Ashley et al. 2006 Neuroscience Letts. 410, 165-168; Ashley et al. 2007 Brain Res. 1166, 47-54.

Chemically responsive nociceptors



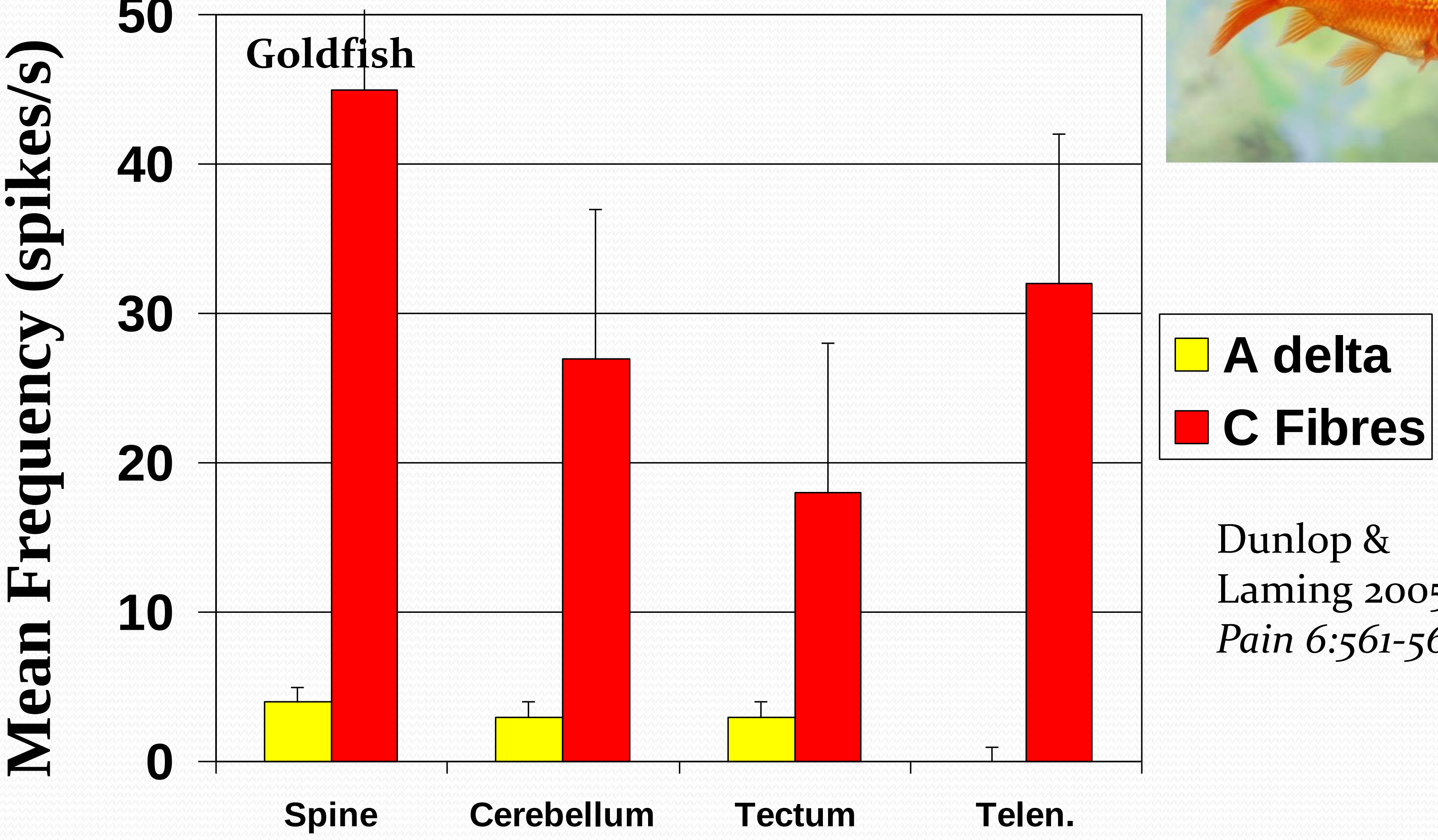
Acetic Acid

Carbon dioxide

■Citric acid, # citric acid phosphate buffer

Mettam, McCrohan & Sneddon, 2012, J. Exp. Biol. 215, 685-693

Neuronal activity in the brain



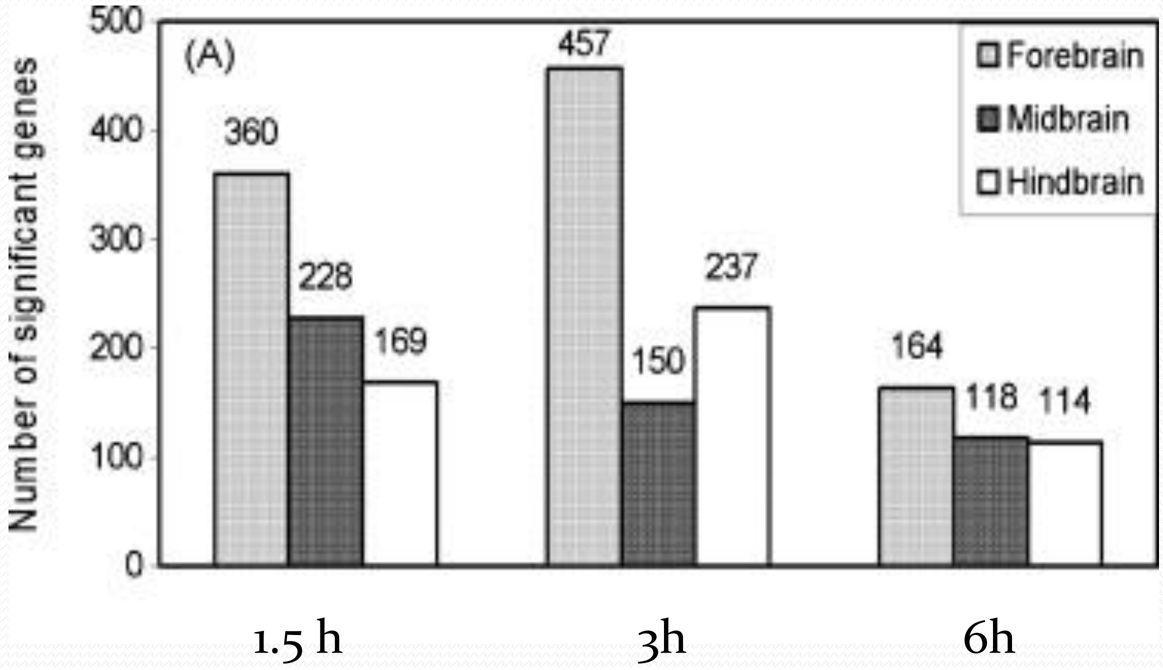
Dunlop & Laming 2005. *J Pain 6:561-568.*

