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Research paper

Can the way pigs are handled alter behavioural and physiological measures of affective state?

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ABSTRACT

Research on human-animal relationship in animal production has been mainly focused on its effect on stress, productivity and meat quality. Only few studies have assessed its effects on the animals' affective state. In the present study, the influence of positive and negative handling (pH and NH, respectively) on affective state and fear as assessed by the cognitive bias test, the novel object test and the defence cascade test was studied in 56 pigs. Serum, saliva and hair were sampled during the study for the analysis of cortisol concentration. Results showed no differences between pH and NH pigs in the behavioural tests, which may be either due to the lack of previous handling effect on the test results, the lack of validity or the low sensitivity of these tests or a combination of them. Moreover, no differences were found in cortisol concentrations between handling groups. However, correlations between tests were found ($p < 0.05$) suggesting that there are individual factors such as the fear level, the motivation or the coping style, that have a similar effect on the response to these tests. Moreover, pigs who were more fearful had higher ($r = 0.37$; $p = 0.014$) levels of serum cortisol at slaughter.

1. Introduction

Exposure to humans is one of the potentially most frightening events that many farm animals experience in their life (Waiblinger et al., 2006). It has been reported that human-animal relationship does not only affect animal welfare, as shown by increased corticosteroids concentrations (Hemsworth and Barnett, 1991; Probst et al., 2013), but also productivity (Hemsworth, 2003; Paterson and Pearce, 1992; Rushen et al., 1999) and meat quality (Geverink et al., 1998). There is, also, plenty of studies focused on assessing the effect of handling on "animal response to humans tests", mainly measuring the approach-avoidance response (de Passillé and Rushen, 2005; Hemsworth et al., 1996; Hemsworth et al., 1986b). Those studies demonstrate that negative handling leads to more avoidance response. However, to our knowledge, only one study focused on the assessment of its effect on the animal affective state (Brajon et al., 2015), not only with the presence of humans but also in other situations.

Recently, methods based on the study of cognitive changes have been used to assess emotion and mood states in non-human animals. One of these methods is the cognitive bias test (CBT), and although there are different tests focused on assessing the effect of cognition on emotions, the judgment bias test is the most used in animals. This test is based on the premise that subjects in negative affective states make more negative and pessimistic judgements about ambiguous stimuli than subjects in positive affective states (Mendl et al., 2009). Before carrying out the test session with the ambiguous stimuli, animals are exposed to two different type of training sessions: one with the presence of a stimulus associated with a reward (e.g. food), called positive stimulus, and the other with the presence of another stimulus with the absence of the reward (e.g. no food) or with a punishment (e.g. electric shock), called negative stimulus. Once animals are able to discriminate between the positive and the negative stimuli they are submitted to the test session where a stimulus intermediate between the previous two, called the ambiguous stimulus, is presented and the response of the animals is assessed. The latency to go to the ambiguous stimulus is the most used measure to assess the cognitive bias of the animal.

Since Harding et al.'s (2004) work, a large number of studies on cognitive bias have been carried out in different species (i.e. dogs,

Abbreviations: pH, positive handling; NH, negative handling; CBT, cognitive bias test; DCT, defence cascade test; NOT, novel object test; A, sessions with access to chopped apples; NA, sessions without access to chopped apples

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monkeys and rats; Gygax, 2014; Mendl et al., 2010; Müller et al., 2012; Rygula et al., 2012), including several farm livestock species (Baciadonna and McElligott, 2015). Different results have been found, but some of them (to see a summary of the results Gygax, 2014), suggest that, according with the hypothesis, animals in a better welfare state have more positive cognitive bias than the other animals.

Brajon et al. (2015) compared the influence of previous positive and negative handling (pH and NH, respectively) on the affective state of piglets using a CBT and found that pH induced a more optimistic response in front of the ambiguous stimulus than NH. In contrast, Briefer Freymond et al. (2014) found that mares trained with negative reinforcement were more optimistic when facing an ambiguous stimulus than the ones trained with positive reinforcement. Authors suggested that judgement bias of NR horses as positive compared to PR horses and concluded that it could be due to higher feeding motivation or the release from the negative event.

There is a large number of behavioural tests focused on assessing emotions. Some of them are focused on particular emotions, such as the novel test, which is focused on assessing fear, and others, such as the Qualitative Behaviour Assessment (QBA) are focused on assessing the general mood (Murphy et al., 2014). In order to assess its reliability the results of two groups with different treatment (i.e. different housing conditions, different type of management…) are usually compared. Moreover, those results can be compared with the results of other validated physiological and behavioural measures.

