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Effect of gender and halothane genotype on cognitive bias and its relationship with fear in pigs

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ABSTRACT

Cognitive bias (CB) has been recently proposed as a tool to study emotions by assessing the cognitive function through behaviour observation. It is based on the premise that subjects in a negative affective state perform more negative judgements about ambiguous stimuli than subjects in positive affective state. This study aimed at investigating if halothane genotype (homozygous Hal-free, NN vs. heterozygous Hal carrier, Nn) and gender (gilts, G vs. entire males, EM) affect the CB in pigs. Moreover, the results of the CB test (CBT) were compared with the results of a novel object test (NOT) in order to assess the influence of fear in the decision taken by pigs during the CBT. The results of both tests were contrasted with the concentration of brain neurotransmitters in four different brain areas in order to analyse the involvement of the dopaminergic and serotonergic pathways on the pigs' affective state and fear. A total of 48 pigs, in terms of 12Hal-free gilts (NNG), 12Hal carrier gilts (NnG), and 12Hal-free entire males (NNEM) and 12Hal carrier entire males (NnEM) were put on the CBT at the age of 20 weeks and on the NOT four days later. After two days, pigs were slaughtered and four brain structures (amygdala, prefrontal cortex, hippocampus and hypothalamus) were dissected for the analysis of brain neurotransmitters. The CBT and NOT results did not show any effect of the genotype and gender or their interaction on pigs' emotional response ($p > 0.10$). However, the CBT correlated positively with the NOT ($r = 0.49$; $p = 0.0005$), with pigs classified with a negative CB tending to be more fearful in front of the novel object than those with a positive CB ($p = 0.05$). Moreover, the pigs that took longer to get in contact with the novel object in the NOT also had lower ($p = 0.013$) concentration of dopamine in the prefrontal cortex and increased DOPAC/dopamine ratio in the hypothalamus ($p = 0.003$). These results suggest that fear level plays an important role in the decision taken by the pig dealing with ambiguous stimuli.

1. Introduction

The assessment of the affective state in non-human animals is a crucial goal in the study of animal welfare which has to be unequivocally achieved through behavioural and physiological measures. Several authors have reported a relationship between cognition and emotional state in animals (Boissy and Lee, 2014; Dantzer, 2002; Paul et al., 2005). The concept of cognitive bias (CB) refers to the influence of emotional state on cognition. Whereas, judgement bias, which is the most widely used technique to assess cognitive bias in animals, refers to the predisposition of an individual to show behaviours indicating anticipation of relatively either positive or negative outcomes in response to ambiguous stimuli (Mendl et al., 2009). Individuals in a negative affective state tend to perform more negative judgements about ambiguous stimuli than individuals in a positive affective state (Eysenck et al., 1991).

Comparing the CB test (CBT) with other techniques used for the assessment of the emotional state it provides a better measure of the valence of the emotion and allows distinguishing between positive and negative emotional states. Moreover, helps avoid the confounding effects of the novel environment on animal behaviour during the test as animals are habituated in a test arena before being tested, and is not invasive (Mendl et al., 2009; Murphy et al., 2014b). However, the CBT is not easily repeatable over time since its

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ambiguity for pigs is short-lived (Doyle et al., 2011), is time consuming and is difficult to perform in non-experimental, uncontrolled conditions (Murphy et al., 2014b; Seehuus et al., 2013).

A large number of studies have been carried out on CB in several species (Gygax, 2014) based on the methodology for its assessment in non-human animals developed by Harding et al. (2004). As yet, the CB has been used to assess the affective state of farm animals in response to extrinsic factors, such us environmental enrichment (Douglas et al., 2012; Wichman et al., 2012), isolation (Düpjan et al., 2013; Salmeto et al., 2011), restraint (Doyle et al., 2011; Murphy et al., 2013) and handling practices (Daros et al., 2014; Neave et al., 2013; Sanger et al., 2011), and to intrinsic factors, such as genotype, breed or gender.

Several studies have assessed if genotype that is related to anxiety or depression like behaviour have an effect in the response to CBT in rodents (Boleij et al., 2012; Enkel et al., 2010; Kloke et al., 2014; Richter et al., 2012). Other studies assessed the response to CBT between different genders of goats (Briefer and McElligott, 2013) and dogs (Müller et al., 2012). In pigs, the CBT was used to assess the effects of breed and birth weight on their emotional response (Murphy et al., 2013, 2014a). However, to our knowledge, the effects of the gender and the Hal gene on the pigs' response to the CBT are unknown.

The Halothane gene, referred to as the porcine stress syndrome gene, causes malignant hyperthermia, which is usually triggered by stress being more sever in pigs both Nn (heterozygous Hal carrier) and nn (homozygous Hal carrier) than in NN (homozygous Hal-free) (Rosenvold and Andersen, 2003). Indeed, the Hal carrier genotypes have been associated with greater stress sensitiveness in pigs. Moreover, Fàbrega et al. (2004) studied the effect of the Hal genotype in an open field test and demonstrated that Nn pigs performed more locomotive activity compared with NN pigs.