Microarray analysis

Differentially expressed compared with saline treated carp

Known Genes
Kainite glutamate receptor
BDNF
CB1

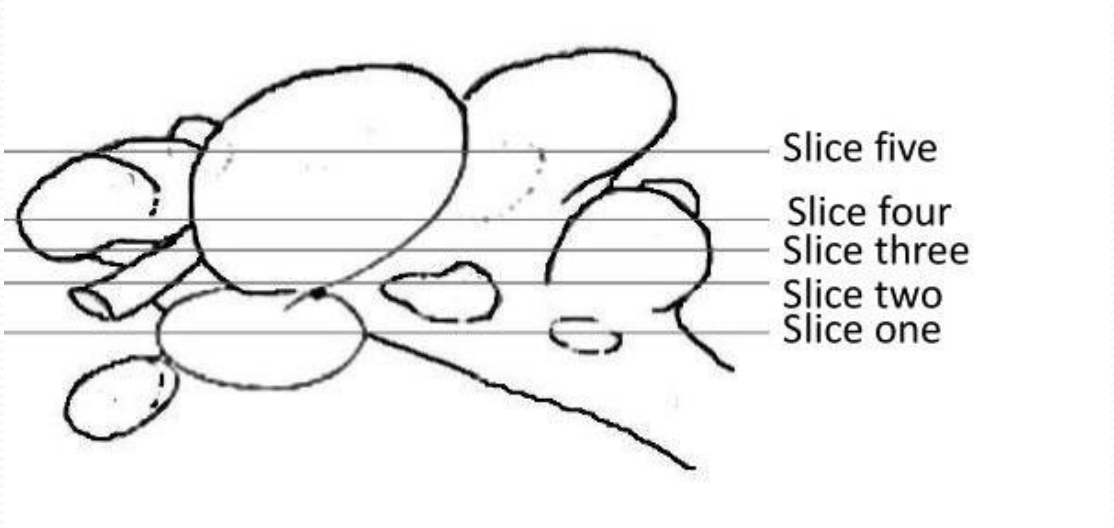
Novel genes

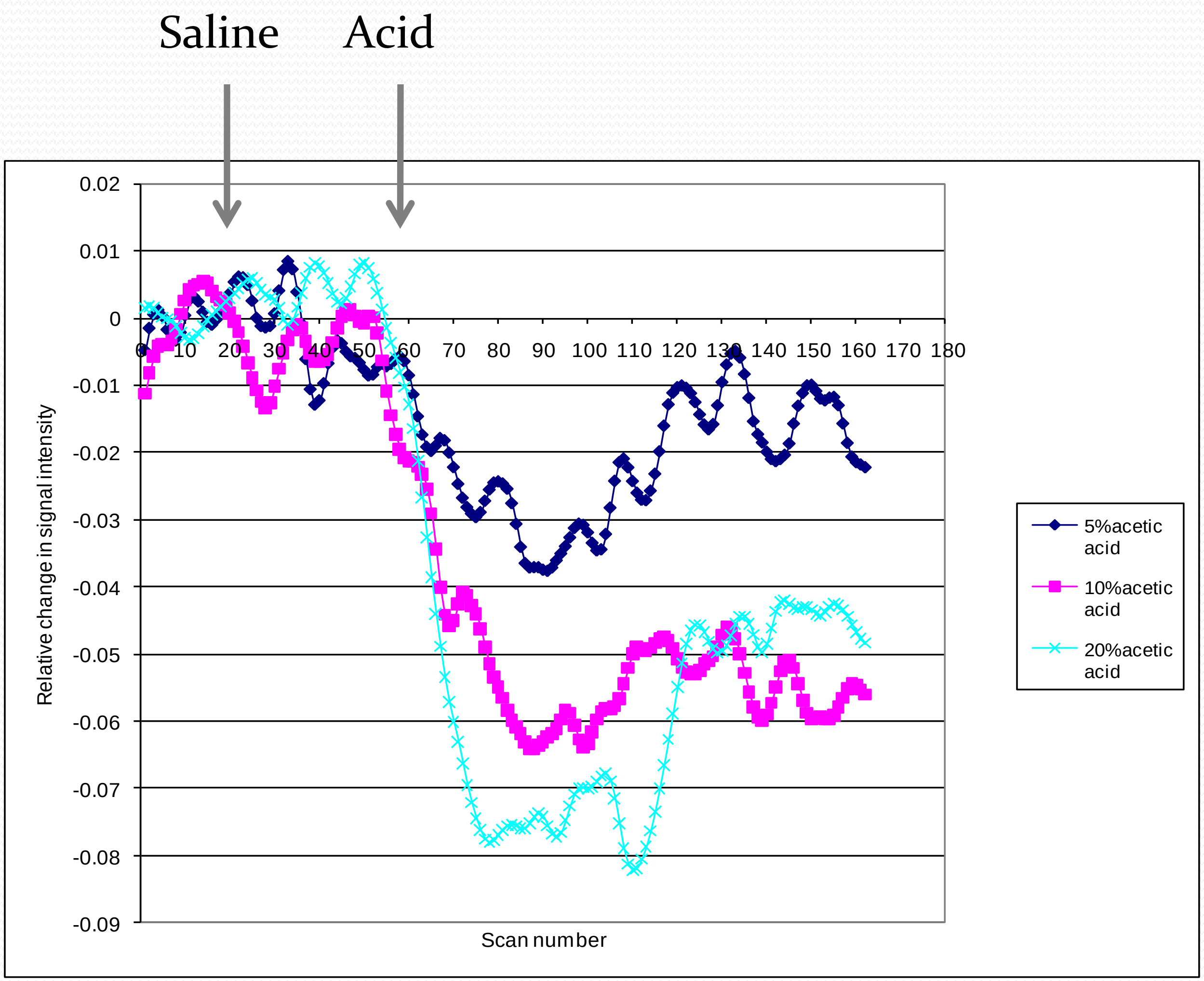


Reilly et al. 2008, Neurosci. Letts. 437, 135-138.

