

RESEARCH PAPER

## The effect of social isolation, gender and familiarity with the experimental procedure on tests of porcine nociceptive thresholds

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### Abstract

**Objective** To investigate the effects of habituation and isolation on mechanical nociceptive thresholds in pigs at the pelvic limbs and at the tail.

**Study design** Prospective randomized multifactorial study.

**Animals** Thirty-two healthy castrated male (experiment 1), and 12 castrated male and 12 female (experiment 2) Danish Landrace × Yorkshire pigs, weighing  $63.5 \pm 0.8$  kg and  $55.4 \pm 0.6$  kg (the mean  $\pm$  SD, experiment 1 and 2, respectively).

**Methods** Mechanical nociceptive thresholds were quantified with a von Frey anesthesiometer applied to two distinct anatomical regions (tail and pelvic limbs). Pigs receiving the mechanical challenge in the pelvic limbs were tested inside a cage, whereas pigs exposed to stimuli at the tail region were tested in an open arena. For both experiments, the effect of familiarity to the procedure was evaluated by comparing thresholds of nociception in habituated versus naïve pigs. The presence of a companion animal was also evaluated in pigs receiving stimuli at the pelvic limbs.

**Results** Pigs tested inside the cage were affected by the habituation to the procedure as indicated by the

increase in willingness and time spent by the animals in the test cage. This effect was reflected in the lower mechanical nociceptive thresholds (medians with 25–75 percentiles) recorded for familiar pigs compared with naïve animals [495 g (302–675) versus 745 g (479–1000), respectively;  $p = 0.026$ ]. Mechanical nociceptive thresholds measured at the tail of the pigs in the open arena were not affected by the familiarity of the animals with the experimental procedure.

**Conclusions and clinical relevance** The current results reiterate the value of habituation in research involving animal behaviour. Further characterization of the methodology is needed to allow its application in the evaluation of clinical conditions in pigs.

**Keywords** behaviour, mechanical thresholds, nociception, pigs.

### Introduction

Quantification of mechanical nociceptive thresholds in large animal species can be performed via the use of hand-held devices delivering noxious mechanical stimuli at specific anatomical regions (Haussler & Erb 2006; Stubbsjoen et al. 2009; Nalon et al. 2013). Instruments operated by hand provide the advantage of targeting distinct anatomical regions with

flexibility. However, the stability of this approach is affected by potential operator bias (Love et al. 2011), as well as experimental factors such as the degree of restraint of the animals and the level of familiarity of the animals with the protocol. Restraining the animals often leads to stress responses and may hinder the expression of the natural behavioural endpoints following noxious challenges, thus limiting the validity of the measurements (Rushen & Ladewig 1991; King et al. 2003, 2007; Herskin et al. 2007). Exposure of the animals to a novel environment has also been associated with increased thresholds of nociception (Herskin et al. 2004). Habituation to the experimental protocol has been reported to increase the sensitivity of tests of nociception in rats regardless of the degree of restraint (Taiwo et al. 1989; Anseloni et al. 2003). Recent results by Raundal et al. (2013) have shown that pretest habituation of loose-housed dairy cows before quantification of mechanical nociceptive thresholds increased the reliability of a hand-held method. In pigs, only a few studies focused on the evaluation of experimental factors such as habituation, restraint and social isolation. Owing to their highly social nature (Swindle 2007), the isolation of individual pigs constitutes a source of acute and long-term stress (Ruis et al. 2001), with possible consequences to their responsiveness in tests of nociception (Dantzer et al. 1986). Hence, allowing social interactions throughout the testing procedure might improve the validity of the measurements.

The present report comprises two experiments aimed at gaining new methodological knowledge about the quantification of mechanical nociceptive thresholds in pigs, focusing on effects of familiarity with the experimental set-up, including two anatomical regions (pelvic limbs and tail) and two levels of confinement.

## Materials and methods

### Animals and housing

Thirty-two clinically healthy castrated male (experiment 1), and 12 castrated male and 12 female (experiment 2) Danish Landrace × Yorkshire pigs from the resident herd at AU-FOULUM, Aarhus University, Denmark were used as experimental animals. At the start of the experiments, the animals weighed  $63.5 \pm 0.8$  kg and  $55.4 \pm 0.6$  kg (experiment 1 and 2, respectively) and were of  $16 \pm 0.5$  and  $16 \pm 1$  weeks of age. The pigs were housed in

standard pens ( $4.40 \times 4.40$  m) with *ad libitum* access to water and feed. All the male pigs had been castrated surgically in accordance with Danish legislation within 2–7 days of life, and all pigs were tail docked (leaving half a tail on the body) within 2–4 days of life. The pigs did not receive any pharmacological treatment during these procedures. Experiment 1 was conducted in the autumn of 2009 and experiment 2 in the spring of 2010. All animal procedures were evaluated and approved by the Danish Animal Experiments Inspectorate (Journal nr 2009/561-1650) and adhered to the guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983).

### Experimental design

#### *Experiment 1: noxious stimulation at the pelvic limbs while confined in a cage*

The experiment was designed as a  $2 \times 2$  factorial design with habituation to the experimental procedure (habituated to the procedures during 3 days *versus* naïve to the procedure) and social isolation (tested alone *versus* accompanied by pen mate during testing) as main factors and consisted of four successive blocks of eight pigs. Each experimental block of pigs (four males and four females) was housed in one standard pen comprising a total of 15 pigs for a minimum of 1 week prior to the start of the experiment. Out of the seven non-experimental pigs, four were used as companion animals and three as supplemental animals. For each experimental pig, the companion animal was randomly selected from non-experimental pen mates of the same gender. Each block corresponded to a treatment group, the allocation of which was determined at random using a shuffled deck of cards (i.e. a total of four combinations of red/black seeds and even/odd numbers).

#### *Experiment 2: noxious tail stimulation in an open arena*

The effects of gender and habituation to the experimental procedure on nociceptive responses were investigated in a  $2 \times 2$  factorial design. The effect of habituation was examined comparing responses of pigs that were either naïve or habituated to the experimental procedure. The 24 experimental pigs were distributed in six pens, each comprising a total of 12 pigs. Every home pen included the four experimental animals together with four companion animals selected out of the eight non-experimental

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