

**USING COGNITIVE  
PARADIGMS TO  
MEASURE EMOTION  
IN PIGS**



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# USING COGNITIVE PARADIGMS TO MEASURE EMOTION IN PIGS

**Het meten van emoties bij varkens met behulp van cognitieve paradigma's**  
(met een samenvatting in het Nederlands)

## **Proefschrift**

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*I gcuimhne ar mo  
dheartáir*



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# **CHAPTER 01**

## **GENERAL INTRODUCTION**



In this thesis I aim to develop an appropriate method to measure emotion in pigs based on recently proposed cognitive paradigms. Welfare of pigs in production environments and, more recently, as model animals in biomedical research, is of increasing concern in society. However, what constitutes good welfare is not always clear. Under E.U. legislation, protection is afforded to farm animals as sentient beings, and unnecessary 'suffering' and 'distress' ought to be avoided. Thus the emotional component of welfare is emphasized - however few validated measures of emotion in animals exist. In this thesis I develop and try to validate novel cognitive tasks for pigs, using biases in judgement and decision making as proxy measures of the valence (positive/negative) of emotion in pigs. This introduction will discuss what is meant by welfare, and why the welfare of pigs is important, as well as how welfare and emotion are commonly studied in animals. I will provide some theoretical basis for using cognitive paradigms to measure emotion in animals, and present an outline of the current thesis.

## **THE USES OF THE PIG**

The domestic pig (*Sus scrofa domestica*) is likely to have been first domesticated in the Near East around 9000 years ago (Giuffra et al., 2000). Pig production now has become a hugely valuable farming industry. The EU is the second biggest producer of pork in the world and the largest exporter (European Commission, Agriculture and Rural Development) - the population of pigs in farming in the EU28 countries was over 146 million animals (<http://epp.eurostat.ec.europa.eu/>). Alongside its role in agriculture, the pig is gaining importance as a model animal to biomedical research (Lind et al., 2007). The use of the pig in this research area is still relatively low - pigs make up less than 1% of laboratory animals (van Zutphen et al., 2001). The pig model has been proposed to bridge the gap between more common animal models (e.g. rodents) and humans (Vodicka et al., 2005). Due to similarities to humans in anatomy and physiology, particularly the cardiovascular, urinary, integumentary, and digestive systems (Swindle et al., 2012), as well as brain anatomy, growth and development (Gieling et al., 2011; Lind et al., 2007), the popularity of pig models is growing. However, funding opportunities for pig research are poor (Reynolds, 2009).

## **PIG WELFARE**

In the developed world, pig farming is one of the most intensive production systems (Marchant-Forde, 2009) and over half of the pig-meat consumed globally comes from intensive systems (CIWF.org.uk). Inherent in modern farming systems are welfare problems due to a number of reasons, including the mismatch between natural behaviour of the animal and provisions within the production environment as well as selection pressures for increased production. Concern for pig welfare has been raised at all levels of pig production, from early weaning, barren housing for fattening pigs, restriction of sow movement for farrowing, transport and slaughter methods. For example, while wild/feral pigs, as well as those kept in free-range systems, engage in foraging and rooting behaviour for much of their active time (Špinka, 2009). If the opportunity to express such behaviours is thwarted, by the lack of provision of adequate rooting material and/or

the restriction of movement, this is associated with increased aggression and stereotypic behaviours (Studnitz et al., 2007; Tuytens, 2005). The selection for higher growth rates means that breeding sows are maintained on a restricted diet in order to avoid obesity, which can lead to the development of stereotypic behaviour and aggression (Lawrence and Terlouw, 1993; Spoolder et al., 1995; Terlouw et al., 1991). Along with restricted feeding in sows, selection for increased litter sizes has led to an increase in number of low birth weight piglets (Beaulieu et al., 2010; Meunier-Salaün et al., 2001; Milligan et al., 2002), which is, in turn, associated with higher mortality (Baxter et al., 2008; Quiniou et al., 2002).

In biomedical research, as with production housing and environmental conditions are important for good pig welfare. However, in laboratory situations, many experimental procedures require far more handling and restraint of the pigs than in farm situations (Ellegaard et al., 2010), which can be a cause of stress for the animals (Hemsworth et al., 1981; Hemsworth, 2003). Regular and positive handling of minipigs in laboratories can reduce stress at handling for the pig as well as difficulty of handling for the experimenter (Tsutsumi et al., 2001). The actual procedures used to collect physiological samples and the health of pigs used for biomedical studies may also lead to welfare problems (Ellegaard et al., 2010). Furthermore, minipigs, selected for their smaller size to suit laboratory environments, are often fed very restricted diets as they will become obese if given ad libitum access to food (Bollen and Ritskes-Hoitinga, 2007). To evaluate the extent of these issues for pig welfare on farm and in the laboratory, what constitutes good welfare ought to be defined and appropriate tools for assessing the impact of these problems on pigs' welfare ought to be developed and validated.

Both in agriculture and in biomedical research, concern for pig welfare is increasing. The welfare of pigs on farms and in laboratories is covered by general laws in the EU which address specific aspects of housing, space allowance, food and drink provision, transport and slaughter. Furthermore, the legislation relating to laboratory animals frequently makes reference to the reduction and/or avoidance of unnecessary 'pain', 'suffering', 'distress' and 'lasting harm' (Directive 2010/63/EU); while legislation relating to pigs in farming focuses more generally on 'welfare', 'needs', 'suffering' and 'injury' (Directive 98/58/EC; Directive 2008/120/EC). However, what constitutes suffering and distress, or what the particular welfare requirements and behavioural needs of the pig are not defined in legislation.

A variety of different concepts as to what constitutes good welfare has been discussed in the literature. Common definitions of welfare differ according to what they consider to be the most important aspect, including the animal's ability to cope with the environment, the animal's feelings or experiences, naturalness, behavioural needs and desires, health and normal functioning (Fraser et al., 1997; Phillips, 2009). Many of the early definitions focus on the absence of negative states and not the promotion of positive states. The Five Freedoms (Brambell Committee, 1965), for example, propose that welfare should be considered in terms of the freedom 1) from hunger and thirst, 2) from discomfort, 3) from pain, injury or disease, 4) to express normal behaviour, and 5) from fear and distress. More recently, dynamic definitions have been proposed

where not only the absence of negative events is an important constituent of positive welfare, but also that an animal has the ‘freedom to react appropriately and adequately to’ negative events (Ohl and van der Staay, 2012), or has the capacity to change or anticipate challenges to maintain stability through change (Korte et al., 2007), or *‘the freedom to display normal behavioural patterns that allow the animal to adapt to the demands of the prevailing environmental circumstances and enable it to reach a state that it perceives as positive’* (Ohl and van der Staay, 2012).

Until recently, it was thought that the study of affective states in animals was unscientific (Fraser, 2009). However, the role of affective states in determining what constitutes positive welfare is of increasing interest to animal welfare science. Dawkins (2004) suggests that animal welfare can be assessed by asking firstly if the animal is healthy (physical well-being) and secondly whether it has what it wants (mental well-being).

## **HOW DO WE KNOW WHAT AN ANIMAL WANTS?**

The term ‘affect’ covers a variety of mental phenomena, including preferences, attitudes, emotion, mood, and affect dispositions (personality) (Scherer, 2005). Many of these phenomena are of interest to animal welfare science for understanding what an animal wants.

Preference, motivation and aversion tests have been commonly used to establish what an animal wants or how hard it is willing to work for something or to avoid something. Kirkden and Pajor (2006) provide a thorough review of these approaches to study feelings in animals, and list a number of advantages to these methods; preference and motivation tests allow animals to express what is important for them, and such tests can be used to compare a wide range of treatments (Kirkden and Pajor, 2006). However, what an animal wants may not always be what is best for the animal in the long term, and requirements may change depending on many factors, such as current need, environmental conditions, pregnancy, health status, etc. Fraser and Nicol (2011) suggest that the traditional short preference tests are not sufficient to account for these many influences. Furthermore, other aspects such as the familiarity of options, the animal’s ability to plan in the long term, or immediacy of the stimulus may influence an animal’s current preferences (Fraser and Nicol, 2011), as well as individual differences. For example, sows need time to adjust to unfamiliar flooring types before using them, and sow’s preference for floor types changes during and immediately after the farrowing period, reflecting situational-dependent requirements (Phillips et al., 1996). The strength of a preference is also an important factor to consider when evaluating the importance of an animal’s wants with respect to its welfare. For example, pigs will continue to work for food as the work load increases, but will reduce working for social contact as the demand increases (Matthews and Ladewig, 1994).

Assessing emotion and mood in animals is another approach to understanding animal’s wants. The measurement of feelings in animals had long been dismissed by science due to the subjective nature of such experiences. However, our inability to measure subjective states in animals does not imply that they do not experience them (Würbel,

2009). Many authors have stressed the importance of mental states, particularly positive emotions, to animal welfare (Balcombe, 2009; Boissy et al., 2007a; Boissy et al., 2007b; Désiré et al., 2002; Paul et al., 2005; Yeates and Main, 2008). Good welfare implies the absence of negative states as well as the presence of positive states (Duncan, 1996). Yeates and Main (2008) call the measurement of mental states in animals '*a critical goal*' for assessing animal welfare.

## **MEASURING 'FEELINGS' IN ANIMALS**

While both emotion and mood can be grouped under the heading of affect, they are said to differ in terms of direction and duration (Schnall, 2010). Mood is seen as a free-floating affective state of long-lasting duration and lower intensity, while emotion tends to be short-lasting, higher in intensity and directed at a stimulus (Dantzer, 1988 in (Boissy et al., 2007b)). Some researchers suggest that these affective states, in humans, are experienced as conscious feelings (Russell, 2003) while others argue for levels of emotional experience. For example de Waal (2011) proposed three levels in relation to the experience or feeling of an emotion ranging from (i) unconscious emotions to (ii) experienced emotions to (iii) reflected-upon feelings. Without the use of language, it is impossible to directly study how, and to what extent, an animal actually experiences an emotion, but it is assumed that animals which show behavioural and physiological responses indicative of pleasure or pain, also have some subjective experience of the emotion (Dantzer, 2002).

There is still much debate about the nature of emotions. Some propose the existence of discrete emotions which are distinct 'natural kinds', while others propose that emotion and mood can be categorised by differences in components along a dimension (for opposing viewpoints see Barrett, 2006; Izard, 2007). This dimensional viewpoint proposes that core affect is a neurophysiological/psychological state which can be described through both valence, positive or negative, and arousal, high or low (Duncan and Feldman Barrett, 2007; Russell, 2003).

A componential view of an emotional response is proposed by Moors (2009) in which each component is linked to a specific function. Firstly the cognitive component serves to evaluate and appraise the stimulus. The feeling component allows for monitoring and self-regulation of the emotion. Next, a motivational component (action tendencies) and a somatic component (responses in the CNS and PNS) prepare the individual for action. Finally a motor component produces an appropriate behavioural response, an action (Moors, 2009). A variety of methods have been applied to the study of emotion in animals, each addressing the specific components of an emotional response.

### **Physiology**

Traditionally, studies of animal welfare have used the concept of 'stress' to measure welfare, as the term 'emotion' was seen as too subjective. However both the terms stress and emotion have many aspects in common, and have often been used synonymously in some of the animal literature (McMillan, 2008). Stress was originally defined as Seyle (1973) as '*the nonspecific response of the body to any demand made*'. Thus stress has an

adaptive value - the stress response allows an individual to react to and potentially adapt to a challenge. The main focus of stress studies in animals has been on activity of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenocortical axis (HPA axis) (Mormède et al., 2007), i.e. physiological activation.

ANS activation is most commonly recorded by electrodermal (e.g. skin conductance) and cardiovascular (e.g. heart rate, blood pressure, heart rate variability) responses (Mauss and Robinson, 2009). William James (1884) originally proposed that different patterns of ANS activation produced different emotions. While there is some evidence for certain patterns of ANS activation correlating with specific emotions in human research (Kreibig, 2010), such results have not been found to be consistent (Mauss and Robinson, 2009) and may in fact reflect level of arousal (Hagemann et al., 2003). Cortisol, the 'stress hormone', is the primary active hormone of the HPA axis which is activated in aversive situations (Mormède et al., 2007). Measures of cortisol in blood or saliva are commonly used in studies of stress in animals. However, while higher doses of cortisol may be an indication of stress, there are a number of other factors which influence the amount of circulating cortisol such as the time of day/night, physical activity, health, feeding or temperature and humidity. Furthermore, the relationship between the amount of cortisol and the apparent severity of the stress in animals is not linear (Mormède et al., 2007). While ANS and HPA axis activity can indicate of stress in an animal, i.e. an indication of negative affect, it may not always accurately reflect the intensity of the emotion or even simply the valence of an emotion. The physiological response to stress is more complex, requiring the measurement of many physiological parameters, and can differ between individuals, breeds or species as well as be modified by adaptive responses to past experience (Blache et al., 2011).

## **Behaviour**

According to Moors' (2009) componential view of an emotional response, outlined above, a behavioural response is the ultimate outcome of an emotional episode. Behaviour, like physiological responses, allows an animal to react and adapt to changes in the environment, and therefore can offer information about the emotional state of an animal (Olsson et al., 2011). Behaviour can be seen as the outward expression of an emotion or as a measure of the emotionality of an animal in response to specific events. Behaviour is often been used as a read-out for emotion in welfare studies. A wide variety of behavioural methods to study emotion have been proposed, many of which focus on behavioural responses in assumed negative situations. Chapter 02 provides an in-depth overview of the different ways in which behaviour has been used as a measure of emotion in pigs. Few of the current methods used to assess pig behaviour are ethologically relevant for the pig, and few are designed to assess positive states (Murphy et al., 2014).

## **Emotion and cognition**

Historically, the study of emotion and the study of cognition were considered separate disciplines. In more recent years however, the interaction between the two processes has been increasingly studied in both human and animal research. Cognitive processes are

those that involve some form of information processing in the brain, such as memory, attention, problem-solving and planning (Pessoa, 2008). Some authors divide cognition into lower and higher level cognitive processes with the higher level ones including judgement, reasoning and decision-making (Blanchette and Richards, 2010). Since the '*functional relationships between cognition and emotion are bidirectional*' (Lazarus, 1991), the link between emotion and cognition can be studied in different ways; how emotion affects cognitive processes or how cognitive processes can impact upon emotional responses.

### **The influence of cognition on emotion**

Cognitive processes play a role in determining emotional reactions to events. We do not react in a uniform way to events, and furthermore even within the individual, emotional responses can differ to the same stimulus in different situations. Appraisal theory, which was first put forward by Arnold (1960), proposes that the emotional response to a stimulus is determined by the evaluation/appraisal of the stimulus according to a fixed set of criteria. Thus, it is the appraisal of the event that determines the specific subjective emotion experienced as well as the intensity, and the same emotional response is elicited when the outcome of the appraisal process is the same, but not necessarily when the stimulus is the same. Different theorists have proposed different criteria for appraisal, including aspects such as the novelty vs. familiarity of the event, whether it is intrinsically positive or negative, or the level of control the individual has towards the stimulus (for a full review see Scherer, 1999). According to this theory, at the most basic level, for example, a stimulus which is sudden and unpredictable would result in a fearful appraisal, while if the stimulus is also unfamiliar, the result of the appraisal could be anger. Thus cognitive processes, such as memory, can affect the emotional response to a stimulus. According to appraisal theories, emotion is an on-going process whereby the situation, along with the initial emotional response, is re-evaluated and generates further emotions in a continuous process (Lazarus, 1991). Appraisal theory has recently been applied to the study of emotion in farm animals (Désiré et al., 2006; Greiveldinger et al., 2009; Greiveldinger et al., 2007; Veissier et al., 2009).

### **The influence of emotion on cognitive processes**

There has been a recent surge in interest studying how affective states can influence cognitive processes such as memory, decision making, judgement, etc. in humans. Numerous studies have shown that these processes can be influenced by mood and emotion. When the effects of emotion lead to systematic alterations in cognitive processes, these are known as emotion-induced cognitive biases. For example, emotion has been found to influence memory processes; recall of emotional events is better than that of neutral ones in humans (Dolan, 2002). However, while recall of events central to the emotion may be better, peripheral information may be lost. This selective memory may occur because emotion facilitates recall of information relevant to currently active goals (Levine and Edelman, 2009). Emotion is also known to influence attentional processes – anxious individuals pay more attention to potentially threatening stimuli

than non-anxious individuals (Bar-Haim et al., 2007). These examples illustrate the likely adaptive function of emotional influences on cognitive processes, whereby such biases help individuals to ‘attend to, memorise and make judgements’ using relevant information in the environment (Paul et al., 2005).

## **EMOTION AND DECISION MAKING UNDER UNCERTAINTY**

The influence of emotion and mood on decision making processes has been widely studied in humans. In the majority of real-world decision making, the outcomes of the various choices are not always clear. Decisions can be made by weighing up the relative risks of each option, or if the outcome is ambiguous, the choice is made by judging the likelihood of different outcomes. Peters et al. (2006) suggest four functions for the influence of emotional processes on judgement and decision making. Emotion, based on experience, can act as a guide in the decision making process when the outcomes are uncertain, and can form a ‘common currency’ with which to weigh up different decisions. The strength of the emotion and the valence can alter the focus onto salient information to further guide the decision, and may even motivate the decision making process (Peters et al., 2006).

In human research, anxiety is associated with mood congruent biases in both decision-making under risk and judgement under ambiguity (Blanchette and Richards, 2010; Hartley and Phelps, 2012); higher levels of anxiety increase risk aversion and lead to more pessimistic judgements of ambiguous stimuli. However, positive moods can also increase or decrease risk aversion depending on the level of risk, while other negatively-valenced states can lead to increased risk taking (Isen, 2008). Judgement, on the other hand, appears to be much more consistently affected by the valence of emotional state – positive moods lead to positive judgements of ambiguity, while negative moods lead to negative judgements (Blanchette and Richards, 2010; Mendl et al., 2009). Emotional valence in animals may also be expressed by systematic biases in judgement of ambiguous stimuli. Cognitive bias paradigms have been proposed as a potential way to measure emotional valence in animals (Harding et al., 2004; Mendl et al., 2009; Paul et al., 2005).

## **EMOTION AND DECISION MAKING UNDER RISK: DECISION MAKING PARADIGMS IN ANIMALS**

One of the most commonly used tasks for assessing decision making in humans is the Iowa Gambling Task (IGT) (Bechara et al., 1994). This task requires subjects to choose between four options. Unbeknownst to participants two options are primed to be more advantageous as they offer low wins but also a low probability of loss, and two are disadvantageous as they offer high wins but even higher chance of loss. In normal decision making, subjects at first choose at random from the four options, but increasingly choose from the advantageous options as time goes on. The IGT has demonstrated decision making deficits in a number of clinical populations, e.g. pathological gamblers and drug addicts, and is associated with damage to the prefrontal cortex and amygdala (Buelow and Suhr, 2009). Choices in the IGT however, are not just based on rational

decision making, there is increasing evidence that emotional aspects influence decision making in the IGT (Buelow and Suhr, 2009). The IGT is said to rely more on intuition than cognitive reasoning; performance in the IGT is not disrupted by the performance of a secondary-task involving executive function (Turnbull et al., 2005). Positive mood states are associated with an earlier preference for advantageous choices in humans (de Vries et al., 2008), while negative moods and trait anxiety are associated with riskier choices (De Visser et al., 2010; Miu et al., 2008; Suhr and Tsanadis, 2007). Thus the IGT may prove a suitable task for measuring emotional state in animals.

The IGT has already been successfully adapted to look at decision making in rodents in a number of studies (Homberg et al., 2008; Koot et al., 2010; Koot et al., 2013; van den Bos et al., 2006). Van den Bos et al. (2006) demonstrated that, similarly to humans, both rats and mice show an increasing preference for the advantageous choices as testing progresses. Various rodent versions of the IGT have been proposed and each includes a number of key features of the original task (de Visser et al., 2011) such as the occurrence of rewards and punishments, and the conflict between short- and long-term gains. In rodent versions of the IGT, similar to findings from human studies, anxiety has been found to lead to more risk-prone behaviour (De Visser et al., 2011). Decision making under risk has not been previously studied in pigs and may offer an opportunity to investigate emotional valence through risk preference.

## **EMOTION AND DECISION MAKING UNDER AMBIGUITY: THE JUDGEMENT BIAS PARADIGM IN ANIMALS**

Judgement bias is the most common emotion-linked cognitive bias measured in animals. While no standardised judgement bias task exists, recent studies have followed a similar pattern to that originally proposed by Harding et al (2004). In this study, rats were trained to lever press in response to a tone-cue associated with a positive outcome, the delivery of reward, and to withhold responding to a second tone-cue associated with an aversive event, a burst of white noise. To measure judgement bias, rats' responses (proportion of lever presses, latency to lever press) to intermediate and previously unheard 'ambiguous' tone-cues were recorded. While the exact methodology has differed across studies using the judgement bias paradigm, the basic principles have been the same; subjects are first trained on a discrimination task to distinguish a 'positive' cue, predicting reward, from a 'negative' cue, predicting no reward, a lesser reward, or a negative outcome. Subjects are then presented with 'ambiguous' cues and responses are scored as resembling responses towards the positive or negative cues i.e. indicative of expectation of a positive or negative outcome. This expectation is then interpreted as a proxy measure of background emotional state.

To date the paradigm has been used with a wide variety of mammals (including, mice: Boleij et al., 2012; dogs: Burman et al., 2011; sheep: Doyle et al., 2011a; rats: Harding et al., 2004; and calves: Neave et al., 2013), as well as birds (starlings: Brilot et al., 2010; chickens: Salmeto et al., 2011) and even insects (honeybees: Bateson et al., 2011). In a review by Mendl et al. (2009) the authors find that many studies have found judgement

biases in the direction predicted. Since then, a number of papers have been published using the judgement bias paradigm. A more recent review by Gygas (2014) found that while many studies have found biases, these biases were not always consistent across ambiguous cues, some were not in the direction predicted, and some were ascribed to alternative explanations than the original predictor. With respect to the lack of consistent responses to ambiguous cues, it has been argued that more pessimistic biases may occur both because of increased expectation of negative events, more common in anxiety, or because of low expectation of positive events, more common in depression, (Burman et al., 2009; Miranda and Mennin, 2007). Depending on the underlying cause of the bias different responses may occur to ambiguous cues closer to the positive and closer to the negative end of the scale.

Tasks have differed in the cue-type used, e.g. spatial (e.g. location of goal-pot, Burman et al., 2008; Doyle et al., 2010; Doyle et al., 2011b), auditory (e.g., noise, Douglas et al., 2012; tone, Enkel et al., 2010), olfactory (Bateson et al., 2011; Boleij et al., 2012), tactile (e.g. grade of sandpaper, Brydges et al., 2011), or visual, (e.g., shade and colour, Brilot et al., 2010; Neave et al., 2013). Tasks have also differed in the required response – while many tasks have used a Go/No-Go style response, where subjects are trained to ‘go’ for a positive cue and refrain from responding for a negative cue (e.g., Burman et al., 2008; Sanger et al., 2011), others have used an active-choice method where subjects are required to make a different active response to both positive and negative cues (e.g., Brilot et al., 2009; Enkel et al., 2010). Go/No-Go style tasks use the latency to respond and/or the proportion of Go-responses as outcome measures, while active choice tasks use the proportion of responses for the positive choice. While some studies have used treatments to induce alterations in affective state, targeting both long-term mood (e.g., enrichment, Brydges et al., 2011; Douglas et al., 2012; Matheson et al., 2008; Richter et al., 2012), and short-term emotion (e.g., pharmacological stimulation, Doyle et al., 2011a; Rygula et al., 2014; restraint, Doyle et al., 2010; pain, Neave et al., 2013), others have relied on natural variation in the population (e.g. stereotypes, Brilot et al., 2010; separation anxiety, Mendl et al., 2010).

Despite the above differences in methodology, results of the studies are promising. Of the studies that have found a consistent difference in judgement of ambiguous stimuli as predicted (according to the review of Gygas, 2014), a wide variety of treatments have been assessed, suggesting that the method has the potential to assess emotional responses across contexts and in a variety of species. Optimistic biases are associated with environmental enrichment in rats (Brydges et al., 2011; Richter et al., 2012), pigs (Douglas et al., 2012), and rhesus macaques (Bethell et al., 2012), as well as with playful handling in rats (Rygula et al., 2012). Pessimistic biases are associated with chronic environmental- and social-stress treatments, as well genetic models of depression in rats (Enkel et al., 2010; Chaby et al., 2013; Papciak et al., 2013), separation anxiety in dogs (Mendl et al., 2010) and restraint handling in rhesus macaques (Bethell et al., 2012). Furthermore, the paradigm has been successfully back-translated to study affective judgement biases in humans (Anderson et al., 2012).

The judgement bias paradigm has been proposed as one of the few measures of emotion in animals which explicitly measures the valence of an affective state, and has the potential to answer questions about what an animal wants by understanding the conditions that can lead to reduced negative and increased positive states. However, at the commencement of this thesis, no suitable method to study judgement bias in pigs had been published. Since commencing, only three studies have been published (Douglas et al., 2012; Döpjan et al., 2013; Scollo et al., 2014) besides the work presented in this thesis.

## AIM AND OUTLINE OF THE THESIS

The aim of this thesis is to develop and validate suitable tasks to study emotion in pigs using the cognitive paradigms discussed above. We wish to demonstrate that these tasks are more suitable for the measurement of emotion in pigs than existing measures, and we want to develop a task that can be used with pigs from different contexts, e.g. laboratory or farm. By choosing treatment groups expected to differ in emotion and mood, we hope to validate our tasks, and we aim to demonstrate the independence of responses in our tasks from each other, as well as other measures of cognitive function.

**Chapter 02** provides an in-depth review of the current state of behavioural measures of emotion in pigs. This chapter defines a list of important criteria for behavioural tests of emotion, and assesses the most commonly used tests for pigs according to these criteria, as well as providing a list of specific behaviours or behavioural patterns which may be useful in measuring emotional responses in pigs. In this chapter we highlight the fact that many commonly used tests do not tap into ethologically relevant behaviours of the pig, and further, are not performed in a standardised manner across studies.

**Chapter 03** describes the development of a suitable judgement bias task for pigs to assess decision making under ambiguity (see also **Appendix A**). The types of discrimination learning necessary for both go/no-go and active-choice judgement bias task designs are compared. An active-choice conditional discrimination task was ultimately developed using tone-cues to signal positive and negative outcomes which were differentially rewarded. The benefits of using such an active-choice task are discussed.

In **Chapter 04**, we apply the active-choice judgement bias task developed in Chapter 03 to assess and compare the responses of common farm and laboratory breeds of pigs to ambiguity. While differences in learning are found between the two breeds, both show similarities in responses to the ambiguous cues and across repeated testing.

**Chapter 05** (and **Appendix B**) cover the development of a decision-making task to assess decision-making under risk in pigs, based on rodent models of the Iowa Gambling Task, and responses are compared between our Pig Gambling Task and our previously developed judgement bias task. In an attempt to validate both tasks we compare the responses of low-birth-weight (LBW) and normal-birth-weight (NBW) pigs which are expected to differ in long-term mood states.

In **Chapter 06** we again use the LBW pig model. By treating sows with Allopuriol, a drug proposed to prevent some of the neurological damage associated with prenatal

hypoxia leading to LBW, in the last trimester of gestation, we wish to investigate whether such treatments can also alleviate the potential emotional problems associated with LBW as measured by responses to ambiguity in our active-choice judgement bias task. Furthermore, physiological measures of acute and chronic stress are used in an attempt to validate the LBW pig model and compare to behavioural responses to ambiguity.

In **Chapter 07**, in order to investigate the independence of responses to ambiguity from memory and learning in pigs, comparisons are made between responses in the judgement bias task and behaviour of the same pigs in a cognitively complex spatial holeboard task designed to assess both reference and working memory in pigs.

Finally, in **Chapter 8** the combined results of our studies measuring emotion using cognitive paradigms in pigs are discussed in a broader context.



# CHAPTER 02

## A REVIEW OF BEHAVIOURAL METHODS TO STUDY EMOTION AND MOOD IN PIGS, *SUS SCROFA*

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## ABSTRACT

The study of emotions in animals is of increasing importance to a number of disciplines such as animal welfare science and affective neuroscience. Pigs are a common farm animal species, most often reared in intensive systems. Moreover, they are increasingly being used in laboratories. To accurately understand the welfare needs of these animals, we need to be able to study emotion, the assumption being that positive emotional states contribute to good welfare, while negative states result in reduced welfare. A variety of methods have been proposed to study emotions in animals through behaviour, many of which have been applied to pigs. This review will focus on the methods by which behaviour can be used to study emotion in pigs. Firstly we discuss the variety of behavioural tests that have been applied to study emotion and mood in pigs. We propose a list of criteria with which to evaluate the behavioural tests and discuss each test with respect to these criteria as well as any behavioural, physiological or pharmacological validation. Secondly, we look at specific behaviours or behaviour patterns that may also be indicative of emotion and mood in pigs. We find a number of issues with the more commonly used behavioural tests, including the lack of ethologically valid test designs, and the need for greater standardisation of design which would facilitate comparison of results across studies. Furthermore, behaviours measured are often not specific to emotion, or sensitive to subtle differences in emotion. Suggestions for improvements to the current methods are given with a focus on species relevant behaviour and the potential for assessing both positive and negative emotions.

