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Measuring cutaneous thermal nociception in group-housed pigs using laser technique—Effects of laser power output[☆]

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ABSTRACT

Nociceptive testing is a valuable tool in the development of pharmaceutical products, for basic nociceptive research, and for studying changes in pain sensitivity is investigated after inflammatory states or nerve injury. However, in pigs only very limited knowledge about nociceptive processes are available, especially methodology which is applicable for pigs kept in group-housing without disturbing the daily routines of the animals.

To validate a laser-based method to measure thermal nociception in group-housed pigs, we performed two experiments observing the behavioural responses toward cutaneous nociceptive stimulation from a computer-controlled CO_2-laser beam applied to either the caudal part of the metatarsus on the hind legs or the shoulder region of gilts. In Exp. 1, effects of laser power output (0, 0.5, 1, 1.5 and 2 W) on nociceptive responses toward stimulation on the caudal aspects of the metatarsus were examined using 15 gilts kept in one group and tested in individual feeding stalls after feeding. Increasing the power output led to gradually decreasing latency to respond ($P < 0.001$) and affected the types of responding (less non-responding ($P < 0.01$), less moving leg ($P = 0.07$), less lifting leg ($P < 0.01$) and more kicking ($P < 0.001$)). Furthermore, the occurrence of tail flicking during laser stimulation was increased ($P < 0.001$). In Exp. 2, effects of laser power output (0, 0.8, 1.5, 2.2 and 3 W) on nociceptive responses toward stimulation on the shoulder region were examined in 10 gilts kept under the same conditions. Again, increasing the power output led to gradually decreasing latency to respond ($P < 0.0001$) and affected the types of responding (less non-responding ($P < 0.001$), less moving shoulder ($P < 0.001$), less moving body ($P < 0.001$), higher occurrence of muscle twitch ($P = 0.09$), and higher occurrence of rubbing shoulder ($P < 0.01$)).

In conclusion, the results of the two experiments suggest that behavioural responses to nociceptive cutaneous laser stimulation are a valid measure of nociception in group-housed gilts which are tested with a minimal disturbance of daily routines, and both when applied to the hind legs and to the shoulder region. Furthermore, porcine nociceptive responses appear to be graded, to include new types of behaviour as the nociceptive input increases and to show elements of site-specificity.

1. Introduction

Despite the large number of pigs kept in production systems and the increasing number of pigs used as experimental animals, little is known about nociceptive responses and sensitivity in this animal species.

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The importance of behaviour as an indicator of animal pain has often been highlighted (e.g. Bateson, 1991; Short, 1998). The scientific focus on porcine nociceptive physiology is increasing (Lynn et al., 1995; Rukwied et al., 2008), and in order to ensure correct interpretation it is important to acquire more knowledge about the nociceptive responses and capacities of pigs.

When nociceptive responses are studied, thermal stimulation is considered one of the preferred sensory modalities as heat is a natural stimulus, is one of the three fundamental nociceptive systems in mammals, and because the intensity of heat stimuli correspond with subjective painful experience in humans (Arendt-Nielsen and Chen, 2003). One thermal nociceptive experimental stimulus is radiant heat, whereby it is possible to test animals in their home environment without handling or fixation, which in itself might affect nociceptive responses (Porro and Carli, 1988; Herskin et al., 2007). Laser is one type of radiant heat, which in humans induces an immediate stinging sensation followed by burning lasting for seconds (Bromm and Treede, 1984; Plaghki and Mouraux, 2003). Laser technique has been used successfully to measure nociceptive thresholds in humans (Arendt-Nielsen and Bjerring, 1988), laboratory rodents (Fan et al., 1995) and also other farm animals (Veissier et al., 2000; Herskin et al., 2003, 2007) and possesses a number of advantages compared to other types of radiant heat, e.g. elicits specific activity without contamination from mechanosensitive receptors and is applicable to different skin areas (Arendt-Nielsen and Bjerring, 1988; Svensson et al., 1991; Arendt-Nielsen and Chen, 2003; Plaghki and Mouraux, 2003). Taken together, the use of laser stimulation is advantageous compared to most other types of nociceptive stimulation, and might be especially relevant as a nociceptive stimulus for animals kept in groups.