fMRI in carp

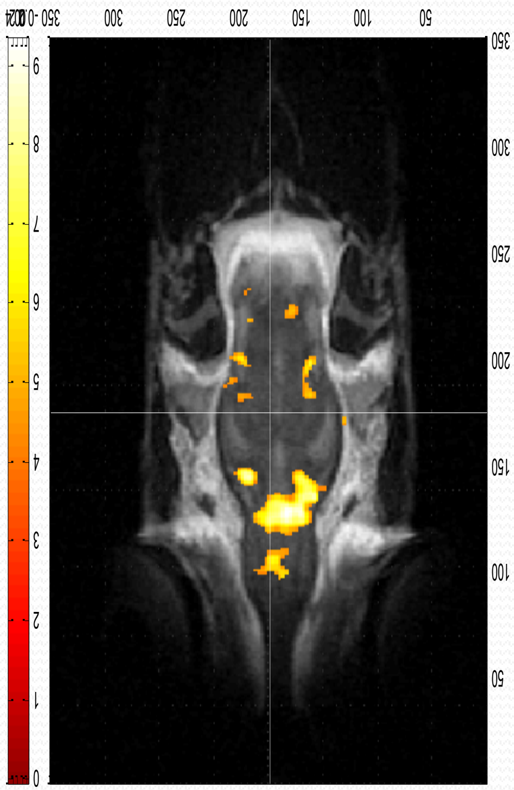
- Apply saline or acetic acid to carp's face
- 3-5 horizontal brain slices every 20 s




Saline
Acid
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
Relative change in signal intensity
Scan number
5%acetic acid
10%acetic acid
20%acetic acid