## Keywords

Pig; Emotion; Behaviour; Open Field; Vocalisation; Play

List of abbreviations used	
ACTH	Adrenocorticotrophic hormone
BT	Back Test
CBT	Cognitive Bias Test
EPM	Elevated Plus Maze
ET	Emergence Test
GABA	Gamma-Aminobutyric Acid
HAT	Human Approach Test
HIT	Human interaction test
L/DT	Light/Dark Test
NOT	Novel Object Test
OD/ET	Open-Door/Emergence Test
OFT	Open Field Test
PCA	Principal Components Analysis
PTZ	Pentylentetrazole
QBA	Qualitative Behaviour Assessment
TI	Tonic Immobility
WRT	Withdrawal Response Test

# INTRODUCTION

## Aim

The aim of this review is to give an overview of the methods by which emotion and mood have been studied through behavioural responses in pigs. After a brief introduction, this review is divided into two sections; *The first section* deals with the numerous behavioural tests that have been used to assess emotional responses in pigs, both through spontaneous behavioural responses and conditioned responses, while *the second section* deals with behaviours which have been studied in pigs in relation to emotion and mood, but which are not specific for a particular test. The theories behind these methods are discussed as well as their relevance as measures of emotion and mood in pigs.

## Defining Concepts

Scherer (2005) lists a number of mental phenomena which fall under the heading of affect, such as preferences, attitudes, emotion, mood, and affect dispositions (personality). In this review we restrict our study to measurements of emotion and mood. Unfortunately, there is no agreement as to the definition and nature of emotion (Cabanac, 2002). According to Moors (2009), emotion theorists disagree on several points; 1) the number and nature of components which form an emotional episode, 2) which of these components form the emotion itself as opposed to the wider emotional episode, 3) whether the components occur sequentially in an emotional episode, and whether this order is fixed, 4) the boundary between “emotions” and non-emotional mental-states, and finally, 5) nature of emotions, like being discrete vs. dimensional. Such disagreement complicates giving a comprehensive definition of emotion.

We use a simple definition from Schnall (2010); Emotions are “*specific, intense, and short responses to stimuli*”, and moods are “*longer, more ambiguous, and nonattributable affective feelings of lower intensity*” (Schnall, 2010). Both emotion and mood can be categorized by valence (positive/negative) and arousal (low/high) (Mendl et al., 2009; Paul et al., 2005). An individual’s core affective state (Russell, 2003) is a combination of background mood and current emotional response (Mendl et al., 2010), i.e. both emotion and mood can influence each other. Also important for this review is the concept of emotional contagion, “*an automatic motor mimicry of emotional expressions which... can generate physiological and subjective components of the emotion being mimicked*” (Edgar et al., 2012)” which forms the basis for empathy (de Waal, 2011). Simply put, emotional contagion is the transfer of emotion or mood states between individuals.

## The Importance of Studying Emotion in Pigs

The European Commission recognises animals as sentient beings, and as such they are provided with protection under animal welfare legislation. Moreover, many modern definitions of welfare include a reference to psychological state or emotion. Positive emotional states are assumed to contribute to good welfare, while negative emotional states can reduce welfare. Pig production systems are most commonly intensive systems; environments where many animals are produced at a minimum cost. Such environments

often do not meet the basic needs of the animals in terms of behavioural expression and cognitive challenge. These environments therefore are assumed to have a negative impact upon the pigs' emotional state and thus their welfare. Furthermore, since pigs are social animals, and are generally group housed, emotional contagion between individuals may also impact upon welfare (Reimert et al., 2013); the spread of emotion, whether positive or negative, within a group increases the number of animals affected by the emotion (Špinka, 2012). Therefore, accurate measurement of emotional state and emotional contagion in pigs is necessary in order to properly assess welfare needs. Research into animal emotion and mood is not restricted to the field of animal welfare science, and is of increasing interest to disciplines such as affective neuroscience, psychopharmacology and pain research (Mendl et al., 2010). Pigs, most often miniature breeds, have become a common biomedical model species used to investigate various aspects of human biology from surgical techniques, disease of organs, wound healing to xenotransplantation (Swindle and Smith, 2008). The increase in pig models can be attributed to a trend of moving away from primate models, but also to the greater homology between humans and pigs in terms of organ physiology, size and development (Lunney, 2007), when compared with common rodents. Recently, there has also been an increase in the use of pigs as models in neuroscience (Lind et al., 2007) due to the greater similarity in brain growth and development to humans when compared with the more common rodent models.

### **Why Focus on Behaviour?**

Among emotion theorists most agree that emotions and emotional episodes are componential in nature, and there is some consensus on which components are included (2009). Possible components often included are a cognitive component, a feeling/subjective component, a physiological/somatic component, a motivational component and a motor/behavioural component (Frijda, 2009; Moors, 2009; Paul et al., 2005). It is thought that emotions evolved from basic abilities that allowed animals to avoid danger and seek resources (Panksepp, 1982) and is still considered to play a role in motivating and directing behaviour (Rolls, 2000). The behavioural component allows the animal to respond to the emotion eliciting stimulus and therefore measurement of this may give some indication of the intensity and the valence of the underlying emotion; behaviour may provide a useful proxy measure of the emotion itself. Two useful reviews of behavioural methods to study emotion in pigs exist, one with reference to the usefulness of the methods to animal welfare science (Forkman et al., 2007) and the second with respect to neuroscience (Lind et al., 2007) both of which focus on a few common behavioural tests. The present review aims to provide more complete coverage of tests used in pigs, including both measures of negative and positive emotions, as well as behaviours which can potentially be measured in a non-test environment.

## BEHAVIOURAL TESTS OF EMOTION IN PIGS

In the following subsections, while the discussion of each test follows a similar pattern, some tests are discussed in more detail than others, depending on the test performances in the various studies. Some tests, such as the Open Field Test and Novel Object Test, have been performed in such a variety of ways that it is difficult to make direct comparisons across studies. Other tests, such as the Elevated Plus Maze or Conditioned Discrimination tests have been performed following similar methods, allowing to describe in more detail the methodology used. Tests are described regarding the theoretical principle behind it, its origin and the typical methodology and measures used. The inherent assumptions underlying these tests will be discussed with respect to their relevance for pigs, following which, results from pig research will be discussed with respect to behavioural, physiological or pharmacological validation where available.

There are no formal criteria for behaviour tests of emotions in pigs, but we suggest the following list (adapted and extended from (Gieling et al., 2011)) and these criteria will be referred to by number hereafter:

- i. The task should be ecologically valid (Koolhaas et al., 2006) and draw upon species-relevant emotional responses. This may preclude the one-to-one implementation of tests, validated for one species, to other species without further validation.
- ii. If the task involves unconditioned responses, the animal should be free to display its natural emotion-related behaviours (Ohl, 2003).
- iii. Ideally, the task should specifically and unambiguously capture emotion-related behaviours. For example, results can be confounded by differences in learning capacity if a task is too complex, or by differences in activity levels or motivation.
- iv. The task should be sensitive enough to capture subtle differences between emotional responses, for example differences in levels of arousal and valence.
- v. The task should allow investigation of changes over time – in response to both experimental interventions and developmental and/or aging processes – and should consequently be suited for repeated testing and longitudinal studies.
- vi. Unless the task aims to specifically address responses to novelty or handling, animals should be well habituated to all procedures before testing to prevent potential stressors influencing the animal's behaviour.
- vii. The task itself and variables recorded should be standardized, allowing for comparisons to be made between studies (van der Staay et al., 2010).
- viii. A task should, where possible, be automated to remove any effect of the experimenter (van der Staay et al., 2009b), provided the system is sensitive enough to subtle changes in behaviour (Ohl, 2003).

### Open Field Test

#### *Test Description: Theory & Measurements*

Developed by Hall (1934) as a test of emotionality in rodents, the Open Field Test (OFT) wasn't used with pigs until the 1960s (Beilharz and Cox, 1967) by which time it was (and still is) one of the most commonly used behavioural tests of locomotion, exploration and anxiety in rodents (Prut and Belzung, 2003; Walsh and Cummins,

1976). The simplicity of the test, as well as its well-established use in laboratory studies, has led to its use with species outside the laboratory including farm animals and birds. In a typical OFT an animal is placed in an unfamiliar arena for a period of time, and the behavioural response is recorded. Of most interest to the study of emotion are the behaviours said to be indicative of anxiety in the OFT: elimination, time spent in the centre, or close to the walls of the OFT arena, and the level of activity in the first 5 minutes (Gould et al., 2009).

### ***Assumptions of the OFT***

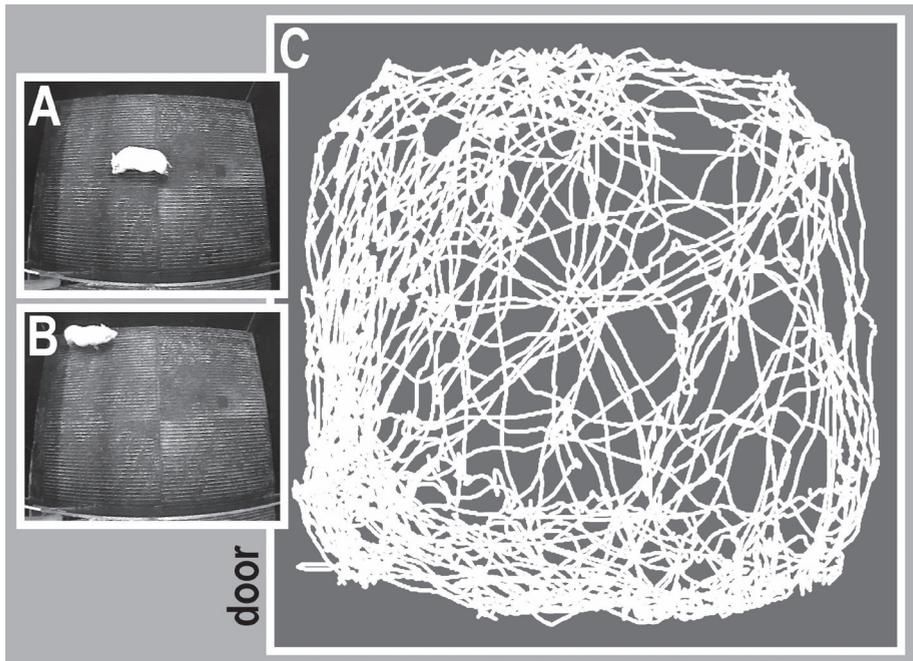
According to Prut and Belzung (2003) there are two aspects to the OFT which are likely to be a cause of anxiety for the animals: social isolation and agoraphobia. These factors are only salient for group-living animals and those that show fear of open spaces. The novelty of the OFT arena is likely a stressor as pigs will move gradually into an unfamiliar open area (Špinka, 2006). A number of pig studies do report that social isolation is a stressor as measured by alterations in behaviour, physiology and the brain (Herskin and Jensen, 2000; Kanitz et al., 2005; Poletto et al., 2006; Ruis et al., 2001b; Tuchscherer et al., 2004). No evidence of thigmotaxis (wall-hugging behaviour) commonly seen in rodents (Ohl, 2003) could be found for domestic pigs. Wild boar prefer to forage close to the forest edge, move along narrow landscape elements, such as hedgerows, in winter and spring, and avoid the centres of arable fields (Thurfjell et al., 2009). However, the average distance at which they forage from the edge of the forest is 54 m, a distance far bigger than a typical OFT arena.

### ***Results of tests: OFT and Pigs***

The OFT is generally performed in pigs in a square or rectangular arena unfamiliar to the animal, ranging from about 3 m<sup>2</sup> to 36 m<sup>2</sup> in area (Fraser, 1974; Puppe et al., 2007) although circular arenas have been used (Andersen et al., 2000a; Andersen et al., 2000b). Test times are generally between 4 to 10 minutes, but tests of 1 hour have also been carried out (Jones et al., 2000; van der Staay et al., 2009a). The OFT is usually performed with individual animals, though it has also been used for testing pairs (Donald et al., 2011) and groups (Magnani et al., 2012; Ruis et al., 2000) of pigs.

Looking at the measures that have been used in a sample of OFT tests performed with pigs, we find a wide variety of behaviours recorded using a number of methods (see Supplementary Data, Table 1).

*Location within the OFT:* Although a tendency of pigs to spend more time in the periphery of the OFT arena than in the centre has been reported (Donald et al., 2011), this could be explained by the location of the entry door along the periphery, or that there is more to explore with both wall and floor surfaces available (Fig. 1). Measures of location were not related to other behavioural tests of emotion, such as an Elevated Plus Maze (EPM) and a Light/Dark Test (L/DT) (Andersen et al., 2000a), although in rodents correlations among such measures are not always found (Ramos et al., 2008). In pigs, the time spent in the centre of the OFT does not seem to be sensitive to various forms of enrichment (Beattie et al., 1995; Siegford et al., 2008) or treatment with the



**Fig. 1** Screenshot of an adult Göttingen minipig boars in the centre (A) and along the edge (B) during an OFT. Movement of a minipig in an OFT captured using Ethovision video-tracking software (Noldus, Wageningen, The Netherlands) (C) shows that while higher concentrations of movement are around the edges of the OFT arena, the pig frequently enters the middle of the arena. Furthermore, the highest concentration of movement of the pig is near the entrance/exit of the arena.

anxiolytic diazepam (Andersen et al., 2000b), while pigs treated with another anxiolytic, Azaperone (Stressnil), surprisingly spent more time in the periphery of the OFT than saline treated controls (Donald et al., 2011; Prut and Belzung, 2003).

*Movement and Exploration:* As locomotor activity and exploratory behaviour are not always clearly distinguished, these categories have been grouped for the purpose of discussion. The variable reduction techniques Principal Component Analysis (PCA) and Factor Analysis have indicated that a common measure of locomotion of pigs in OFT (lines crossed/squares entered) does not mirror anxiety (Andersen et al., 2000a; Jensen et al., 1995). Another study found activity measures to load on a component along with avoidance measures, which they interpreted as a measure of neophobia (Donald et al., 2011). Thodberg et al. (1999) distinguished a factor relating to exploration and activity and a factor relating to non-exploratory activity. A PCA carried out by Giroux et al. (2000) found that, within the same factor, activity measures loaded highly positively while exploration measures loaded highly negatively. Andersen et al. (2000a) found that movement in the OFT was directly related to the number of open arm entries in the EPM and entries into the light in a L/DT, suggesting high activity in OFT means low anxiety.

The finding that pigs from barren environments scored higher on locomotion measures ((Beattie et al., 1995), but see Pearce and Paterson (1993), for a failure to demonstrate this) seems in line with this interpretation. It seems that activity and exploration in an OFT are separate types of behaviour, and general activity need not reflect anxiety per se. Different methodological factors and animal characteristics are known to influence the activity of pigs in the OFT. Repeated testing on the same animals has been reported to result in a reduction in activity, locomotion or exploratory behaviours with both relatively short (Donald et al., 2011; Kanitz et al., 2009; Sumner et al., 2008) and relatively long periods between tests (Désautés et al., 1997; Rutherford et al., 2006; von Borell and Ladewig, 1992). Large White pigs, which have naturally lower levels of circulating cortisol and ACTH than Meishan pigs, were found to have the higher locomotion scores of the two breeds (Désautés et al., 1999; Mormède et al., 1994). Artificially increased cortisol in sows in early or late, but not mid, gestation led to higher locomotion scores in their offspring (Kranendonk et al., 2006), while administration of ACTH to sows in mid or late gestation did not produce differences in locomotion or exploration behaviours of the offspring (Andersen et al., 2000b; Donald et al., 2011; Otten et al., 2007).

*Escape Behaviours:* Escape behaviours like jumping or pushing at OFT entrance are not always part of OFT observation protocols, and some report low instances of such behaviours (Jensen et al., 1994). Administration of ACTH to sows in late gestation led to more escape attempts in offspring tested at 24 days old (Otten et al., 2007). Across repeated OFT, in some studies escape attempts increased (Kanitz et al., 2009; Otten et al., 2007; Rutherford et al., 2006), where others show a reduction of escape attempts (Kanitz et al., 2009; Puppe et al., 2007), or no effect (Kanitz et al., 2004). Escape behaviours may be more pronounced in some experimental setups than others, e.g. a moveable entry gate may encourage pigs to push it. Age at testing may also play a role.

*Elimination and Vocalisation:* Elimination in the OFT has been shown to have little relation to other common tests of emotion (Andersen et al., 2000a). Both elimination and vocalization decrease with repeated testing (Désautés et al., 1997; Fraser, 1974; Kanitz et al., 2009). Piglets from sows which were administered cortisol in early- and mid-gestation vocalised more than control piglets (Kranendonk et al., 2006), however this result was not replicated when sows were administered ACTH in mid and late gestation (Otten et al., 2007). Vocalisation as a measure of emotional state is discussed in detail later on.

*Physiological Correlates:* Both heart rate and cortisol measures have been found to increase during the OFT (Désautés et al., 1999; Hessing et al., 1994; Loijens et al., 2002; Ruis et al., 2001a).

### ***Discussion: OFT and Pigs***

We agree with Forkman et al. (2007) and Lind et al. (2007) that measures of location in the OFT are not ethologically valid indicators of emotion in pigs (i). Two factors, social isolation and an unfamiliar environment, may induce an emotional response in pigs. How best to measure the response may depend on which of these factors is more salient. For example, vocalisations may reflect attempts to regain contact with group-

mates, while exploration measures may reflect response to novelty. Prior habituation to social isolation or group habituation to the environment may allow us to tease out the effects of isolation vs. novelty in the OFT (vi).

While the OFT does allow the pig to express a wide range of emotion related behaviours, some emotions may trigger an escape response which cannot be performed (ii). Very young pigs may all experience strong negative emotions in an OFT, but if given a chance to return to “safety” might show more differences in levels of exploration. Erhard and Mendl (1999) argue that the behaviour resulting from forced confrontation with novelty is different from neophobia itself. Furthermore, it is important to know what behaviours are relevant to the research question at hand; few studies provide specific hypotheses about which behavioural responses should be altered in the OFT and the measures recorded may be too general for detecting subtle differences (iv). Often, there are no beforehand assumptions on the interpretation of behavioural responses and what some use as measures of pure activity, others use as indications of anxiety (iii). As the OFT has been carried out to date, there is no standardization (vii) of the arena design nor variables recorded to allow direct comparisons across studies. Furthermore, the test is subject to habituation effects with reductions in activity/exploration measures upon repeated testing, creating difficulties for longitudinal research (v). Once these issues have been teased out, automation of the test could be considered (viii).

## **Elevated Plus Maze**

### ***2.2.1 Test Description: Theory and Measurements***

The Elevated Plus Maze (EPM) in its current form was developed by Handley and Mithani (1984), based on work by Montgomery (1955), to study fear-induced behaviour in the rat. The EPM consists of a raised plus-shaped maze, with opposing arms which are either closed (walled by barriers) or open (without walls). The motivation to explore the EPM (approach) is said to conflict with the fear of the unprotected open arms (avoid). The EPM is a popular test of rodent anxiety (Carobrez and Bertoglio, 2005). The test has pharmacologically been validated in rodents through the use of both anxiogenic and anxiolytic substances (Hogg, 1996; Walf and Frye, 2007). Anxiety in the EPM is commonly measured by the number of entries onto and the time spent on the open and closed arms.

### ***Assumptions of the EPM***

The EPM has two main assumptions: it is an ethological animal test for anxiety, as aversion of the open arms in the EPM has a direct ecological relevance for the animal, and it is an unconditioned test as the animal has an innate aversion to the open arms and dangerous drop (Carobrez and Bertoglio, 2005; Walf and Frye, 2007). Evidence from studies of transport and loading in pigs show that pigs prefer solid and mesh barriers on a ramp, i.e. more closed sides, as opposed to horizontal or vertical bars i.e. more open sides (Phillips et al., 1988). The fear of falling off the ramp may be more important here than agoraphobia as there is little evidence for thigmotaxis in pigs (see *Open Field Test*), and it is assumed that openness is not an ethologically

relevant aversive stimulus for them. A study on the depth perception in (only 2) six week-old piglets revealed that they avoid the “deep” side of a visual cliff (Walk and Gibson, 1961). The lack of more convincing studies leaves it uncertain to what extent maze elevation is aversive for pigs, which is questioned even for rodents (Treit et al., 1993).

### ***Results of tests: EPM and Pigs***

A similar EPM apparatus was used in six studies with pigs (Andersen et al., 2000a; Andersen et al., 2000b; Donald et al., 2010; Janczak et al., 2000; Janczak et al., 2002a; Rutherford et al., 2012), with a maze elevation of 1m, arms of 0.6m X 0.3m in size and transparent closed arms. Transparent barriers were used to create closed arms. Test duration was consistent at 5 minutes, and 5- to 8-week-old pigs of both sexes were tested. A significant though low correlation was found between time spent on the open arms and light side entries in a light/dark test (L/DT) (Andersen et al., 2000a). Other correlations between behaviour on the EPM and measures of L/DT and OFT likely reflect activity levels rather than anxiety. Two studies found that pigs do not show unconditioned avoidance of the open arms, one of the main assumptions of the EPM (Donald et al., 2010; Janczak et al., 2002a). Rodent behaviour in the EPM can be affected by minor alterations in the design of the apparatus as well as transport and handling prior to testing (Hogg, 1996). Surprisingly, in pigs, EPM behaviour was found not to be susceptible to effects of pre-handling or transport (Janczak et al., 2000; Janczak et al., 2002b).

Effects of anxiolytic compounds on EPM behaviour in pigs differ across studies. Compared to saline-treated controls, pigs treated with diazepam spent more time on, and more frequently entered, the open arms (Andersen et al., 2000b), while those treated with Azaperone spent more time in the closed arms, and entered those more often (Andersen et al., 2000b; Donald et al., 2010; Rutherford et al., 2012). Perhaps the differences reflect the different mechanisms of action of the compounds, being strongly GABA-ergic vs. dopaminergic, respectively (Rutherford et al., 2012).

### ***Discussion: EPM and Pigs***

The EPM is proposed as one of the few tests in which fear responses can be separated from general measures of activity (Andersen et al., 2000a), as activity on the open arms can be compared with activity on the closed arms (iii). Furthermore, it allows animals to display a wide variety of emotion related behaviours, including some level of escape or avoidance behaviours with the presence of the closed arms (ii), and in pigs is not affected by pre-test procedures (vi). The methods used so far with pigs have been fairly standardised, but not yet automated (vii, viii). Measures commonly recorded in the EPM have been described as too general and more specific behaviours may be more subtle indicators of emotional state (Carobrez and Bertoglio, 2005) (iv). No information on repeatability is yet available (v). Most importantly though, pigs do not show unconditioned avoidance of the open arms (Donald et al., 2010; Janczak et al., 2002a), and it is likely, as with the OFT, that the novelty of the environment as well as

the social isolation play a large part in determining the response of the pig in the test. We conclude that the EPM is not an ethologically valid test of anxiety for pigs (i).

## **Light Dark Test**

### ***Test Description: Theory and Measurements***

The Light/Dark test (L/DT) was first developed to investigate the anxiolytic effect of benzodiazepines in rodent species (Crawley and Goodwin, 1980), and it is still a commonly used test of anxiety. Test subjects are placed in either an OFT-style lighted compartment or an adjacent, smaller, dark compartment. Behaviours commonly recorded include latency to move from the light to dark (or vice versa) and number of transitions between compartments, as well as the time spent, and the behavioural activity, in both compartments (for full review see Hascoët et al., 2001). In mice, the L/DT has been validated pharmacologically with benzodiazepines. Explorative behaviour in the test has also been increased through use of other anxiolytic compounds (Bourin and Hascoët, 2003).

### ***Assumptions of the L/DT***

Similarly to the OFT and the EPM, the L/DT paradigm is based on the assumption that motivation to explore a novel environment will conflict with the test subject's inherent aversion towards the open space and bright lighting of the light compartment (Hascoët et al., 2001). Unlike mice, rats and even wild boar, domestic pigs sleep mostly during darkness (Špinková, 2009). Light has actually been shown to be rewarding to pigs under various testing conditions (Baldwin and Meese, 1977; Baldwin and Start, 1985) and transportation studies have shown that pigs move readily from dark to light (van Putten and Elshof, 1978), suggesting brightly lit areas are not inherently aversive. Regarding the "openness" of the light compartment, while there is ample evidence to suggest that pigs have a high motivation to explore (*see Novel Object Test*), open spaces are not aversive to pigs as they do not show thigmotaxic behaviour (*see Open Field Test*).

### ***Results of tests: L/DT and Pigs***

In the few L/DT studies conducted in pigs, the light and dark compartments were of equal size, and smaller than typical OFT arenas used with pigs. Andersen et al. (2000a) found that 7-week-old piglets spent more time in the dark (start) compartment, and 27% of subjects remained in the dark compartment for the duration of the test. In a set-up with a brighter light compartment (2100 and 51 lx in the light and dark compartment, respectively, compared to 90 and 2.5 lx in the foregoing study), 1-week-old piglets showed a shorter latency to move from the dark compartment to the light than vice versa, and moved sooner between compartments if both were lit than when both were dark (Tanida et al., 1996).

Time spent in the light compartment was found to have low positive correlations with measures of activity in the EPM and OFT (EPM: entries into open and closed arms, OFT: lines crossed) (Andersen et al., 2000a). PCA analyses revealed a direct relationship between time spent in the light compartment and on the open arms in the

EPM (Andersen et al., 2000a). Diazepam treated pigs showed no differences from saline treated controls on latency, duration and number of entries into the light compartment (Andersen et al., 2000b).

### ***Discussion: L/DT and Pigs***

Since so few studies of L/DT responses in pigs have been performed it is difficult to evaluate the test for use with pigs with regard to standardisation and automation (vii, viii), habituation/handling effects (vi) and repeatability (v). Similarly to the EPM, the L/DT allows animals to display a wide range of emotion related behaviours, including escape/avoidance of the “more aversive” lit compartment (ii), and the test design used with pigs potentially allows for the separation of activity measures from emotion measures by providing a “safe” dark and an “aversive” light compartment of equal size (iii). However, the behaviours recorded may again be too broad to assess subtle differences (iv). The main problem with using the L/DT with pigs is that there is not enough evidence to suggest that the test is ethologically valid (i); as discussed with respect to the OFT, pigs do not seem to be averse to brightly lit places. Agreeing with Andersen et al. (2000b) we conclude that the L/DT is not suitable for testing emotional responses in pigs.

## **Novel Object Test**

### ***Test Description: Theory and Measurements***

The Novel Object Test (NOT) is commonly used to assess fear or anxiety responses to unfamiliarity. Although the test is performed in a variety of ways, the latency to explore an unfamiliar object is always measured, often along with the duration and frequency of object exploration. Avoidance of the stimulus, indicated by higher latencies to approach /investigate and less time spent investigating the stimulus, is taken to indicate higher levels of anxiety or fear.

### ***Assumptions of the NOT***

Similarly to the OFT and EPM, the NOT assumes that animals undergo a conflict between their motivation to explore and to avoid when confronted with an unfamiliar object. Neophobia, which can be defined as *a stilted or avoidant behavioral response to novel physical objects*, is present in many animal species and said to underlie the traits *proactive/reactive coping* and *exploration/curiosity* in pigs (Cavigelli, 2005). Neophobia can describe short-term responses to a change in environment, or a more stable personality trait (Cavigelli, 2005). Neophobic responses are commonly used indicators of negative emotions such as fear or anxiety.

There are two types of exploration: goal-directed exploration (extrinsic exploration) and exploration motivated by curiosity (intrinsic exploration). It has been argued that both types are present in pigs (Day et al., 1995; Studnitz et al., 2007; Wood-Gush and Vestergaard, 1991); that is, pigs are motivated to explore their environment both to seek resources (for about 50% of the time Špinka, 2009), but also out of an intrinsic need to explore. There is ample evidence to suggest that pigs have a high motivation to explore novelty; pigs have a preference for novel objects over familiar ones (Wood-Gush and

Vestergaard, 1991), will work to gain access to exploratory (rooting) material (Pedersen et al., 2005), and prefer environments where they have to explore/forage for food versus those with food freely available (de Jonge et al., 2008b).

### ***Results of tests: NOT test and Pigs***

Since the earliest reference to use of the NOT with pigs, studying the effect of diet restriction on exploratory behaviour (Barnes et al., 1976), it has been used frequently to assess responses to novelty and fear responses in pigs. While mostly similar measures are recorded across studies – principally the latency, frequency, and duration of contacts with the novel object – both the novel objects themselves and the method of presentation has differed between studies (see Supplementary Data, Table 2). The novel object is sometimes present in the arena before the pig enters, sometimes introduced slowly after the pig is present, and sometimes introduced suddenly. Other differences include testing pigs individually or in groups, or in the home pen versus a novel environment, often with little habituation.

No correlations were found between latency to touch the object and latency measures in a Human Approach Test (HAT) and Open Door/Emergence Test (OD/ET) when pigs were group tested in their home pen at 23 weeks of age (Brown et al., 2009). Approach scores in the NOT performed by van Erp-van der Kooij et al. (2002) were moderately correlated with scores on a HAT and OD/ET in pigs. Inconsistent NOT responding and low across test correlations have been found in studies including repeated testing (Brown et al., 2009; Brown et al., 2009; van Erp-van der Kooij et al., 2002). Janczak et al. (2003a) suggest that age may play a part in NOT as they found positive correlations between duration and frequency of exploration of a suddenly-introduced object and frequency and duration of exploration of the human in a HAT in 8-week-old pigs but not when the animals were retested at 24 weeks.

