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RESEARCH PAPER

Challenges of thermal nociceptive threshold testing in the donkey

Nicola J Grint*,†, Helen R Whay†, Thierry Beths*, Kathy Yvorchuk* & Joanna C Murrell†
*Ross University School of Veterinary Medicine, St Kitts, West Indies
†Animal Welfare and Behaviour Group, University of Bristol, Bristol, UK

Correspondence: Nicola J Grint, Cave Veterinary Specialists, Georges Farm, West Buckland, Wellington, Somerset, TA21 9LE, UK. E-mail: nicki.grint@yahoo.co.uk

Abstract

Objective To evaluate a thermal nociceptive threshold (TNT) testing device in the donkey, and the influence of potential confounding factors on TNTs.

Animals Two groups (Group 1 and Group 2) of eight castrated male donkeys aged 4–9 years, weighing 105–170 kg.

Methods TNTs were measured by heating a thermal probe on skin until an end-point behaviour (threshold temperature) or a cut-out temperature (51 °C) was reached. The withers and the dorsal aspect of the distal limb were used as sites for TNT testing. The effects on TNT of different confounding factors: the limb tested; rate of heating; and ambient temperature were evaluated. Data were analyzed using general linear models, and Mann-Whitney tests, $p < 0.05$ was considered significant.

Results End-point behaviours (skin twitch or donkey looking at test device) when the thermal probe heated the withers were observed in approximately half of tests. TNT was (mean ± SD) 46.8 ± 2.85 °C. Subsequently the limb was evaluated as the test site in Group 1 followed by Group 2 donkeys; end-point behaviour being a foot-lift. In Group 1, 72% of tests ended in an end-point behaviour but the response rate was lower in Group 2 (20%), although TNTs were similar [(47.6 ± 3.3) and (47.3 ± 3.0) °C respectively] for responding animals. Rate of heating, ambient temperature and laterality (right or left) did not affect thresholds, but mean TNT was significantly higher in the forelimb (48.5 ± 2.8 °C) than the hind limb (47.4 ± 2.8 °C) ($p = 0.012$).

Conclusions When a thermal probe cut-out temperature of 51 °C was used in TNT testing in the donkey a high proportion of tests did not produce an identifiable end point behaviour. Higher cut-out temperatures damaged the skin. Under these conditions, thermal nociceptive threshold testing appears not be an appropriate analgesiometry technique in the donkey.

Clinical relevance TNT testing under these conditions is not suitable form of analgesiometry for donkeys.

Keywords donkey, equine, nociceptive threshold testing, thermal.

Introduction

The donkey has a reputation of stoicism with more subtle behavioural pain expression than horses. Current pain assessment techniques in animals depend heavily on behavioural cues, making assessment of pain difficult in donkeys. Nociceptive threshold testing (NTT) is a technique used to quantify nociceptive thresholds and analgesic efficacy, and has been used in various species of animals including horses. In equidae, three modalities of noxious stimuli have been applied; mechanical (Love et al. 2012) thermal (Robertson et al. 2005; Love

et al. 2012) and electrical (Spadavecchia et al. 2003). Delivery of more than one type of noxious stimulus is recommended in order to fully evaluate the functional status of the nociceptive pathways (Nielsen et al. 2009).

In laboratory animals, thermal NTT is widely employed in classical experimental tests such as the tail flick test, tail withdrawal, paw withdrawal or hot plate test (Le Bars et al. 2001). Either the time taken (latency) for an animal to respond to an applied constant temperature, or the temperature at which an animal responds (if there is a ramped change in temperature) is measured. Thermal nociceptive threshold (TNT) testing has not been evaluated in the donkey previously, although the modality has been used in horses to evaluate the effectiveness of alpha-2 adrenoceptor agonists (Wegner et al. 2010), local anaesthetics (Robertson et al. 2005) and opioids (Love et al. 2012).

An ideal NTT stimulus should fulfil certain criteria. The stimulus should be easy to apply and repeatable, the behavioural response should be clear and easily identifiable and the stimulus should produce no lasting harm to the animal (Beecher 1957).

In this study we aimed to evaluate a thermal probe-based TNT device that had been developed for horses (Wegner et al. 2010; Love et al. 2012), in the donkey. The initial probe location to be evaluated was the withers (Study 1), and subsequently the limb (Studies 2 and 3). We investigated whether experimental factors such as whether right or left side of the body (laterality) (Studies 1 and 2), limb tested (Study 2), presence or absence of a companion (Study 2), level of distraction (Study 2), rate of heating (Study 2) and ambient temperature range experienced (Study 3) influenced the TNTs. Data collected in two groups of donkeys separated by 12 months were also compared. Certain experimental conditions (i.e. a companion present, limb tested, rate of thermal probe heating) were standardized as per Study 2, although location and level of prior training of the donkeys were not identical.

Materials and methods

Ethical approval

This study received ethical approval from the University of Bristol (UB/10/019) and Ross University Institutional Animal Care and Use Committee.

Animals

Two groups of eight adult castrated male donkeys were studied. Group 1 donkeys were aged between four and eight years, and weighed between 105 and 160 kg. This group was studied in November and December 2010. Group 2 donkeys were aged between four and nine years and weighed between 152.5 and 170.5 kg. Group 2 donkeys were studied in November 2011. Donkeys in each group were kept together at grass as part of a larger research herd. Donkeys were healthy, based on clinical examination, and were receiving no concurrent medication. The groups contained the same animals used for mechanical nociceptive threshold testing described by Grint et al. (2014). Mechanical nociceptive threshold tests had been completed a minimum of four days before TNT testing commenced.

Testing environment

Testing was conducted in one of three possible locations at Ross University School of Veterinary Medicine (RUSVM) on the island of St Kitts in the West Indies. One location was a shaded pen (3×3 m) bedded with sand at the large animal facility (LAF). The second location was a shaded, concrete floored pen (3.3×3.7 m) at the outdoor large animal research park (LARP). The third location was an indoor laboratory (LAB) with the donkey penned in a corral (3.6×3.6 m) constructed of metal fence panels. Flooring was non-slip rubber mats. To produce a 'cool' environment in the LAB, the doors were closed and the air conditioning system used to produce low ambient temperatures. To produce the 'warm' environment, the double doors at the two sides of the LAB were opened to the outside. Donkeys were tested unrestrained in all accommodations, either alone or with another donkey from the research herd (companion). Ambient temperature was monitored continuously using a room thermometer (Radiometer Spares, UK). Fly repellent (Absorbine Supershield Red fly repellent; WE Young Inc, RI, USA) was applied to the donkeys' coats at the beginning of every test day. Drinking water was available during testing.

Device description and instrumentation

The TNT testing device (WTT1; Topcat Metrology Ltd, UK) comprised a thermal probe that contained