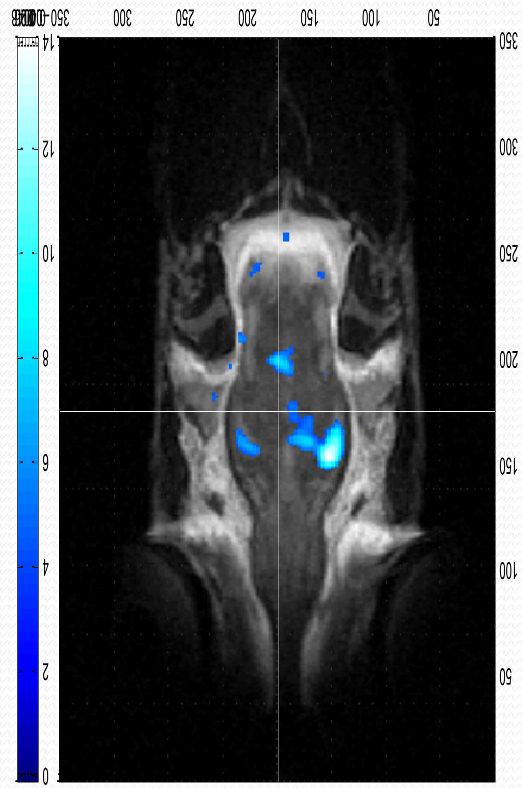
Results

SLICE 4 – 10% Acid



R L

Acid > Saline



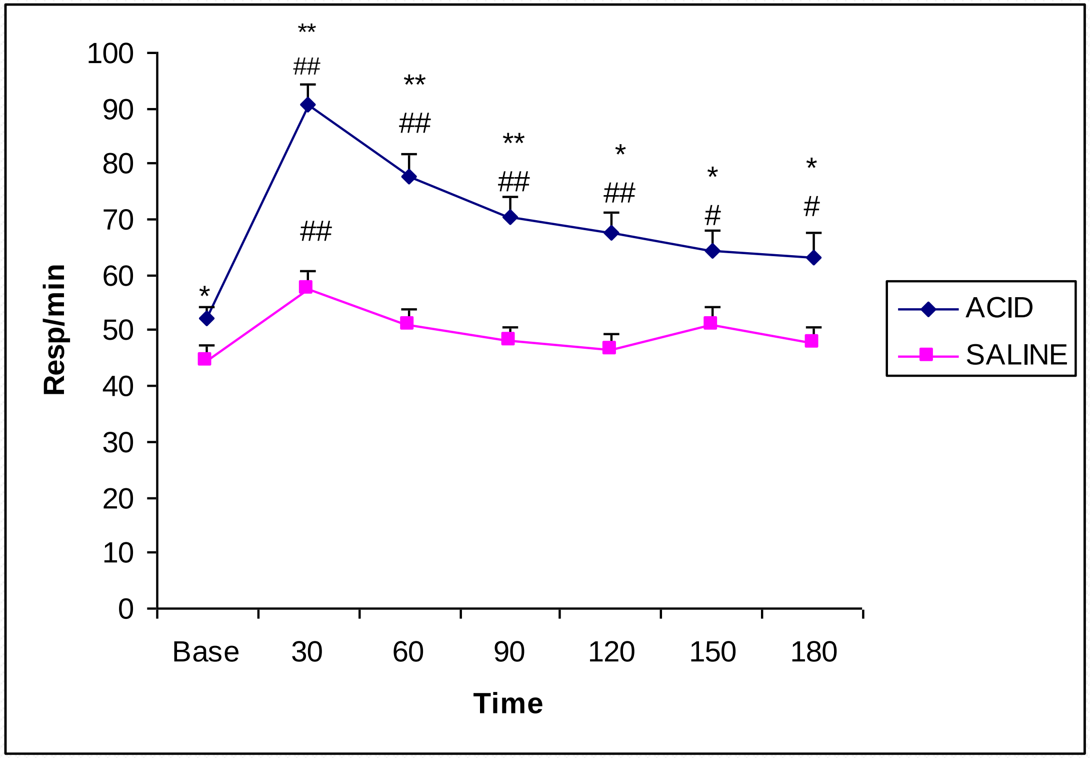
R L

Acid < Saline

Marleen Verhoye et al. & Sneddon MS submitted;
University of Antwerp

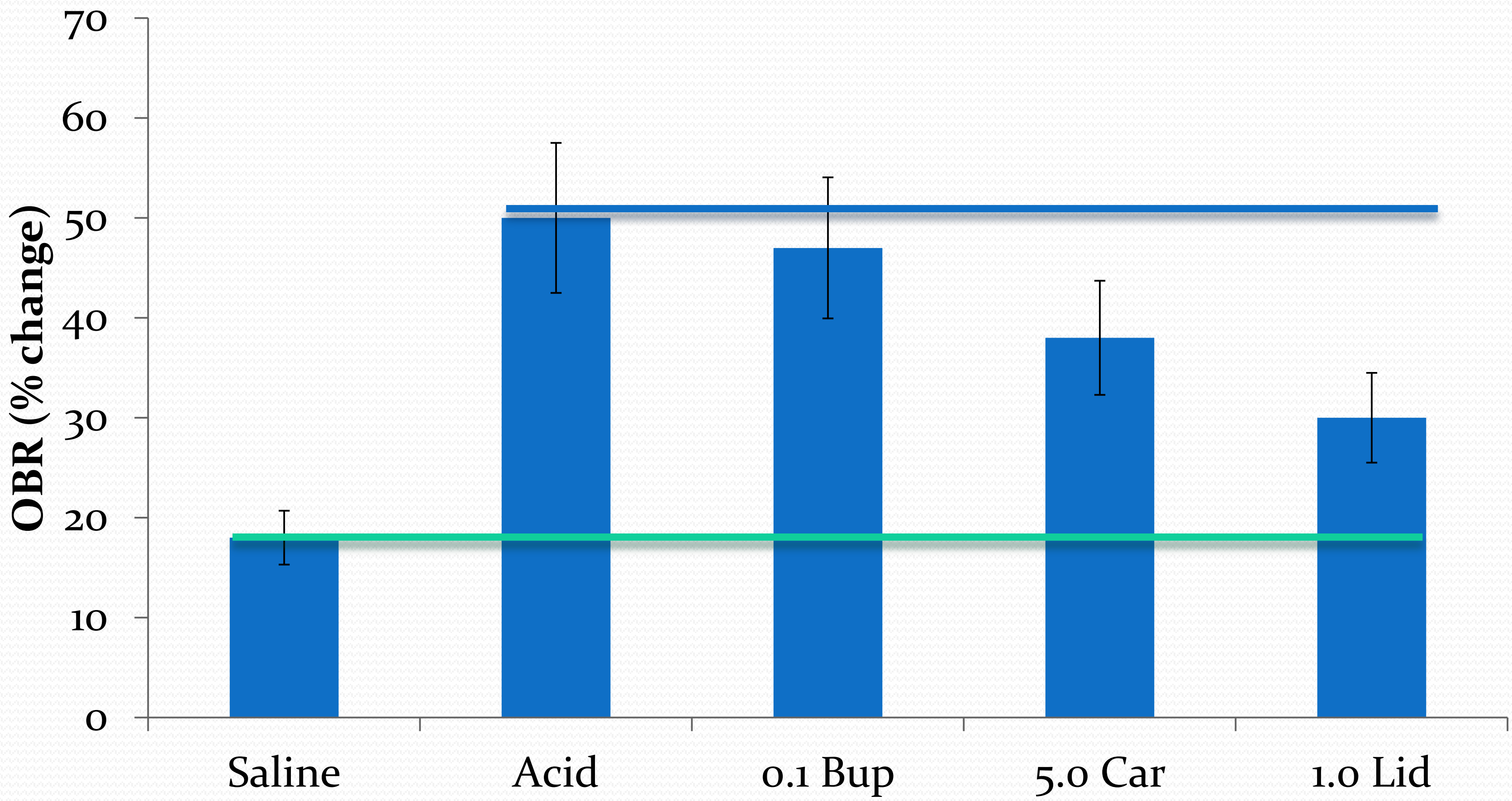
Opercular beat rate

