Impact of study blinding on outcome of behavioural studies in rat pain models

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Pain in laboratory animals may be assessed by measuring an evoked behavioural response following stimulation, e.g. paw withdrawal threshold (PWT) in rats to a punctuate stimulus such as a von Frey filament (tactile allodynia).

We demonstrate here the impact of adopting fully-blinded protocols on the variability of tactile allodynia in studies on reference analgesic agents in rat models of tibial nerve transection (TNT, neuropathic pain) and mono-iodoacetate injection to the knee (MIA, osteoarthritis pain). We previously employed a protocol where the observer was blinded to the identity of the treatment group, but all the rats in the group received the same treatment (single blind). We then adopted a protocol in which a colleague randomly allocated treatments and carried out the dosing. The observer measuring the behavioural end-point was fully blinded to treatment (fully-blind).

PWT in normal rats is approximately 8g. Following TNT surgery, PWT decreases to ≤4g indicating tactile allodynia is present. In a vehicle-controlled single blind study of the effects of gabapentin, Median PWT (measured using the Field method Pain 1999, 80; 391-398) in the post-vehicle group at +2h was 3g (range 2-4g). Median PWT at +2h after gabapentin 100mg/kg p.o. increased to 10g (P<0.001, Mann-Whitney U test, n=6,). In contrast, in a fully blinded study of pregabalin, the same vehicle produced PWT ranging from 1 to 8g at +2h (median 2g). This level of variability which had been masked by the single-blinded protocol resulted in an inability to detect the effect of pregabalin 10mg/kg po (PWT median 8g,(P>0.05, Mann-Whitney, n=9, +2h) in the fully blind study.

The pregabalin study was repeated in a fully blinded protocol using the up-down method (Chaplan et al. 1994, J. Neurosci. Methods 53:55-63) to measure 50% PWT. Good control of vehicle group variability was observed at +1.5h (50% PWT 2.5g mean +/- 0.6g SEM, n=7) and clear effects of pregabalin were detected (50% PWT 6.1g +/-1.1g, P<0.01 ANOVA). Furthermore, similar findings were obtained in single- and fully-blinded studies of valdecoxib in the rat MIA model.

These results suggest that the true variability of evoked pain behavioural responses in rat models may be masked by the use of inappropriate study blinding procedures. Consequently, rat studies on novel analgesic agents may give ‘false positive’ predictions of efficacy in clinical studies. Observers measuring pain behavioural responses in the rat need to be fully-blinded to treatment if animal data is to be interpreted reliably.