The defence cascade test has been proposed as a test to assess affective state. The term “defence cascade response” has been defined as a continuum of innate, hard-wired, automatically activated defence behaviours (Kozlowska et al., 2015). However, the behaviours included in the assessment of the defence cascade response vary between studies. Gratton et al. (2007) included freezing, flight or fight, and tonic immobility, while Kozlowska et al. (2015) included arousal, flight or fight, freezing, and tonic, collapsed and quiescent immobility. Recently, defence cascade response has been proposed as a tool to assess the emotional state in pigs (Statham et al., 2013), as chronic stress, which can be induced by handling (e.g. Hemsworth et al., 1986a), can increase fearfulness, and thus, alter its affective sate. Indeed, the defence cascade response has been suggested to reflect affective state in both humans and rodents (Lang et al., 1990, 2000). Statham et al. (2015) proposed a test to assess the defence cascade response (DCT) in pigs which involves the assessment of an initial response (magnitude of startle), an evaluation phase (duration of freeze) and a final response (defensive or resume behaviour) and found that pigs raised in barren conditions decreased the magnitude of startle in their initial response compared with pigs raised in enriched conditions.

Another test focused on assessing emotions is the novel object test (NOT), it is a widely used technique to assess fear or anxiety responses to unfamiliarity in pigs (for a review see Murphy et al., 2014). Murphy et al. (2014) suggested that exposure to novelty is an ethologically valid test of emotional response in pigs. The latency to contact the novel object is the most usual measure to assess fear in the NOT, but other measures, such as, the number of times the animal contact with the novel object during the test, or the time the animals is in contact with the novel object. Hemsworth et al. (1996) found that positively handled pigs were quicker to approach the novel stimulus in the NOT than minimally handled pigs. However, the same study failed to find differences in the NOT in cows with different handling experience.

Moreover, Wichman et al. (2012) found that laying hens which were more pessimistic in a CBT (took longer to approach an ambiguous location) also showed more fear in an NOT (took longer to approach the object), suggesting the role of the fear in the decision making in laying hens.

Cortisol concentrations have been extensively used to assess stress, and hence the affective state, in different species, including pigs (Mormède et al., 2007). The effects of negative handling and human-animal interaction on serum and saliva cortisol concentrations have been studied extensively, finding the expected results, that is, animals with unpleasant handling having higher cortisol concentrations in some studies (e.g. Bergamasco et al., 2010; Hemsworth et al., 1986a) and no differences between handling groups were found in some others (e.g. Paterson and Pearce, 1992). However, both serum and saliva samples provide a measurement of the cortisol concentrations at a single point in time (Russell et al., 2012). Although a multi-sampling over time is possible for saliva and serum, animals have to be subjected to invasive collection techniques and thus it can alter the results. Hair cortisol has recently been proposed as a biomarker of chronic stress to overcome this issue (Stalder and Kirschbaum, 2012). The effect of human animal interaction in serum (e.g. Hemsworth et al., 1986a,b; Paterson and Pearce, 1992) and saliva (e.g. Bergamasco et al., 2010) samples has been measured in several studies. However, to our knowledge, the effect of human animal interaction has not been assessed in hair cortisol in any animal species.

When assessing how well behaviour is measured, the validity and the sensibility refer to the relation between what a parameter is supposed to measure and what it really measures (Martin and Bateson, 1993).

There are some individual factors, such as temperament, motivation and copying style that can affect the validity and sensibility of the behavioural test masking the differences between treatment groups. The temperament is referred as the combination of mental, physiological and emotional traits of an individual (Wemelsfelder et al., 2001). The motivation concerns things animals want and how much they want them (Kirkden and Pajor, 2006). The coping styles hypothesis has recently received much attention on studies investigating individual differences in personality. This hypothesis states that animals have consistent physiological and behavioural responses that can be characterised on a continuum from reactive to proactive (Janczak et al., 2003).

The present study aimed at assessing the effect of pH vs. NH on the pigs’ affective state, as assessed by the CBT, and on the fear response, as assessed by the NOT. Furthermore, a DCT was performed to assess the fear response presumably influenced by the affective state. Another objective of this study was to investigate the effects of handling on the variation of cortisol concentration along the tests and correlate it with the CBT, NOT and DCT behavioural observations.

The hypothesis of this study was that pigs with a pH experience have a more optimistic response in the CBT, are less reactive to a sudden stimulus and resume the activity faster in the DCT, are less fearful in the NOT and have lower concentrations of cortisol than pigs with a NH experience.

2. Material and methods

2.1. Animals and housing conditions

In this study, 56 female pigs ((Landrace x Large White) x Piétrain) were used. At 9 weeks of age, pigs were transported from a commercial farm to the experimental facilities of IRTA (Monells, Spain) and housed in two rooms of four pens with seven pigs each. Pens (5 × 2.7 m) had fully slatted floor and were under natural light conditions at a constant environmental temperature (22 ± 3 °C). Each pen was provided with one steel drinker bowl (15 × 16 cm) connected to a nipple and a concrete feeder (58 × 34 cm) with four feeding places. Pigs had water and food ad libitum. Pigs were inspected daily and no health problems were observed during the experimental period. At 27 weeks of age, pigs were transported to the experimental slaughterhouse of IRTA (1.2 km trip). On arrival, pigs were immediately driven to a CO_2 stunner and exposed to 90% CO_2 for 3 min before exsanguination.

The study was approved by the Institutional Animal Care and Use Committee (IACUC) of IRTA.