- Trout



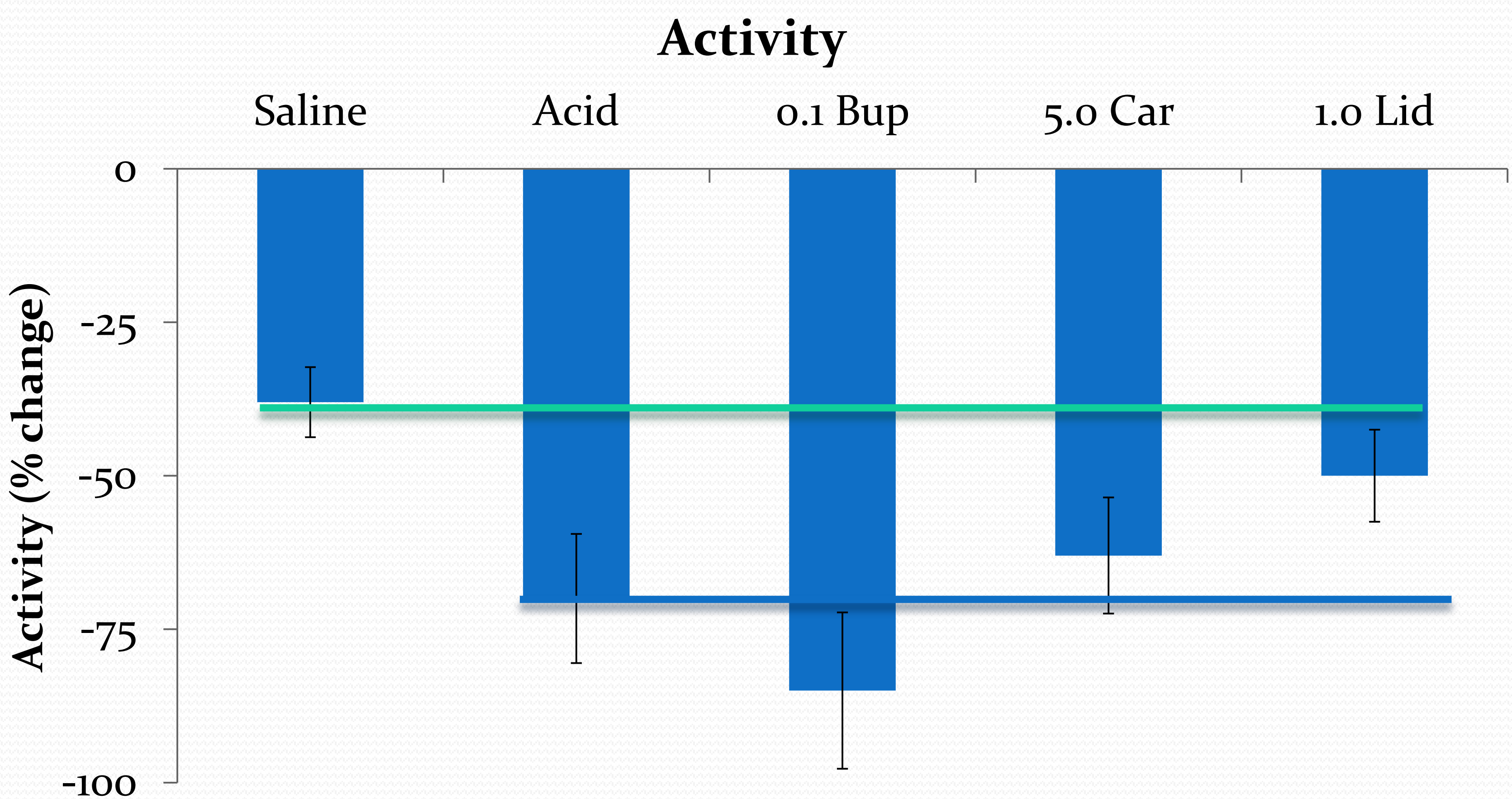
Ashley et al. 2009 Anim. Behav. 77, 403-410; Reilly et al. 2008 Applied Animal Behaviour Science 114, 248–259.

Testing of analgesics



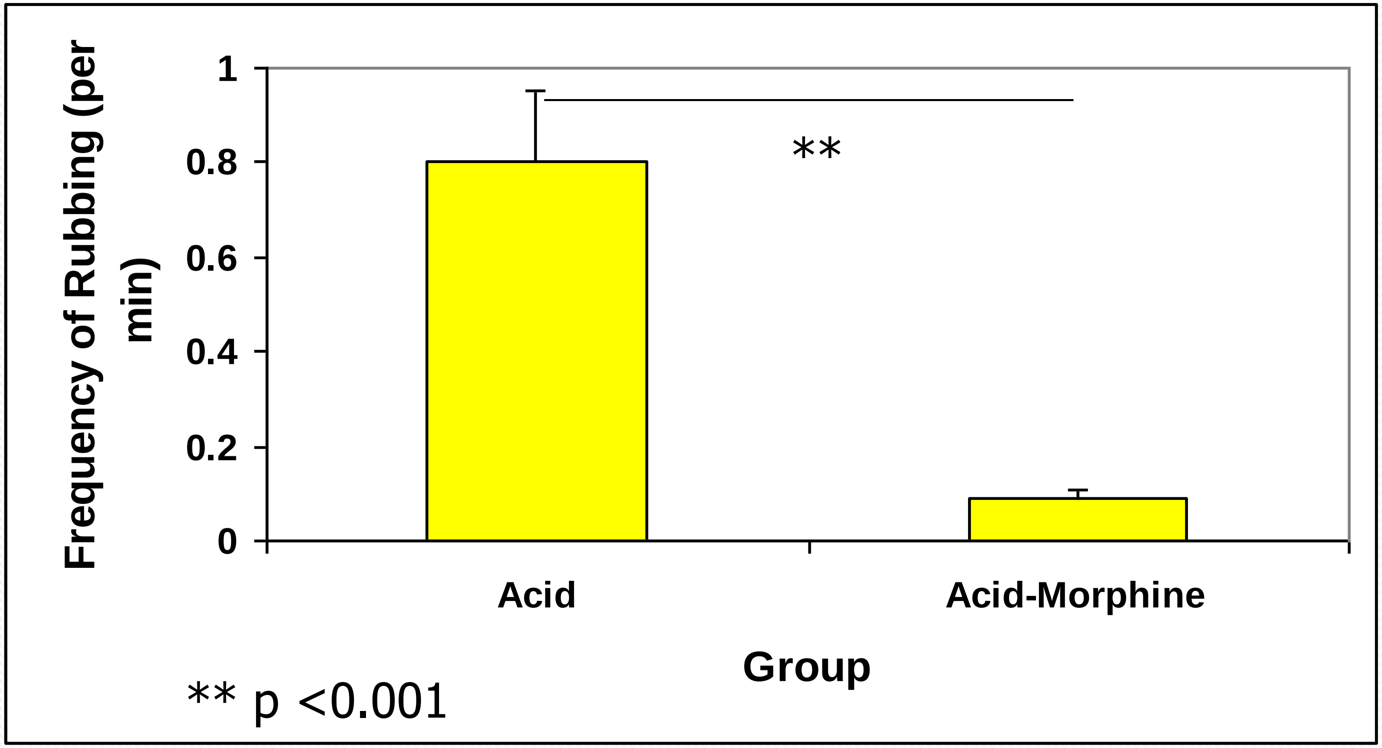
Mettam et al. 2011 Appl. Anim. Behav. Sci. 133, 265-274.

