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Mechanical nociceptive threshold (MNT) testing in rats: effects of probe tip configuration and cage floor characteristics for electronic von Frey (EvF) compared to traditional filaments (Fil)

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Background

Devices to evaluate nociceptive sensitivity measure threshold to withdraw or a latency to respond.

von Frey filaments (Fil) are still the "gold standard" method for measurement of mechanical nociceptive thresholds (MNT) in rodents. However, increasing forces are applied in discrete, non-linear steps, and the MNT can only be derived.

A further disadvantage of traditional filaments is that several replicate stimuli are required for each data point where fewer are required when using an electronic ramped stimulus.

Aim

In order to refine MNT testing in rats we evaluated the effects of probe and flooring characteristics on MNT measured using a rat-specific EvF (RatMet, Topcat Metrology Ltd) and traditional von Frey Filaments (Stoelting).

Methods

This study was performed in accordance with the Animal (Scientific) Procedures Act 2013 (EU Directive 2010/63/EU) UK Home Office regulations project licence (PPL 30/3156).

Twelve adult male (Wistar and Sprague Dawley) rats (420 ± 27g) were studied. They were randomly assigned for withdrawal threshold testing (both hind paws) using four different EvF probes: 9L (0.9 mm diameter, long); 9S (0.9 mm diameter, short); 5L (0.5 mm diameter, long); 5F (0.5 mm diameter, flexible) as well as von Frey filaments (Fil) using three floors: mesh (12 x 12 mm squares), wide spaced bars (16 mm apart) or narrow spaced bars (6 mm apart) (Fig. 1).

Triplicate EvF readings were taken and the up-down method used for Fil (Chaplan et al., 1994). Within group comparisons were made with 1-way repeated measures ANOVA, and between group comparisons with 2-way ANOVA.

Experimental set up

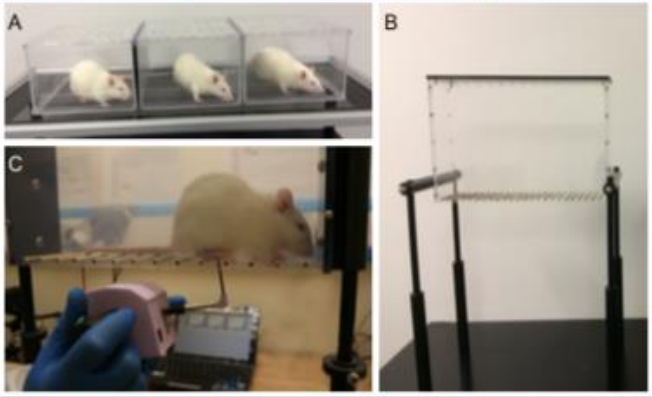

Figure 1. (A) Acclimatisation of animals prior to testing with Fil; (B) Perspex box for use of EvF with narrowly spaced bars (6 mm); (C) EvF in use with rat on wide spaced bars (16 mm).

Results

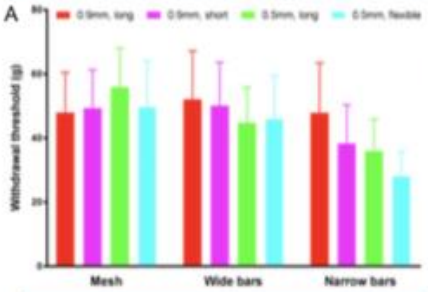

Figure 2. Hind paw MNT using different EvF probes and testing surfaces (A). There were no significant differences ($p > 0.05$, 2-way ANOVA) in MNT's with different floor surfaces however the mesh (B) was technically easier for accurate application of EvF probes and the rats appeared more comfortable than on bars (C & D; wide spaced bars)

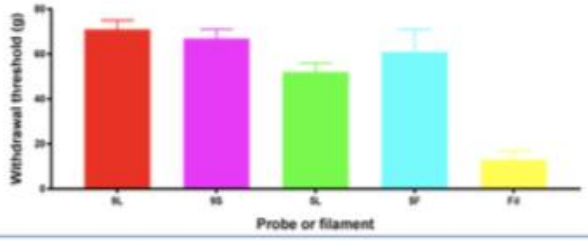

Figure 3. EvF probe length did not influence MNT. However MNT's with 0.9 mm diameter (9L, 9S) probes were significantly higher (1-way ANOVA, Tukey's post-tests, $p = 0.0001$) than 0.5 mm diameter (5L, 5F) and all EvF MNT's were significantly higher (Tukey's post-tests, $p < 0.0001$) than von Frey filaments (Fil).

Conclusions

- Consistent with previous RatMet data (Pang & Shuster, 2015) larger diameter probe tips produces higher MNT; probe flexibility and length are less influential.
- Mesh flooring allows more natural posture for MNT testing.
- Fil result in lower MNT than EvF with similar probe tip diameter (~0.5 mm). However, filaments bend at their maximum applied force so the contact area is considerably less than filament diameter (and not precisely known; Fig. 4).

Figure 4. (A) Filament touching skin; full cross section in contact; (B) Force applied to filament, bends; edge only of filament in contact. Area much less: higher pressure (Adapted from Bove, 2006).

References

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Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L. (1994) Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods*, **53**, 55-63.

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