In pigs, only one study has reported nociceptive behavioural data based on this technique, where single-housed sows were stimulated while in a recumbent position (Jarvis et al., 1997). There are reports quantifying pain sensitivity in pigs using other methods, e.g. Dantzer et al. (1986) measured latency to respond to nociceptive electric stimulation in young pigs and Rushen et al. (1990, 1993) measured pain sensitivity using thermal contact stimulation on the rumps of crated sows and gilts. The same technique has been used to quantify changes in pain sensitivity in sows after nose slinging (Rushen and Ladewig, 1991). However, none of these methods are applicable on group-housed animals, without considerable disturbance of the daily routines of the animals.

This paper presents data from two experiments examining whether responses toward laser stimulation is valid as a measurement of nociceptive threshold in group-housed pigs, which were minimally disturbed in their daily routines by the testing procedures, and stimulated on (1) the caudal part of the metatarsus of the hind legs and (2) the shoulder region.

2. Materials and methods

2.1. Animals and feeding

Thirty Danish Landrace × Yorkshire gilts from the resident herd at Danish Research Centre Foulum were used as experimental animals, half of them in each of two successively run experiments. For the two experiments, the animals were 179 ± 29 days old (range: 145–248) and 205 ± 17 days old (range: 184–225) and weighed 107 ± 9 kg (range: 96–125) and 92 ± 12 kg (range: 76–114) at the initiation of the experiments. In each experiment, the 15 gilts were kept in one group in a loose-housing pen of approximately 47 m^2 equipped with 16 individual feeding stalls each measuring 52 cm × 225 cm, where all feeding and nociceptive testing took place. The pigs were kept in this pen for at least 12 days before initiation of the experiments. The pigs had *ad libitum* access to water from water nipples placed in each feeding stall as well as four water nipples placed centrally in the pen. The pigs were fed once daily (at 09:00 h) in the feeding stalls with 1.5 kg of commercial concentrate for pregnant sows (chemical composition as percentage of dry matter: wheat 34%, barley 30%, triticale 10%, sunflower meal 8%, oat husk meal 5%, rape seed cake 4%, oat 2%, molasses 2%, palm oil 2%, soy meal 2%, and minerals 1%). During seven days before the initiation of the experiment, the gilts were locked into the feeding stalls for 2 h after feeding in order to accustom them to the testing environment. In this was the nociceptive testing took place with only minimal disturbance of the daily routines of the animals. The experiments took place in the spring of 2005 with room temperature in the pen of 18.0 °C (range: 16.6–18.8) and 19.1 °C (range: 18.1–19.8) for the two experiments, respectively.

All procedures involving animals were evaluated and approved by the Danish Animal Experiments Inspectorate.

2.2. Experimental design

Effects of power output on nociceptive responses were examined in two 5 × 5 Latin square designs (Lehner, 1996) each with five different power outputs (Exp. 1: 0, 0.5, 1.0, 1.5 and 2.0 W; Exp. 2: 0, 0.8, 1.5, 2.2 and 3 W) and five test days (testing interval at least 48 h). In each experiment, each gilt received all five power outputs in a balanced order. The individual animals were tested in the same order each test day after feeding. Testing started immediately after the pigs had finished eating (at 09:10 h) and lasted in total approximately 70 min. After testing, the gilts were released from the feeding stalls.

2.3. Laser equipment

The nociceptive responses of the gilts were assessed by examining their reactions to a laser beam applied to the skin (adapted from Herskin et al., 2003). Before the actual experiments pilot studies were performed in order to establish the cut-off time of 25 s. Especially at the higher laser power outputs some signs of blushing could be seen, but no blistering was observed. In Exp. 1 the gilts were stimulated on the caudal part of the metatarsus of the hind legs, whereas in Exp. 2 the dorsal part of the shoulder