Testing of analgesics



Mettam et al. 2011 Appl. Anim. Behav. Sci. 133, 265-274.

Analgesia in rainbow trout



Sneddon 2003 App. Anim. Behav. Sci., 83, 153-162.

Goldfish: Newby, N.C., Wilkie, M.P. and Stevens, E.D. (2009) Canadian Journal of Zoology 87, 388–399.

Motivational change

- Self-administration of analgesia
- Pay a cost to accessing analgesia
- Selective attentional mechanisms
- Altered behaviour after noxious stimulation - conditioned place avoidance and avoidance learning paradigms
- Relief learning
- Long-lasting change in memory and behaviour
- Avoidance of the noxious stimulus modified by other motivational requirements as in trade-offs
- Evidence of paying a cost to avoid the noxious stimulus

Selective attention strategies

- How important is the experience?
- Divert attention away from the potentially painful experience
- Humans pain dominates e.g. 177 ms slower to recall words on a memory test

Selective attention strategies

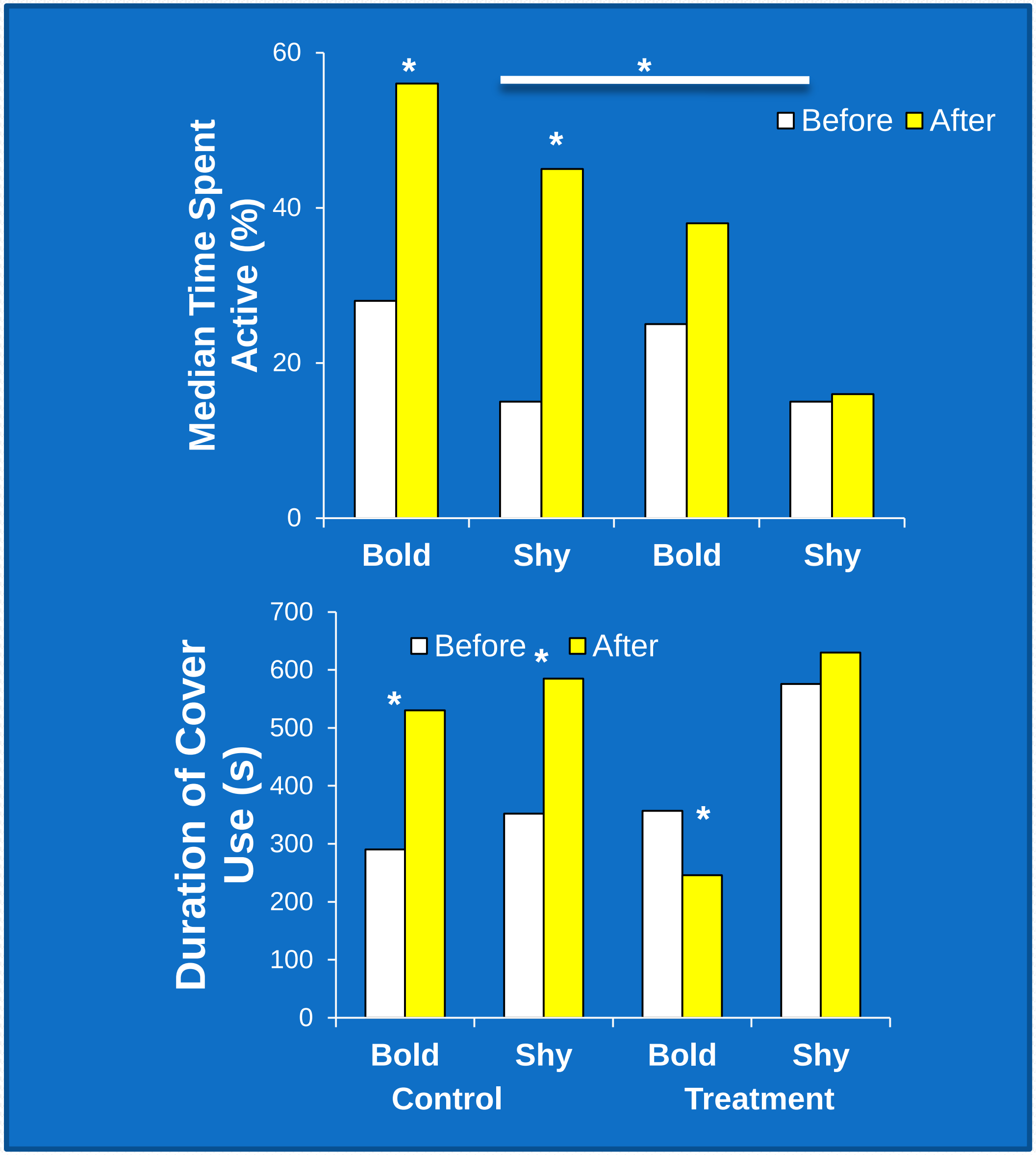
- Predator cue

Predator cue

- Shy-Bold assessment
- Behaviour pre and post treatment
- Saline or acetic acid
- Addition of water or alarm substance

Noxiously treated fish do not show a rise in activity (*P<0.01)

Noxiously treated fish do not show an increase in cover use (*P<0.01)



Ashley et al. 2009 *Anim. Behav.* **77**, 403-410

Principle of triangulation

- Clear indices to assess likelihood of pain
- In isolation do not prove pain
- Multimodal approach
- Combined these criteria suggest pain

	Aves	Amphibia/Reptiles	Agnatha/teleosts
Receptors for analgesic drugs	√	√	√
Physiological	√	√	√
Protective	√	√	√
Self-administration	√	?	√
No response to other stimuli	√/?	?	√
Cost to accessing analgesia	?	?	√
Altered choices/preferences	√	?	√
Relief learning	?	?	?
Rubbing, limping, guarding	√	?	√
Trade offs	√	?	√

Species: Atlantic cod; Atlantic salmon; common carp (koi); goldfish; Nile tilapia; piaçu; rainbow trout; zebrafish

