



Evaluation of a new electronic von Frey system for measurement of mechanical thresholds in mice



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Introduction

Nylon von Frey filaments have been routinely used to measure mechanical nociceptive thresholds and hypersensitivity in animal models of pain and in the development of effective analgesia. Despite widespread use they have a number of inherent limitations.

The filaments often produce marked variation in thresholds because they create unpredictable forces. The thickness and cross section of each filament defines the load at which it buckles, and the tip geometry determines the surface area over which the force is applied when it buckles (Bove 2006). Hand tremor makes it difficult to apply the force vertically in a controlled manner, essential for consistent data, because the filament is stiff in the axial direction until the point at which it buckles (Bove et al. 2003). Filaments provide only an approximate indication of a threshold that exists on a continuum, as each filament provides a discrete force (and not a linear progression), so threshold may fall between different filaments (Harvey 1997). Cage arrangements allow the mouse to move around on a grid which obscures much of the foot. The operator has to chase the mouse round the cage, often from an uncomfortable position. Considerable training is required to achieve consistency within and between operators (Personal observations).

Alternative techniques have been developed (e.g. Ugo Basile Planter von Frey or Somic Electronic von Frey), but the problems still apply. MouseMet (Topcat Metrology Ltd) aims to solve these problems (See Box 1).

Methods

Animals & Surgery: Eighteen male CD1 mice (Charles River, Kent, UK) weighting 29 to 39.4 g underwent bilateral vasectomy* via the scrotal approach under isoflurane anaesthesia with oxygen (Induction: 5% at 2 L min⁻¹, Maintenance: 2.5% at 0.5 L min⁻¹).

Analgesia: The mice received either saline, 20 mg kg⁻¹ Meloxicam subcutaneously 30mins prior to surgery or 5 mg kg⁻¹ Bupivacaine infiltrated into the surgical wound intra-operatively (n=6 per group).

Procedure: Mechanical nociceptive thresholds were recorded using standard calibrated von Frey filaments (Bioseb, Cedex, France: Figure 1) and the recently developed MouseMet (Topcat Metrology Ltd: Figure 2) (0.3mm diameter probe tip). Thresholds were recorded alternately on both hind feet (mean of 6 readings, 3 per foot) in a random order before and 1 hour after surgery with a 5 minute interval between probing the same paw.

Analysis: Data were normally distributed with homogeneous variance. Repeated measures ANOVA was used to compare mean thresholds, between pre and post surgery time points (*within-subject factor*) and analgesic treatment groups (*between-subject factor*). P<0.05 was considered significant.



Figure 1: von Frey filaments



Figure 2: Threshold recording with the MouseMet

Box 1. The MouseMet (UK Patent Application 1105990.4)

- Mouse is caged in long, narrow run - encourages it to remain sideways to the operator
- Run is height adjustable, elbows comfortable on benchtop and mouse feet at eye level
- Floor bars spaced to maximise the plantar area available
- Operator holds MouseMet by two handles to advance the probe to the pawForce is applied by rotating the two handles with measurement arm horizontal (see Figure 3)
- 45° rotation applies the full force (4g), therefore insensitive to hand tremor
- At threshold MouseMet is removed downwards & force ramp displayed on the screen

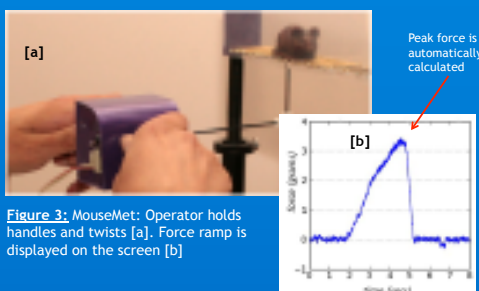


Figure 3: MouseMet: Operator holds handles and twists [a]. Force ramp is displayed on the screen [b]

Results

- The thresholds recorded with the MouseMet were less variable (smaller standard errors) than those recorded with the von Frey Filaments (see Figure 4a & b).
- The operators reported that the MouseMet was easier to use than the filaments.
- Mean thresholds recorded with the filaments were not significantly different pre compared to post operatively or between the analgesic treatment groups (Figure 4a).
- Mean thresholds recorded with the MouseMet were significantly lower post compared to pre operatively (P=0.007), but there was no significant analgesic treatment effects (see Figure 4b).

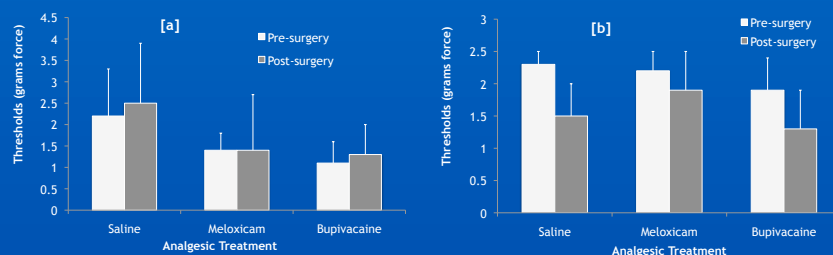


Figure 4: Mean mechanical nociceptive thresholds recorded with von Frey Filaments [a] and MouseMet [b] (Means ± 1SE are displayed).

Discussion

The findings demonstrate that nociceptive thresholds were less variable when measured with the recently developed MouseMet von Frey device compared to standard calibrated von Frey filaments. This is most likely to be because (1) MouseMet provides a linear scale of force measurement rather than the discrete points of the von Frey hairs, (2) the effects of hand tremor, (particularly at the lower forces) are removed, (3) a smaller number of tests are required, (4) greater operator comfort leads to better technique.

As a consequence of the consistent threshold measurement, the Mouse Met was able to identify a significant decrease in nociceptive thresholds following bilateral scrotal vasectomy. The development of secondary hyperalgesia, resulting in reduced thresholds is rarely reported in other studies of mice undergoing surgery, but corresponds to significant increase in the frequency of pain-related behaviours and facial expressions observed from pre to post vasectomy in the same mice (Leach et al. 2011). The failure to detect this difference using currently available methods (e.g. von Frey filaments) is likely to be due to poor accuracy in measuring sensory thresholds.

The lack of difference in thresholds observed post-vasectomy between the analgesic groups is likely to relate to local anaesthetic infiltration and NSAID administration being shown to have minimal or no effects on peripheral hyperalgesia.

Conclusions

- The two methods of recording mechanical nociceptive thresholds appear to differ in their sensitivity.
- MouseMet showed less variability than the von Frey Filaments.
- Surgery-induced hyperalgesia was observed.
- But was unaffected by administration of either 20 mg kg⁻¹ Meloxicam (SC) or 5 mg kg⁻¹ Bupivacaine (*local infiltration*).

References

1. Bove et al. (2003) J. Neurosci Methods 123: 23-30.
2. Bove G (2006) Pain 124: 13-17.
3. Harvey LO (1997) Spat Vis 11: 121-128.
4. Leach et al. (2011) PLoS One: In Press.

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