Pigs reared in enriched environments showed greater avoidance of a suddenly-introduced novel object into the home pen and had a longer latency to make contact than barren-housed pigs (Olsson et al., 1999). Pigs with marginally enriched conditions tested alone in a separate arena tended to spend more time in contact with a suddenly-introduced object and touched the object more frequently than did more barren housed-pigs (Tönepöhl et al., 2012). When novel object tests were carried out in a separate arena comparing pigs housed with or without substrate no differences in object directed behaviours were found (Wemelsfelder et al., 2000). However when the test was carried out in the home pen, barren housed pigs spent more time chewing the object and were more likely to chew or nose the object (Wemelsfelder et al., 2000). Individual pigs' responses to novelty have been found to be fairly stable (Janczak et al., 2003a; Spooler et al., 1996), but handling can impact on responses in the test (Hemsworth et al., 1996a) and the aforementioned findings indicate a strong influence of the setting in which the novel object is presented.

In response to the sudden introduction of an object, Back Test (BT) resistant (proactive) pigs showed a greater increase in heart rate than non-resistant (reactive) pigs (Hessing et al., 1994). Pair-housed gilts that were tested alone also showed a greater increase in heart rate responses to a slowly-introduced object compared with gilts that were individually

housed (Ruis et al., 2001a). Post-weaning pigs treated with the anxiolytic midazolam spent more time compared to saline-treated controls in proximity of a trough in which a novel stimulus of various modalities (visual, auditory, or olfactory) was presented, though this difference was not found for the visual stimulus in finishing pigs (Dalmau et al., 2009). Pigs treated with Azaperone spent more time close to a ball during an OFT than saline treated pigs (Donald et al., 2011). Pigs' heart rate responses to novel objects and anxiolytic-mediated eagerness to approach them suggests that anxiety does play a role in the pigs' NOT behaviour.

### ***Discussion: NOT test and Pigs***

Boissy (1995) states that one of the most powerful experimental paradigms to provoke a negative emotional response in animals is to expose the animal to novelty. While pigs do show a strong motivation to explore novelty, they also show some initial aversion to novel objects, suggesting that exposure to novelty is an ethologically valid test of emotional response in pigs (i). Tests relying on an exploration-avoidance conflict may not be useful for comparing anxiety in animals that differ in prior experience with exploration. e.g. barren housed-pigs may have a greater need to explore novel objects (de Jong et al., 1998) having had less chance to satisfy this motivation compared with enriched-housed pigs. The NOT allows pigs to display a wide variety of behaviours, including avoiding the novel stimulus (ii), and it produces individual responses that are stable across repeated testing (v). Studies done on NOT with pigs lack standardisation (vii) and for example the manner in which the object is introduced is likely to have a big impact on the pig's responses. Sudden introduction of objects may be perceived as a threat in itself, masking differences in neophobia. Sheep show a greater startle response and short-term increase in heart rate to suddenly introduced objects compared with gradually introduced ones (Désiré et al., 2004; Désiré et al., 2006). In terms of the NOT paradigm being able to register subtle differences in emotions, again, the manner of introduction of the object may also be important (iv) - appraisal theories pose that specific emotional responses to an event depend on characteristics including suddenness, familiarity, and predictability (Veissier et al., 2009).

A further issue with the lack of standardization between studies is the lack of proper habituation to the experimental environment and procedures (vi). Studies which have performed the NOT in a separate test area often provide only minimal habituation to the arena before introducing the object. This confounds responses in the NOT by not habituating the animals to the novel environment and social isolation. Mice will explore a novel object more if they are free to return to a familiar environment than when they cannot (Misslin and Cigrang, 1986), suggesting that perceived control over approach or avoidance of a stimulus plays a role in the level of anxiety it invokes. Issues regarding the generalness of measures (iv) and requirements for automation of test procedures (viii), as raised for the OFT, apply to the NOT also.

## **Human Interaction Tests**

### ***Test Description: Theory and Measurements***

Human interaction tests (HIT) were first used in the 1980s to study the effect of the stockperson and handling on productivity and growth in pigs (Hemsworth et al., 1981a; Hemsworth et al., 1981b). The test takes one of two forms. The voluntary Human Approach Test (HAT) looks at the latency of an animal to approach or contact an unmoving, generally unfamiliar, experimenter within a stated length of time. The Withdrawal Response Test (WRT), focuses on the behavioural reaction of the animal when an experimenter approaches the animal (Marchant-Forde, 2002).

### ***Assumptions of the HIT***

Similar to tests discussed previously, human interaction tests are assumed to induce an approach-avoidance conflict in the animal. Curiosity may be more dominant in the voluntary HAT, while avoidance may be more dominant in the WRT (Waiblinger et al., 2006). The HAT assumes some level of motivation to have contact with the human. When pigs were denied the opportunity to make contact with a human, they showed frustration responses (Terlouw and Porcher, 2005), suggesting an underlying motivation to contact humans. It is unclear if this is governed by a motivation to explore or aggression. Sows classified as more aggressive towards humans also scored higher in “boldness”, a score partly based on approach latency and frequency in a HAT (Marchant-Forde, 2002). The WRT may provide a better measure of fear responses since it is based strongly on avoidance responses.

### ***Results of tests: HIT and Pigs***

As with the OFT and NOT, direct comparisons between experiments using HIT is difficult due to design differences. Many studies test the pigs alone in an unfamiliar arena (e.g. Hayne and Gonyou, 2003; Hayne and Gonyou, 2006; Hemsworth et al., 1994; Hemsworth et al., 1996a; Hemsworth et al., 1996b; Marchant et al., 2001; Marchant-Forde, 2002; Siegford et al., 2008), while others test in groups in the home pen (Brown et al., 2009; van Erp-van der Kooij et al., 2002) or an adjacent corridor (Ruis et al., 2000). Studies also differ in which measures they use. For the HAT, some authors use the latency to approach or make contact with the human, while others look at duration and frequency of contacts with the human. In the WRT, responses are usually categorised on a scale from no to extreme avoidance response. The WRT can be performed with free-moving pigs or when pigs are confined ( e.g. in sow stalls, Scott et al., 2009b). Most studies perform either the HAT or the WRT but some also combine the two (e.g. Marchant et al., 2001; Marchant-Forde, 2002).

Large variation in individual responses in the HAT (e.g. Marchant et al., 2001), makes it difficult to find statistical differences in behaviour between treatment groups. Previous positive experience with humans may encourage approaches in the HAT (Hemsworth et al., 1994; Hemsworth et al., 1996a; Hemsworth et al., 1996b; Tanida et al., 1995). Easy to handle pigs had shorter latencies to approach in a HAT (Hemsworth et al., 1994) and had weaker avoidance reactions in a WRT (Clouard et al., 2011; Lawrence et al., 1991).

This contrasts with the findings of Marchant-Forde (2002) mentioned above where gilts which showed more stockperson directed aggression had lower approach latencies.

Some authors report (low) correlations between latency measures in the HAT and other behavioural tests such as the NOT (van Erp-van der Kooij et al., 2002) and Pen Door Test, ODT (Brown et al., 2009; van Erp-van der Kooij et al., 2002) as well as between WRT responses and ODT latency measures (Lawrence et al., 1991) in group-tested pigs. In pigs tested individually, however, Janczak et al. (2003b) found that NOT and HAT responses loaded onto separate factors. Vocalizations of gilts tested alone before and during the entry of the human in an unfamiliar arena correlated with lower contact latencies and more frequent contact in a HAT (Marchant et al., 2001), which is interesting as vocalization is often taken as an indicator of negative arousal in tests such as the OFT.

Only low correlations were seen between scores in HAT tests separated by 3-4 days (Brown et al., 2009), and no correlations were seen when the test and retest were separated by 5 or 14 weeks (Ruis et al., 2000; van Erp-van der Kooij et al., 2002). Responses of sows tested individually to approaches by the experimenter in a WRT have higher repeatability than responses in the HAT, and were also found to be only weakly or not at all related to HAT measures (Scott et al., 2009b), suggesting that the HAT and the WRT measure different aspects of responses towards humans.

Heart rate responses to the sudden entry of a human, and average heart rates during the human presence in the pen, were higher in pigs housed individually than pair-housed (Ruis et al., 2001a). Outdoor-housed pigs had a lower average heart rate while the human was present, but did not differ from indoor-housed pigs in their heart rate response to the entry of the human (Marchant-Forde et al., 2003). Sows which were aggressive to the stockperson had a reduction in heart rate in a WRT while sows which showed piglet-directed aggression had an increase (Marchant-Forde, 2002). Higher heart rate measures were also associated with the performance of squeals in a HAT, but with a reduced contact latency (Marchant et al., 2001). Low-Resistant pigs (Back Test) had higher contact latencies in a HAT (performed as part of a NOT) as well as higher cortisol levels than High-Resistant pigs (Ruis et al., 2000).

### ***Discussion: HIT and Pigs***

The key assumption of both the HAT and WRT is that a pig faces an approach-avoidance conflict when confronted with an unfamiliar person, and that more anxious pigs will be less likely to approach (HAT) or show a greater avoidance response when approached (WRT). We know that pigs are motivated to make contact with people (i) but that the cause of the motivation may differ; pigs may approach out of a motivation to explore (indicative of lower anxiety) or may approach out of aggression (iv). The common measures used lack the sensitivity to differentiate between these two motivations (iii). While the HAT allows for more free display of natural responses, the WRT, due to the nature of the test design, can limit the pigs' ability to respond fully (ii). As with previous tests, the lack of standardisation across studies using both the HAT and WRT makes comparisons difficult (vii). While some evidence of stability in responses to repeated

WRTs has been found, the HAT seems more susceptible to effects of repeated testing (v). Age seems important as older animals are likely to have experienced more handling. As with the NOT, pigs ought to be well habituated to the test environment (vi). This task is not suitable for automation as it involves responses to a human (viii) but the behaviour of the person during the test should be standardised.

## **Open Door/Emergence Test**

### ***Test Description: Theory and Measurements***

The Open Door Test (ODT), also known as the Emergence Test (ET), has been used to assess emotion in rats as early as the 1930s (Anderson, 1938). It is said to be a test of “timidity” (Archer, 1973) and a measure of neophobia (Paré et al., 2001). With rodents, the OD/ET is often performed by placing the animal in an unfamiliar start box and measuring the latency to emerge into another unfamiliar arena.

### ***Assumptions of the OD/ET***

As with the OFT and NOT, the main assumption in the OD/ET is that the motivation to explore beyond the open door will conflict with the motivation to avoid the unfamiliar. As discussed in previous sections, pigs have a strong drive to explore but are cautious in the face of novelty.

### ***Results of tests: OD/ET and Pigs***

The majority of OD/ET studies performed with pigs have looked at the latency to emerge from the home pen in groups ((Brown et al., 2009; de Jong et al., 2000; Lawrence et al., 1991; Purslow et al., 2008; Ruis et al., 2000; van Erp-van der Kooij et al., 2002) but see (Erhard and Mendl, 1999; Jones et al., 2000; Ruis et al., 2001a)). Measures include latency to emerge (Brown et al., 2009; Ruis et al., 2000), scores grouping pigs into categories based on latency to emerge (Purslow et al., 2008), and actions required to get the pig to leave the home pen (Lawrence et al., 1991).

While van Erp-van der Kooij et al. (2002) found some, generally low, correlations between the OD/ET and HAT, Brown et al. (2009) did not. Both studies found (generally low) correlations of responses in repeated testing of the OD/ET over relatively short (consecutive days) and longer (5-7 weeks vs. 10-12 weeks) inter-test intervals. When animals were tested alone in an unfamiliar start box, emergence times were found to decrease after the first test (Ruis et al., 2001a). Erhard and Mendl (1999), on the other hand, found emergence latencies to increase after the first test, but since pigs in this study were subjected to a Tonic Immobility test immediately upon exit, this may have resulted in a greater reluctance to exit. Unsurprisingly, pigs scored as “willing” to exit in the OD/ET are more likely to be involved in piling incidents at slaughter plants, while those scored as “reluctant” receive more prodding or hitting from handlers (Purslow et al., 2008).

### ***Discussion: OD/ET and Pigs***

The OD/ET test is based on the approach-avoidance conflict, which we consider to be valid for pigs (i), but is subject to significant procedural differences across studies (vii). An OD/ET with the option to stay in a familiar area or enter a novel area cannot be interpreted in the same way as when both the start and emergence area are unfamiliar. The former gives the pig control over the situation and allows it to return to a familiar “safe” area (ii), while the latter is essentially an adapted OFT.

Some response consistency over time has been found (v) but the measure of latency to exit may not be sensitive enough to differentiate different emotions (iv) – the manner in which a pig approaches the exit may say more about its emotional state than the speed at which it exits. The latter may be confounded with activity level, the motivation to return to the home pen, or the behaviour of other pigs if animals are tested as a group (iii). For group tested animals, the relative order of emergence may also provide a better measure than latency as the behaviour of one pig (e.g. blocking the exit) is less likely to impact order than latency. If the test is not performed in the home pen, it is important to habituate the pigs to the starting environment and to being alone as these may both influence behavioural responses in the test (vi). Automation of the test seems feasible, but has yet to be implemented (viii).

### **Cognitive Bias Test (CBT)**

#### ***Test Description: Theory and Measurements***

Emotion and mood can lead to biases in cognitive processes such as memory, decision making, attention and judgement. With judgement in particular, there is evidence of mood congruent biases in humans, that is, positive moods leading to positive judgements and negative moods leading to negative judgements (Blanchette and Richards, 2010). These biases have been proposed as a proxy measure of the valence of emotion (Harding et al., 2004; Mendl et al., 2009). Early research in this area was carried out with rats (Burman et al., 2008; Harding et al., 2004) and starlings (Bateson and Matheson, 2007; Matheson et al., 2008), and the method has since been applied to a variety of other species.

As a relatively new measure, there is no standard cognitive bias task but the aims of the tests are similar; subjects are first trained to associate one cue with a positive outcome (positive cue) and a second cue with a negative/neutral outcome (negative cue). In the test the animal is then presented with a third, novel and ambiguous cue and responses are interpreted as being either positively or negatively biased depending on whether they most resemble behaviour after presentation of the trained positive or negative cues. This bias is then said to reflect a positive or negative underlying mood state (Mendl et al., 2009).

Two test designs are commonly used. Firstly, a Go/No-Go, where the animal is trained to respond to the positive cue and refrain from responding to the negative cue (e.g. Burman et al., 2009; Doyle et al., 2010a). Latency to respond or Go-responses to the ambiguous cues are the main measures of judgement bias. Secondly, Active-Choice designs, where the animal has to perform a similar response to both the positive and

negative cue but in a different location (e.g. Enkel et al., 2010; Matheson et al., 2008). In this case, the choice made by the animal in response to the ambiguous cues is the main measure. Cues used have been spatial, visual, auditory and olfactory. Positive cues generally predict food rewards, negative cues may predict no-reward or a punishment, and ambiguous cues can be unrewarded, rewarded or partially rewarded.

### ***Assumptions of the CBT***

The cognitive bias paradigm assumes that emotion interacts with cognitive process, such as memory, judgement etc., in animals in a similar way as in humans, and the same mood congruent biases should therefore be produced. Mendl et al. (2009) suggest that this is likely to be the case if we see emotional influences on cognition as adaptive. In an environment where threat is high, it is safer to interpret ambiguous stimuli as potentially threatening, but in an environment where the threat is low, interpreting ambiguous stimuli as potentially positive may result in fitness benefits to the individual, e.g. something novel may be a potential food source.

### ***Results of tests: CBT and Pigs***

At the time of writing, only four studies of cognitive bias in pigs have been published, each with a different set-up. Douglas et al. (2012) trained gilts in a Go/No-Go style task using auditory cues where the positive cue signalled a reward for a Go response, while following a negative cue a Go response resulted in an aversive event (shaking of a plastic bag). Döpjan et al. (2013) and Scollo et al. (2014) both used a Go/No-Go style task with spatial cues where a goal box on one side of the arena (positive cue) signalled the presence of an accessible reward while a goal box on the other side of the arena (negative cue) signalled the presence of no reward. Murphy et al. (2013), on the other hand, used an Active-Choice task with auditory cues, where one tone signalled the presence of a large reward in one goal box, while a second tone signalled the presence of a small reward in a second goal box.

Douglas et al. (2012) used only one ambiguous cue and found that when enriched-housed, gilts showed a more positive judgement bias (higher proportion of go-responses and lower latency to respond to the ambiguous cue) than when barren-housed. Similar effects of enrichment on judgement bias have been found in rats (Brydges et al., 2011). Neither repeated isolation in piglets, nor high and low stocking densities affected responses to the ambiguous cues (Döpjan et al., 2013; Scollo et al., 2014). Murphy et al. (2013) showed that pigs of conventional farm breeds and miniature lab breeds perform similarly on the task, and that when ambiguous cues are unrewarded in an Active-Choice design, repeated testing of pigs results in a drop in optimistic choices.

### ***Discussion: CBT and Pigs***

A number of experiments report biases in various species in the direction predicted (Mendl et al., 2009), suggesting the paradigm is ethologically valid across species (i); the predicted effects of enrichment on mood in pigs was found (Douglas et al., 2012) but not for social isolation (Döpjan et al., 2013). More work is needed to validate the CBT for

use with pigs and determine how the varying methodologies in current CBT protocols may affect the results (vii). The CBT is not a test of unconditioned responses (ii), and therefore may be limited in its sensitivity to subtle differences between emotions (iv). Held et al. (2009) suggest that it may be easier to interpret the results of experiments in which an arbitrary action can be used as a measure of self-report of an emotional state. The CBT is one of the few tests specifically designed to distinguish positive from negative emotions, and could be quite simply automated once a valid design has been established (viii). Issues with repeated testing have been reported for both pigs and sheep (Doyle et al., 2010b; Murphy et al., 2013) (v), where an animal quickly learns the outcome of the ambiguous cues, rendering the cues no longer ambiguous. Furthermore, in Go/No-Go designs activity may confound measures of emotion where latency is the sole measure of bias; active choice designs should reduce this problem by having the animal make a response to all cues (iii). The cognitive bias paradigm may seem useful to study valence of emotion and mood in pigs, but requires further validation and optimization regarding repeated testing.

## **Conditioned Discrimination**

### ***Test Description: Theory and Measurements***

The drug discrimination paradigm has been used since the 1970s to look at the subjective effects of drugs in laboratory animals (Colpaert, 1999). Typically, an animal is trained to perform a particular operant response under one condition, e.g. the presence of a drug in its system, and to perform a second response under another condition, e.g. the absence of the drug (Glennon et al., 1983). Different concentrations of the substance can then be tested to see which dose can be detected by the animal.

### ***Assumptions of the Conditioned Discrimination***

The main assumption of a conditioned discrimination paradigm with respect to the study of emotion is that if the animal can detect the presence of the drug in its system, it does so based on the effect of the drug on its emotional state i.e. that the animal can identify changes in its internal state. Furthermore, it assumes that the action of the drug produces changes within the animal that accurately reflect alterations in emotional state.

### ***Results of tests: Conditioned Discrimination and Pigs***

A series of experiments by Carey and Fry in the 1990s (1992; 1993; 1995) used this method to study the ability of pigs to differentiate the effects of an anxiogenic, pentylenetetrazole (PTZ) from a saline injection. Caution should be used when interpreting the results of these studies, as the numbers of pigs used was low (max. 4 pigs), but the approach may offer a method to study the animal's perception of its internal state. In the initial experiment (Carey et al., 1992), all four pigs could discriminate between PTZ and saline in an operant task using lever pressing. In a second experiment (Carey and Fry, 1993), a tone cue previously associated with mild shock also resulted in a preference for the PTZ lever, an effect not seen after pre-treatment with the anxiolytic diazepam. Finally, some environmental changes (apparatus design, presence of a novel

object, ambient temperature) affected responses of some individual pigs, while other changes (lighting, apparatus colour, odours, transport) had little or no effect (Carey and Fry, 1995).

### ***Discussion: Conditioned Discrimination and Pigs***

The small number of pigs used in studies on conditioned discrimination makes it impossible to draw strong conclusions. Pigs appeared to discriminate the effects of an anxiogenic compound from an injection of saline, but it is not clear that these effects were due to experienced changes in emotional state or sensations without a hedonic component (i, iii). Environmental stressors failed to induce the same behavioural response as an anxiogenic (PTZ). Any change from normal, positive or negative, may result in a switch away from the saline response, or changes in responding may be sensitive to other factors such as novelty of a stimulus or pre test handling (vi). As a test of conditioned responses, like the CBT, the range of behaviours that can be recorded is limited (ii, iv). The conditioned discrimination task is suitable for repeated testing, unlike the CBT, (v), and could be easily standardised and automated (vii, viii). A weakness of the conditioned discrimination paradigm is its assumption that an animal can reliably detect alterations in its internal emotional state, and that different stimuli induce similar alterations in emotional state.

## **GENERAL BEHAVIOURS**

As discussed, many of the behavioural tests that have been used to study emotion or mood in pigs lack the ability to disambiguate emotional behaviours from others such as general activity, as well as to distinguish subtle differences in emotions. Furthermore, few tests are designed to specifically measure positive emotion. The recording of general behaviours that mirror emotion and mood in pigs may help to overcome such shortcomings and are discussed next.

### **Play**

#### ***Play and Emotion***

It is commonly accepted that play and play behaviours are associated with positive emotions, or feelings of pleasure. Play can be social, involving conspecifics, or non-social (locomotor-rotational and/or object play). Social play in rats has been shown to be rewarding, and involves neurotransmitter systems associated with other rewarding behaviours such as eating or sex, as well as the same brain regions associated with emotions (Vanderschuren, 2010). Play is said to have long-term adaptive benefits, such as providing training for future skills or unexpected situations (Pellis and Pellis, 2009; Špinka et al., 2001), as well as immediate benefits, such as conflict management, stress reduction and learning about other conspecifics (Palagi et al., 2004; Pellis and Pellis, 2009). Play-associated pleasure may occur either during or shortly after the occurrence of play behaviour, and is directly related to the fitness benefit of play (Fraser and Duncan, 1998). Play has been suggested as an indicator of positive states as it generally occurs when all other needs have been met, and will reduce/cease in situations of threat (Boissy

et al., 2007). Furthermore, play is contagious behaviour, thus pleasure associated with play may be transferred between individuals (Held and Špinka, 2011).

### ***Play in Pigs***

Defining and distinguishing play behaviours from real conflict can be difficult. Many authors use “play markers” (Chalmers and Locke-Haydon, 1981), i.e. indicators in a behavioural sequence which signify that the behaviour is playful. Common play markers in pigs’ solitary play, often termed locomotor play (Newberry et al., 1988), and object play include waving/tossing of the head, scampering, jumping, hopping, pawing, pivots, flops, gambolling and shaking or carrying an object (Bolhuis et al., 2005; Chan et al., 2011b; Dudink et al., 2006; Newberry et al., 1988; O’Connor et al., 2010). In terms of social play, studies have included any reciprocal contact between two pigs over 5 s duration (Dobao et al., 1985), play-fighting (Kelly et al., 2000) or pushing/nudging another pig, biting or lifting another pig, and self-handicapping postures (Chaloupková et al., 2007; Donaldson et al., 2002).

Most studies of play behaviour in pigs have looked at the effect of environment. Pigs housed in enriched environments showed increased locomotor play (Bolhuis et al., 2005), and piglets showed more play if the arrival of enrichment was signalled compared to piglets which received un-signalled enrichment (Dudink et al., 2006). Pigs with an enriched pre-weaning environment tended to have higher levels of locomotor and social play than piglets in a standard farrowing crate (Chaloupková et al., 2007). However, Wood-Gush et al. (1990) found barren-housed pigs to show more play than enriched pigs when given access to a novel pen. Exposure to high levels of ammonia resulted in reduced levels of play in 3-week-old piglets (O’Connor et al., 2010).

Past experience can also influence later play. Pigs given the opportunity to play with unfamiliar pigs at a young age, showed more social play behaviours than those with limited play opportunities when mixed post-weaning (Donaldson et al., 2002). Pigs that, pre-weaning, were given access to a playpen while music was playing, post-weaning showed more play behaviours when exposed to music than control pigs, although music also increased play somewhat in the control group (de Jonge et al., 2008a).

Play decreases with age after weaning (Bolhuis et al., 2005; O’Connor et al., 2010) but not in the pre-weaning period (Chaloupková et al., 2007). In the days immediately post weaning, locomotor and social play decreased in piglets, and only locomotor play increased again (Donaldson et al., 2002). The peak period for play behaviour in pigs is between 2 and 6 weeks (Newberry et al., 1988). Environmental and social change post-weaning affect play behaviour separate from the effect of the removal of the sow: piglets kept in the same environment post weaning showed more play than those that were both moved to a new environment and mixed (Colson et al., 2012).

### ***Discussion: Play as a Measure of Emotion in Pigs***

Burghardt (2005) lists a number of criteria for a behaviour to be classified as play including that the behaviour in itself is rewarding, and only occurs when the animal is in a non threatening environment. It is difficult to measure a pig’s intrinsic motivation

for solitary/locomotor play, but authors have looked at motivation for object-directed play. As discussed with respect to the NOT pigs have a high motivation to explore. They show a preference for exploring a novel object over a familiar one (Wood-Gush and Vestergaard, 1991). Play occurs more under better housing conditions. Pigs housed in enriched environments play more than barren housed pigs, but given access to an enriched environment, pigs in barren housing will play more, most likely due to the fact that the motivation to play has been frustrated in their regular housing. So, while there is an intrinsic motivation to play, it is facilitated by a stimulating environment.

Held and Špinka (2011) suggest that not all social play is necessarily pleasurable, such as play fighting where there is a potential to escalate into real conflict. In pigs, it has been suggested that some forms of social play are used to establish dominance relationships (Dellmeier and Friend, 1991) and therefore may not be inherently pleasurable for all participants. Social play may increase during periods of social stress as a means of reducing social tensions (Held and Špinka, 2011), so it may not always be an indicator of positive states but a means of coping with potential negative situations. Behavioural indicators might be used to discriminate between social play which is pleasurable and that which is related to hierarchy formation or onset of aggression. In rats, vocalisations have been used as such (Burgdorf et al., 2006).

Locomotor-rotational and object play may be a better gauge of emotional state in pigs. Many of the studies of solitary/locomotor play in pigs use similar play markers based on those established by Newberry et al. (1988). Play could be useful for the study of emotion in pigs either as a method to induce a positive state, or potentially its occurrence could be used as a measure of an existing positive state.

## **Qualitative Behaviour Assessment**

### ***QBA and Emotion***

In Qualitative Behaviour Assessment (QBA) human observers are asked to judge an animal's state using one-word descriptors they generate themselves from watching sample clips (Wemelsfelder et al., 2001). A (number of) dimension(s) upon which behaviour of any one animal can be scored is created based on consistency in semantic meaning of the generated terms. This score is taken as a measure of an animal's state. This method assumes that our perception of the animal's state reflects the actual state of the animal.

### ***QBA in Pigs***

QBA has been used in a number of studies with pigs, and naïve observers show a high degree of intra- and inter-observer reliability in terms of the semantic descriptors used (Wemelsfelder et al., 2001). Background cues from video recordings only slightly affect observer's ratings of pigs (Wemelsfelder et al., 2009). Extensively reared pigs were scored as happier and more content than pigs from more intensive conditions (Temple et al., 2011). While no study has looked at physiological correlates of QBA ratings in pigs, Stockman et al. (2011) demonstrated that cattle rated as more "agitated" by observers also had higher heart rate and body temperature, among other measures. Furthermore,

pigs treated with an anxiolytic, Azperone, were rated by observers as more confident and curious than saline treated pigs in both an OFT and an EPM (Rutherford et al., 2012).

### ***Discussion: QBA as a Measure of Emotion in Pigs***

This approach has been suggested as a possible method for studying positive emotions on farm (Boissy et al., 2007). However, it has also received criticism for being too anthropomorphic, for assuming that our interpretation of an emotional state in animals reflects the actual emotional state. Würbel (2009) suggests that while it may seem adaptive to be able to read the expression of emotions in animals, as it may allow us to better predict their behaviour, this ability is greatly affected by our level of similarity with the species in question. More work needs to be done in order to validate the method as a way to measure emotion or mood in pigs. By correlating observers' ratings with physiological measures and other valid behavioural methods we may learn more about the potential of QBA for studying emotion in pigs. It may also be interesting to look at what specific behaviours or postures of an animal influence the observers' ratings, and to compare expert and inexperienced observers on more subtle behavioural differences.

## **Vocalisation**

### ***Vocalisation and Emotion***

Vocalisations can communicate different messages. For example, a call may be used to signal readiness to mate, to warn conspecifics of a predator, to keep in touch with other members of the group, or it could be an expression of pain or need. Vocalisation can express an emotional state, or its nature may be altered by physical changes in the vocal apparatus due to an emotional state (Briefer, 2012). Vocalisations influence the behaviour of others (Bachorowski and Owren, 2008) and in humans studies on neural lesions and imaging show that specific brain areas are activated by non-linguistic emotional vocalisations e.g. laughter or screaming, of others (Scott et al., 2009a). In animals too, it is suggested that vocalisation can be an expression/communication of an emotional state or reaction to an event, and eliciting emotional states in others (Seyfarth and Cheney, 2003). Thus, analysis of vocalisations has been suggested as a non-invasive method for studying the emotional state of an animal (Manteuffel et al., 2004).

