Comparison of mechanical threshold testing in mice using von Frey filaments and the MouseMet electronic system

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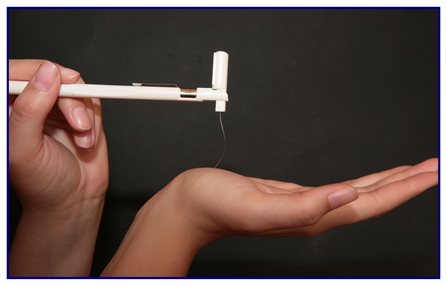
Background

Mechanical nociceptive threshold (MNT) testing using von Frey filaments (vF) has been the standard method for testing sensory and nociceptive thresholds in pain research for many years. However, this method has a number of disadvantages (1):

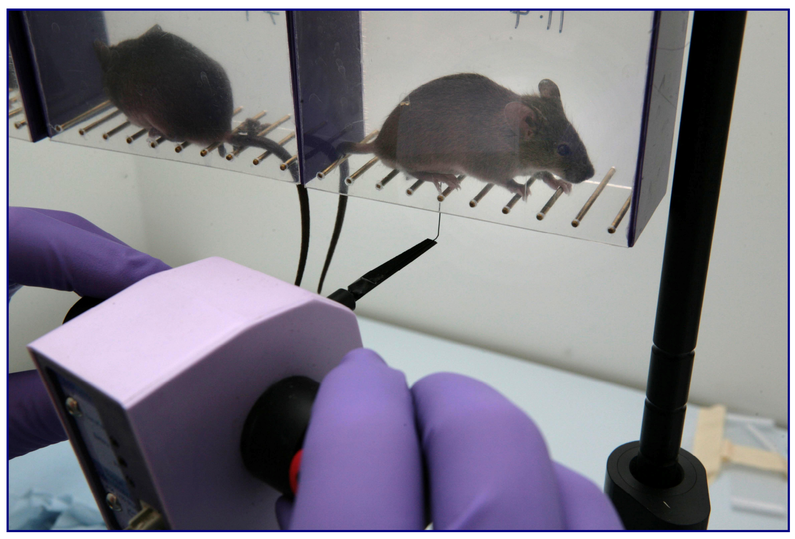


Each von Frey filament provides a constant force once it has buckled

•The cross-sectional area of each filament is different, and the contact area and profile change as the filament buckles. This makes it almost impossible to infer the tissue pressure.
•Increasing forces are applied in discrete, non-linear steps, necessitating the application of repeated stimuli for one MNT data point. Only an approximation of the MNT is achieved and the data are non-parametric.

Electronic ramped force systems produce continuous data and reduce the number of stimuli required. However those using stiff force transducers are unsuccessful in mice where the low forces are dwarfed by hand tremor and the effects of side-loading on the force transducer.

We compared MNT collected using von Frey filaments and MouseMet, a novel electronic system employing a “soft” transducer to remove the effects of hand tremor.



MouseMet, the electronic von Frey system with a 7gF range, designed specifically for mice

Methods

MNT were measured in 39 mice in three unrelated studies to assess mechanical hypersensitivity.

Data were collected from:
• 7 male 10-12 week old 129OlaHsd mice during investigation of streptozotocin-induced diabetes (Graph A)
• 24 adult female B6 mice during an investigation into the effects of a novel analgesic on complete Freund’s adjuvant (CFA)-induced hypersensitivity (Graph B)
• 8 male 10-12 week old 129OlaHsd mice with nerve injury-induced hypersensitivity (Graph C)

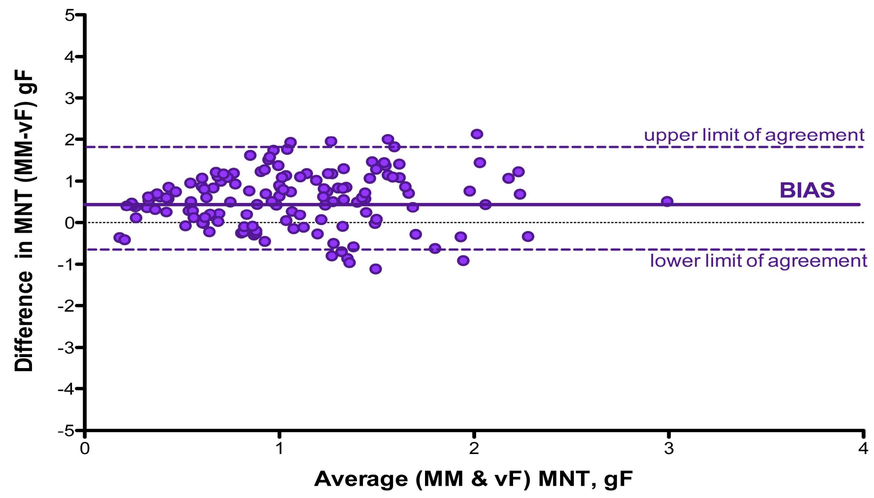
MNT were measured using vF and MouseMet (Topcat Metrology Ltd) (MM), an electronic system based on a soft spring force transducer (0.25mm tip). Measurements were also made on 24 of the mice (Graph D) using an improved (even softer) MouseMet system to eliminate reactions to the initial touch-on of the tip.

