Refinements in thermal and mechanical nociceptive threshold testing in mice

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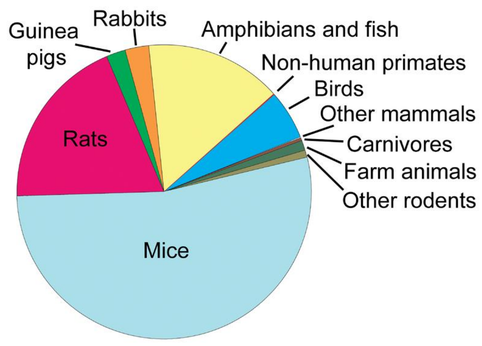
PM Taylor, MJ Dixon, JR Deuis, I Vetter,
Topcat Metrology Ltd, UK; University of Queensland, Australia.

The University Of Queensland AUSTRALIA | IMB
Institute for Molecular Bioscience

Background

Thermal and mechanical nociceptive threshold testing (NTT) in rodents is widely used for investigation into mechanisms and treatment of pain.

The most common methods used are tail flick, hot plate or Hargreaves systems for thermal testing and von Frey filaments (vF) for mechanical trials.



Animals used in research

Mechanical nociceptive threshold testing in mice using von Frey filaments

Hargreaves thermal testing system

Von Frey filaments

Filaments leave much to be desired (1):

- Each data point requires a number of stimuli followed by mathematical derivation of threshold force (usually the "up-down" method or percent response)
- The filaments have a range of diameters and therefore the contact area changes; data are artificially spread out and therefore non-parametric (often ignored!)
- Electronic ramped vF systems avoid these problems but usually employ an inappropriate force range for mice

Hot plate thermal testing

Hargreaves thermal testing is widely accepted and uses a ramped stimulus, but:

- It requires 30 minutes or more acclimatization time
- It cannot be performed in the same cage as mechanical testing

Moving to a new environment

Moving the test subjects to a new environment is stressful. They need to acclimatize - to explore and then settle in each new envrionment. This takes time.

However well executed – handling is always stressful to mice.

Need for refinement!

Test both modalities in one cage

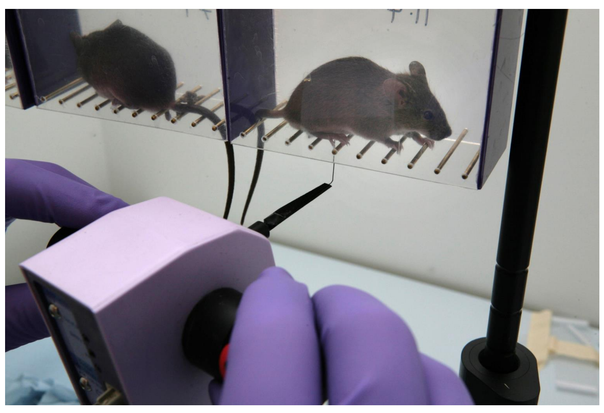
- Less stress
- Saves time

Use electronic ramped mechanical stimuli with force range suited to mice

- Fewer stimuli per data point
- Less interference, less stress
- No mathematical derivation of data required
- Saves time

We evaluated methods that allow both thermal (TT) and mechanical (MT) thresholds to be measured in the same environment, removing the need to move the mice for the second modality

Novel systems for thermal & mechanical testing
(Topcat Metrology Ltd)



Mechanical ←

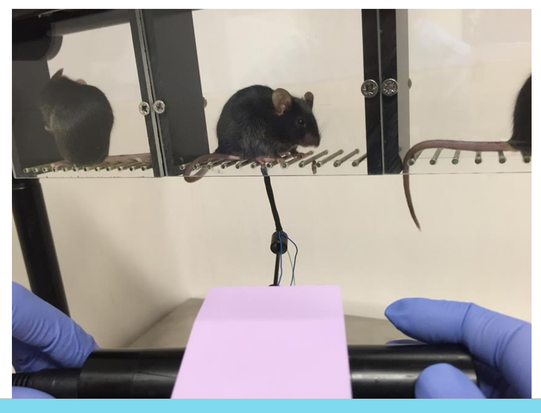
MouseMet electronic von Frey

- *0.1- 7g force range*
- *0.3 mm diameter probe*
- *Rotary 'soft' transducer eliminates hand tremor*
- *MT (gf) automatically recorded at foot withdrawal*

Thermal →

MouseMet Thermal

- *Mouse-specific thermal probe*
- *Rounded 2.5 mm diameter metal probe*
- *Mounted on same style rotary transducer*
- *Heating triggered on contact*
- *TT (° C) automatically recorded at foot withdrawal*



Methods

Adult male C57BL/6J mice (n=12, groups of 4-8) were tested before and after: Sensitisation with carageenan, capsaicin or burn injury & Treatment with opioid analgesics

The testing environment

The mice were transferred from their home cage to dedicated one-dimensional runs *(Topcat Metrology Ltd)*

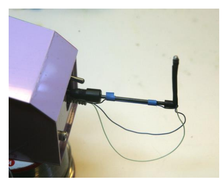
- The mouse sits sideways to the operator
- Foot bars are carefully spaced

