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Development of a mechanical stimulator and force measurement system for the assessment of nociceptive thresholds in pigs

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ABSTRACT

A mechanical stimulator and force measurement system was developed to quantify withdrawal thresholds to noxious mechanical stimulation of the foot in young pigs. The device and associated PC software have design and control features not previously used in other mechanical stimulators. The device, capable of delivering stimulus rates between 2 and 17 mm/s, maximum force 27 N, was validated in a cross-over study on 8 juvenile pigs (6–8 weeks of age) to check the repeatability and reliability of force threshold measurement and assess its ability to measure changes in force threshold following an inflammatory challenge. Threshold force measurements were obtained over several time periods before and after the pigs received a 0.25 ml subcutaneous injection of 3% carrageenan in 0.01 M phosphate buffered saline (PBS) or PBS in the hind foot. Consistent withdrawal thresholds were measured in injected (ipsilateral) and contralateral feet, 24 h and 30 min prior to injection (mean 8.4; 95% CI 7.1–9.7 N). Carrageenan injection, but not PBS injection, induced a significant decrease in withdrawal thresholds 90 min after injection (4.6 ± 0.9 N) which remained reduced for 6 h after injection. The testing system provided reliable and reproducible measurements of foot withdrawal thresholds to noxious mechanical force in young pigs (weight range 32–39 kg), and was capable of detecting and monitoring changes in threshold sensitivity following the induction of acute local inflammation in the foot. The system is suitable for studying nociceptive mechanisms in pigs.

1. Introduction

Obtaining accurate and repeatable measurements of sensory thresholds in freely behaving animals presents a considerable challenge in pain research. In the absence of verbal communication in animals, pain assessment is largely based upon either physiological or behavioural responses, the latter being regarded as more pain specific (Le Bars et al., 2001). A wide range of tests have been developed, primarily for use in rodents for assessing responses to noxious and non-noxious stimuli, and their use has been well documented (Randall and Selitto, 1957; Hargreaves et al., 1988; Vinegar et al., 1990; Chaplan et al., 1994; Yeomans and Proudfit, 1994; Ren, 1999; Gibbs et al., 2006). The most commonly used approaches in rodents are the tail flick and hot plate tests (thermal) and paw withdrawal test (mechanical) as described by Randall and Selitto (1957) and Hargreaves et al. (1988). The types of mechanical stimulators available for use in laboratory species deliver relatively low forces and are therefore often not appropriate for use in larger species, where the stimulation forces required to elicit a behavioural response are much greater. Several devices for delivering noxious mechanical stimuli have been developed for use in domesticated and farm animals such as sheep (Nolan et al., 1987; Ley et al., 1989; Welsh and Nolan, 1994, 1995a,b; Main et al., 1995), dogs (Hamlin et al., 1988; Lascelles et al., 1997, 1998), horses (Chambers et al., 1990, 1994), cats (Slingsby et al., 2001; Dixon et al., 2007), and cattle (Ley et al., 1996; Kemp et al., 2008). For the most part, these devices have incorporated the use of a blunt pin head positioned against the skin of a lower limb, which is pneumatically driven into the skin and the force transmitted onto the underlying tissue, until a behavioural response (commonly leg lift) is evoked. The device is attached to the limb by a cuff. These devices have proved reliable and have been reported to work well on relatively large animals whose demeanour accommodates attachment of the cuff.

As part of a study to develop robust and reliable methods for measuring nociceptive responses in juvenile pigs, devices based on the above design were evaluated, but they proved ineffective, pri-

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marily due to the difficulty in handling the animals to attach the cuff, thus supporting the premise that the development of any test must be tailored to the individual species (Dubner, 1994). Consequently it was considered appropriate to develop a species-tailored means of assessing noxious mechanical thresholds in unrestrained pigs. Initial work to develop a device that required attaching a stimulating section to the animal, similar to that done for sheep, cattle and horses indicated that such an approach would not produce reliable responses. Consequently an approach commonly used in rodents which involves mechanical stimulation of the plantar surface of the foot was adopted. The design of the stimulation device described in this paper was developed from the commercially available Dynamic Plantar Aesthesiometer (Model 37400, Ugo Basile, Comerio, Italy) which is used to assess touch sensitivity on the plantar surface of rodents (Arévalo et al., 2003; Anderson et al., 2005; Dolan and Nolan, 2007). However, unlike the touch stimulator which can only generate force loads up to 0.5 N, the device described was developed to deliver force loads up to 27 N, covering a force range equivalent to that used previously by others working in sheep and cattle (Nolan et al., 1987; Ley et al., 1989; Main et al., 1995; Welsh and Nolan, 1995a,b; Kemp et al., 2008), but without requiring the attachment of the apparatus to the animal. Other novel design and control features were incorporated into the device and its associated PC software. These features are fully described in Section 2.

