Mechanical nociceptive threshold testing in mice: evaluation of the errors incurred using the up-down method to analyse von Frey filament data

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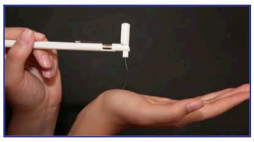
Background

Von Frey filaments 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.



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

The two most common ways of extracting the data are the up-down and % response methods. The up-down method, originally described by Dixon (1965) for analysing all-or-none (yes-no) responses in small data sets, is the most commonly used to derive MNT from the filament data (Chaplan et al 1994).

The up down method

Filaments of different force ratings are used in succession; if a response is elicited, then the next lower rated filament is used; if there is no response, the next higher rated filament is used. Testing normally continues until 4 measurements have been made after the first change in direction.

gf	Mouse 1						
6							
4							
2			1				1
1.4		0		1		0	
1	0				0		
0.6							
0.4							

gf	Mouse 2							
6								
4				1		1		1
2			0		0		0	
1.4		0						
1	0							
0.6								
0.4								

gf	Mouse 3					
6						
4						
2						
1.4				1		1
1	1		0		0	
0.6		0				
0.4						

gf	Mouse 4								
6					1				
4				0		1			
2			0				1		1
1.4		0						0	
1	0								
0.6									
0.4									

Record of "yes-no" documented during testing: gf is filament size; 1 = response, 0 = no response

A factor k is generated from these up-down data using Dixon's (1965) table appropriately reproduced by Chaplan et al (1994). The MNT is calculated from $10^{(X+kd)}/10^4$ where, in $\log_{10}$ units, X is the value of the last filament used and d is the average increment ($\log_{10}$) between filaments. Dixon's up-down method advises equal increments between filaments with d = sigma, the standard deviation of the dataset ($\log_{10}$) ± 50%.

Practical limitations of the up-down method with commercial filaments

1) The filaments are not equally spaced
   - an average (d = 0.22) is commonly used
2) The filaments are incorrectly labelled in log units
3) The method assumes that the first filament used is close to the mean value of the data set (which is unknown) *see note*
4) Sigma has never been defined (as it could not be measured) so the log increment may not be equal to sigma *see note*

Correct progression	Actual progression	Correct log of actual	Number on handle
8	8	4.9	4.93
5.6	6	4.78	4.74
4	4	4.6	4.56
2.8			
2	2	4.3	4.31
1.4	1.4	4.15	4.17
1	1	4.0	4.08
0.7			
0.5	0.6	3.78	3.84
0.35	0.4	3.6	3.61
0.25			
0.18	0.16	3.2	3.22
0.1			
0.08	0.07	2.84	2.83

Diff 0.15 (log units)

Correct progression & actual labelling on commercially available filaments

Commercially available von Frey filaments - force range used for mice

More on point 3) - by example: take a typical mouse MNT as 2g – based on real data collected using a ramped electronic von Frey system (MouseMet, Topcat Metrology Ltd). The 1st filament should be 1 or 2g. However, starting low is common: if the start is at 0.16g or 0.4g, several zeros are generated before the first response (and therefore the 1st change in direction). This may lead to use of the wrong coefficient which will generate the wrong answer!

More on point 4) – this may be considered a limitation: a property of the data set (the SD) *generated* by the method is required to *implement* the method. Fortunately, testing for a set of readings often requires only 3-6 filaments – this indicates that the spacing is probably appropriate for the sigma of the data set.

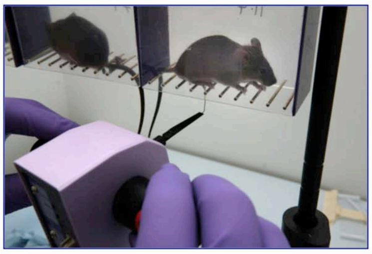
However, robust analog data are needed to generate sigma; without a ramped system with the right force range (0-7g), sigma cannot be measured in mice.
MouseMet is the first analogue instrument enabling accurate MNT measurement in this force range.

Methods

Measurement in mice

A novel electronic von Frey instrument designed for the low forces required for mice (MouseMet, Topcat Metrology Ltd) was used to obtain the data needed to calculate sigma.

Baseline, pre-treatment MNT was measured in three groups of 10-24 mice from independent studies. The average of 3-5 measurements from each animal was taken as the MNT for that mouse. This generated sigma of 0.15g ($\log_{10}$) in all groups.



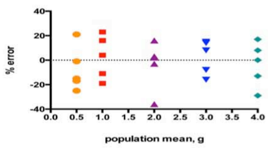
The MouseMet electronic von Frey has a 7gf range designed specifically for mice

Evaluation of the up-down method

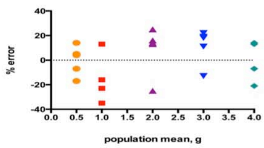
This knowledge of sigma was used in mathematical simulation:
A hypothetical data set of normally distributed thresholds ($\log_{10}$) with sigma 0.15g was generated in MS Excel with means of 0.5, 1, 2, 3 & 4 g using first the ideal case: a true logarithmic progression of hypothetical filaments of 0.15 ($\log_{10}$) intervals.
Test cases were then used to simulate the problems outlined:

1) The common filament values of 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0 g, correctly logged, with d = 0.22
2) These values using the log units on the filament handle.
3) Testing started a long way from the mean, with all "no"s (zeros) factored in when extracting k from the table

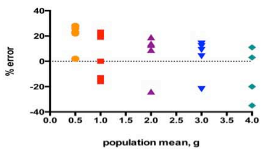
Results



The ideal case - true logarithmic progression of hypothetical filaments: errors of ±20%



The common commercially available filament values: errors of ±30%



Values on the handle: errors of ±40%

Up down progression	Factor k	% difference
0XXXX0	1.60	
0000XXXX0	2.00	25%
0X0XXX	0.61	
0000X0XXX	0.76	25%
0XX0XX	0.30	
0000XX0XX	0.50	40%

Effect on k of using a stream of leading zeros (000) due to starting too far below the mean

Further simulations suggested that in certain cases the errors may be cumulative.

Conclusions

The spacing of commercially available filaments is usually appropriate. The up-down method, although widely accepted, gives only an approximation of MNT. Commercial filaments are not ideally spaced nor correctly labelled. Ramped stimuli from an electronic system with an appropriate force range is preferred.

References

Dixon WJ (1965) The Up-and-Down Method for Small Samples. *J Am Stat Assoc* **60** 967-978

Chaplan SR (1994) Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53** 55-63

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