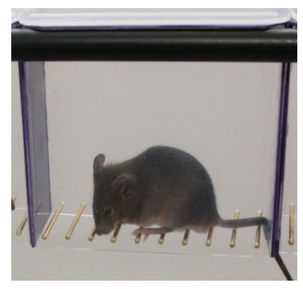
Five minutes were allowed for acclimatization before the first test

All stimuli were applied to the plantar surface of the hind paw

Thermal testing

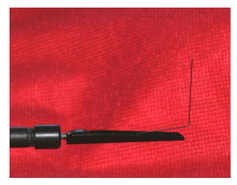
MouseMet Thermal probe
Probe heating starts at 37° C
Heats at 2.5° C/sec
Automatic cut-off at 60° C
Average of 3-5 measurements = TT



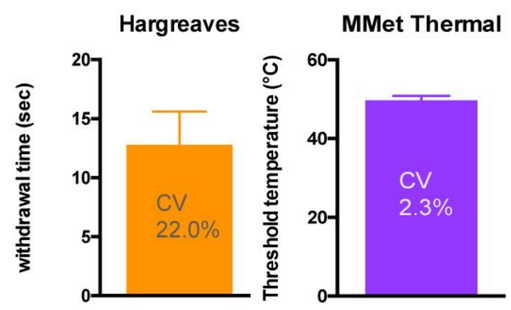


Mechanical testing

MouseMet electronic von Frey system
Average of 3-5 measurements = MT

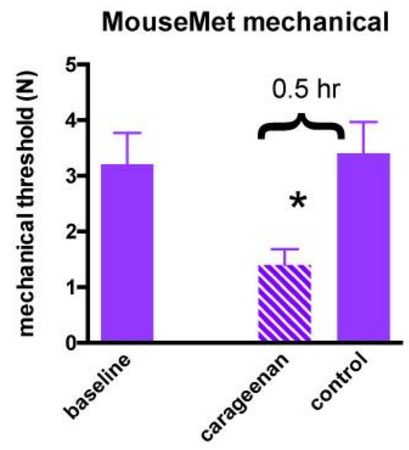


Results

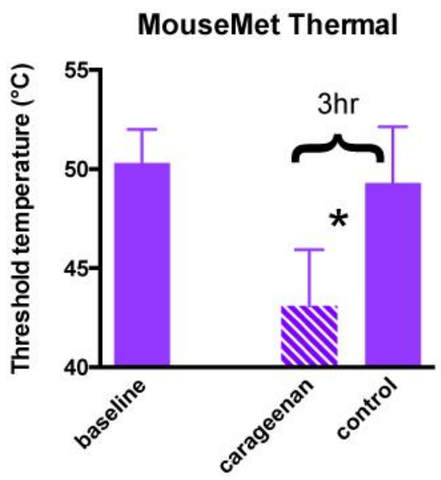


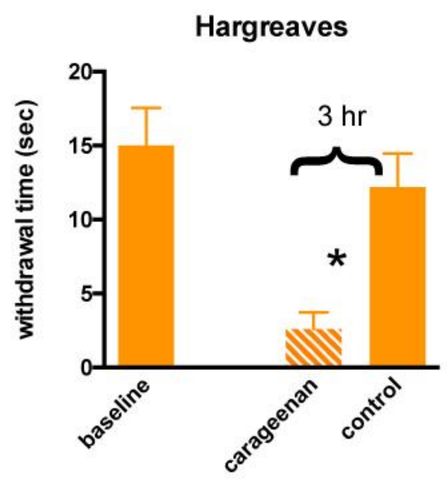
Naïve C57 mice (n=8) (mean ±SEM)

MT in 8 mice after carageenan



TT in 8 mice after carageenan





← *Foot withdrawal time in 8 mice after carageenan*

- TT were similar after capsaicin (control; treated) (49.7±0.6; 44.8±1.2° C) and burn injury (50.8±0.5; 43.2±0.6° C).
- Comparable to the Hargreaves test (11.8±1.5; 4.2±0.8 sec) and mechanical testing after burn injury (3.9±0.7; 1.1±0.2 gf)
- Capsaicin-induced thermal allodynia and opioid mediated analgesia were also detected

Conclusions

- Thermal and mechanical NTT performed in the same environment detected allodynia and analgesia in accordance with previous reports
- Mechanical thresholds were consistent with previously reported data and TT were statistically indistinguishable from Hargreaves data
- This method refines NTT and enhances animal well-being since testing is completed more quickly and the mice are not stressed by extra handling. Tighter data lead to reduction as fewer mice are needed

References

1) Bove G (2006) Pain 124: 13-17
2) Deuis J, Vetter I (2016) Temperature 3: 1-9

Ethical approval

Ethical approval for in vivo experiments in animals was obtained from the University of Queensland animal ethics committee. Experiments involving animals were conducted in accordance with the Animal Care and Protection Regulation Qld (2012), the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th edition (2013) and the International Association for the Study of Pain Guidelines for the Use of Animals in Research

SEB conference June 2016: Improving experimental approaches in animal biology: Implementing the 3Rs