Methods of thermal stimulation were also investigated as part of the study into developing methods of nociceptive assessment in pigs. The use of thermal stimulation (CO_2 infra-red laser) has been reported in previous studies on pigs to determine nociceptive thresholds in pregnant sows (Jarvis et al., 1997), however, this method was considered to be unworkable under the test conditions employed in this study, and those likely to be encountered in future studies. Thermal stimulation was evaluated using a hand-held radiant heat device, previously used in studies evaluating the effects of castration on hyperalgesia in calves (Molony et al., unpublished results-web link http://www.link.vet.ed.ac.uk/animalpain/Pages/Moviepages/Moviethermalstimcalf.htm). This device was found to be unsuitable for use on juvenile pigs due to their high basal levels of activity and reactivity to application of the thermal stimulation device.

2. Methods

2.1. Test device and platform

The key components of the mechanical stimulation and force measurement system are illustrated in Fig. 1. The stimulation and force measurement head is mounted on three rounded nylon feet which provide smooth movement across the lower flat surface of a platform. A control arm, hinged to the head unit, allows the operator to move the stimulator easily into position using the image of the probe against the underside of the perforated sheet as shown on the video monitor, and to control and initiate the stimulus via the control panel mounted on the outer end of the control arm. The assembly is linked to a laptop PC for data acquisition, storage and display.

A rigid platform was constructed with a perforated standing surface made from mild steel sheet 1 × 0.5 m (1 × *w*) and 3 mm thick with 5 mm diameter holes on an 8 mm hexagonal grid with "nearest neighbour" lines of holes aligned parallel to the long axis of the sheet and welded to the outer edges of the rigid frame to minimise deflection due to the weight of the animal. A feed/drink dispenser was located at the end of the platform opposite the access ramp. A large piece of polished hardboard was affixed below the perforated grid to provide a smooth and rigid surface to allow the stimulator to be positioned accurately and quickly under a suitable hole beneath the pig's foot.

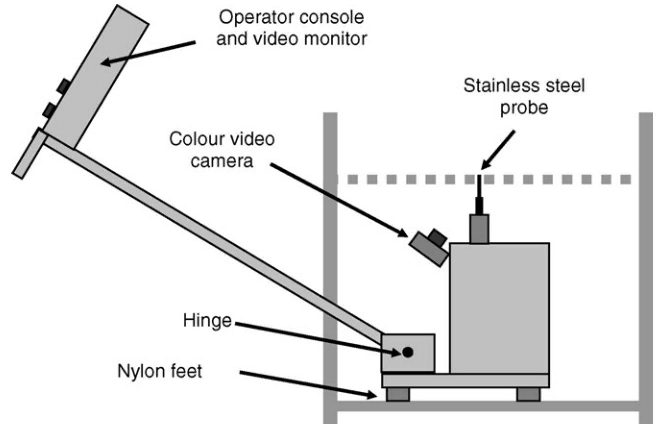

Fig. 1. Diagrammatic representation of the mechanical force delivery and measurement device and test platform.

2.2. Stimulator and force measurement device

The stimulus probe was made from a 70 mm × 4 mm stainless steel rod polished to reduce friction, with the upper 30 mm turned down to 2 mm diameter with a hemispherical tip. The overall length was such that, with the probe fully retracted, the probe tip was level with the top of the probe guide. The probe was moved by a stepping motor linear actuator (26DBM10D2U-L) which moved a platform vertically along a linear track. The probe was mounted (free standing) on a force transducer (Honeywell FSG-15N1) mounted on top of the platform that pushed the stimulus probe upwards and measured the force imparted through the probe upon contact with the pigs foot. The whole process of mechanical stimulation was controlled by a microcontroller chip which provided all the calibration and measurement features of the stimulator. The weight of the probe was automatically compensated for by the microcontroller (Microchip PIC16F876A). The mechanical arrangement of the stimulator load mechanism is shown in Fig. 2.

Software was written to facilitate the storage, display and subsequent analysis of the acquired data on a laptop PC linked to the stimulation device. The microcontroller has a LCD data display which provides the operator with all the information required to run the system for calibration and data collection and also provided a number of other useful support facilities such as locating the vertical position of the perforated plate relative to the stimulator. The operator communicates with the microcontroller system through a menu displayed on the LCD display via three "soft" press switches,

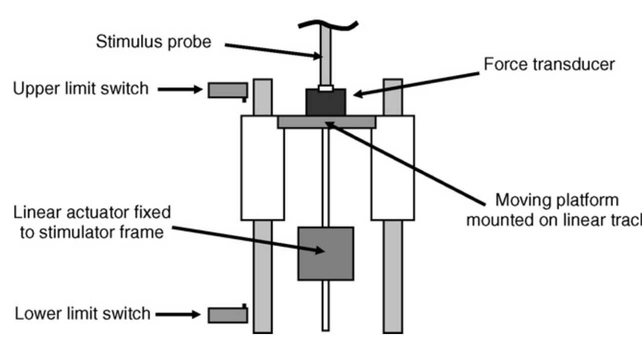

Fig. 2. Diagrammatic representation of the mechanical arrangement of the stimulator load mechanism.