### ***Vocalisation in Pigs***

The main assumption is that vocalizations are honest signals i.e. that they truly reflect the animal's state. Weary and Fraser (1995) found evidence for this in that piglets with greater need (lower than average weight or those that missed a feed) had a higher rate of calling and used calls which differed in frequency and duration than piglets with less need (higher weight, no missed feed). Pig calls are generally classified as either contact calls, generally low-pitched "grunts", or calls reflecting level of arousal, generally high pitched "squeals" and "screams" (Held et al., 2009; Manteuffel et al., 2004). Some automatic measurement techniques and software have been developed to detect these high-pitched vocalisations (Manteuffel and Schön, 2004; Moura et al., 2008; Schön et al., 2004). The "bark", a low-pitched atonal call of short duration (Chan et al., 2011b),

occurs both during play and in a situation of alarm (Newberry et al., 1988). Chan et al. (2011a) found subtle acoustic differences in peak frequencies between barks used in contexts of play and alarm.

A number of studies have reported an increase in high frequency screams in reaction to assumed painful experiences like castration in piglets (Marx et al., 2003; Puppe et al., 2005; Taylor and Weary, 2000; Weary et al., 1998). Piglets weaned after 12 days of age had fewer high-frequency vocalisations in an OFT than older weaned piglets, but did not differ in lower-pitched grunts (Sumner et al., 2008). Piglets trained to associate different tone-sequences with either a positive or a negative outcome were more likely to give high-frequency vocalisations during the anticipation period before the negative situation (Imfeld-Mueller et al., 2011). However, hungry sows can also make high-pitched vocalisations in anticipation of food (Xin et al., 1989). Subtle differences in vocalisations have been found when pigs are exposed to a physical stressor, electric shock, and a mental stressor, anticipation of a shock (Düpjan et al., 2008).

There are few studies examining the emotion-related effects of vocalisation on the perceiver. The behaviour of isolated pigs exposed to recordings of high-frequency distress calls did not differ from responses to a control sound, but they did reduce their own vocalisations more during presentation of the distress calls than when presented the control sound (Düpjan et al., 2011). Juvenile pigs were more likely to show fleeing responses and freezing in response to the barks of adult sows than to those of other juveniles or control sounds (Chan et al., 2011b). Furthermore, depending on the context, playback of alarm barks and play barks can have a greater effect on behaviour than playback of grunts; Chan et al. (2011) found that pigs were more responsive to play barks than grunts when in a playful context, but that in a neutral context, pigs responded more to alarm barks than to grunts.

The relationship between different vocalisations and stress hormones has shown that higher levels of adrenaline are related to the production of more “squeal-grunts”, while increased cortisol is associated with fewer “grunts” (Scott et al., 2009a). High frequency vocalisations, as induced by decreasing ambient temperature, did not correlate with levels of salivary cortisol in gilts tested in groups (Hillmann et al., 2004). “Squeals” have been associated with arousal in gilts as measured by heart rate in a HAT (Marchant et al., 2001). Artificially stimulated cortisol release in boars resulted in higher behavioural activation and higher rates of grunting than in boars administered with saline (Parrott et al., 2000). Pharmacological stimulation of the amygdala in pigs using acetylcholine produced high-pitched vocalisations similar to the screams associated with distress (Manteuffel et al., 2007). Pigs treated with an anxiolytic, Azaperone, vocalised less in an OFT than non-treated pigs (Donald et al., 2011).

### ***Discussion: Vocalisations as a Measure of Emotion in Pigs***

While vocalisations have often been studied in pigs with regard to the level of arousal, there have been few studies on vocalisations that signal positive affect, probably as these are more rare (Briefer, 2012). Rats produce ultrasonic vocalisations during play which can be induced by anticipation of the positive experience of play (Knutson et al., 1998; Knutson

et al., 2002). The bark in pigs could be a possible indicator of positive affect, provided the play bark can be easily differentiated from the alarm bark. In a study by Reimert et al. (2013), pigs barked during a rewarding event but not an aversive one. This difference was not significant, however, and Imfeld-Mueller et al. (2011) did not find any vocalisations specific to anticipation of a positive event in pigs.

Vocalisations could be used as an indicator of arousal or intensity of emotional state, but more work needs to be done to investigate their valence. Looking at vocalisations within context, and using other behavioural or physiological measures which concur with the vocalisation, may allow valence to be inferred (Manteuffel et al., 2004). Furthermore, factors other than emotion, such as motivation, may also influence the nature of a vocalisation, making it difficult to interpret (Briefer, 2012). Little work has been done with pigs on the effect that a vocalisation has on conspecifics and further research on emotional contagion by means of vocalizations is recommended.

## **Anticipation behaviours**

### ***Anticipation and Emotion***

Since signalling the arrival of reward can result in the release of endorphins and dopamine, anticipatory behaviours have been proposed as a possible measure of positive states in animals (Spruijt et al., 2001). Anticipatory behaviours after reward is signalled are associated with dopaminergic activity (Boissy et al., 2007) and are associated with an increase in behavioural transitions in a number of species (Spruijt et al., 2001). When it comes to aversive events, animals consistently prefer signalled aversive events over randomly occurring aversive events (Bassett and Buchanan-Smith, 2007). The ventral striatum, which is activated during the anticipation of reward in humans, has also been found to be active during the anticipation of aversive stimuli (Jensen et al., 2003) and it is suggested that this activation leads to the behavioural response.

### ***Anticipation in Pigs***

Only a few studies looked at anticipation behaviours in pigs, and the methods vary. Pigs appear to show mild differences in behavioural response and vocalization to positive and negative signalled situations (Düpjan et al., 2008; Imfeld-Mueller et al., 2011; Reimert et al., 2013), but do not appear to differ in physiological responses (Imfeld-Mueller et al., 2011).

### ***Discussion: Anticipation behaviours as a Measure of Emotion in Pigs***

Too few studies of anticipations behaviours in pigs have been carried out to fully evaluate its usefulness as a measure of emotion. The specific behavioural response may vary depending on the intensity of the positive and negative outcome signalled. Thus there may not be a consistent behavioural pattern, but individual behaviours may be indicators of the valence of a response, and the presence or absence of some behaviours, such as vocalisations, may also be indicators of the intensity of the response.

## **Other behaviours**

Risk assessment behaviours in test animals confronted with novelty are well defined in rodents (Ohl, 2003), but no such behaviours or behavioural sequences have been described for pigs. Startle and freeze responses in pigs – “*any general massive body flexion in animals* (Blackshaw et al., 1998)” – are common reactions to loud or sudden stimuli. Paul et al. (2005) suggest that the startle response may be useful as a measure of emotional valence since the response is enhanced when the subject is in a negative emotional state, but reduced when the subject is in a positive state. Tail movement and position have been suggested as possible indicators of positive emotion in pigs, whereas freezing, elimination, high frequency vocalisations and escape attempts were found to occur in aversive situations (Reimert et al., 2013).

## **GENERAL DISCUSSION AND CONCLUSIONS**

A variety of methods have been applied to the study of emotion and mood through behavioural responses in pigs. We have outlined a list of criteria which we feel are important when evaluating behavioural tests of emotion in pigs. Using these criteria we have come to a number of conclusions. As with rodent studies (Ohl, 2003), the ethological validity of behavioural tests of emotion for pigs is generally overlooked, though elements incorporated in anxiety tests do appear to be aversive for pigs, e.g. social isolation and novelty. Regarding tests of spontaneous behaviour, these should be designed so that an animal is able to perform its full repertoire of emotional responses.

Many common tests rely on quite broad behavioural measures, which makes it difficult to define which responses specifically reflect emotion, and discriminate between different types of emotional responses. We have given some examples of behavioural patterns which may provide more specific and subtle measures of emotional responses, in particular, detailed analyses of vocalisations. Until recently, the focus in the literature has generally been on negative emotion, particularly in the majority of behavioural tests. However, positive emotions may be of greater importance for welfare than the absence of negative emotions (Boissy et al., 2007). More recent interest in cognition and emotion, play behaviours, vocalisations, and anticipation behaviours provide the opportunity to measure positive emotions. Emotional contagion has received little attention in pigs, but may be a useful area of future research.

One difficulty we repeatedly found in evaluating the most common behavioural tests used to measure emotion in pigs is the lack of any standardisation of design and measurements. While in the literature, papers can be grouped under the various test headings, with vast differences in the designs of the tests these are unlikely measuring the same thing. Furthermore, few tests are suitable for repeated testing making it difficult to measure emotional responses in the same individual before, during and after interventions.

As was concluded by Forkman et al. (2007), we would suggest that the common tests ought to be reassessed and redesigned with greater ethological relevance for pigs, and they ought, as much as possible to try to fulfil the criteria we have proposed. Without suitable tests of emotional responses for pigs, we cannot accurately validate their use in biomedical research or assess welfare needs.

## SUPPLEMENTARY MATERIAL

**Table 1** Measures of OFT behaviours of pigs recorded in 33 experimental papers\*. Within these 33 papers, 55 different behaviours were recorded using 92 separate methods for scoring the behaviour.

Behavioural Category	Behaviours Recorded	Scoring of behaviour (number of papers) <sup>‡</sup>						
		Dr	F	L	S	O	Ds	W
Movement/ Posture	Line/Square Crossing		17					
	Posture	8	1		2			
	Locomotion	5	2	1			1	
	Lying (Ventral/Lateral)	7				2		
	Walk	7			1			
	Start Box/ Pen Exit	1		5				
	Sit	5						
	Alert	3	2					
	Inactive	3						
	Run/Trot	2				1		
	Immobile	1			1			
	Head Shake		1				1	
	Rear		1				1	
	Play		1				1	
	Wobble		1				1	
	Kneel	1						
	Idle/Doze	1						
	Look					1		
	Active					1		
Walk/Run					1			
Vocalisation	Vocalisation		11	1	2			
	Grunt	1	4					
	Scream/Squeal	1	3					
	Low-Pitched		2					
	High-Pitched		2					
	Long Grunt		1					
	Short Grunt		1					
	Bark						1	
Other Vocalisation		1						
Location within the OF	Entry into centre	8	4	3				
	In Periphery	5	1					
	Near Centre	1	1					
	Location	1						
	Novel Location Visit		1					
	Start Box Visit	1						
	Next to Feeder	1						
Next to Object	1							

**Table 1** Continued

Behavioural Category	Behaviours Recorded	Scoring of behaviour (number of papers) <sup>‡</sup>						
		Dr	F	L	S	O	Ds	W
Explorative	Explore	6	1		4			
	Root	3	1					
	Wall Contact	3	1					
	Floor/Substrate Contact	3						
	Arena Explore	1	1					
	Object Contact	2						
	Object Sniff	1						
Elimination	Defecation		9	1				3
	Elimination	3	1		2			
	Urination		1					
Escape	Escape Attempt	3	2		1	1		
	Exit directed behaviour	3	2	1				
Ingestion	Chew	2						
	Feeder contact	2						
	Drinker contact	1						
	Graze	1						
	Ingestion				1			
Other	Other	4	3					

<sup>‡</sup>(Dr = Duration; F = Frequency; L = Latency; S = Scan Sampling; O = Occurrence; Ds = Distance; W = Weight)

\* Papers found on the database Science Direct using the search: “Pig” AND “Open Field” OR “Novel Environment” ANDNOT “Guinea” in an Abstract, Title, Keywords search. This search generated 52 papers, 19 non-experimental or irrelevant papers were excluded: Andersen et al., 2000a; Andersen et al., 2000b; Beattie et al., 1995; de Jong et al., 2000; de Sevilla et al., 2009; Désautés et al., 1997; Désautés et al., 1999; Donald et al., 2011; Fraser, 1974; Giroux et al., 2000; Hession et al., 1994; Jensen et al., 1994; Jensen et al., 1995; Jones et al., 2000; Jones and Nicol, 1998; Kanitz et al., 2009; Kanitz et al., 2004; Kranendonk et al., 2006; Loijens et al., 2002; Magnani et al., 2012; Mormède et al., 1994; Otten et al., 2007; Pearce and Paterson, 1993; Puppe et al., 2007; Ruis et al., 2000; Ruis et al., 2001; Rutherford et al., 2006; Rutherford et al., 2012; Siegford et al., 2008; Sumner et al., 2008; Taylor and Friend, 1986; Thodberg et al., 1999; von Borell and Ladewig, 1992.

**Table 2** NOT studies of pigs divided by method of object presentation, social situation in which the test is performed, and the location in which the test is performed from in 32 experimental papers generated from the database Science Direct, searching in Abstract, Title and keywords for the occurrence of both “Pig” and “Novel Object”.

<b>Method of Presentation</b>	<b>Social Situation</b>	<b>Home Pen</b>	<b>Separate Arena</b>
<b>Slow</b> Lowered, Slowly introduce	<b>Alone</b>	None	(de Sevilla et al., 2009; Hemsworth et al., 1996; Jensen, 1994; Kranendonk et al., 2006; Lawrence et al., 1991; Lind et al., 2005; Pearce and Paterson, 1993; Ruis et al., 2001; Siegford et al., 2008)
	<b>Group</b>	None	None
<b>Sudden</b> Dropped, Thrown, Curled	<b>Alone</b>	(Olsson et al., 1999)	(Dalmau et al., 2009; Hessing et al., 1994; Janczak et al., 2003a; Janczak et al., 2003b; Jones and Nicol, 1998; Tönepöhl et al., 2012)
	<b>Group</b>	(Brown et al., 2009; Smulders et al., 2006)	(Magnani et al., 2012)
<b>Placed by person</b> Put, Left, Placed	<b>Alone</b>	(Burne et al., 2001; Wemelsfelder et al., 2000)	(Hayne and Gonyou, 2003; Morrison et al., 2007)
	<b>Group</b>	(van Erp-van der Kooij et al., 2002)	None
<b>Already present</b>	<b>Alone</b>	None	(Dalmau et al., 2009; Wemelsfelder et al., 2000; Wemelsfelder et al., 2009)
	<b>Group</b>	None	None
<b>Unclear</b> Introduced, Presented, Confronted with	<b>Alone</b>	(Forkman et al., 1995; Spake et al., 2012)	(Jensen et al., 1994; Jones and Nicol, 1998; Puppe et al., 2007; Ruis et al., 2002; Rutherford et al., 2006)
	<b>Group</b>	(Bracke and Spoolder, 2008)	None
<b>Combination</b> Brought in by & dropped	<b>Alone</b>	None	(Zupan et al., 2012)
	<b>Group</b>	None	None





# CHAPTER 03

## SUCCESSIVE AND CONDITIONAL DISCRIMINATION LEARNING IN PIGS

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## **ABSTRACT**

We studied the ability of pigs to discriminate tone cues using successive and conditional discrimination tasks. Pigs (n=8) were trained in a successive discrimination Go/No-Go task (Experiment 1) to associate a Go-cue with a reward at the end of a runway and a No-Go-cue with the absence of reward. Latency to reach the goal-box was recorded for each cue-type. Learning of a conditional discrimination task was compared between low-birth-weight (LBW, n=5) and normal-birth-weight (NBW, n=6) pigs (Experiment 2), and between conventional farm (n=7) and Göttingen miniature (n=8) pigs (Experiment 3). In this Active-Choice task one cue signalled a response in a right goal-box was correct, a second cue signalled a response in a left goal-box was correct. Cues were differentially rewarded. The number of sessions to learn the discrimination and number of correct choices per cue-type were recorded. In Experiment 1, 4 out of 8 pigs showed learning on the task, i.e. a higher latency to respond to the No-Go-cue, within 25 sessions. In Experiment 2, 8 out of 11 pigs learned the discrimination within 46 sessions. LBW learners learned faster than NBW learners. In Experiment 3, all 15 pigs learned the task within 16 sessions. Göttingen miniature pigs learned faster than conventional farm pigs. While some methodological issues may improve the Go/No-Go design, it is suggested that an active-choice task yields clearer and more consistent results than one relying on latency alone.

### **Key Words**

Learning; Successive Discrimination; Conditional Discrimination; Go/No-Go task; Low-Birth-Weight; Pig

## INTRODUCTION

Pigs are increasingly being used as model animals in neuroscience due to the similarity to humans in anatomy, physiology and organ development (Lind et al., 2007; Lunney, 2007). Furthermore, assessing learning and cognition in pigs is also of interest to applied ethology. In order to understand the neurobiological processes behind both normal and abnormal behaviour, appropriate behavioural tasks are crucial (Lind et al., 2007). Appropriate learning tasks for pigs can be used to assess the impact of husbandry conditions and management systems on emotion and cognition (Mendl, 1999). However, there is a lack of standardisation and validation of learning and memory tasks that can be used with pigs (Gieling et al., 2011a).

Many tests typically used to assess learning in rodents or primates have rarely been applied to pigs, such as a Go/No-Go task (Lind and Moustgaard, 2005; Moustgaard et al., 2005). A Go/No-Go task is a simple (successive) discrimination task in which one cue signals a Go response, while another cue signals that a No-Go response is correct (Moustgaard et al., 2005). The task has been used to look at impulsive behaviour and deficits in action inhibition associated with a number of disorders, particularly ADHD, and involves decision making processes (Eagle et al., 2008). More specifically, this task is said to be a measure of action restraint, the inhibition of a premeditated motor response before it has begun (Schachar et al., 2007). Strong comparability of the test has been found from rodents to primates to humans (Eagle et al., 2008), but not yet for pigs.

While discrimination tasks have been commonly used in pigs to assess sensory capacities (Croney et al., 2003), as well as social, object, and human recognition learning (Koba and Tanida, 1999), little research has been done on conditional discrimination tasks i.e. tasks in which the pig has to learn *If X then response 1, if Y then response 2*. Active-choice tasks are used to study conditional discrimination learning where one cue signals a response at point A is correct, while another cue signals a response at point B is correct. Responses may be operant, e.g. lever pressing or go-left, go-right. Only one study specifically addressing conditional discrimination learning in an active choice task has been performed with pigs, where Göttingen miniature pigs (hereafter called minipigs) had to choose the correct lever based on visual cues present in the test arena (Moustgaard et al., 2005).

More recently, both Go/No-Go style tasks and Active-Choice tasks have been used to investigate cognitive bias as a measure of emotion in a number of animal species (Mendl et al., 2009). Go/No-Go style tasks tend to require less training and are therefore quicker to perform, but arguments have been proposed that it may be difficult to separate a No-Go response from an omission (Enkel et al., 2010), or from anhedonia brought on by, for example, depressive like states (Matheson et al., 2008). Performance of animals in these two types of tasks may be of interest to research on emotion in animals.

In order to investigate learning of pigs in successive and conditional discrimination tasks we carried out three experiments. Since auditory perception in pigs exceeds that of humans, with a range from 42Hz to 40.5kHz (Heffner and Heffner, 1990), and previous research has demonstrated that pigs can successfully discriminate between tone sequences signalling the availability of food (Ernst et al., 2005), and predicting

positive and negative situations (Imfeld-Mueller et al., 2011), tones served as cues (discriminative stimuli) in all three experiments.

In the first experiment, we used a Go/No-Go style task in a runway to investigate successive discrimination learning in pigs. The pigs were trained to associate one tone cue with the presence of reward in a goal-box at the end of a 10 m long runway and a second cue predicted the absence of reward in the goal-box. This was not a strict “Go/No-Go” task, since there was no penalty for an incorrect response, i.e. no incentive to encourage a No-Go response other than the lack of reward, unlike a previous Go/No-Go task used with pigs by Moustgaard et al. (2005). The latency to respond to the cues was the main measure and not strict Go or No-Go responses. We expected that latency to reach the goal-box would be lower for Go-cues than for No-Go-cues as pigs have been shown to respond faster when a positive outcome is signalled than a negative one (Imfeld-Mueller et al., 2011).

In the second experiment, we trained pigs on an active-choice conditional discrimination task. The tone cues, same as used previously, signalled in which of two goal-boxes an operant response would result in reward. The pigs were required to perform an active response to both cues and correct responses to both cues were differentially rewarded. A correct response to a ‘positive’ cue resulted in a large amount of reward, while a correct response to a ‘negative’ cue only resulted in a small amount of reward. Incorrect responses were ‘punished’ with a time-out where no responses were possible. Learning of the discrimination was compared between piglets born at low-birth-weight (LBW) and normal-birth-weight (NBW) controls. In humans, LBW is associated with cognitive impairments later in life (O’Keeffe et al., 2003; Silva et al., 2006), and a study investigating the effect of LBW in piglets on a cognitive holeboard task, found that LBW piglets initially performed worse, in terms of working memory, in a reversal task than NBW controls (Gieling et al., 2011b). Thus, in the current experiment, it was hypothesised that LBW pigs would perform worse in a conditional discrimination task than NBW controls.

In a third experiment, we trained pigs in a similar active-choice conditional discrimination task as in the second experiment. We tried to speed up acquisition of the discrimination by increasing the difference between the ‘positive’ and ‘negative’ tone cues. As few studies directly compare the performance of different breeds of pigs in cognitive studies, we compared learning in conventional farm pigs and minipigs to look at breed differences. Minipigs are increasingly being used in biomedical research to bridge the gap between rodent research and humans (Lind et al., 2007). Pigs used in laboratories and conventional farm pigs face very different challenges due to different environmental conditions and usages. While it is assumed that these different breeds of pig are quite similar (Holtz, 2010), few direct comparisons of behaviour or learning have been made (Gieling et al., 2011a). As results from our group show that Göttingen minipigs were slower to learn a spatial learning task than conventional pigs (Gieling et al., 2013), we hypothesized slower acquisition of the active-choice task in Göttingen minipigs compared to conventional pigs.

## MATERIALS & METHODS

### Ethical Standards

This study was reviewed and approved by the local ethics committee (DEC, diereperimenten-commissie), and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All efforts were made to minimize the number of animals used and avoid suffering.

### Experiment 1:

*Animals:* In this experiment, 8 female piglets from 4 litters (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) born at the commercial breeding farm of Utrecht University were used. At the age of 6 weeks they were moved to our experimental facility.

*Housing:* Housing was similar for all three experiments. All pigs were group-housed in straw bedded pens (15 m x 3 m) and provided with a nest area and enrichment. Water was available ad libitum, and animals were not food restricted. Instead, they received 25% of their normal allowance in the mornings and the remaining 75% in the evening after testing.

*Apparatus:* Testing was conducted in a separate area to the housing area. The testing apparatus was a 10 m long runway with a start box and a goal-box attached at either end (Fig. 1). Access from the start box to the runway could be controlled remotely by the experimenter via a guillotine door. The goal-box contained a food bowl (15 cm deep and raised 25 cm from the ground so that pigs could not see its contents until they reached the goal-box), which had a false perforated base under which inaccessible food rewards were placed to control for odour cues. During training and testing the experimenters stood outside the apparatus on either side of the runway.

Tone cues, 200 Hz and a 500 Hz pure tone, each 5s long, were generated using the open source software Audacity (<http://audacity.sourceforge.net/>). The cues were played via Windows Media Player (Microsoft) through two speakers (Trust SP-2200 2.0, Trust International B.V., Dordrecht, The Netherlands) attached above the start box. For overview of training and testing schedule, see Fig. 2.

*Habituation to experimenter and rewards:* Upon arrival, pigs were left to settle for a day. Over 4 days, pigs were habituated to the two experimenters and the rewards.

*Group habituation to apparatus:* One week after arrival pigs were brought to the testing area as a group. Over the next week, group sizes were reduced to 4 pigs (8 sessions) and to 2 pigs per group (8 sessions), on average 3 sessions per day. During this period, pigs were also habituated to waiting in the start box for up to 30 s before gaining access to the runway. Rewards were initially scattered on the floor of the runway to encourage exploration and as sessions continued, concentrated only around and within the goal-box.

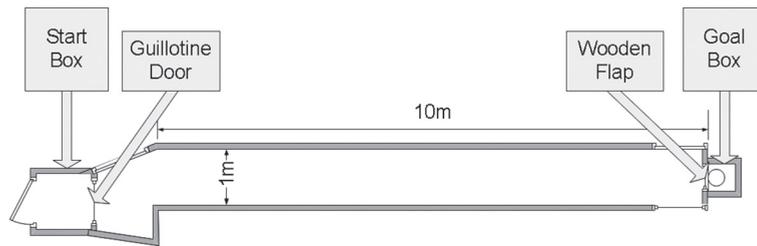
*Individual training in apparatus:* Over 4 weeks, pigs were individually trained to perform trials in the apparatus, and rewards were only available in the goal-box. A trial consisted of a holding period in the start box, after which the guillotine door was opened, providing access to the runway, and the pig could retrieve the reward in

the goal-box. A trial ended once the pig had accessed the goal-box, or when 150 s had elapsed, whichever event occurred first. To avoid the stress of social isolation reducing the pigs' motivation to work for food (Pedersen et al., 2002), individual training was gradual whereby each animal received on average 2 sessions per day starting with 1 trial per session and increasing to 6 trials per session. Once the number of trials per session increased above 6, each animal was trained for 1 session per day and the number of trials was gradually increased to 12 per session. During this period the holding time in the start box was also gradually increased up to 60 s to allow the experimenter to replace the rewards and prepare for the next trial.

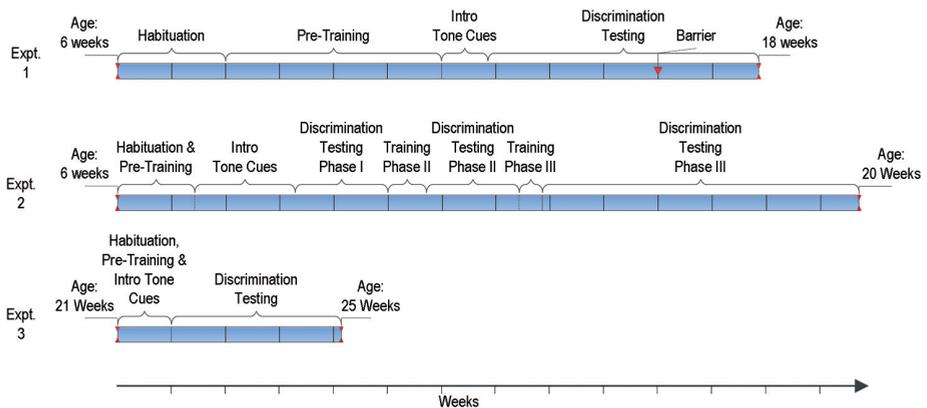
*Introduction of tone cues & associated outcomes:* Each pig received 3 sessions, with 12 trials per session. The holding period in the start box was kept at 60 s. As the pig came within 1 m of the goal-box, a 5 s tone-cue was played. A "Go-cue" signalled the presence of reward, a piece of apple and a peanut M&M, a "No-Go-cue" signalled the absence of reward. The tone cues were balanced for meaning (Go/No-Go) across animals. During these sessions, 6 Go (G) and 6 No-Go (NG) cues were presented in the following order: G G – NG NG – G G – NG NG – G G – NG NG. After each trial, pigs were returned to the start box for the next trial. Each animal was trained and tested in the same order every day to minimise any effects of time of day on motivation.

*Discrimination Testing:* Pigs received 25 sessions of 12 trials. After the 60 s holding period, a 5 s tone cue was played in the start box. Pigs were then given access to the runway and a trial ended when the pig's head entered the goal-box or after 150 s. Trials were presented in a pseudorandom order (max. 2 consecutive trials of the same type) with 6 Go- and 6 No-Go-cues per session. After session 15, the testing apparatus was slightly modified; a wooden flap was introduced in front of the goal-box (Fig. 1), as the effort used to access a resource has been shown to be a function of the importance of that resource (Matthews and Ladewig, 1994). After this modification, pigs were each given a session of 5 Go trials to learn to use the flap. The following day, discrimination testing continued at session 16.

*Data Recording & Analysis:* Latency to reach the goal-box was recorded using a stopwatch (Fastime 6, Fastime, Leicestershire, UK). Mean latencies to reach the goal-box after presentation of Go- and No-Go-cues were calculated per session per pig. A repeated measures ANOVA with the between subjects factor Pig (1 – 8) and two within subjects factors, Sessions (1 – 25) and Trial-Type (Go, No-Go), was performed to look for differences between latency to respond to Go- and No-Go-cues across sessions. In addition, the data were analysed per pig. An ANOVA with the two within subjects factors, Sessions (session 1 – 25) and Trial-Type (Go, No-Go), was performed to reveal which animals eventually discriminated between Go and No-Go trials. In order to further explore the data of "learners", difference scores were calculated per pair of No-Go and Go trials within a session i.e. 1st No-Go trial latency minus 1st Go trial latency, etc. (six difference scores per session). One-sample t-statistics were used to determine in which sessions mean difference scores differed from zero for individual learners. All data analysis was carried out using SAS 9.2 (SAS Institute, Cary, NC, USA).



**Fig. 1** Scale drawing of Go/No-Go apparatus used in Experiment 1.



**Fig. 2** Schematic timeline for each experiment showing time taken for habituation, training and testing of subjects in all three experiments. Each block represents 5 days (1 week excluding weekends).

### Experiment 2:

**Animals:** In this experiment, 6 normal-birth-weight (NBW) and 5 low-birth-weight (LBW) male piglets were chosen from 3 litters (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) born at the commercial breeding farm of Utrecht University were used. Selection criteria of LBW and NBW piglets were defined according to Gieling et al. (2011b). At the age of 6 weeks they were moved to our experimental facility.

**Housing:** Animals were housed as in Experiment 1. When the pigs were 3 months old the area was doubled by connecting two adjacent pens.