Mice were tested before treatment and on another 2-3 occasions up to 26 days after induction of hypersensitivity. The mean of 3-5 MM measurements taken at ≥20 sec intervals and 1-3 series of up/down vF measurements (2) was taken as the measurement for one time point.

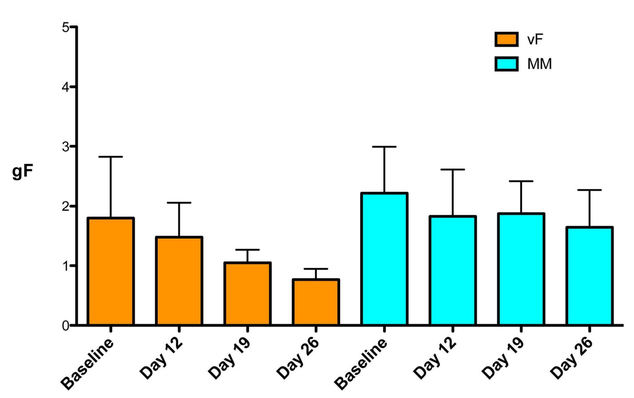
All measurements were made on a hind paw, with vF before MM. 140 duplicate measurements were made at the same site. Data were examined using Bland-Altman plots (3).

Results

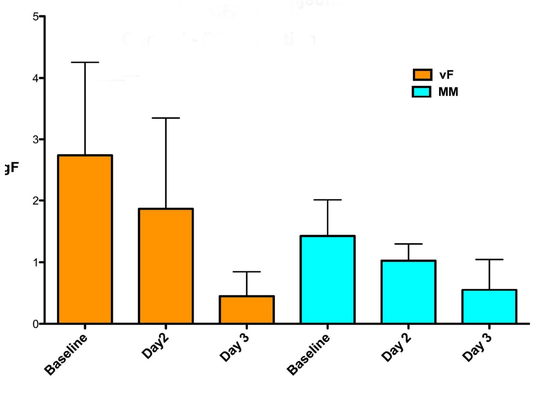
Mean (SD) vF MNT were 1.06 (0.94) (range 0.002 - 4.06) gF and MM were 1.51 (0.80) (range 0.30 - 4.07) gF. The mean (SD) bias was 0.57 (0.67) gFand 95% limits of agreement -0.75 - 1.88 gF. MM was easy to use and required fewer tests per animal (≤5) than vF (≥6).



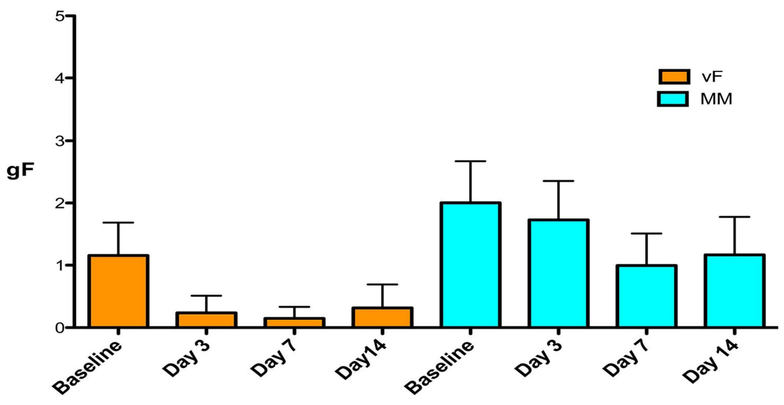
Bland Altman plot of MNT measured with vF and MM in 39 mice



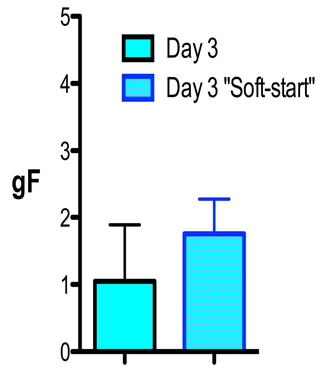
Graph A, MNT in 7 diabetic mice



Graph B, MNT in the control group (N=4) from 24 mice



Graph C, MNT in 8 nerve injured mice



Graph D, MNT in 24 mice, post treatment, (6 groups combined) comparing standard with improved (softer) MouseMet

Conclusions

Data from MM and vF were sufficiently comparable for the electronic system to be accepted as a robust alternative to vF filaments for pain research in mice. Testing time and the number of nociceptive stimulations may therefore be reduced, leading to REFINEMENT. As a result of this study, improvements have been made to the MouseMet electronic system to reduce variability, particularly at low forces. This should lead to a REDUCTION in the number of animals required.

References

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(2) Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1986) Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 53: 55-63
(3) Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 327: 307-310