



Original Research

Analysis of Behaviors Observed During Mechanical Nociceptive Threshold Testing in Donkeys and Horses



Nicola J. Grint^{a,*}, Thierry Beths^{b,1}, Kathy Yvorchuk-St Jean^b, Helen R. Whay^a,
Joanna C. Murrell^a

^aSchool of Veterinary Sciences, University of Bristol, Bristol, UK

^bRoss University School of Veterinary Medicine, Basseterre, St Kitts, West Indies

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ABSTRACT

The aims of the study were to analyze and compare behaviors in horses and donkeys observed during nociceptive threshold tests with a mechanical stimulus applied to the limb. The purpose was to identify end point behaviors suggesting the animals had perceived the stimulus to be noxious. Six male castrated horses (aged 3–4 years, weighing 415–503 kg) and eight castrated male donkeys (aged 4–9 years, weighing 152.5–170.5 kg) were studied. Video data recorded during mechanical nociceptive threshold test were analyzed by a single observer. Behaviors were classified into short-duration event behaviors and longer duration activity/state behaviors. Frequency of behaviors within a test (event behaviors) and percentage time spent during the test (activity/state behaviors) were calculated. Data were compared between horses and donkeys using Mann–Whitney tests (nonparametric data) or *t*-test (parametric data). Significance was taken as $P < .05$. Behaviors during the tests were observed which could indicate the animals perceived the stimulus as noxious. These included flattening ears back against the head, and turning the head (horses) and chewing (donkeys) although these were not consistent across both species. Foot lifts were often preceded by other behaviors which suggests that the foot lift was not purely a reflex withdrawal response. A shift in weight toward the contralateral limb was a consistent prodromal sign for an end point foot lift.

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1. Introduction

In recent years, there has been increasing interest in behavioral expression of pain in donkeys. Regan et al [1] constructed an ethogram that was used to record behaviors in working donkeys. Certain behaviors changed in response to the administration of a nonsteroidal anti-inflammatory drug which suggested that these behaviors may be an expression of pain. Olmos et al [2] used a checklist of pain-related behaviors that correlated with

abnormal and potentially painful lesions found on post-mortem examination of donkeys in a donkey sanctuary. The findings of Regan et al [1] and Olmos et al [2] do suggest that donkeys may exhibit a wider repertoire of pain behavior than previously described in the literature [3], although the behaviors appear to be more subtle than those exhibited by other equidae.

To compliment behavioral assessments, nociceptive threshold testing (NTT) has been evaluated in the donkey [4–7], aiming to objectively measure the functional state of the nociceptive system. Nociceptive threshold testing is an objective method for investigation of threshold responses to different noxious stimuli and evaluates the somatosensory system in its entirety, including nociceptors, peripheral nerves, the spinal cord, brain stem, thalamus, and cortex [8]. When choosing a stimulus, it should be

* Corresponding author at: Nicola J. Grint, Georges Farm, Wellington, Somerset, UK.

E-mail address: ngrint@cave-vet-specialists.co.uk (N.J. Grint).

¹ Present address for T Beths: University of Melbourne, School of Veterinary Medicine, Werribee, Victoria, Australia.

repeatable, reliable, and easy to apply without producing lasting harm to the animal [9]. When evaluating different NTT modalities, end point behaviors need to be established. These are clear behavioral responses performed in response to the noxious stimulus, indicating that the animal has perceived the stimulus to be noxious.

Difficulty in interpreting end point behaviors in donkeys was found when developing different NTT methodologies. In thermal threshold testing using the withers site and visceral NTT using a rectal balloon model, testing was discontinued after initial pilot studies, in part due to the difficulty of interpreting and recognizing end point behaviors [5,7]. Mechanical and thermal NTT using the limb site were both initially more successful models, with foot lifts seen as end point behaviors in all tests where the animals responded [4,6,7]. The foot lift response has also been used in other species as an end point in mechanical NTT limb testing, for example, cattle [10], horses [11], and sheep [12]. This may represent a “complex” behavioral response to noxious stimuli, suggesting that perception of the stimulus has taken place or some may regard this response as a withdrawal reflex.