**Apparatus:** The testing apparatus, adjacent to the home pens, consisted of a start box (1.2 m<sup>2</sup>) connected to a rectangular test chamber (3.6 m x 2.4 m) (Fig. 3b). A set of swing doors, which could be locked or released by the experimenter, separated the start box from the test chamber. At the back wall of the test chamber, to the left and right, were the “goal-boxes” in which a pig had to perform an operant response to receive a reward (Fig. 3a). Each goal-box (0.4 m wide) contained a bowl, covered by a large, hard-plastic ball (Fig. 3c). A correct “ball-lift” response required the pig to raise the ball to a height of 20 cm to break a photobeam. Goal-boxes could be opened or closed

using guillotine doors operated remotely by an experimenter. A pipe, attached to the wall between the two goal-boxes was connected to two containers which allowed for remote delivery of large or small food rewards into a central food bowl. Tone cues, as for Experiment 1 (200 Hz, 500 Hz), were played via an mp3 player (Archos 18 Vision 4gb, Archos GmbH, Grevenbroich, Germany) through speakers, as for Experiment 1, attached above the start box.

Rewards used throughout the experiment were M&M chocolates.

For overview of training and testing schedule, see Fig. 2.

*Habituation to experimenter and rewards:* Performed as for Experiment 1.

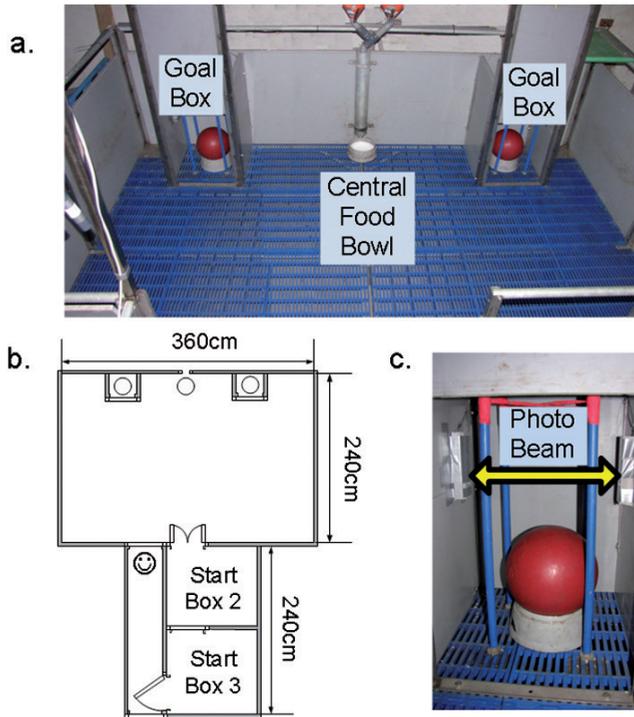
*Group habituation to apparatus:* One week after arrival, pigs were brought to the testing area as a group and allowed to explore. Access to the goal-boxes was closed. Rewards were periodically dropped into the central food bowl. Over 4 days, habituation was carried out 3 times per day. Group sizes were reduced to 6 pigs (4 sessions), to 3 pigs (2 sessions), to 2 pigs per group (2 sessions) and finally pigs were habituated individually (4 sessions).

*Operant response training:* Alongside the group habituation, pigs were individually trained to perform the ball-lift operant response in an empty pen. Pigs were clicker trained and shaped the raise the ball to the required height to receive one reward in 8 sessions of 10 trials.

*Individual training in apparatus:* Over 4 days (8 sessions) pigs were individually trained in the test apparatus to obtain one reward in the central food bowl. After consuming the bait, a pig was returned to the start box and the next trial started. Each training session consisted of 5 trials.

*Introduction of tone cues & associated outcomes:* In 10 sessions (12 trials per session) pigs were held in the start box for 10 s before being given access to the test area. Pigs learned to perform their previously learned ball-lift response in order to receive rewards in the central food bowl. In the first 4 sessions, tone cues were played as pigs performed the response. In the other 6 sessions tone cues were played for 5 s in the start box before the pig was given access to the test area. In each trial, only the correct (associated with the tone cue) goal-box was available, i.e. they were forced trials. Tone cues were differentially rewarded; a “positive-cue” signalled that 4 rewards would be delivered after performance of the ball-lift response while a “negative-cue” signalled that only one reward would be delivered. During these sessions, 6 Positive (P) and 6 Negative (N) cues were presented in the following order: (Sessions 1-4: P P – N N – P P – N N – P P – N N; Sessions 6-10: pseudorandom with a maximum of 2 consecutive cues of the same type). The meaning of cues (P/N) and their associated goal-boxes (left/right) were balanced across pigs. In 3 further sessions (12 trials per session) pigs received 2 forced trials as above and 10 “free” trials where both goal-boxes were open. To teach the pigs that there was always a reward available, they could respond in both goal-boxes in the free trials, but only a response in the correct goal-box would be rewarded. Cues were presented in the pseudorandom order as above.

*Discrimination Testing:* Pigs received up to 46 sessions of 12 trials per session. Each session consisted of 2 forced trials (1 P, 1 N) followed by 10 free trials (5 P, 5 N). Trial



**Fig. 3** Operant apparatus used in experiments 2 & 3 (a), Scale drawing of testing apparatus where ☺ indicates location of experimenter during training and testing (b), and close-up of a goal-box showing location of the photobeam (c).

order was pseudorandom as before and pigs had 30 s in which to make a response. Correct choice of goal-box in free trials resulted in the delivery of the associated large or small reward, while incorrect choices or omissions (failure to respond within 30 s) resulted in both goal-boxes being closed and a 90 s time penalty before commencement of the next trial. The criterion chosen to indicate learning of the discrimination was correct responses in 80% of negative and 80% of positive free trials in 3 out of 4 consecutive sessions.

Discrimination testing occurred in 3 phases. In Phase I (8 sessions) the 5 s tone cue was played in the start box before the pig was released into the testing area. In Phase II (8 sessions) the length of tone cues was increased; the cue was played as the pig was released from the start box until a response was made or for a maximum of 30 s. In Phase III (Sessions 17 – 46) the location of reward delivery was moved; rewards were placed in the bowl underneath the ball in the correct goal-box. As in Experiment 1, odour cues were avoided by placing inaccessible rewards in the base of the food bowl. Between Phases I and II pigs received 4 sessions of 12 forced trials, and between Phases II and III pigs received 2 sessions of 12 forced trials to habituate them to the alterations in the testing procedure.

*Data Recording & Analysis:* The choice of goal-box (correct, incorrect, or omission) was recorded per trial. The number of sessions taken to reach criterion was determined, as well as the number of pigs reaching criterion within the maximum of 46 sessions. The ability of LBW and NBW pigs to learn the discrimination was compared using Fisher's exact probability test. Two-sample t-statistics were carried out on SAS 9.2 to investigate group differences in the number of sessions needed to learn the discrimination.

### **Experiment 3:**

*Animals:* In this experiment, 8 female Göttingen minipigs from Ellegaard, Denmark (<http://www.minipigs.dk/>) and 7 female conventional farm pigs born at the commercial farm of Utrecht University (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) were used. All pigs had been previously used in our facility in a cognitive-holeboard task to assess spatial learning and memory (Gieling et al., 2013). For the present experiment, at the age of 5 months they were moved to new housing in a different section of the same stables where they had been housed for the holeboard study.

*Housing:* Animals were housed as in Experiment 1. Conventional pigs and minipigs were housed in separate, identical adjacent pens.

*Apparatus:* The apparatus used was the same as for Experiment 2. The start box was moved (see Fig. 3) and access to the test arena was controlled via a guillotine door. Pigs now had to first enter a smaller area, and then push open the swing doors before reaching the goal-boxes. It was noted that in Experiment 2, some pigs appeared to exit the start box in the direction of the positive goal-box, i.e. the goal-box associated with the larger reward, regardless of the cue presented. This modification gave the pigs longer to process the cue before they were able to move in the direction of either goal-box. This study used tone cues with a greater difference (200 Hz and 1000 Hz) played via an mp3 player (as Experiment 2) through new speakers (Logitech z-313, Logitech Europe S.A., Morges, Switzerland) attached at the back of the testing area between the two goal-boxes.

Rewards used throughout the experiment were M&M chocolates as for Experiment 2. For an overview of the training and testing schedule, see Fig. 2.

*Habituation to Experimenter and rewards:* As these animals had previously been used in another experiment they were already used to handling.

*Group habituation to apparatus:* On the 4th day after arrival in their new housing, pigs were brought to the testing area in groups. Access to the goal-boxes was closed. Rewards were periodically placed in front of each goal-box. As these pigs were used to being alone in a testing environment, habituation was kept to a minimum. Pigs were habituated in groups of 4 (2 sessions) and groups of 2 (2 sessions), all in the same day.

*Individual training in apparatus:* As the previous holeboard experiment (Gieling et al., 2013) involved a similar ball-lift response, training was kept to a minimum. Pigs were brought into the apparatus for individual habituation, 2 sessions of 8 forced trials, with equal numbers of rewarded left and right goal-boxes presented in a semi-random order, as before. Between trials pigs were returned to the start box.

*Introduction of tone cues:* In 3 sessions, of 12 forced trials per session, tone cues were played as the pig was given access to the testing area until a response was made or 60 s had elapsed. During these sessions, 6 Positive (P) and 6 Negative (N) cues were presented in the following order, 'PP NN PP NN PP NN', and the associated large or small rewards were available within the goal-box (as for Experiment 2, Phase III). The meaning of cues (P/N) and their associated goal-boxes (left/right) were balanced across pigs. In 2 further sessions (12 trials per session) pigs received 2 forced trials as before and 10 free trials (pseudorandom order) where in which they were allowed to respond in both goal-boxes as in Experiment 2.

*Discrimination Testing:* Pigs received up to 16 sessions of 12 trials per session. Each session consisted of 2 forced trials (1 P, 1 N) followed by 10 free trials (5 P, 5 N). Trial order was pseudorandom as before and pigs had 30 s in which to make a response. As for Experiment 2, correct choices in free trials resulted in the delivery of the associated large or small reward, while incorrect choices or omissions (failure to respond within 30 s) resulted in both goal-boxes being closed and a 90 s time penalty before commencement of the next trial. The criterion chosen to indicate learning of the discrimination was stricter than Experiment 2. Pigs had to respond correctly to 80% of negative and 80% of positive free trials in 3 consecutive sessions.

*Data Recording & Analysis:* The choice of goal-box (correct, incorrect, or omission) was recorded per trial. The number of sessions taken to reach criterion was determined, as well as the number of pigs reaching criterion within the maximum of 16 sessions. Two-sample t-statistics were carried out on SAS 9.2 to investigate breed differences in the number of sessions needed to learn the discrimination.

## RESULTS

### Experiment 1:

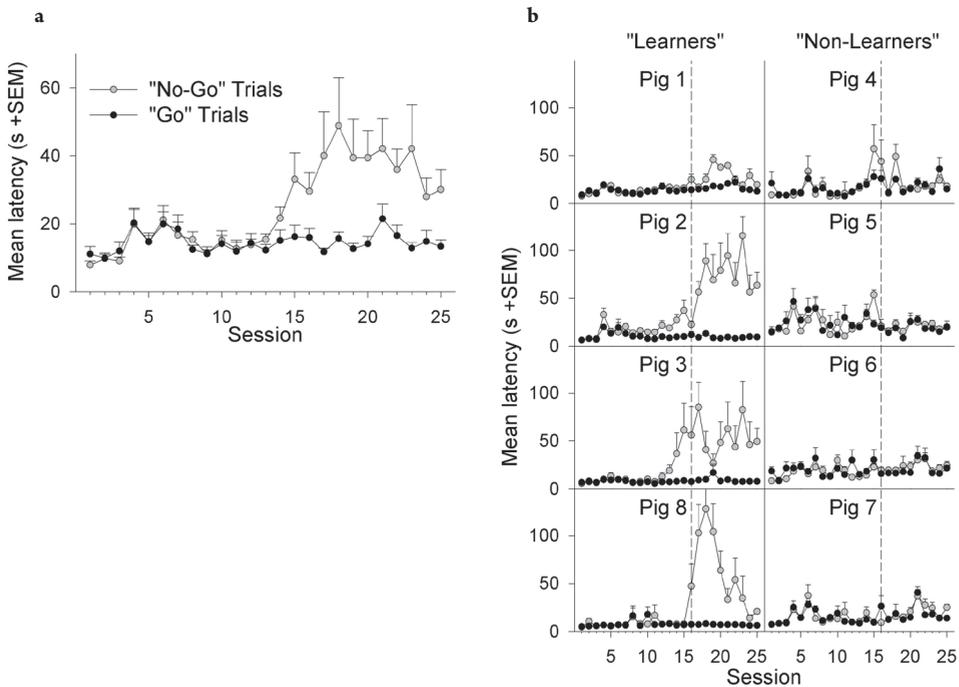
Pigs differed in latency responses to the cues and across sessions (Pigs,  $F_{7,40} = 4.20$ ,  $P = 0.0015$ ). The mean latencies increased across sessions (Sessions,  $F_{24,960} = 13.68$ ,  $P < 0.0001$ ), and the latencies in the No-Go trials were, on average, higher than those of the Go trials (Trial-Type,  $F_{1,40} = 122.81$ ,  $P < 0.0001$ ) (Fig. 4a). Second and third order interactions indicated that there were strong differences in learning between the pigs (Pigs by Sessions interaction,  $F_{168,960} = 3.25$ ,  $P < 0.0001$ ; Pigs by Trial-Type interaction,  $F_{7,40} = 23.12$ ,  $P < 0.0001$ ; Sessions by Trial-Type interaction,  $F_{24,960} = 9.87$ ,  $P < 0.0001$ ; Pigs by Sessions by Trial-Type interaction,  $F_{168,960} = 2.57$ ,  $P < 0.0001$ ) (see Fig. 4b).

Since there were significant differences between pigs, we performed additional within subjects ANOVAs on the data of individual pigs (within subjects factors: Session & Trial-Type). Analysis of the learning curves of the individual pigs revealed that only four out of the eight pigs successfully discriminated between Go- and No-Go-cues as measured by an increased latency to respond to the No-Go-cue i.e. showed learning on the task (Table 1). A series of one-sample t-tests performed on the mean difference scores per session of individual "learners" revealed that even within "learners" responses across sessions to the No-Go-cue were highly variable (See Fig 4b).

**Table 1** *F*-values and associated *P*-values for the analyses for individual pigs show that some animals learned the task as indicated by effects of session as well as a interactions between session and trial type.

Pig	Session	Trial-Type	Session X Trial-Type
	$F_{24,120}$	$F_{1,5}$	$F_{24,120}$
1	5.97 ***	8.83 *	1.98 **
2	5.76 ***	57.39 ***	6.14 ***
3	2.41 ***	36.65 **	2.36 **
4	4.18 ***	0.52	0.95
5	4.17 ***	0.03	1.05
6	2.91 ***	0.17	0.85
7	6.24 ***	0.15	1.54
8	6.87 ***	62.44 ***	6.43 ***

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$

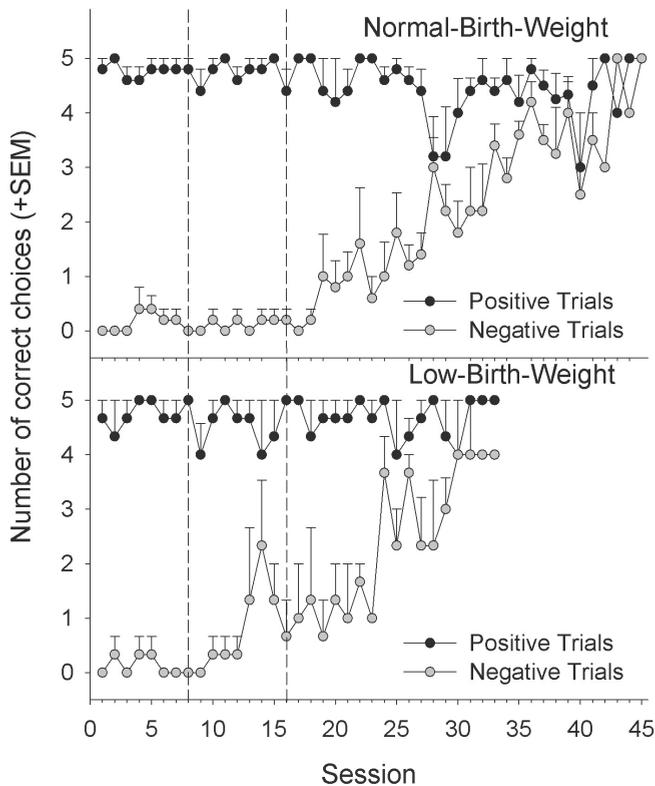


**Fig. 4** Mean latency to respond ( $\pm$  SEM) in rewarded and unrewarded trials for all 8 pigs across sessions (a), and mean latency to respond ( $\pm$  SEM) for rewarded and unrewarded trials for each individual animal (b). For clarity, only the positive SEMs are shown. The dashed-line indicates the point from which the barrier was introduced in front of the goal-box.

## Experiment 2:

For Experiment 2, data from all 3 discrimination testing phases are presented together. Of the 11 pigs used in this study, 8 reached the criterion level of performance, i.e. learned the discrimination, in Phase III within a maximum of 46 trials (mean±SEM: 36.88±1.73 sessions). Of the 8 learners, 5 were from the NBW group, 3 from the LBW group.

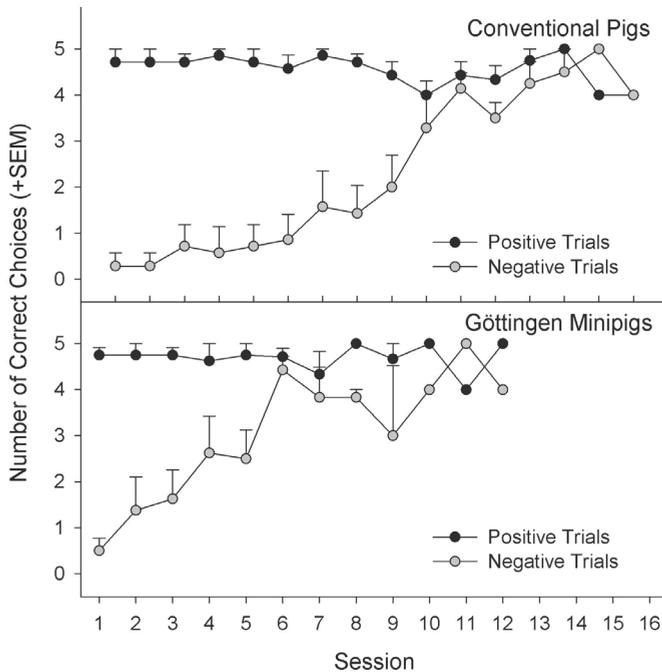
A Fisher's exact probability test revealed that birth weight group did not affect whether a pig was able to learn the discrimination within the number of sessions given ( $P=0.342$ ). However, the Learners of the LBW group acquired the tone discrimination faster than NBW pigs, i.e. needed fewer sessions to reach criterion (mean ± SEM: LBW, 32±1.00 sessions; NBW, 39±1.53 sessions;  $t_6=3.60$ ,  $P=0.01$ ) (Fig. 5). Looking at the responses of learners only to the modifications made in the 3 phases of the discrimination testing, it appears that while some increase in correct responses occurred in Phase II, the pigs still did not come close to reaching criterion until Phase III (Fig. 5).



**Fig. 5** Mean number of correct choices of goal-box for positive and negative cues per session (maximum score possible per cue type is 5) of NBW and LBW Learner pigs in Phase I (sessions 1-8), Phase II (sessions 9-16), and Phase III (sessions 17+) in discrimination testing. For clarity, only the positive SEMs are shown. All pigs show a strong bias for the positive goal-box, i.e. the goal-box containing the large reward, early in testing, and have to learn to approach the negative goal-box with repeated testing.

### Experiment 3:

All 15 pigs reached criterion for learning on the task within 16 sessions. Minipigs learned the discrimination faster than conventional pigs, i.e. reached criterion within fewer sessions than the conventional pigs (mean±SEM: minipigs, 8.38±0.80 sessions; conventional pigs, 13.14±0.67 sessions;  $t_{13}=4.49$ ,  $P=0.0006$ ) (Fig. 6).



**Fig.6** Mean number of correct choices of goal-box for positive and negative cues per session (maximum score possible per cue type is 5) for Conventional pigs (n=7) and Göttingen Minipigs (n=8). For clarity, only the positive SEMs are shown.

## DISCUSSION

Three experiments were carried out to investigate successive and conditional discrimination learning in pigs using tone cues predicting outcomes of different value. The results highlight that the different methods can affect the number of pigs which were able to learn the discrimination and the time taken for pigs to learn.

### Successive Discrimination Learning – Experiment 1

In Experiment 1 we demonstrated that only some pigs were capable of learning the Go/No-Go style task using tone cues to signal the appropriate response within the maximum number of 25 sessions and 300 trials given. Of the 8 animals trained on the task, only 4 were found to have discriminated between the cues as measured by an increased latency

to respond to the No-Go-cue. However, in these learners the performance was far from stable once they had acquired the discrimination. Furthermore, animals were tested for 25 sessions only so it is possible that more pigs would eventually have shown with continued training.

Our data shows that group mean response latencies masked individual differences and huge intra-subject variations; even for learners, responding to the No-Go-cue differed across and within sessions. A similar “Go/No-Go” style task with rats, using location of a goal pot as the cue, also reported changes in group mean latency responding (Burman et al., 2008). However, intra-subject variations were far lower than in the present study. Our results make choosing an index of learning which is applicable across subjects difficult.

Pigs have previously been shown to discriminate between tone cues (Ernst et al., 2006; Imfeld-Mueller et al., 2011) but the present experiment found that only half of the pigs appeared to do so. Cues in previous studies consisted of tone sequences, so perhaps pigs have more difficulty distinguishing individual tones. It may be that the cues we used were harder to distinguish or that the 5 s exposure was too short and/or too removed from the outcomes, particularly as the speakers were situated in the start box. Experiments by Neill and Harrison (1987) suggest that the proximity of the source of an auditory cue (i.e. the speaker) with respect to the response point can influence the rate of learning in a Go/No-Go task. Rats learned the discrimination faster when the source was adjacent to the response point than when the source was remote. Some of these issues were addressed in Experiments 2 and 3.

A previous study of Go/No-Go responses to visual cues in minipigs showed that it took up to 16 sessions of 100 trials per session for most animals to reach criterion (Moustgaard et al., 2005), (Lind and Moustgaard, 2005). While we found some pigs showed learning within 25 sessions i.e. 300 trials, we did not find response latencies to be consistent. As latency may be influenced by a variety of factors such as hunger, anhedonia and motivation to return to the home pen, strict Go/No-Go responses ultimately provide a clearer index of learning.

In the present Go/No-Go experiment, there was only a small energy cost to making an incorrect response. If we had increased the penalty for an incorrect response, e.g. an aversive noise (Kanitz et al., 2005; Talling et al., 1996), perhaps pigs might have shown more strict Go/No-Go responses to the cues. Pairing the No-Go cue with a definite negative outcome rather than just no outcome, might have increased the number of learners. Trapold (1970) showed that spatial discrimination learning in rats is faster if the associated outcomes also differ, a phenomenon known as the “differential outcome effect”, i.e. the outcome of stimulus-response discrimination learning can have bearing on the learning itself (Urcuioli, 2005). Alternatively, the effort needed to perform a Go response regardless of cue type could be increased. Pigs will continue to work hard for food on an incremental fixed ratio schedule, but will reduce working for other resources (Matthews and Ladewig, 1994).

Pigs in the present study may have learned to terminate their own trials, as a trial ended once pigs performed a Go response, which may explain inconsistent responses

within learners. Returning to the home pen has been used as a reward with pigs in a study by Siegford et al. (2008). Furthermore, remaining in the test apparatus may be considered aversive, even after the extended habituation. In future experiments, using a fixed inter-trial interval or introduction of a time penalty for an incorrect response could solve this issue.

## **Conditional Discrimination Learning – Experiment 2**

In Experiment 2, 8 out of 11 animals learned the discrimination within the maximum of 46 sessions given. Contrary to expectation, learning was not affected by birth-weight. In fact, of the pigs which learned the discrimination, LBW pigs actually learned faster than NBW pigs. However, if testing had continued perhaps more animals would have learned, and it is possible that this difference would have disappeared.

Some issues with the methodology used in Experiment 1 discussed above were avoided in Experiment 2. Firstly, defining the point at which learning occurred was far easier in Experiment 2 as an active-response to both cues was required as opposed to relying on latency measures. By extension, categorising pigs as Learners and Non-Learners was also clearer. The cost of making an incorrect response was much greater in Experiment 2; pigs received a 90 s time-out for incorrect responses or omissions, which prevented pigs from terminating their own trials. Thus, we expected these modifications to increase the number of animals learning the task and reduce the number of trials required to learn. However, it is difficult to make direct comparisons between the methods used in Experiment 1 and Experiment 2, as pigs in the latter were given up to 46 sessions (of 10 trials) in which to learn the discrimination, while those in Experiment 1 only received 25 sessions (of 12 trials).

In a previous study of conditional discrimination in Göttingen minipigs using visual cues (Moustgaard et al., 2005), all pigs were able to learn the task within 26 sessions with 20 trials per session i.e. within 520 trials. In the present study, the maximum number of trials reached was 460. However since pigs have better hearing than visual abilities (Gielsing et al., 2011a) it would be expected that an auditory task would be learned faster than a visual one. Harrison (1984) showed that rats could acquire a conditional discrimination using auditory cues within one session if the source of each cue was located adjacent to the associated response point. However, in the studies of Harrison, the cue was the location of the source and not the sound itself. With the present set-up, the length of time taken to acquire the discrimination might be reduced if the positive and negative cues were played from different speakers, each located adjacent to the associated goal-boxes.

The modifications in the different phases of testing in Experiment 2 were designed to simplify the task, without changing the fundamental discrimination required from the pigs. While learning in Phase III may be simply due to a longer exposure to the cues and their outcomes, the steep increase in correct responses in Phase III suggests that the procedural modifications had a greater effect. In Phase I, the cue was only played for 5 s and a more complex arbitrary ball-lift response was required, i.e. the required operant response had no direct relevance to the outcome. In Phase II, the cue length was

increased but still an arbitrary response was required. In a conditional discrimination task with rats, the proximity of the cue to the reinforcer has been found to have an effect on learning (Modo et al., 2000). When the cue (different floor type predicting left or right arm rewarded in a Y-maze) was presented in the start box, none of the rats reached criterion, however, when the cue was presented in the arms of the Y-maze all rats were able to learn the discrimination. Thus, the increase in length of the tone cue and the presentation of rewards in the correct goal-boxes most likely both contributed to the ability of the pigs to learn the conditional discrimination in Phase III.

It is surprising that, contrary to expectation based on the findings of Gieling et al. (Gieling et al., 2011b), we found that low-birth-weight Learner pigs actually learned the discrimination faster than the normal-birth-weight pigs. Martínez and colleagues (2012) found that the differential outcome effect had a greater impact on learning of human children born preterm with a very LBW than on NBW children. LBW children performed better in conditional discrimination task when they were trained with differential outcomes than when they were trained with non-differential outcomes, while the nature of the outcome did not affect learning of the task in NBW children. Other factors, e.g. hunger motivation may have contributed to LBW pigs' faster learning. While pigs in the present study were not food restricted, larger pigs may have eaten more than their fair share of the feed allowance, leaving the LBW pigs hungrier.

### **Conditional Discrimination Learning – Experiment 3**

In Experiment 3, the final method established in Experiment 2 was further simplified by increasing the difference between the positive and negative cues. With these changes, all of the pigs from both the conventional group and the minipig group were able to learn the discrimination within 16 sessions. This is far less than the 520 trials needed by Göttingen minipigs to discriminate visual cues in the study by Moustgaard et al. (2005). Additionally, minipigs in the present study performed even better, all learning within 120 trials.

The difference in learning between the two breeds in our study contrasts with results found on memory and learning in a spatial holeboard task, using these same pigs (Gieling et al., 2013). In the holeboard, the minipigs were initially slower to learn, but eventually reached the same level of performance as the conventional pigs. As few comparisons have been made between these breeds, there may be a number of explanations for these contrasting findings. Firstly, the nature of the task is different; perhaps minipigs are better at auditory discrimination than spatial discrimination. Secondly, while both breeds were the same age, they were not at the same stage in life. Minipigs reach puberty at a younger age than conventional pigs (minipig gilts: 4 months; conventional gilts: 7 months (Holtz, 2010)) so that in the initial discrimination training, it was likely that minipigs had reached puberty, but that conventional pigs had not. It has been shown that rats exposed to a stressor show enhanced learning in a conditioning paradigm during puberty, but not prior to puberty (Hodes and Shors, 2005). Perhaps, in the present study, avoiding the time penalty gave the minipigs a greater incentive to learn.

## **CONCLUSIONS**

The three experiments in this study approached assessment of discrimination learning in pigs in two ways. Firstly a successive discrimination task was developed which required suppression of a response in order to demonstrate learning. Secondly, two experiments developed a conditional discrimination task using differential rewards which required an active response to both cues. While some issues with the methodology of the successive discrimination are discussed, it is suggested that the conditional discrimination method requiring an active response provides a clearer index of learning than relying on latency alone, and appears to result in more consistent responding in pigs. Particularly with respect to cognitive bias research which depends on mastering the discrimination task before ambiguous cues can be presented, we expect that the active choice conditional discrimination task yields clearer results than the Go/No-Go style successive discrimination task.