Regarding the gender, van Erp-van der Kooij et al. (2000) found that female piglets were more active than males in a backtest. Moreover other studies have related the gender with differences in emotional behaviour (Gray, 1971) and in the propensity to take risks (Felton et al., 2003).

According to Wichman et al. (2012) and Seehuus et al. (2013), CBT results should be interpreted with caution as they may be confounded by individual parameters, such as fearfulness. Fearfulness may, in fact, have an effect on the perception of the individual animal towards the test, thus influencing the final result of the CBT. An individual in an anxiety or fear-inducing situation may benefit from making 'safety first' judgements when facing ambiguous stimuli (Mendl et al., 2009), which may lead to the interpretation that the response of fearful and anxious individuals to such stimuli may be excessive.

The novel object test (NOT) is commonly used to assess fear or anxiety responses to unfamiliarity (Murphy et al., 2014b). Wichman et al. (2012) found a positive correlation between the results of the CBT and the level of fear assessed with a NOT in laying hens. To our knowledge, such a comparison was never made in pigs.

Brain areas, such as the prefrontal cortex and the amygdala, and the dopaminergic and serotonergic activities are involved in the judgement of ambiguous stimuli (Berridge, 2007; Schultz, 1997) and in the fear response (D'Angio et al., 1988; Davis et al., 1994). The animal's affective state or mood may modulate some or all activities of these brain areas (Mendl et al., 2009; Ruhé et al., 2007). For example, in mice studies, high brain dopamine levels have been associated with positive affective state (Ashby et al., 1999; Burgdorf and Panksepp, 2006), while the absence of D4Rs, a dopamine receptor, has been related to increased avoidance behaviour to novel stimuli (Dulawa et al., 1999; Falzone et al., 2002). To our knowledge, the involvement of brain neurotransmitters (NT) in the pigs' emotional state in response to the CBT or NOT has never been studied.

The overall objective of this study was to determine the CB in pigs of different gender and carrying or not carrying the Hal gene. In addition, the study aimed to assess to what extent pigs consider ambiguous the stimulus triggered by the contact with a novel object (known object but situated in a new location) in the CBT and the relationship between affective state and fear. Another goal was to assess the involvement of the dopaminergic and serotonergic pathways in the response to judgement and fear.

2. Material and methods

2.1. Animals and housing conditions

In this study, 48 crossbred pigs (Landrace × Large White sows sired with Piétrain boars) were divided into four groups of 12 pigs each. Each group either consisted of Hal-free gilts, Hal-free entire males (NNG and NNEM), Hal carrier gilts and Hal carrier entire males (NnG and NnEM).

At 9 weeks of age, pigs were transported from a commercial farm to the experimental facilities of IRTA (Monells, Spain) and housed separately by gender and genotype in 8 pens (6 pigs per pen). Pigs were kept in pens (5 × 2.7 m) on fully slatted floor under natural light conditions and at a constant environmental temperature of 22 ± 3 °C. Each pen was provided with one steel drinker bowl (15 × 16 cm) connected to a nipple and with a concrete feeder (58 × 34 cm) with four feeding places. Pigs had water and feed ad libitum. Pigs were inspected daily and no health problems were observed during the experimental period. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of IRTA.

2.2. Cognitive bias test

At 19 weeks of age, pigs were trained and tested individually for the CB according to the methodology described by Carreras et al. (2015). Twelve training sessions were performed, 6 with access and 6 without access to chopped apples (A and NA sessions, respectively). During the first training session all pigs had access to chopped apples for 10 min. If the pig did not eat them during the first and second A sessions, the apples were put 0.5 m in front of the bucket for 2 min in order to encourage the pig to eat them and learn the association between the bucket and the food. The remaining sessions finished 30 s after the pig ate (in the A sessions) or tried to eat the chopped apples, i.e., contacted the wire mesh with the snout (in the NA sessions) or 90 s after entering the test pen if the pig did not eat or try to eat. Pigs were subjected to two training sessions per day (from 07:00 a.m. to 04:00 p.m.) during 6 consecutive days. During the following two days, two additional A and NA reminder sessions were performed before the test session. The latency to contact the bucket, defined as the time pigs took from entering the test pen to the contact with the bucket, was recorded in all sessions. The pigs that did not learn to discriminate between the two cues after the reminder sessions were excluded from the CBT. The criterion used to exclude pigs from the CBT is described in Carreras et al. (2015). Briefly, pigs were excluded from the study if the mean time during the two A sessions was higher or equal than in the two NA sessions.

After the reminder sessions, each pig was individually subjected to a CBT that finished when the pig ate the chopped apples or 90 s after the pig had entered the test pen. At the end of the CBT, the test pen door was opened and the pig returned to the housing pen. The latency to contact the bucket was also recorded as in the previous sessions.