Given the subtlety of behavioral expression of pain in the donkey compared with the horse [3,13], one possibility is that other behaviors, which were cues that the animal had perceived the stimulus as noxious and therefore should have been interpreted as an end point behavior, were missed or misinterpreted. There have been no comparative studies between donkeys and horses analyzing their behavioral responses to identical painful stimuli. Pain, as defined by the International Association for the Study of Pain, is an “unpleasant sensory and emotional experience.” The measurement of nociceptive thresholds tests the sensitivity of the somatosensory pathways and can be standardized across the two species, but NTT does not measure any emotional experience that accompanies nociception. Such emotional experiences cannot be measured directly [14], although indices such as behavioral analysis can be used to try and identify the affective state of the animal along with the presence or absence of pain.

This study describes the analysis of data generated from videotaped behaviors during the application of the noxious mechanical stimulus to the limbs of horses and donkeys. The first aim of the study was to analyze behaviors observed during mechanical NTTs to try to identify behaviors other than a foot lift that may have suggested the donkey had perceived the stimulus to be noxious. This would in turn help identify alternative end point behaviors for future NTT in the donkey and establish whether the end point foot lift is a withdrawal reflex or involves higher cognitive function. The second aim of the study was to compare behavioral responses to mechanical NTTs in horses and donkeys.

2. Materials and Methods

2.1. Ethical Approval

This study received ethical approval from the University of Bristol (UB/10/019) and Ross University School of

Veterinary Medicine (RUSVM) Institutional Animal Care and Use Committee.

2.2. Animals

Six male castrated horses (aged 3–4 years, weighing 415–503 kg) and eight castrated male donkeys (aged 4–9 years, weighing 152.5–170.5 kg) were studied at the Large Animal Research Park (LARP) at RUSVM on the island of St Kitts in the West Indies. The donkeys had been at the LARP facility for at least 6 months and were habituated to handling. The donkeys had been part of a teaching herd, having been exempt from any procedures for a minimum of 4 months. The horses were retired race horses. They were imported to RUSVM and housed at the LARP 2 months prior to the start of testing. The horses were habituated to handling but had not been used for any studies or procedures at RUSVM. All animals had been assessed by a veterinary surgeon before the study started and were deemed healthy based on clinical examination. Both horses and donkeys were kept at grass in between testing and fed supplementary Guinea grass (all animals) and concentrates (horses) twice daily.

2.3. Mechanical Nociceptive Threshold (MNT) Testing

Each test was conducted in one of two identical outdoor pens at the LARP. The pens were 3.3 m × 3.7 m in size, with concrete floors. They were enclosed with wooden slatted sides and a wooden roof. Water, but not food, was available to the animals during the testing procedure.

Each test involved the pressurization of a pneumatically driven actuator (Top Cat Metrology, Suffolk, UK) that housed three round-ended pins in a triangular formation (each 2.5 mm diameter, total pin surface area of 15 mm²) onto the dorsal aspect of either the metacarpus or metatarsus of the animal. The pin formation, contour, and surface area were identical between the actuators for the two species; however, the convexity of the plastic mounting and the brushing boot used to secure the actuator against the limb differed between species due to limb conformation and size. In both donkeys and horses, on the contralateral limb, a sham actuator (of a similar shape and weight but without the pins) was secured in the same place with an identical brushing boot to that used to secure the test actuator.

A 60-mL air-filled syringe was attached to the actuator using a plastic extension tube. The syringe was pressurized manually to apply force to extrude the pins, at a rate of 0.8 N/s. One test was defined as the application of force until a behavioral end point response was seen (foot lifted off the floor or turning to look at the leg being tested) or until a maximum cutoff force of 25 N was reached. Foot lifts that occurred at forces less than 4 N were disregarded, and the test continued until an end point behavior was observed or the cutoff force was reached. Four repeats of a test with intervals of at least 15 minutes between tests produced one test series. Within a test series, the limb tested was kept constant.

Fly repellent (Ultrashield Red, Absorbine, MA) was applied at the beginning of each test series. Donkeys and horses were acclimatized to the testing procedures for 1

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