# CHAPTER 04

## RESPONSES OF CONVENTIONAL PIGS AND GÖTTINGEN MINIATURE PIGS IN AN ACTIVE CHOICE JUDGEMENT BIAS TASK

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## ABSTRACT

Pigs are commonly kept in intensive farming systems. Their use as model animals in biomedical research has increased. Both conditions may impact upon their welfare. Recent definitions of welfare emphasize the importance of emotion. Mood congruent biases in judgement have been proposed as proxy measures of emotion in animals; optimistic responses to ambiguous cues are said to reflect positive emotional states while negative biases reflect negative emotional states. We developed a novel active-choice task to measure judgement bias in conventional farm pigs ( $n=7$ ) and Göttingen minipigs ( $n=8$ ). Pigs were trained to distinguish a positive tone-cue, from a negative tone-cue, signalling the location (goal-box) of large or small rewards respectively. After learning this discrimination pigs were presented with a series of ambiguous tone-cues and the percentage of choices for the positive goal-box recorded as optimistic responses (Phase I). After a 4-week break, pigs were retrained and challenged with a restraint treatment to induce a negative emotional state. Judgement bias was again measured (Phase II). After another 2-week break, a third test was performed to assess the effect of repeated testing (Phase III). Minipigs learned the initial discrimination faster than conventional pigs but there were no differences in subsequent relearning (Breed:  $F_{1,13} = 21.47$ ,  $P = 0.0005$ ; Phase:  $F_{2,26} = 121.56$ ,  $P < 0.0001$ ; Breed\*Phase:  $F_{2,26} = 10.53$ ,  $P = 0.0004$ ). Both groups responded similarly in our judgement bias task ( $F_{1,13} = 2.70$ ,  $P = 0.1241$ ) and both showed a reduction in optimistic responses after the first test ( $F_{1,28,16.59} = 15.08$ ,  $P = 0.0007$ ). This reduction was likely due to learning about the outcomes of ambiguous cues, which potentially masked any effect of the restraint treatment. The comparable performance between the two breeds suggests that the task is suited for both breeds, and that research from one these breeds can be generalized to the other, across farm and laboratory housing and management conditions. Advantages of an active choice task for assessing judgement bias are discussed, and questions are raised about the suitability of this task for repeated and longitudinal assessment of judgement biases in pigs.

## Keywords

Emotion; Judgement Bias; Pig; Discrimination learning; Animal Welfare

## INTRODUCTION

Pig production is one of the most intensive farming systems in the developed world (Marchant-Forde, 2009) with large numbers of animals produced at the lowest cost. Many aspects within this system may compromise the welfare of the animal. Pigs are also increasingly being used in laboratories to model issues related to human health. They have been proposed as suitable animal for modelling brain disorders due to the greater similarity between pigs and humans in terms of brain anatomy, growth and development than established rodent models (Gieling et al., 2011; Lind et al., 2007). This has led to the development of commercially available miniature breeds of pigs, which are more suitable to laboratory environments, both due to their small size and their standardised breeding and rearing. Pigs used in laboratories and conventional farm pigs face very different challenges due to different environmental conditions and usages. These challenges are very likely to impact upon their behaviour and welfare. The vast majority of welfare research on pigs has been carried out on farm breeds, while laboratory breeds are more often used in biomedical research. To understand if the results of research from one of these breeds can be generalized to the other breed, across farm and laboratory housing and management conditions, comparisons of responses in different tasks is important. Until recently however, no direct comparisons of behaviour in conventional farm breeds and miniature laboratory breeds have been performed, except for a study by Gieling et al. (2013) who demonstrated that conventional farm pigs and Göttingen minipigs perform similarly in a cognitive holeboard task assessing learning and memory.

Welfare has been defined in many different ways, but more recent attempts to define welfare agree on the importance of psychological state and emotion (Désiré et al., 2002; Rushen, 2003), particularly positive emotions (Boissy et al., 2007b; Ohl and van der Staay, 2012). Emotion has traditionally been studied using specific behavioural tests or physiological measures which have generally focussed on negative states (Mendl et al., 2009). More recently however, the link between emotional and cognitive processes has become of interest to researchers in animal welfare (Boissy et al., 2007a; Dantzer, 2002; Paul et al., 2005).

Cognitive processes can influence emotion, but emotion can also influence cognitive processes. Evidence from human psychology suggests that affective states can influence processes such as attention, memory, decision making and judgement. Judgement and risk perception in particular, are influenced in a consistent way by the valence of an emotional state (Blanchette and Richards, 2010). Positive states are said to induce more optimistic judgements of ambiguous stimuli while negative states result in more pessimistic judgments. In animal welfare research, the judgement bias paradigm, first proposed by Harding et al. (2004), uses an animal's judgement of ambiguous stimuli as proxy measures of the valence of its background emotional state.

The effect of emotion on judgement has been the most investigated bias in animal studies to date. It has been studied in a variety of species including rodents (Burman et al., 2009), farm animals (Doyle et al., 2010a), birds (Brilot et al., 2010), and even insects (Bateson et al., 2011). The supposition that emotional state induces mood congruent effects on judgement has been supported in a number of studies. For example, removal

of enrichment led to more pessimistic judgements by starlings (Bateson and Matheson, 2007), while rats provided with enrichment showed more optimistic judgements (Brydges et al., 2011). Only one study to date has looked at judgement bias in pigs and found that enrichment, as with rats, led to more optimistic judgements while removal of enrichment led to more pessimistic judgements (Douglas et al., 2012). While most studies of cognitive bias in animals have focused on short term manipulations of emotion, more stable long term differences, such as personality, may also influence mood and judgements. Dogs that are more anxious when separated from the owner also show more pessimistic biases (Mendl et al., 2010). Starlings which develop stereotypies (Brilot et al., 2010) and capuchins that exhibit some types of stereotypic behaviour (Pomerantz et al., 2012) both show more pessimistic biases compared with non-stereotyping conspecifics.

In this experiment we aimed to measure baseline judgement bias in two breeds of pigs – conventional farm pigs and Göttingen minipigs – as well as alterations in judgement bias in response to a mild stressor. Early life conditions differed for each breed but reflected the standard conditions for farm and laboratory pigs. While most studies of judgement bias in animals use Go/No-Go type responses, we developed a novel Active Choice judgement bias task for pigs. From a previous experiment with a Go/No-Go design (Murphy et al., 2013), we found that Active Choice tasks are easier to interpret and they differentiate between an omission of a response and a pessimistic response. Furthermore, reliance on latency assumes that a longer response to an ambiguous cue is due to a more pessimistic judgement of that cue. However, a delay in response may be a reaction to the novelty of an ambiguous cue prior to and unrelated to any judgement of its meaning, and a rapid response may be an optimistic response but could also be a form of impulsive action (Mar and Dalley, 2010). Cyders and Smith (2007) suggest that both positive mood and negative mood can lead to rash action. Personality factors may also influence latency responses; Bolhuis et al. (2004) found that pigs with more high resistant coping styles are less behaviourally flexible compared with more low resistant pigs. Thus the response latency to an ambiguous cue may not always accurately reflect the pig's judgement but an inability of some pigs to alter their behaviour in response to a change. In an Active Choice task animals do not have to inhibit a response and instead perform a similar response to all cues. Active Choice tasks have been previously used with starlings (Brilot et al., 2010) and rats (Brydges et al., 2011; Enkel et al., 2010).

Pigs were presented with two goal-boxes and were trained that a “positive” tone-cue signalled the presence of a large reward in the “positive” goal-box while a “negative” tone-cue signalled the presence of a small reward in the “negative” goal-box. Once this discrimination was learned, pigs were given a baseline judgement bias test (Phase I) whereby ambiguous tone-cues were presented among the learned positive and negative cues and choice of positive or negative goal box was recorded. After a four-week break, pigs were retrained on the positive/negative cue discrimination. In order to induce a negative emotional state, pigs were subjected to a restraint treatment whereby they were individually placed in a weigh-crate for 15 minutes before and after training. Restraint in a “mini-crate” has been used previously to challenge pigs (Jarvis et al., 2006). Pigs

were then subjected to a second judgement bias task performed concurrently with the restraint treatment (Phase II). Finally, after a shorter two-week break, pigs were retrained without the restraint treatment and given a final judgement bias task as before (Phase III).

## **METHODS**

### **Ethical Note**

The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

### **Subjects and Housing**

We used eight female Göttingen minipigs (hereafter minipigs) obtained from Ellegaard, Denmark (<http://www.minipigs.dk/>) selected from seven different litters and seven female conventional farm pigs (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace), also from different litters. All pigs were weaned at 4 weeks of age and then transferred to our research facility. Minipigs were transported to our facility in the Netherlands from Denmark in an air-conditioned van while conventional pigs were transported into the research stables adjacent to the farrowing stables. All pigs were 5 months old at the start of the study and had previously been subjected to a spatial holeboard task assessing learning and memory (Gielsing et al., 2013). Conventional pigs and minipigs were group-housed separately in two straw-bedded pens (5 x 3 m). They had access to water ad libitum, and were fed twice per day, with 25% of the allowance in the morning before testing, 75% in the evening after testing. Both groups of pigs were fed according to the recommendations of their breeders and pigs were weighed weekly to ensure that weight gain was within the normal range for each breed.

### **Apparatus**

A detailed description of the testing apparatus (Fig. 1) can be found in Murphy et al. (2013). In short, the apparatus consisted of a start box connected via an antechamber to a testing arena. Exits from the start box to the testing arena were controlled using a guillotine door. In the testing arena were two goal-boxes, each of which contained a food-bowl covered with a large hard-plastic ball which could be raised in order to gain access to the food bowl. Each goal-box was associated with either a large or small food reward (counterbalanced across animals). Only one goal-box was rewarded per trial and tone-cues were used to indicate which goal-box contained a reward. Tone-cues were generated using the open source software Audacity (<http://audacity.sourceforge.net/>). The cues were played via an MP3 player (Archos 18 Vision, 4 GB, Archos GmbH, Grevenbroich, Germany) through speakers (Logitech z-313, Logitech Europe S.A., Morges, Switzerland) attached at the back of the testing area. The training tone-cues used were a 200 Hz and a 1000 Hz pure tone, each 30 s long and counterbalanced for consequence across animals (Waveform: Sine; Amplitude: 1). The “positive” tone-

cue signalled the presence of a large reward (4 M&M® chocolates) in the food bowl in the “positive” goal-box while the “negative” tone-cue signalled the presence of a small reward (1 M&M® chocolate) in the “negative” goal-box (Fig. 2). Three ambiguous tone-cues (AmbigNeg, AmbigMid and AmbigPos, with AmbigNeg and AmbigPos counterbalanced for meaning across pigs) were also generated at equal intervals between the training tone-cues on a logarithmic scale: 299.07 Hz, 447.21 Hz, and 668.74 Hz.

All pigs were tested on three separate occasions (Phase I, Phase II, and Phase III). After each test, pigs were given a break from training before being retrained to criterion again (Fig. 3).

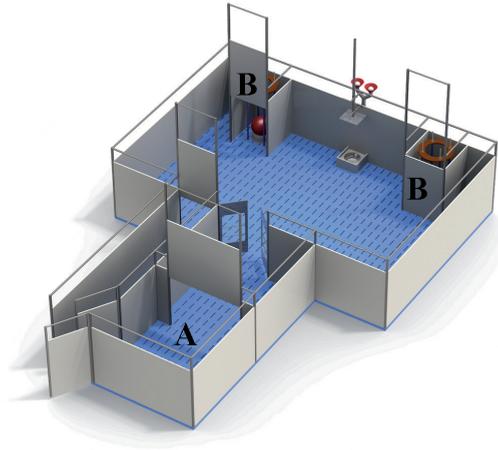
## **Phase I**

*Habituation & Training:* Pigs were first trained to discriminate between the positive and negative training tone-cues. A detailed description of the habituation and training procedure has recently been published (Murphy et al., 2013). Training sessions consisted of 13 trials of which three trials were “forced-trials”, where only the correct goal-box was available (1xPositive; 2xNegative), followed by 10 “free-trials”, where both goal boxes were available (5xPositive; 5xNegative). The order of free-trials differed daily and was pseudorandom with no more than two consecutive presentations of the same trial type. Incorrect choices in free-trials lead to a time-out penalty of 90 s before the pig was allowed to return to the start box for the next trial. During the time-out period, the pig remained in the testing arena but access to the goal-boxes was closed via a guillotine door at the entrance to each. Each pig received one training session per day and training with each animal continued until they had reached the predetermined criterion level of performance (max. 16 sessions): three consecutive sessions with four out of five correct choices for both positive and negative tone-cues.

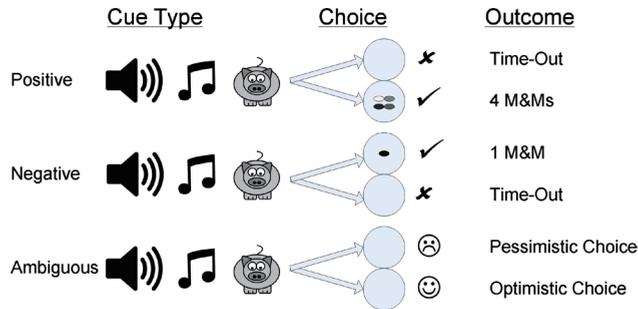
*Judgement Bias Test:* Once pigs had reached criterion, they were tested over four sessions. Test sessions consisted of 16 trials; three forced-trials and 10-free trials, as before, along with three ambiguous trials (trial numbers 6, 11, and 16). Ambiguous trials were similar to the positive and negative trials except that the tone-cue played was one of the three ambiguous tone-cues and neither goal-box contained a reward (see Fig. 2). Trial 6 was always the AmbigMid-trial so that the mid tone-cue was the first presented ambiguous cue. AmbigNeg and AmbigPos cues were played in trials 11 and 16 (counterbalanced across test sessions and pigs). Trial orders in test sessions were designed such that pigs had equal numbers of presentation of positive and negative cues before presentations of each ambiguous cue to counteract any effect of previous cue on responses in ambiguous trials.

## **Phase II**

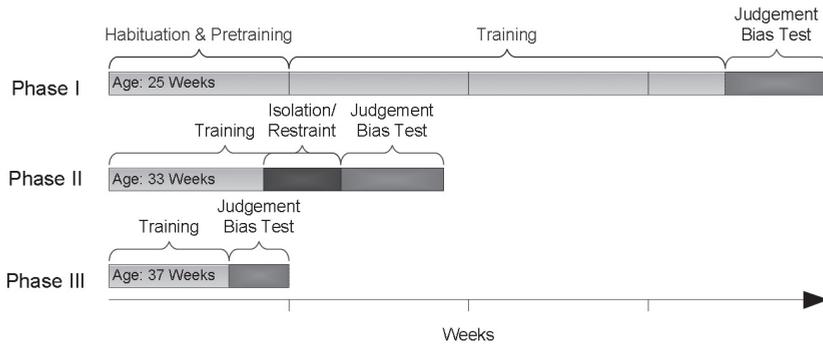
*Training:* After a 4 week break without training, pigs were retrained to discriminate the positive and negative training tone cues. Training continued for nine sessions even if pigs reached criterion before this in order to standardise the restraint treatment. In training session 5, pigs were placed in a restraint crate for 1 minute before and after each training session. In session 6, this was repeated for 5 minutes, and for 15 minutes before and after



**Fig. 1** Active-choice Judgement Bias testing apparatus indicating the location of the start box (A) and each goal-box (B).



**Fig. 2** Schematic overview of judgement bias task. Training tone-cues signalled the location of large and small food rewards. A correct choice to a positive cue resulted in a large reward, while a correct choice for a negative cue resulted in a small reward. An incorrect choice to either cue started a 90 s time-out penalty. In testing, ambiguous probe tone-cues were presented. The choice of goal-box for ambiguous cues was taken as a pig's expectation of a large (optimistic) or small (pessimistic) reward.



**Fig. 3** Overview of experimental timeline. After Phase I, pigs received a 4-week break from training before commencing Phase II. After Phase II, pigs received a 2-week break before commencing Phase III.

training for sessions 7 – 9. Two restraint crates were used, each of which had just enough room for the pig to stand-up and lie-down but not to turn around (conventional pig crate: 150 x 55 x 93 cm; minipig crate: 85 x 27 x 45 cm). Doyle et al. (2010a) found that sheep restrained prior to testing showed a positive bias and concluded that release from restraint induced a positive emotion. To avoid this we decided to subject the pigs to the restraint treatment both before and after training so that the expectation of restraint would be present during testing.

*Judgement Bias Test:* Pigs were again tested as described for Phase I for four sessions. During testing pigs continued to be placed in the crate for 15 minutes before and 15 minutes after test sessions.

### **Phase III**

*Training:* After a 2 week break without training, pigs were again retrained to criterion to discriminate the positive and negative training tone cues for five sessions.

*Judgement Bias Test:* Pigs were again tested, as described for Phase I, for two sessions.

### **Data Recording & Analysis**

For each training period (Phases I – III), the number of sessions needed to reach criterion was recorded per pig, and was used as a measure of discrimination learning. For each judgement bias test (Phases I – III), the choice of goal-box was recorded per trial (negative, positive or omission). Optimistic Choice (OC; %) was calculated per pig as the percentage of choices for the positive goal-box for each trial-type (Negative, AmbigNeg, AmbigMid, AmbigPos, or Positive) within each test phase. Using the Optimistic Choice data, the Area Under the Curve (AUC) was calculated per pig for each test phase using the linear trapezoidal method. The mean percentage of optimistic choices between each pair of consecutive cue types was calculated (Negative + AmbigNeg; AmbigNeg + AmbigMid; AmbigMid + AmbigPos; AmbigPos + Positive). To obtain the overall mean AUC per test, the mean of these mean scores was calculated.

The mean latency (s) to respond to each cue type for free trials during test phases was determined per pig. The latency to respond to each of the ambiguous cues was combined into a mean ambiguous latency.

All analyses were carried out using the statistical package SAS 9.2 (SAS Institute, Cary, North Carolina, USA). All data was checked for normality. Learning (Sessions to Criterion) and Judgement Bias (AUC and response latency) data were analysed for breed differences and for the effect of training phase using an analysis of variance (ANOVA) with the factor Breed (conventional pigs, minipigs) and the repeated measures factor Test Phase (I, II, and III). For latency the repeated measures factor Cue-Type (negative, ambiguous, and positive) was also included in the analysis. Where the assumption of sphericity was not met, the Greenhouse-Geisser Epsilon correction was applied.

To check for relationships in responses across tests, individual pigs' AUCs for all three test phases were analysed using a Pearson's Correlation. A Pearson's Correlation was also used to check for any relationship between judgement bias (AUC) and discrimination learning (sessions to criterion) in Phase I.

Optimistic choice, pessimistic choice and omission data was analysed for breed differences using Wilcoxon's-Mann-Whitney statistics. The data was then analysed for the effect of test phase (I, II, III) using a Friedman's ANOVA for repeated measures. To look for within test learning in Phase I, mean AUCs from the first two test sessions and for the last two test sessions were calculated. Grouping the first two and the last two test sessions ensured that these measures included a presentation of each ambiguous cue immediately following presentations of both positive and negative trials. These data were analysed for breed differences using a Wilcoxon Mann-Whitney statistics and session grouping differences (first two/ last two) using a Wilcoxon Signed-Rank test.

## RESULTS

### Learning and Memory

*Mean number of sessions taken to learn the discrimination (see also Table 1):* Overall effects of breed and phase, as well as an interaction between breed and phase was found on learning (sessions needed to reach criterion) (ANOVA; Breed:  $F_{1,13} = 21.47, P = 0.0005$ ; Phase:  $F_{2,26} = 121.56, P < 0.0001$ ; Breed\*Phase:  $F_{2,26} = 10.53, P = 0.0004$ ). In the initial discrimination training (Phase I), all pigs reached criterion for learning on the task within a maximum of 16 sessions. As is reported in Murphy et al. (2013), minipigs learned the discrimination in fewer sessions than conventional pigs, but in subsequent retraining (Phase II & III) there were no breed differences. Both breeds relearned the initial discrimination of positive and negative cues faster in each subsequent training period (Table 1).

### Judgement Bias

*Area under the curve (see Fig. 4c):* There was no effect of breed nor any interaction between breed and test phase, but there was an effect of test phase on AUC measures (ANOVA; Breed:  $F_{1,13} = 2.70, P = 0.1241$ ; Phase:  $F_{1,28,16,59} = 15.08, P = 0.0007$ ; Breed\*Phase:  $F_{1,28,16,59} = 1.55, P = 0.2361$ ). AUC measures decreased after Phase I but did not differ between Phases II and III (ANOVA; I vs. II  $F_{1,13} = 18.05, P = 0.0010$ ; I vs. III  $F_{1,13} = 15.87, P = 0.0016$ ; II vs. III  $F_{1,13} = 1.74, P = 0.2103$ ). As the AUCs from Phase I (Fig. 4c) suggest that there may be a breed difference, we calculated an effect size using the G\*Power software (Faul et al., 2007). This revealed a relatively high effect size ( $d = 0.83$ ) suggesting that, provided this is a real difference, then a sample size of 24 pigs per breed group ( $\alpha = 0.05$ ;  $\beta = 0.80$ ) would be necessary to confirm this difference statistically.

*Consistency across tests:* There was a very strong correlation between AUCs in Phases I and II for conventional pigs (Pearson's Correlation:  $r = 0.87, P = 0.0119$ ), but not with Phase III. For minipigs, there was no correlation between AUCs in any of the testing phases.

*Learning vs Judgement Bias:* There was no relationship between pigs' AUCs in Phase I and the number of sessions needed to learn the discrimination in Phase I for either conventional pigs (Pearson's Correlation:  $r = 0.24, P = 0.6075$ ) or minipigs (Pearson's Correlation:  $r = -0.15, P = 0.7181$ ).

*Optimistic choices* (see Fig. 4a,b; Table 2): There was no effect of breed on Optimistic Choice for any of the cue-types but there was an effect of test phase on all three ambiguous cues (Friedman's test; AmbigNeg:  $X^2(2) = 12.67, P = 0.0018$ ; AmbigMid:  $X^2(2) = 11.76, P = 0.0028$ ; AmbigPos:  $X^2(2) = 10.84, P = 0.0044$ ). There was also an effect of phase on Optimistic Choice for both negative and positive training-tones (Friedman test; Negative:  $X^2(2) = 8.55, P = 0.0139$ ; Positive:  $X^2(2) = 7.00, P = 0.0302$ ). As with AUC measures, Optimistic Choice was lower after Phase I for all ambiguous cues, but did not differ between Phases II and III (Fig.4a, b). In response to the first presentation of an ambiguous tone, the AmbigMid tone, in Phase I, 3 out of 7 conventional pigs (42.8%) and 5 out of 8 minipigs (62.5%) chose the positive goalbox.

*Latency to choose* (Table 3): Overall effects of breed, phase and cue, as well as interactions between breed and cue, and phase and cue were found on latency responses in test phases (ANOVA; Breed:  $F_{1,13} = 7.98, P = 0.0143$ ; Phase:  $F_{1,15, 14,93} = 27.19, P < 0.0001$ ; Cue:  $F_{1,61, 20,91} = 23.40, P < 0.0001$ ; Breed\*Cue:  $F_{1,61, 20,91} = 4.10, P = 0.0390$ ; Phase\*Cue:  $F_{1,54, 20,05} = 11.20, P = 0.0011$ ). There was no interaction between phase and breed, nor between phase, breed and cue (ANOVA; Phase\*Breed:  $F_{1,15, 14,93} = 0.12, P = 0.7693$ ; Phase\*Breed\*Cue:  $F_{1,54, 20,05} = 0.11, P = 0.8425$ ).

Exploration of the effects of breed, cue and phase (Table 3), revealed that minipigs responded faster than conventional pigs to all cue-types in phase I and II, but there was no difference in phase III. Both breeds increased their latency to respond to most cues-types in repeated tests (Phases II & III), particularly so for the ambiguous cues (Fig. 5). Furthermore, both breeds responded faster to positive cues than negative cues, a difference which was greater for conventional pigs. Responses between trained cues (positive, negative) and ambiguous cues varied (Table 3).

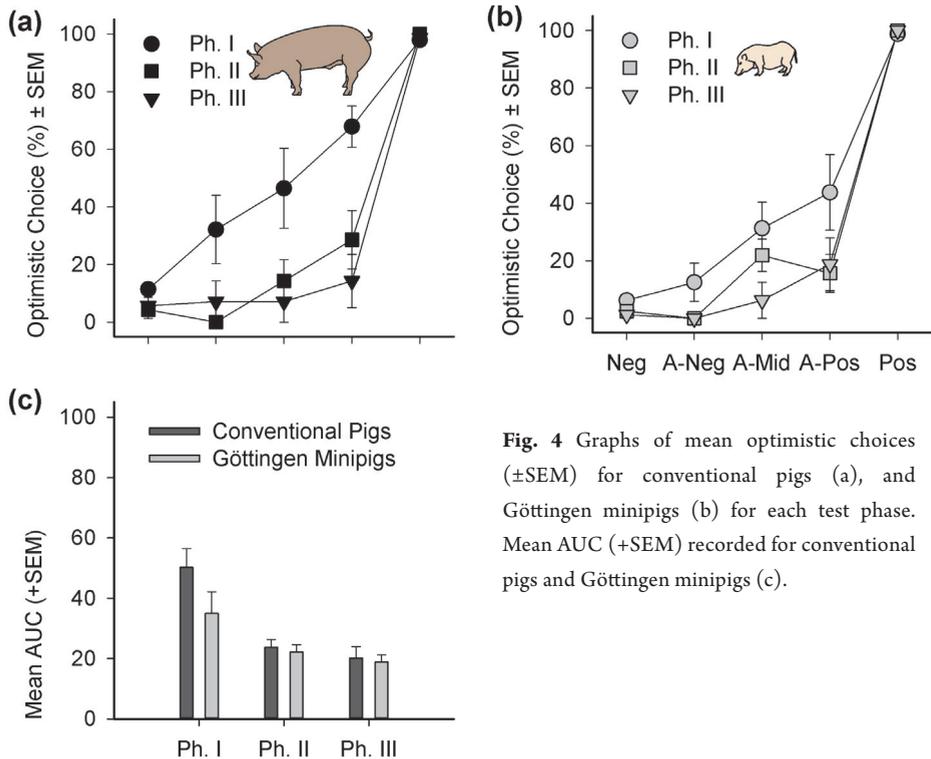
*Pessimistic choices and omissions* (Tables 4, 5): There was no effect of breed on the percentage of pessimistic choices or omissions for any of the cue-types except that conventional pigs made more omissions to the negative training cue in Phase II than minipigs (Tables 4, 5). Pessimistic choices increased in Phase II for both the AmbigNeg and AmbigPos Cues, while the percentage omissions increased in Phase III for all three ambiguous cues (Friedman's test; Pessimistic Choices: AmbigNeg:  $X^2(2) = 9.00, P = 0.0111$ ; AmbigMid:  $X^2(2) = 3.87, P = 0.1443$ ; AmbigPos:  $X^2(2) = 7.58, P = 0.0225$ ; Omissions: AmbigNeg:  $X^2(2) = 14.44, P = 0.0004$ ; AmbigMid:  $X^2(2) = 11.47, P = 0.0032$ ; AmbigPos:  $X^2(2) = 7.43, P = 0.0244$ ) (Tables 4, 5).

*Within Phase Learning (Phase I)*: Within Phase I, there were no breed differences in mean AUCs for the first two test sessions while minipigs tended to have lower AUCs than conventional pigs for the last two test sessions (Wilcoxon Mann-Whitney; FirstSess:  $W_s = 66.00, z = 1.18, P = 0.2386, r = 0.30$ ; LastSess:  $W_s = 71.00, z = 1.75, P = 0.0809, r = 0.45$ ). The mean AUCs of the last two test sessions was found to be lower than that of first two test sessions (mean $\pm$ SEM: Sess12 =  $49.33\pm 4.97$ ; Sess34 =  $35.33\pm 5.98$ ; Wilcoxon Signed-Rank;  $S = 43.5, P = 0.0106$ ) suggesting some learning about the ambiguous cues even within Phase I.

**Table 1** Mean number of sessions taken to learn to discriminate positive and negative cues ( $\pm$ SEM) in training, Phases I - III. All data was analysed using a repeated measures ANOVA.

Sessions to Criterion			
Breed	Phase I	Phase II	Phase III
Conv.	13.14 $\pm$ 0.67	5.57 $\pm$ 0.64	3.29 $\pm$ 0.18
Mini	8.38 $\pm$ 0.80	4.25 $\pm$ 0.37	3.00 $\pm$ 0.00
Effect of Breed (Conventional vs. Mini)			
	$F_{1,13} = 20.16$ $P = 0.0006^{***}$	$F_{1,13} = 3.36$ $P = 0.0897$	$F_{1,13} = 2.77$ $P = 0.1197$
Effect of Test Phase			
	I vs. II	I vs. III	II vs. III
Conv.	$F_{1,6} = 247.85$ $P < 0.0001^{***}$	$F_{1,6} = 178.54$ $P < 0.0001^{***}$	$F_{1,6} = 11.29$ $P = 0.0152^*$
Mini	$F_{1,7} = 16.75$ $P = 0.0007^{***}$	$F_{1,7} = 45.10$ $P = 0.0003^{***}$	$F_{1,7} = 11.67$ $P = 0.0112^*$

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$



**Fig. 4** Graphs of mean optimistic choices ( $\pm$ SEM) for conventional pigs (a), and Göttingen minipigs (b) for each test phase. Mean AUC ( $\pm$ SEM) recorded for conventional pigs and Göttingen minipigs (c).