The detection, assessment and alleviation of pain in laboratory zebrafish

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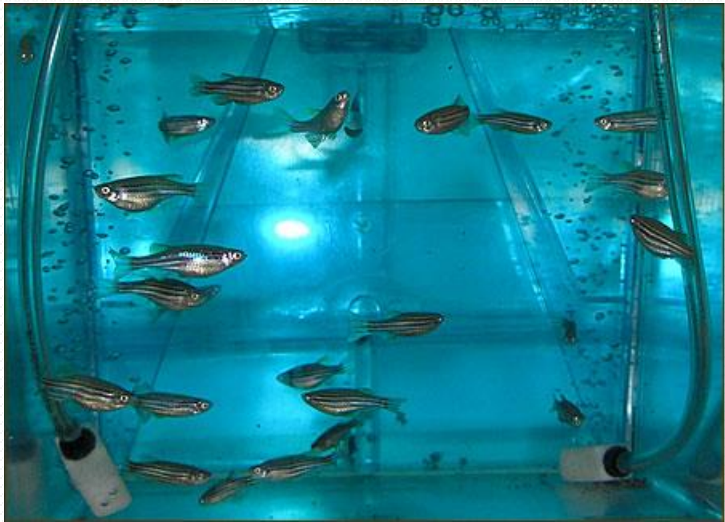
The importance of fish as experimental models

- Fish are second most popular model in the UK
- Some 300,000 fish used at the University of Washington, USA
- Vital that we can reliably assess their health and welfare
- Automatic monitoring would be a major step forward and important refinement
- Allow researchers and carers to intervene and improve health and welfare

The challenge of assessing welfare in fish

- With approximately half a million fish used in the UK alone, assessing welfare is a priority
- Improve lab animal welfare in an important model organism
- Procedures that may compromise health or cause pain which is not the objective of the study
- Reduce pain by testing analgesics

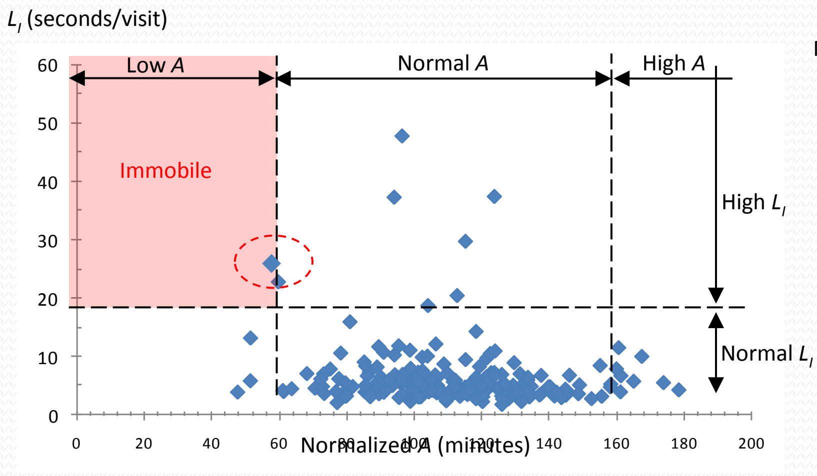


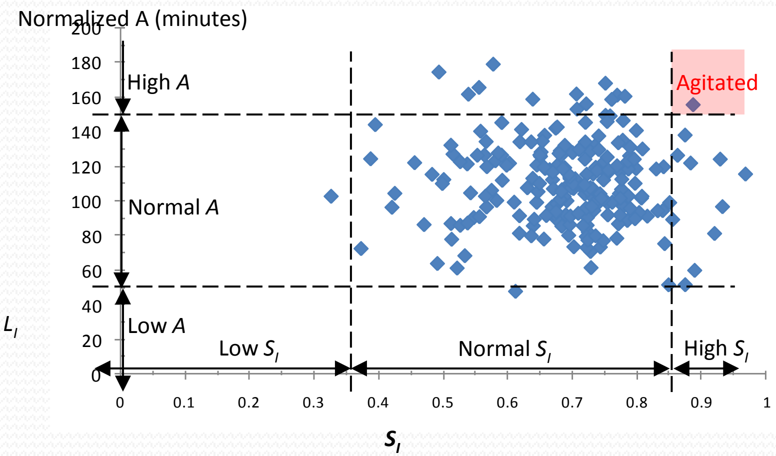
Objectives

- Developing the automated detection and assessment of pain in zebrafish
- Assessing the efficacy of analgesia

