Refinements in thermal and mechanical nociceptive threshold testing in mice

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

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.

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 environment. This takes time.

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

Results

MT in 8 mice after carageenan

TT in 8 mice after carageenan

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

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


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 Regulations Qld (2010), the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, the internet (2013) and the International Association for the Study of Pain Guidelines for the Care of Animals in Research.