**Table 2.** Percentage of Optimistic Choices in response to each cue type ( $\pm$ SEM) for each test phase. Wilcoxon Mann-Whitney tests were used to look for breed differences; while post hoc analyses of the effect of test phase were performed using Wilcoxon Signed-Ranks test.

Optimistic Choice (%) $\pm$ SEM						
Phase	Breed	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I	Conv	11.43 $\pm$ 2.10	32.14 $\pm$ 11.85	46.43 $\pm$ 13.83	67.86 $\pm$ 7.14	97.86 $\pm$ 1.01
I	Mini	6.25 $\pm$ 2.06	12.50 $\pm$ 6.68	31.25 $\pm$ 9.15	43.75 $\pm$ 13.15	98.75 $\pm$ 0.82
II	Conv	4.29 $\pm$ 2.97	0.00 $\pm$ 0.00	14.29 $\pm$ 7.43	28.57 $\pm$ 10.10	100.00 $\pm$ 0.00
II	Mini	2.50 $\pm$ 0.94	0.00 $\pm$ 0.00	21.88 $\pm$ 5.66	15.63 $\pm$ 6.58	100.00 $\pm$ 0.00
III	Conv	5.71 $\pm$ 2.97	7.14 $\pm$ 7.14	7.14 $\pm$ 7.14	14.29 $\pm$ 9.22	98.57 $\pm$ 1.43
III	Mini	1.25 $\pm$ 1.25	0.00 $\pm$ 0.00	6.25 $\pm$ 6.25	18.75 $\pm$ 9.15	100.00 $\pm$ 0.00

Optimistic Choices - Breed Differences					
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I	$W_s = 69.00$	$W_s = 67.00$	$W_s = 63.50$	$W_s = 67.00$	$W_s = 51.00$
	$z = 1.58$	$z = 1.37$	$z = 0.90$	$z = 0.99$	$z = -0.71$
	$P = 0.11$	$P = 0.17$	$P = 0.37$	$P = 0.19$	$P = 0.48$
	$r = 0.41$	$r = 0.35$	$r = 0.23$	$r = 0.34$	$r = -0.18$
II	$W_s = 54.00$	$W_s = 56.00$	$W_s = 48.50$	$W_s = 64.00$	$W_s = 56.00$
	$z = -0.26$	$z = 0.00$	$z = -0.94$	$z = 1.31$	$z = 0.00$
	$P = 0.79$	$P = 1.00$	$P = 0.34$	$P = 0.32$	$P = 1.00$
	$r = -0.07$	$r = 0.00$	$r = -0.24$	$r = 0.26$	$r = 0.00$
III	$W_s = 65.00$	$W_s = 60.00$	$W_s = 56.50$	$W_s = 53.50$	$W_s = 52.00$
	$z = 1.34$	$z = 1.07$	$z = 0.10$	$z = -0.35$	$z = -1.07$
	$P = 0.18$	$P = 0.29$	$P = 0.92$	$P = 0.72$	$P = 0.29$
	$r = 0.35$	$r = 0.28$	$r = 0.03$	$r = -0.09$	$r = -0.28$

Optimistic Choice - Effect of Test Phase (I, II, III)					
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I vs. II	$S = 21.0$	$S = 18.0$	$S = 21.5$	$S = 42.5$	$S = -7.5$
	$P = 0.0313^*$	$P = 0.0078^{**}$	$P = 0.0313^*$	$P = 0.0042^{**}$	$P = 0.0625$
I vs. III	$S = 30.5$	$S = 16.0$	$S = 27.5$	$S = 36.0$	$S = -4.5$
	$P = 0.0563$	$P = 0.0703$	$P = 0.0020^{**}$	$P = 0.0098^{**}$	$P = 0.5313$
II vs. III	$S = -0.5$	$S = -0.5$	$S = 14.5$	$S = 4.0$	$S = 0.5$
	$P = 1.000$	$P = 1.000$	$P = 0.1133$	$P = 0.6953$	$P = 1.0000$

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

**Table 3.** Latencies ( $s \pm SEM$ ) to respond to negative, ambiguous and positive cues. Effects of breed, test phase and cue-type are explored. All analyses were performed using a repeated measures ANOVA with the between subjects factor Breed and the within subjects factors Test Phase and Cue-Type.

		Latency		
Test Phase	Breed	Negative	Ambiguous	Positive
I	Conv	11.27 $\pm$ 0.88	9.18 $\pm$ 0.84	7.32 $\pm$ 0.30
	Mini	6.78 $\pm$ 0.45	6.55 $\pm$ 0.84	6.03 $\pm$ 0.45
II	Conv	12.33 $\pm$ 1.20	11.15 $\pm$ 1.21	7.80 $\pm$ 0.41
	Mini	6.99 $\pm$ 0.45	8.02 $\pm$ 0.75	6.16 $\pm$ 0.44
III	Conv	15.57 $\pm$ 2.11	17.77 $\pm$ 2.08	8.42 $\pm$ 0.53
	Mini	10.24 $\pm$ 1.77	15.87 $\pm$ 2.83	7.24 $\pm$ 0.72
<b>Effect of Breed (Conventional vs. Mini)</b>				
I		$F_{1,13} = 22.14$ $P = 0.0004^{***}$	$F_{1,13} = 8.63$ $P = 0.0116^*$	$F_{1,13} = 5.29$ $P = 0.0386^*$
	II	$F_{1,13} = 19.41$ $P = 0.0007^{***}$	$F_{1,13} = 5.15$ $P = 0.0409^*$	$F_{1,13} = 7.16$ $P = 0.0191^*$
III	$F_{1,13} = 3.81$ $P = 0.0729$	$F_{1,13} = 0.28$ $P = 0.6065$	$F_{1,13} = 1.67$ $P = 0.2181$	
<b>Effect of Test Phase (I, II, III)</b>				
I vs. II		$F_{1,13} = 2.30$ $P = 0.1532$	$F_{1,13} = 16.01$ $P = 0.0015^{**}$	$F_{1,13} = 5.22$ $P = 0.0398^*$
I vs. III		$F_{1,13} = 9.54$ $P = 0.0086^{**}$	$F_{1,13} = 27.34$ $P = 0.0002^{***}$	$F_{1,13} = 16.58$ $P = 0.0013^{**}$
II vs. III		$F_{1,13} = 8.24$ $P = 0.0131^*$	$F_{1,13} = 21.03$ $P = 0.0005^{***}$	$F_{1,13} = 10.26$ $P = 0.0069^{**}$
<b>Effect of Cue Type (Negative, Ambiguous, Positive)</b>				
		<b>Neg. vs. Pos.</b>	<b>Neg. vs. Ambig.</b>	<b>Pos. vs. Ambig.</b>
I	Conv	$F_{1,6} = 34.21$ $P = 0.0011^{**}$	$F_{1,6} = 28.17$ $P = 0.0018^{**}$	$F_{1,6} = 7.52$ $P = 0.0337^*$
	Mini	$F_{1,7} = 7.25$ $P = 0.0310^*$	$F_{1,7} = 4.22$ $P = 0.0790$	$F_{1,7} = 4.41$ $P = 0.0740$
II	Conv	$F_{1,6} = 27.56$ $P = 0.0019^{**}$	$F_{1,6} = 2.10$ $P = 0.1978$	$F_{1,6} = 9.69$ $P = 0.0208^*$
	Mini	$F_{1,7} = 19.95$ $P = 0.0029^{**}$	$F_{1,7} = 5.24$ $P = 0.0558$	$F_{1,7} = 13.82$ $P = 0.0075^{**}$
III	Conv	$F_{1,6} = 17.63$ $P = 0.0057^{**}$	$F_{1,6} = 0.65$ $P = 0.4515$	$F_{1,6} = 24.36$ $P = 0.0026^{**}$
	Mini	$F_{1,7} = 6.07$ $P = 0.0433^*$	$F_{1,7} = 4.29$ $P = 0.0772$	$F_{1,7} = 10.30$ $P = 0.0149^*$

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

**Table 4.** Percentage of Pessimistic Choices in response to each cue type ( $\pm$ SEM) for each test phase. Wilcoxon Mann-Whitney tests were used to look for breed differences; while post hoc analyses of the effect of test phase were performed using Wilcoxon Signed-Ranks test.

Pessimistic Choice (%) $\pm$ SEM						
Phase	Breed	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I	Conv	85.00 $\pm$ 4.08	67.86 $\pm$ 11.85	53.57 $\pm$ 13.83	32.14 $\pm$ 7.14	2.14 $\pm$ 1.01
I	Mini	93.75 $\pm$ 2.06	87.50 $\pm$ 6.68	68.75 $\pm$ 9.15	56.25 $\pm$ 13.15	1.25 $\pm$ 0.82
II	Conv	92.14 $\pm$ 3.25	100.00 $\pm$ 0.00	85.71 $\pm$ 7.43	67.86 $\pm$ 8.99	0.00 $\pm$ 0.00
II	Mini	97.50 $\pm$ 0.94	96.88 $\pm$ 3.13	75.00 $\pm$ 4.72	81.25 $\pm$ 6.25	0.00 $\pm$ 0.00
III	Conv	90.71 $\pm$ 2.54	71.43 $\pm$ 14.87	78.57 $\pm$ 10.10	78.57 $\pm$ 10.10	1.43 $\pm$ 1.43
III	Mini	96.88 $\pm$ 1.62	56.25 $\pm$ 14.75	68.75 $\pm$ 9.15	56.25 $\pm$ 14.75	0.00 $\pm$ 0.00

Pessimistic Choices - Breed Differences						
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive	
I	$W_s = 41.00$	$W_s = 45.00$	$W_s = 48.50$	$W_s = 45.00$	$W_s = 61.00$	
	$z = -1.78$	$z = -1.37$	$z = -0.90$	$z = -1.31$	$z = 0.71$	
	$P = 0.07$	$P = 0.17$	$P = 0.37$	$P = 0.19$	$P = 0.48$	
	$r = -0.46$	$r = -0.35$	$r = -0.23$	$r = -0.34$	$r = 0.18$	
II	$W_s = 46.00$	$W_s = 59.50$	$W_s = 66.50$	$W_s = 47.00$	$W_s = 56.00$	
	$z = -1.27$	$z = 0.93$	$z = 1.35$	$z = -1.14$	$z = 0.00$	
	$P = 0.2055$	$P = 0.35$	$P = 0.18$	$P = 0.25$	$P = 1.00$	
	$r = -0.33$	$r = 0.24$	$r = 0.35$	$r = -0.29$	$r = 0.00$	
III	$W_s = 40.50$	$W_s = 62.00$	$W_s = 61.50$	$W_s = 64.50$	$W_s = 60.00$	
	$z = -1.88$	$z = 0.75$	$z = 0.73$	$z = 1.08$	$z = 1.07$	
	$P = 0.06$	$P = 0.45$	$P = 0.46$	$P = 0.28$	$P = 0.29$	
	$r = -0.49$	$r = 0.19$	$r = 0.19$	$r = 0.28$	$r = 0.28$	

Pessimistic Choice - Effect of Test Phase (I, II, III)						
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive	
I vs. II	$S = -17.5$	$S = -14.0$	$S = -20.5$	$S = -36.0$	$S = -7.5$	
	<i>ns</i>	<b><math>P = 0.0156^*</math></b>	<i>ns</i>	<b><math>P = 0.0081^{**}</math></b>	$P = 0.0625$	
I vs. III	$S = -23.0$	$S = 12.5$	$S = -11.0$	$S = -18.0$	$S = -4.5$	
	<i>ns</i>	$P = 0.2754$	<i>ns</i>	$P = 0.2231$	$P = 0.5313$	
II vs. III	$S = 5.5$	$S = 18.0$	$S = 10.0$	$S = 8.5$	$S = -0.5$	
	<i>ns</i>	<b><math>P = 0.0078^{**}</math></b>	<i>ns</i>	$P = 0.5063$	$P = 1.0000$	

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$

**Table 5.** Percentage of Omissions in response to each cue type ( $\pm$ SEM) for each test phase. Wilcoxon Mann-Whitney tests were used to look for breed differences; while post hoc analyses of the effect of test phase were performed using Wilcoxon Signed-Ranks test.

Omissions (%) $\pm$ SEM						
Phase	Breed	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I	Conv	3.57 $\pm$ 2.83	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
I	Mini	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
II	Conv	3.57 $\pm$ 1.43	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	3.57 $\pm$ 3.57	0.00 $\pm$ 0.00
II	Mini	0.00 $\pm$ 0.00	3.13 $\pm$ 3.13	3.13 $\pm$ 3.13	3.13 $\pm$ 3.13	0.00 $\pm$ 0.00
III	Conv	3.57 $\pm$ 1.43	21.43 $\pm$ 10.10	14.29 $\pm$ 9.22	7.14 $\pm$ 7.14	0.00 $\pm$ 0.00
III	Mini	1.88 $\pm$ 1.32	43.75 $\pm$ 14.75	25.00 $\pm$ 9.45	25.00 $\pm$ 13.36	0.00 $\pm$ 0.00

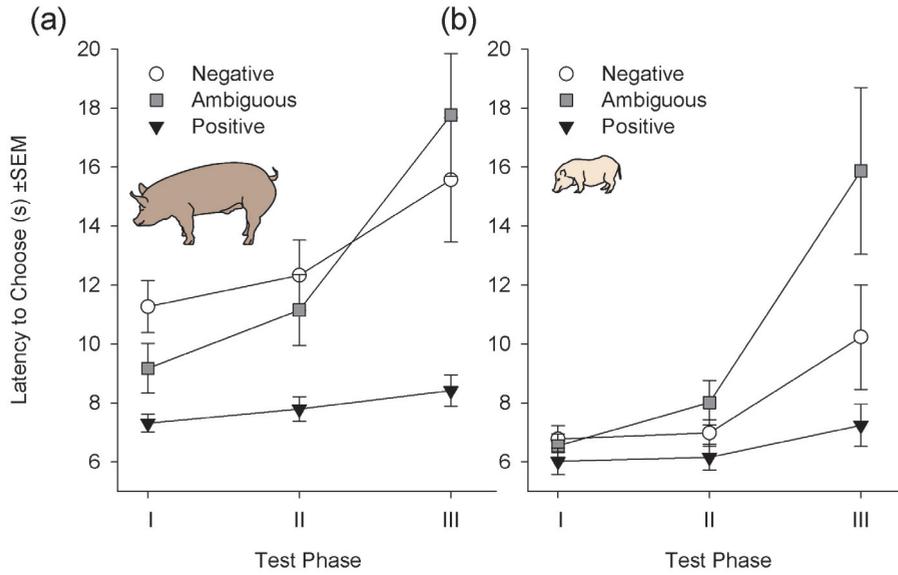
  

Omissions - Breed Differen					
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I	$W_s = 64.00$	$W_s = 56.00$	$W_s = 56.00$	$W_s = 56.00$	$W_s = 56.00$
	$z = 1.56$	$z = 0.00$	$z = 0.00$	$z = 0.00$	$z = 0.00$
	$P = 0.12$	$P = 1.00$	$P = 1.00$	$P = 1.00$	$P = 1.00$
	$r = 0.41$	$r = 0.00$	$r = 0.00$	$r = 0.00$	$r = 0.00$
II	<b><math>W_s = 72.00</math></b>	$W_s = 52.50$	$W_s = 52.50$	$W_s = 56.5$	$W_s = 56.00$
	<b><math>z = 2.39</math></b>	$z = -0.93$	$z = -0.93$	$z = 0.10$	$z = 0.00$
	<b><math>P = 0.02</math></b>	$P = 0.35$	$P = 0.35$	$P = 0.92$	$P = 1.00$
	<b><math>r = 0.62</math></b>	$r = -0.24$	$r = -0.24$	$r = 0.03$	$r = 0.00$
III	$W_s = 64.00$	$W_s = 47.50$	$W_s = 50.00$	$W_s = 49.00$	$W_s = 56.00$
	$z = 1.06$	$z = 1.08$	$z = -0.82$	$z = -1.05$	$z = 0.00$
	$P = 0.29$	$P = 0.28$	$P = 0.41$	$P = 0.29$	$P = 1.00$
	$r = 0.27$	$r = -0.28$	$r = -0.21$	$r = -0.27$	$r = 0.00$

Omissions - Effect of Test Phase (I, II, III)					
Phase	Negative	AmbigNeg	AmbigMid	AmbigPos	Positive
I vs. II	$S = 0.0$	$S = -0.5$	$S = -0.5$	$S = -1.5$	<i>ns</i>
	<i>ns</i>	$P = 1.000$	$P = 1.000$	$P = 0.5000$	
I vs. III	$S = -4.5$	$S = -18.0$	<b><math>S = -10.5</math></b>	$S = -5.0$	<i>ns</i>
	<i>ns</i>	<b><math>P = 0.0078^{**}</math></b>	<b><math>P = 0.0313^*</math></b>	$P = 0.1250$	
II vs. III	$S = -5.0$	$S = -18.0$	<b><math>S = -10.5</math></b>	$S = -5.0$	<i>ns</i>
	<i>ns</i>	<b><math>P = 0.0078^{**}</math></b>	<b><math>P = 0.0313^*</math></b>	$P = 0.1250$	

\*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$



**Fig. 5** Graphs of latency to choose ( $\pm$ SEM) for each cue-type for (a) conventional pigs, and (b) Göttingen minipigs for each test phase. Minipigs choose significantly faster than conventional pigs for all cues types in Phases I & II but not Phase III. Both breeds responded faster to positive cues than negative cues in each test phase (Table 3).

## DISCUSSION

We compared two breeds of pigs – a typical farm breed and a typical laboratory breed – on baseline measures of judgement bias using a novel active choice task. We subjected all pigs to a mild stress treatment (restraint in a crate) in an attempt to induce a negative mood state, and again performed the judgement bias task. To account for the effect of repeated testing, we performed the judgement bias task for a third time, this time without the restraint treatment. We looked at both learning of the task (sessions to criterion) and judgement bias measures (Optimistic Choice and response latencies). We found that minipigs were initially faster learners than conventional pigs, but that once the task was learned, both breeds performed similarly.

Conventional pigs and minipigs did not differ in measures of judgement bias in any of the three tests we performed, and both showed a reduction in optimistic responses in repeated tests. This effect of test phase was most likely due to learning about the outcomes of ambiguous trials, rendering them no longer ambiguous, rather than any effect of the restraint treatment. Minipigs responded faster to all cues in the first two judgement bias tests, but there were no breed differences in the third test. Both breeds showed a preference for the positive reward as demonstrated by a faster latency to respond to positive cues than negative cues, and this difference was greater in conventional pigs. Both breeds also showed an increasing latency to respond to ambiguous cues in repeated tests further indicating some learning about the outcomes of ambiguous trials.

Although we set out to compare behaviour of a typical farm pig to that of a typical laboratory breed on a judgement bias task, a true breed comparison would have required not only that conditions for each group were identical, but also the history of these pigs from birth to arrival at our test facility. While we were able to achieve this as much as possible once the animals arrived at our research facility, several factors which may have influence the results need to be taken into consideration.

Firstly, the groups were bred at different facilities and minipigs were transported a far longer distance to our research facility. There is little information on how minipigs are affected by transport but it is known that they lose 6-8% of their bodyweight (Ellegaard et al., 2010). Most of the research on the effects of transport in pigs has focused on the effects of handling rather than the actual transport for practical reasons (von Borell and Schäffer, 2005). Thus, while both groups in our experiment experienced similar handling, minipigs may have had a more stressful experience due to the length of the transport. Early life stress can have long term consequences for later emotional functioning (Pechtel and Pizzagalli, 2011).

A second factor to consider is potential differences in food motivation between the two groups of pigs. While each was fed according to the recommendations of their breeders, minipigs, particularly females, are fed a naturally restricted diet to control their growth (Ellegaard et al., 2010). Minipigs, therefore, may have had a higher motivation for the food rewards which may explain their faster learning of the original discrimination and shorter latencies to respond to the cues. However, we found that learning was not related to judgement bias measures in Phase I suggesting that food motivation may not play a role in ambiguous cue interpretation.

However, while these factors may have influenced our results, these are the standard procedures for both breeds and therefore need to be taken into account when making comparisons. Göttingen minipigs are only bred at the research facility of Ellegaard and therefore are likely to experience long distance transport before they are used in any research facility. With respect to potential differences in diet, the same reasoning applies; by feeding both breeds according to their breeder recommendations it means our results are more applicable for the normal usages of each breed. Therefore, our definition of breed comparison does not just encompass genetics, but also the inherent differences between the two groups of pigs.

### **Learning and Memory**

Our finding that minipigs learned the initial discrimination of positive and negative cues faster than conventional pigs contrasts with findings by Gieling et al. (2013) using the same pigs. These differences may be age-related as the pigs were older in the present study, or they may be related to the nature of the two tasks – spatial vs. auditory discrimination – as discussed in Murphy et al. (2013). It may also be that, as previously discussed, potential differences in food motivation also influenced learning, although the same feeding schedule was maintained in the present study as that used by Gieling et al. (2013). In subsequent retraining we found no differences between the breeds. Furthermore both breeds needed fewer training sessions to reach criterion demonstrating that, once learned, they were able to retain this discrimination over time. This suggests a well-developed reference memory in both breeds of pigs i.e. after learning they retained the “rules” of the discrimination task (Arts et al., 2009) even after a four-week break from training. There have been few studies of long-term memory in pigs. De Jong et al. (2000) found that enriched housed pigs exposed to a maze task at 11 weeks showed some retention of the task when retested at 20 weeks, but that this retention was impaired in barren housed pigs. The pigs in our study were housed on straw in large pens, with more space allowance per pig than in commercial piggeries. The pens were provided with toys, a condition may be considered as enrichment.

### **Judgement Bias**

We found no differences between the responses of either pig breed on any of the measures of judgement bias analysed for each test phase. Minipigs appeared to have a lower overall AUC than conventional pigs and it is possible that with a larger number of animals this difference would have been significant. Since the training and testing of animals is relatively time consuming, reduction of the within group variation, by for example, increasing the number of exposures to the ambiguous cues, may be a more useful approach. However, increasing the number of presentations of the ambiguous cues with the method as described also has inherent problems as discussed below. Furthermore, the lower AUCS for minipigs in Phase I might also be accounted for by some within test learning about the outcomes of the ambiguous trials as minipigs tended to have a lower mean AUC than conventional pigs in the last two test sessions of Phase I.

Exploration of the causes of the large within group variation may also be of interest. Personality in pigs has traditionally been measured through coping styles (Bolhuis et al., 2005; Hessing et al., 1994) but it may also be that optimistic/pessimistic personality types exist within pigs. We found a strong relationship between responses in Phase I and II for conventional pigs suggesting that there may be optimistic and pessimistic personality types. In minipigs, any such relationship may have been masked by learning about the outcomes of ambiguous cues slightly earlier than conventional pigs, since they appear to be faster learners and showed a tendency for lower AUCS than conventional pigs in the last two sessions within Phase I. The development of stereotypic behaviours, as has been demonstrated in starlings (Brilot et al., 2010) and capuchins (Pomerantz et al., 2012), might relate to optimistic and pessimistic personality types.

In terms of responses across testing phases, we found that both breeds of pigs reduced their choices for the positive goal-box after Phase I. The attempt to manipulate mood state through use of a restraint treatment in Phase II did not produce conclusive results as the reduction of optimistic responses in Phase II was more likely due to some level of learning about the outcomes of ambiguous trials. Learning is further suggested by the fact that both breeds took increasingly longer to make a choice in response to ambiguous cues in repeated tests and choices for the positive goal-box reduced even within Phase I. This same reduction in optimistic responses has been found in sheep tested repeatedly over a three week period (Doyle et al., 2010b), and starlings also appear to learn rapidly that ambiguous cues are unrewarded (Brilot et al., 2010). Conversely, pigs in the experiment by Douglas et al. (2012) did not show an effect of repeated testing on judgement bias in a Go/No-Go style task using unrelated auditory cues. However, pigs in their experiment were exposed to the ambiguous cue only once per test as opposed to four times per test in our experiment. Reducing the number of exposures to the ambiguous cues may reduce the effect of learning, but might also serve to increase the variation.

The cause of the reduction of optimistic responses differed between phases. We found that pigs switched from choosing the positive goal-box in response in phase I to choosing the negative goal-box in phase II, while in phase III omissions in response to ambiguous cues increased. However, the overall number of omissions was still relatively low in comparison to pessimistic choices suggesting that pigs which had probably learned quickly that ambiguous cues were unrewarded, continued to choose, most likely to avoid the time-out, but continued to choose for the negative goal-box.

The effect of learning in repeated tests may possibly be reduced by reinforcing the ambiguous cues as expected; unreinforced cues may stand out more in a task where, by reaching criterion, a pig is receiving a reward in almost every trial – if an expectation is violated, this could result in faster learning as the unexpected event is more strongly encoded in memory (Jamieson et al., 2012). Papini et al. (1997) suggest that unexpected omissions of signalled reward result in similar physiological activation to that which occurs in stress response and induces frustration in an animal. In consequences of experiencing such an unexpected event, an animal will update its knowledge about the environment (Papini, 2003) thus making it more likely that an unexpected event is

remembered. A second option could be to introduce partial reinforcement of control cues once animals have reached criterion, but before the judgement bias test itself, such as in Matheson et al. (Matheson et al., 2008), which would, however, increase the number of sessions needed before the animals could be tested. A third option would be to increase the ratio of control cues to ambiguous cues so that the animals receive only one or two ambiguous cues per session rather than three. However, for this either the number of testing sessions must be increased to achieve the same number of presentations of the ambiguous cues, or the number of individual ambiguous cues must be reduced.

Minipigs responded faster than conventional pigs to all cue-types in test phases I & II. This difference disappeared in test phase III. This again contrasts with results from Gieling et al. (2013) using the same animals. They observed that the conventional pigs initially found the first reward faster in a spatial holeboard task but in overall trial duration the breeds did not differ. This difference may have been due to the potential greater food motivation in minipigs as compared to conventional pigs as discussed previously, or due to the relative weight difference between the breeds. Conventional pigs were much larger than the minipigs in the present experiment and may have had more difficulty moving at speed on the plastic flooring of the testing arena. Both conventional pigs and minipigs responded faster to the positive cue than the negative cue during test sessions. As latency to reach the goal pot is a measure often used in cognitive bias studies to differentiate between optimistic and pessimistic responses (Mendl et al., 2009), this result confirms that both breeds had a preference for the large reward even during test sessions. This difference was more pronounced in conventional pigs which may suggest that the minipigs found the small reward more motivating than conventional pigs.

The perception of the relative difference between the small and large rewards may have an effect on responses to ambiguous cues. A pig which perceives the difference as being large may be more reluctant to choose for the small reward than an individual for which the difference is perceived as less. A study by Melotti et al. (pers. comm) looking at delay-discounting as a measure of impulsive choice in pigs found two distinct response types in pigs – those who switched easily from choosing a large reward option (associated with increasing delays) to choosing a small immediate reward option, and those who appeared to prefer to omit from responding as delays increased rather than switch to the small reward option. This suggests that some pigs may find it more difficult to accept a smaller reward. In a judgement bias task, individuals who show this reluctance could be scored as optimistic. If this is the case we would expect some relationship between learning of the discrimination, which requires a pig to willingly choose for the small reward, and the number of optimistic choices they make. However, we found no relationship between the number of sessions needed to learn the initial discrimination and the AUC from test Phase I.

## CONCLUSIONS

We found some breed differences in learning between the conventional farm pigs and Göttingen minipigs which may have been due to some differences in the standard husbandry of each breed. However, overall both breeds behaved similarly. Both appeared to retain the discrimination of positive and negative cues over time, and both made similar choices in response to ambiguous cues in testing. While minipigs may have been faster to respond to the cues, both breeds also responded similarly to the cues in terms of latency. This suggests that knowledge about the behaviour of farm breeds of pigs can be applied to minipigs and vice versa despite differences in early experiences and standard husbandry. Similarly to findings by Gieling et al. (2013), except for initial differences in learning, both breeds showed a comparable level of performance on this task.

In terms of the task itself, a previous active-choice task by our group (Murphy et al., 2013) and one by Enkel et al. (2010) both failed to get all animals to reach the criterion level of performance. However, in the present study we found that both breeds easily learned the initial discrimination, and maintained this learning over time. In the first test phase pigs showed responses as expected with increasing optimistic choices as the cues neared the frequency of the positive cue. Unlike Douglas et al. (2012) we did not find it overly time-consuming to train the pigs to distinguish two tone-cues.

However, this may be explained by the length of our cues - 30 s as opposed to notes on a glockenspiel used by Douglas et al. Previous attempts to use shorter cues showed these to be more difficult to use for training (Murphy et al., 2013). Our auditory cue allowed us to choose a number of ambiguous cues that were on the same scale as the trained positive and negative cues similar to previous judgement bias tasks where variations in spatial location (Burman et al., 2008) or shades of colour (Bateson and Matheson, 2007) are used as cues. Furthermore, an active-choice task avoids the need for behavioural inhibition, which may be influenced by emotion (Cyders and Smith, 2007). So, the task as described has a number of advantages when it comes to measuring judgement bias in animals. However, the issue of repeated testing limits the use of any judgement bias task in longitudinal studies.