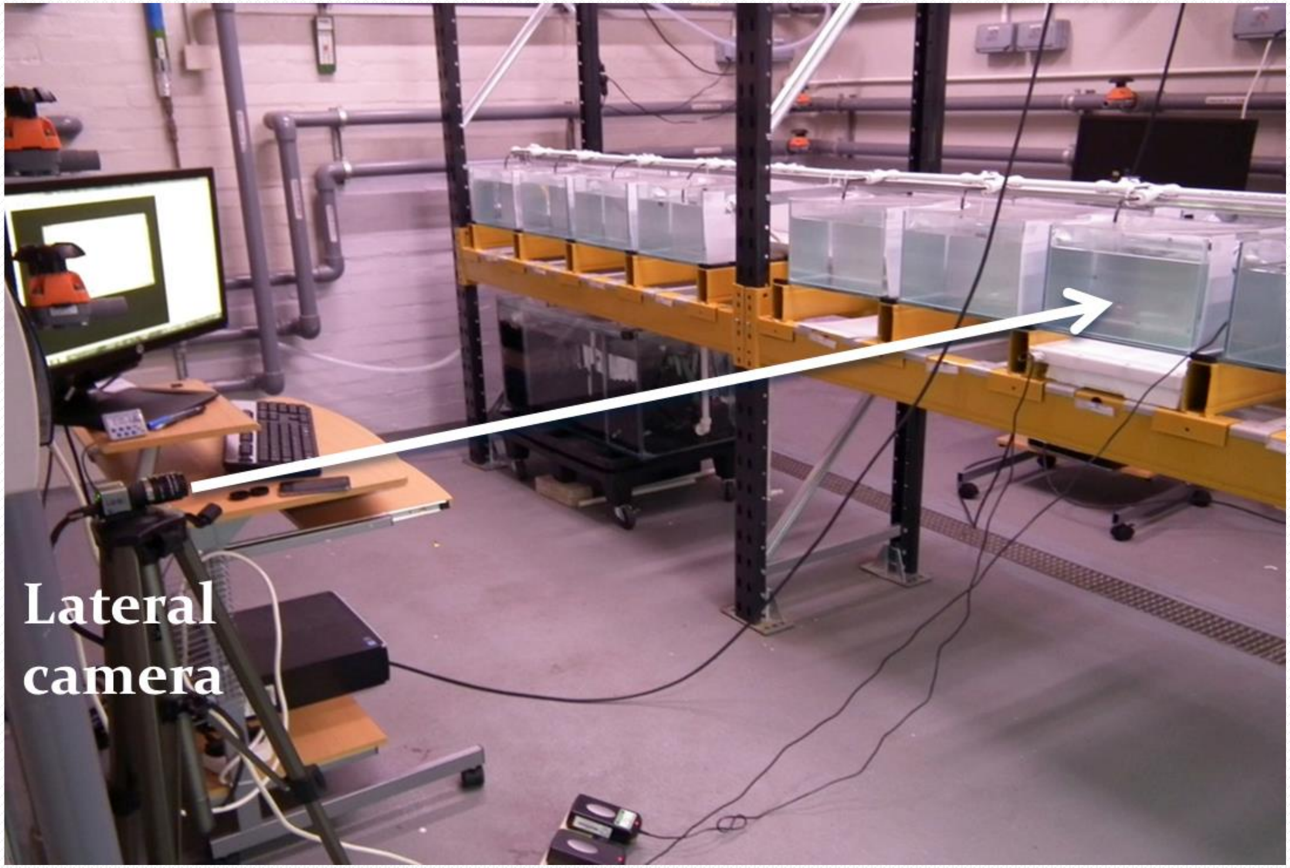
Developing an intelligent monitoring tool

- Collaboration with engineers at Liverpool
- Developed intelligent monitoring software used in geriatric care home
- Determine who needs attention and care



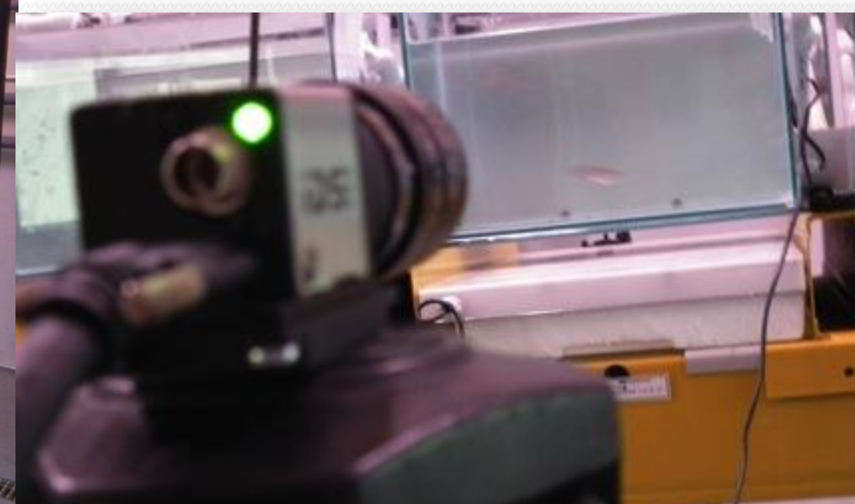


Monitoring of zebrafish



Monitoring of zebrafish

Dorsal cameras



Behaviour assessed:

- Individuals
- Pairs
- Groups

Automatic analysis of behaviour

- **Get real-time information**
- Speed
- Distance travelled
- Acceleration
- Deceleration
- Time spent in specific areas
- Time spent active/motionless
- And many more

Hamza Alzu'bi and Waleed Al-Nuaimy, Electrical Engineering; unpublished data

Recognising signs of pain in zebrafish

Healthy

- Continuous swimming
- Swimming in mid water
- Calm swimming
- Gentle turns

Unhealthy

- Immobile
- Increased use of tank bottom
- Bursts of erratic swimming
- Unusual behaviours

Conclusions

Major advance in diagnosing the symptoms of poor welfare

Automatic monitoring

Testing of analgesics or painkillers to refine protocols

NC
3Rs

Acknowledgements

NC 3Rs
National Centre for the Replacement Refinement & Reduction of Animals in Research

- Co-PIs
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  - Dr Matthew Leach, Newcastle
  - Dr Tom Pottinger, CEH Lancaster
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  - NC3Rs
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Analgesic	Dose	Species	Side effects	Efficacy
Lidocaine	0.1-2mg/kg	Trout Zebrafish	None observed	Very efficient at 1mg/kg or 1mg/L
Morphine	5-50mg/kg	Trout Flounder Goldfish	None observed	Very efficient at 5mg/kg
Buprenorphine	0.01-0.1 mg/kg	Trout	Reduced activity No impact on feeding or ventilation	Not efficient
Carprofen	1-5mg/kg	Trout	Depressed activity Increased ventilation	Reduced time to feed using 2.5mg/kg
Butorphanol	0.25-5mg/kg	Koi carp (0.4) Dogfish		NS Koi – improved behaviour
Ketoprofen	1-4mg/kg	Koi carp (2) Dogfish	No impact on behaviour in Koi	Not efficient

Fish Species	Swimming	Ventilation Rate	Feeding	Plasma Cortisol	Light Preference	Changes in Gill Physiology	Anomalous Behaviours
Rainbow trout	↓	↑	↓	↑	NM	NM	√
Common carp	↔	↔	↓	NM	NM	NM	√
Zebrafish	↓	↑	↓	NM	NM	NM	√
Nile tilapia	↑	NM	NM	↔	↑	↑	NM

Sneddon 2009, ILAR J, 50, 338-342; Sneddon 2011 J. Consciousness Stud. 18, 209-229