# CHAPTER 05

## DECISION-MAKING UNDER RISK AND AMBIGUITY IN LOW- BIRTH-WEIGHT PIGS

*Animal Cognition, In Press*

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## **ABSTRACT**

Low-Birth-Weight (LBW) in humans is a risk factor for later cognitive, behavioural and emotional problems. In pigs, LBW is associated with higher mortality, but little is known about consequences for surviving piglets. Alteration in hypothalamic-pituitary-adrenal axis function in LBW pigs suggests altered emotionality but no behavioural indicators have been studied. Decision-making under uncertain conditions, e.g. risk or ambiguity, is susceptible to emotional influences, and may provide a means of assessing long term effects of LBW in piglets. We tested LBW ( $N = 8$ ) and normal-birth-weight (NBW;  $N = 8$ ) male pigs in two decision-making tasks. For decision-making under risk, we developed a simple two-choice probabilistic task, the Pig Gambling Task (PGT), where an 'advantageous' option offered small but frequent rewards and a 'disadvantageous' option offered large but infrequent rewards. The advantageous option offered greater overall gain. For decision-making under ambiguity, we used a Judgement Bias Task (JBT) where pigs were trained to make an active response to 'positive' and 'negative' tone-cues (signalling large and small rewards, respectively). Responses to ambiguous tone-cues were rated as more or less optimistic. LBW pigs chose more often for the advantageous option in later blocks of the PGT, and were scored as less optimistic in the JBT than NBW pigs. Our findings demonstrate that LBW pigs have developed different behavioural strategies with respect to decision-making. We propose that this is guided by changes in emotionality in LBW piglets and we provide behavioural evidence of increased negative affect in LBW piglets.

### **Keywords**

Pig; Decision-Making; Judgement Bias; Risk; Birth Weight; Animal Welfare

## INTRODUCTION

In humans, children who are considered small for gestational age at birth are at greater risk of cognitive impairments in later life, such as impairments in learning and attention (O’Keeffe et al. 2003), academic performance (Strauss 2000), and executive function (Anderson and Doyle 2004). Low-birth-weight (LBW) is also a risk factor for later behavioural and emotional problems (Hayes and Sharif 2009) such as trait anxiety (Lahti et al. 2010), attention deficit hyperactivity disorder (Mick et al. 2002) and depression (Raikkonen et al. 2008).

In pigs, *Sus scrofa*, as a consequence of the selection pressure for larger litter sizes, there has been an increase in the birth of LBW piglets (Quiniou et al. 2002). LBW in pigs has mostly been studied with regard to production outcomes; LBW pigs have higher mortality rates (Milligan et al. 2002, Quiniou et al. 2002) but little is known about the behavioural, emotional and cognitive development of LBW piglets. Gieling et al. (2011) have demonstrated that LBW piglets showed delayed reversal learning in a spatial holeboard task, a finding that was not, however, confirmed in a subsequent study (Gieling 2013). LBW has been shown to affect how the hypothalamic-pituitary-adrenal (HPA) axis functions in pigs, possibly leading to long-term augmented stress reactivity (Rutherford et al. 2013). Repeated negative experiences can induce more negative long-term mood states (Mendl et al. 2010a), which can influence decision-making when outcomes are uncertain (Mendl et al. 2009). However, Rutherford et al. (2013) suggest that behavioural measures of altered emotionality are necessary before drawing conclusions about the welfare consequences of alterations in HPA axis activity in LBW in pigs.

Many theoretical models of personality differences in animals propose that inter-individual variation arises from differences in state (state differences may refer to morphology, physiology, neurobiology or environment) which lead to adaptive behavioural responses (Dingemanse and Wolf 2010). LBW pigs differ in morphology, physical size, and physiology (see above), compared to their siblings which, according to these models, ought to lead to consistent differences in behaviour. For example, McElreath and Strimling (2006) suggest that when environmental cues about the risk of predation are ‘noisy’ or unclear, smaller individuals, who may be more at risk, ought to be more cautious about foraging while larger individuals, who may be less at risk, may be less cautious. While LBW piglets on farm may not be at risk of predation, they are in constant competition with their larger siblings (Milligan et al. 2002), and thus may develop different behavioural strategies in order to adapt.

Animals constantly have to make decisions throughout their daily life, and to make adaptive decisions, the costs and benefits of each option should be evaluated (Sugrue et al. 2005). Both internal factors (e.g. current need, past experience, emotion) and external factors (e.g. environmental conditions, time constraints) can influence this evaluation process. Normally, decisions are made under uncertain conditions (Kacelnik and Bateson 1997), which can be divided into decisions involving risk (where the probability of each outcome is known) and decisions involving ambiguity (where the outcome is unknown) (Bechara et al. 2005, Krain et al. 2006). There is evidence supporting the notion that

the two processes, decision-making under ambiguity (judgement) and decision-making under risk, involve different neural substrates (Krain et al. 2006).

Decision-making is not just about making rational choices. Recent research has distinguished between 'cold' decision-making involving cognitive reasoning and 'hot' decision-making which is influenced by affective processes (Peters et al. 2006). Decisions made under uncertain conditions may be more susceptible to emotional influences. In human research, anxiety is associated with mood congruent biases in decision-making and judgement (Hartley and Phelps 2012, Blanchette and Richards 2010); higher anxiety increases risk aversion and pessimistic judgements of ambiguous stimuli. However, positive mood may also increase risk aversion whereas the valence of an emotional state (positive/negative) has a more congruent effect on judgement of ambiguity (Mendl et al. 2009, Blanchette and Richards 2010).

In animal studies, the effect of risk on decision-making has been more widely studied than the effect of ambiguity. In typical decision-making tasks, animals choose between two options with the same overall gain, a consistent 'safe' option or a 'risky' option, where risk is manipulated by varying the amount of reward, the time delay until reward, or the probability of a reward occurring. Risk-prone individuals prefer the risky option while risk-averse individuals prefer the safe option (Mazur 1988), although the means by which the risk is manipulated can influence these preferences (Kacelnik and Bateson 1996). More recently, rodent models of the Iowa Gambling Task (Bechara et al. 1994) have looked at decision-making where the overall gain of the 'safe' option (predicting small but frequent rewards) is greater than that of the risky option (predicting large but infrequent rewards) (van den Bos et al. 2006, Homberg et al. 2008, Koot et al. 2010). Interestingly, when the safe option offers greater overall gain, anxiety has been found to lead to more risk-prone behaviour, in both human and rodent models of the IGT (Miu et al. 2008, De Visser et al. 2010, De Visser et al. 2011), while positive mood results in earlier choices for the safe option (de Vries et al. 2008). Some studies report that low birth weight in human infants is associated with lower risk taking behaviours in later life (Hack et al. 2002, Hack et al. 2004, Schmidt et al. 2008), however, in these studies it is difficult to separate out effects of prematurity from birth-weight.

Due to the more congruent findings on the effect of emotion on decision-making involving ambiguity, judgement bias has been proposed as a means to study emotional valence in animals (Paul et al. 2005, Mendl et al. 2009, Harding et al. 2004). Typical studies involve training subjects to discriminate a 'positive' cue (predicting a positive or favourable outcome) from a 'negative' cue (predicting a negative or less favourable outcome). Next, unfamiliar 'ambiguous' cues are presented and responses to these cues are rated as 'optimistic' if they resemble responses to the positive cue, i.e. indicative of expectation of favourable outcome, or 'pessimistic' if they resemble responses to the negative cue, i.e. indicative of expectation of less favourable outcome. Within this framework, optimistic and pessimistic responses are used as a proxy indicator of positive and negative emotional states, respectively. A number of studies in a variety of species have demonstrated that presumed positive situations lead to more optimistic judgements of ambiguous stimuli, e.g. enrichment (Brydges et al. 2011, Douglas et al. 2012), while

others have shown that presumed negative situations lead to more pessimistic judgements, e.g. pain (Neave et al. 2013), separation anxiety (Mendl et al. 2010b).

To study whether LBW piglets have developed different behavioural strategies, and whether these strategies are indicative of altered emotionality, the current experiment aimed to compare decision-making under risk and ambiguity in LBW piglets to NBW sibling controls in two tasks. To look at decision-making under risk, we developed a simple two-choice probabilistic decision-making task, the Pig Gambling Task (PGT), where the 'safe' option offered slightly greater overall gain. To look at decision-making under ambiguity, we tested the same pigs in an active-choice Judgement Bias Task (JBT) similar to that we have previously used with adult pigs (Murphy et al. 2013b), where responses to ambiguous tone-cues were rated as more or less optimistic. We expect that LBW pigs should show more risk aversion than NBW pigs, and that, if LBW pigs demonstrate altered emotionality compared with NBW pigs, they should show a more pessimistic bias towards ambiguity.

## **METHODS**

### **Ethical Note**

The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

### **Subjects and Housing**

We used 16 male piglets (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) bred at the farm of the Faculty of Veterinary Medicine of Utrecht University. We selected 8 male low birth weight (LBW) piglets and 8 male normal birth weight (NBW) piglets according to the criteria of Gieling et al. (2011). In short, all pigs from 9 sows were weighed within 12 hours of birth. Pigs weighing at least 1SD less than the litter mean were considered LBW. NBW pigs were those closest to the litter mean when all pigs classified as LBW were excluded. Only 7 of these litters contained suitable LBW piglets so we selected one of each LBW and NBW piglets from 6 litters and two of each LBW and NBW from 1 litter.

Pigs remained in their litter groups, and were weaned at 4 weeks. Some non-experimental siblings were cross-fostered shortly after birth to make litter numbers more even, standard procedure at the breeding unit. At 5 weeks of age, subjects were moved into the research facility and housed in two straw-bedded pens (5 x 3 m). Each pen contained 4 LBW piglets and 4 NBW piglets. Sibling piglets were housed in separate pens (except in the case of the four piglets from 1 sow in which case 1 LBW and 1 NBW piglet were housed in each pen). Each pen contained a nest area and was provided with toys for enrichment. Water was available *ad libitum*, and animals were not food restricted. Instead, they received 25% of their normal allowance in the morning before testing and the remaining 75% in the afternoon, after all pigs had been tested. Pigs were weighed regularly to monitor weight gain.

## **Apparatus**

The same apparatus was used for both the Pig Gambling Task (PGT) and Judgement Bias Task (JBT) (Fig. 1a). A start box (1.2 m<sup>2</sup>) was connected via an antechamber (1.2 m<sup>2</sup>) to the test arena (3.6 m x 2.4 m). In the test arena were two goal-boxes, each of which contained a food bowl covered by a large, hard-plastic ball. The ball could be raised off the bowl but not knocked off. Guillotine doors operated remotely by the experimenter controlled access to the test arena and goal-boxes. In both the PGT and the JBT, the swing-doors between the antechamber and test arena were kept fully open. In the PGT, the goal-boxes were used purely as response points, while in the JBT, the goal-boxes were both response points and reward points. Rewards used in both experiments were chocolate M&M's<sup>®</sup> (Mars Nederland b.v., Veghel, The Netherlands).

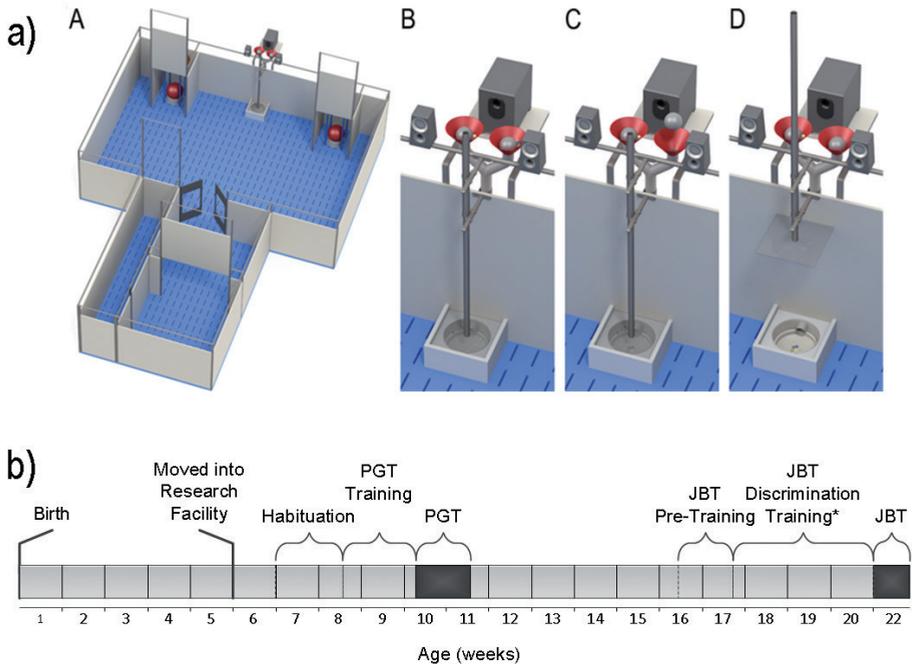
In the PGT, a food delivery system allowing for delivery of large or small rewards, which could be rendered accessible or inaccessible, was located on the back of the test arena, equidistant from each goal-box (see Fig. 1a). Rewards were placed in two funnels connected to a Y-shaped delivery pipe attached at the back of the apparatus which fed into a food bowl in the test arena. The experimenter controlled the release of rewards from either funnel into the food bowl. The food bowl was covered by a transparent perforated Perspex lid which could only be raised or lowered by the experimenter. With this system, a number of cues indicated to the pig that rewards were present in the food bowl – the sound of the M&M's dropping down the reward delivery tube into the central food bowl provided auditory cues, while the lid allowed both olfactory and visual cues.

In the JBT, the central food bowl was not used and was permanently covered with the lid. Instead, rewards were placed directly in each goal-box. Tone-cues, generated using the open source software Audacity (<http://audacity.sourceforge.net/>), were used to signal which goal-box was rewarded. The training tone-cues used were a 200 Hz and a 1000 Hz pure tone (Waveform: Sine; Amplitude: 1), while the ambiguous tone-cues were 299.07 Hz, 447.21 Hz, and 668.74 Hz pure tone (Waveform: Sine; Amplitude: 1). The tone cues were played using an MP3 player (Archos 18 Vision, 4 GB, Archos GmbH, Grevenbroich, Germany) via speakers (Logitech z-313, Logitech Europe S.A., Morges, Switzerland) attached at the back of the testing arena (Fig. 1a).

For an overview of the experimental timeline, see Fig. 1b.

## **Habituation**

After arrival in the research facility, pigs were left to settle undisturbed for one week. Over the following 3 days, pigs were habituated to the presence of the experimenter and to the rewards. Next, over 5 days, pigs were habituated to the testing apparatus, initially in large groups of eight piglets (4 sessions), then in smaller groups of four animals (4 sessions), of two animals (8 sessions), and finally individually (4 sessions). Each session lasted approximately 3 minutes during which the pigs were allowed to explore the apparatus. Rewards were placed in front of each goal-box and periodically dropped into the central food bowl so that pigs learned to associate the sound of food delivery with the availability of rewards in the central food bowl. Pigs were deemed ready for training after this procedure as all rewards were consumed during habituation sessions.



**Fig. 1 a)** Design of experimental apparatus used for both the PGT and the JBT, highlighting the food delivery system used in the PGT to render rewards accessible or inaccessible, **b)** Experimental timeline. \* While the number of sessions necessary to learn the tone discrimination in the JBT varied between individuals, here we present mean number of sessions required by all pigs.

### Pig Gambling Task Training

Pigs were shaped to perform an active response in each goal-box in order to receive rewards in the central food bowl. They were trained to move the ball in either goal-box to obtain rewards in the central food bowl. To keep the response simple, any lift/push of the ball with enough force to cause the ball to move was considered a ‘choice’ and resulted in the delivery of reward. In early sessions some rewards were placed underneath the ball in order to reinforce interactions with the ball. The shaping process was performed over 12 sessions (3 days). Pigs were first trained to approach the goal-boxes in order to receive reward in the central food bowl. As sessions progressed pigs were increasingly only rewarded after touching, then pushing/lifting the balls in either goal-box. Each session consisted of 10 rewarded actions. All pigs underwent the same training procedure after which they were consistently nudging the ball in each goal-box order to obtain rewards in the central food bowl.

Over two further sessions of 10 trials each (1 day), pigs were trained to return to the start box after each choice, once rewards had been consumed, before commencing the next trial. At this point, the lid was placed on the central food bowl and, while rewards were delivered immediately after a choice was made, the lid of the food bowl was only raised by the experimenter when the pig was within 20 cm and facing towards the bowl.

Next pigs were taught that both goal-boxes would yield a reward in two sessions of 20 'forced' trials (2 days), where only one goal-box was available per trial. Left and right goal-boxes were available in a pseudorandom order with a maximum of two consecutive presentations of the same goal-box. Two rewards were delivered in each trial. Since in a pilot study we encountered the problem that pigs did not appear to sample from both options, we gave all pigs a third session of 20 trials where choices in advantageous and disadvantageous goal-boxes were rewarded with two or four rewards, respectively. Advantageous and disadvantageous goal-boxes were counterbalanced across LBW and NBW pigs.

### **Pig Gambling Task**

Over six blocks of 20 trials each (one block per day, total: 120 trials), pigs could choose freely between left and right goal-boxes. While a choice in either goal-box resulted in the delivery of reward into the central food bowl, the quantity and accessibility of reward were governed by a predetermined schedule. A choice for the advantageous goal-box yielded small quantities of reward (two chocolate M&M's), but had a high probability that the rewards would be made accessible (eight 'wins' in every ten trials) i.e. a potential total of 16 rewards per 10 trials. A choice for the disadvantageous goal-box yielded higher quantities of reward (four chocolate M&M's), but there was a low probability of the rewards being made accessible (three 'wins' in every ten trials) i.e. a potential total of 12 rewards per 10 trials. These reward and probability contingencies were previously used in a rodent model of decision-making (Koot et al. 2010).

In win trials, rewards were delivered into the food bowl immediately after a choice was made. When the pig was in position in front of the central food bowl, the lid was raised giving access to the rewards within. In loss trials, rewards were also delivered after a choice was made, but the lid remained closed, rendering the reward inaccessible. In both win and loss trials, pigs were allowed to return to the start box for the next trial 25 s after making a choice. The order of wins and losses differed daily but the probability of wins and losses remained the same within each series of 10 trials. The number of choices for the advantageous goal-box was recorded per pig for each of the six blocks of 20 trials.

### **Judgement Bias Task: Pre-Training**

After a 5 week break where pigs were left undisturbed, training for the JBT began. As the pigs were already familiar with the apparatus, we were able to start with training for the judgement bias task without any habituation sessions. Pigs were trained, similar to the method used by Murphy et al. (2013b), to associate one training tone with the availability of a large reward (4 chocolate M&M's) in one goal-box while the second training tone predicted the availability of only a small reward (1 chocolate M&M's) in the other goal-box. In this way, one training tone and its associated goal-box were labelled 'positive', while the second training tone and associated goal-box were labelled 'negative'. Only one goal-box was rewarded per trial and the meaning (positive/negative) of training tones (200 Hz/1000 Hz) and goal-boxes (right/left) was counterbalanced across animals. Furthermore, advantageous and disadvantageous goal-boxes from the

PGT were counterbalanced as positive and negative goal-boxes for the JBT for both LBW and NBW pigs. Each pig received one training session per day and the order of positive and negative trials differed daily in a pseudorandom order with no more than two consecutive presentations of the same tone-cue.

We commenced training with four sessions of 'forced' trials (5 positive; 5 negative) where only the correct goal-box was available, as predicted by the tone cue, and the associated quantity of reward was available in the goal-box. Pigs were brought into the start box and after a couple of seconds, a tone cue was played. After 1 s of tone cue, the guillotine door between start box and antechamber was raised and the pigs were freely able to enter the antechamber and test arena for 60 s. The tone-cue was stopped once a correct response was performed i.e. a pig had gained access to the rewards present in the goal-box. Following this, pigs received three sessions of 'open-choice' trials. Each session consisted of 13 trials; three forced trials (1 positive; 2 negative), as before, and 10 'open-choice' trials (5 positive; 5 negative) in which both goal-boxes were available but only the correct one, as predicted by the tone-cue, was rewarded. In open-choice trials, pigs were allowed to choose from both goal-boxes until they found the reward so that they could learn that there was always a reward available.

### **Judgement Bias Task: Discrimination Training**

In the final stage of training, pigs were trained until they demonstrated that they had learned to discriminate the positive and negative training tone-cues. Each session consisted of 13 trials; three forced trials (1 positive; 2 negative), as before, and 10 free trials (5 positive; 5 negative), where only a correct choice (a choice for the goal-box predicted by the tone-cue within 30 s) was rewarded. If a correct choice was made both goal-boxes remained open, and once the reward was consumed the pig was returned to the start box. Incorrect choices or response omissions (failure to respond within 30 s) resulted in both goal-boxes being closed and the pig remained in the test arena for a 90 s time-out penalty before commencing the next trial. To qualify for testing, pigs had to respond correctly in 80% of negative and 80% of positive free trials in three consecutive sessions.

### **Judgement Bias Task**

Once a pig had reached the learning criterion, they were tested in the Judgement Bias Task over four sessions, one session per day. Test sessions consisted of 16 trials; three forced and ten free trials, as before, along with three ambiguous trials (Trials 6, 11, and 16). In an ambiguous trial, one of the three ambiguous tone-cues (termed: 'AmbigNeg', 'AmbigMid', 'AmbigPos' depending upon their relationship to the negative and positive training tone-cues) was played instead of a training tone-cue for 30 s, and both goal-box contained their associated quantity of rewards (i.e. one M&M in the negative goal-box and four M&M's in the positive goal-box). We have previously demonstrated that pigs learn very quickly when ambiguous cues are unrewarded and have suggested that this learning can be slowed down by providing the rewards expected by the pig (for discussion see Murphy et al. 2013b). Each ambiguous cue was presented four times (once per test

session). The AmbigMid cue was always presented first each session, while the other two ambiguous cues were presented in a counterbalanced order. The order of trials in test sessions were balanced such that pigs had equal numbers of presentation of positive and negative tone-cues before presentations of each ambiguous tone-cue.

### **Saliva Sampling**

For each pig, in the mornings before test sessions one and two of the JBT, salivary cortisol samples were taken. Pigs were allowed to chew on cotton swabs (Heinz Herenz, Hamburg, Germany, Cotton Swabs 150x4mm WA 2PL) for 60 s until the swabs were wet through. Two samples were taken per pig and placed in centrifuge tubes (Salivette, Sarstedt, Germany) which were then labelled and refrigerated before transportation to the laboratory. Tubes were then centrifuged at 3000g for 5 minutes and the saliva was pipetted into Eppendorf tubes and stored at -20°C until cortisol could be measured. Cortisol concentrations were measured using a Coat-a-Count radioimmunoassay, according to the manufacturer's procedure (Coat-a-Count cortisol TKCO, Diagnostic products cooperation, Apeldoorn, the Netherlands).

### **Data Recording & Analysis**

Analyses were carried out using R version 3.0.2, using the package lme4 (Bates et al. 2012). From this library the functions lmer and glmer were used for the model fitting, the function boot for the bootstrap intervals, and the function MuMIn for information-theoretic model selection (AIC). Correlational analyses were carried out using SAS 9.4 (SAS Institute, Cary, NC).

### **Birth Weight**

To monitor weight development we compared weights at birth, following performance of the PGT, and following performance of the JBT between LBW and NBW piglets. A linear model for the weight with random litter and pigs-within-litter effects was used. Weight-Group (NBW, LBW), Time-Point (Birth, Post-PGT, Post-JBT), and the interaction between Weight-Group and Time-Point were included as fixed effects in the model.

### **Pig Gambling Task**

The number of choices for the advantageous option was calculated per pig for each block of 20 trials. 'Advantageous Choice' was analysed using a logistic regression model with random litter effects and random pig-within-litter effects. Birth-Weight Category (LBW/NBW), Block (1-6), and the interaction between Birth-Weight category and Block were included as fixed effects in the model. To see which of these fixed effects were important, different models with different fixed effects were fitted and compared using Akaike's Information Criterion (AIC), based on information theory whereby the model with the lowest AIC value represents the best approximation, i.e. the model in which the least information is lost (Symonds and Moussalli 2011, Burnham and Anderson 2002). For all fixed effects that were important according to the AIC, confidence intervals were calculated using the parametric bootstrap with 1000 bootstrap samples.

To get an overall measure of decision-making under risk ('Adv. Choice Preference'), orthogonal trend components of the changes over blocks were calculated per animal.

### Judgement Bias Task

*Judgement Bias:* Analyses were carried out using R version 3.0.2. The percentage of choices for the positive goal-box ('Optimistic Choice') was calculated for each cue-type (Negative, AmbigNeg, AmbigMid, AmbigPos, Positive) in test sessions. Optimistic Choice was analysed using a logistic regression model with random litter effects and random pig-within-litter effects. Birth-Weight Category (LBW/NBW), Cue-Type (Negative, AmbigNeg, AmbigMid, AmbigPos, Positive), and the interaction between Birth-Weight Category and Cue-Type were included as fixed effects in the model. To see which fixed effects were important, different models with different fixed effects were fitted and compared with Akaike's Information Criterion (AIC). For all fixed effects that were important according to the AIC, confidence intervals were calculated using the parametric bootstrap with 1000 bootstrap samples.

As an overall measure of judgement bias, the mean Area Under the Curve ('Mean AUC') was also calculated per pig as

$$\text{Mean} \left[ \left( \frac{\text{Neg} + \text{AmbigNeg}}{2} + \frac{\text{AmbigNeg} + \text{AmbigMid}}{2} + \frac{\text{AmbigMid} + \text{AmbigPos}}{2} + \frac{\text{AmbigPos} + \text{Pos}}{2} \right) \right]$$

*Latency:* In test sessions, the mean latency (s) to respond to each cue-type in free trials was calculated per pig. A linear mixed effects model was used on the log10 transformed latency values with random litter effects and random pig-within-litter effects. Birth-Weight Category (LBW/NBW), Cue-Type (Negative, AmbigNeg, AmbigMid, AmbigPos, Positive), and the interaction between Birth-Weight Category and Cue-Type were included as fixed effects in the model. To see which fixed effects were important, different models with different fixed effects were fitted and compared with Akaike's Information Criterion (AIC). For all fixed effects that were important according to the AIC, confidence intervals were calculated using the parametric bootstrap with 1000 bootstrap samples.

*Cortisol:* Cortisol measurements from the mornings before test session one and two were combined to get a 'Mean Cortisol' value for each pig. To check for differences between LBW and NBW pigs we used a linear model on the Log Mean Cortisol, with random litter effects and fixed birth-weight effect. Mean Cortisol was added to the Optimistic Choice model to see whether cortisol levels were an important predictor of Optimistic Choice.

To test for any relationship between cortisol and overall performance in the JBT, Mean Cortisol was compared with Mean AUC using Spearman's correlations conducted separately for LBW and NBW pigs.

## **Decision-making Under Risk & Under Ambiguity**

To check if performance in the JBT predicted performance in the PGT, Mean AUC from the JBT was added to the PGT model. Furthermore, to test for any relationship between overall performance on the PGT and the JBT, Adv. Choice Preference (PGT) was compared to Mean AUC (JBT) using separate Spearman's correlations for LBW and NBW pigs.

## **RESULTS**

All descriptive statistics are presented as mean + SEM.

### **Birth Weight**

LBW piglets weighed  $1.17 \pm 0.08$  kg at birth, while NBW piglets weighed  $1.64 \pm 0.08$  kg. At the end of the PGT LBW pigs weighed  $34.00 \pm 2.35$  kg, while NBW pigs were  $40.88 \pm 2.07$  kg. After completion of the JBT, LBW pigs weighed  $109.63 \pm 5.70$  kg, while NBW pigs were  $122.63 \pm 2.96$  kg (see Fig. 2). The full model with both main effects and the interaction had an AIC of 344.76. The linear model with only the main effects, i.e. without the interaction, had a higher AIC of 348.10, indicating that the interaction is important as the model including the interaction had the lowest AIC. Although the 95% bootstrap intervals would suggest that that the groups did not differ in birth weight (Lower: -6.88, Upper: 5.77), it is likely that the difference we specifically selected subjects for is not picked up by this model due the small differences between the groups relative to the differences at later time points. LBW pigs remained lighter than NBW pigs when weighed after performance of both the PGT (Lower: -13.07, Upper: -0.40) and the JBT (Lower: -19.65, Upper: -6.89).

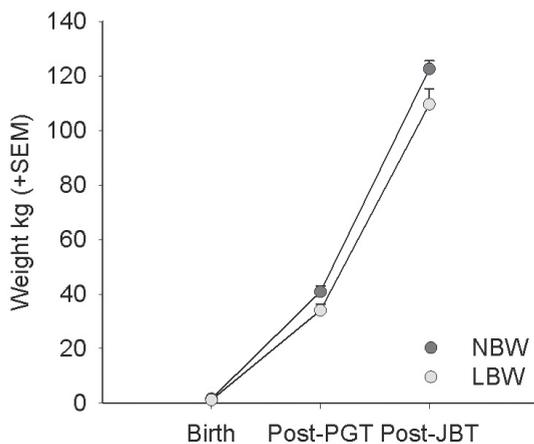
### **Pig Gambling Task**

All pigs made the required 20 choices per block of trials. Fig. 3 shows the percentages of Advantageous Choice as a function of birth weight and trial blocks. The full model, the model with both main effects and the interaction, had an AIC of 714.70. The logistic regression model with only the main effects, i.e. without the interaction, had a higher AIC (721.70), indicating that the interaction is important as the model including the interaction had the lowest AIC. Looking at the 95% parametric bootstrap intervals (Table 1), shows that LBW pigs made more Advantageous Choices than NBW pigs in Blocks 3, 4 and 6.

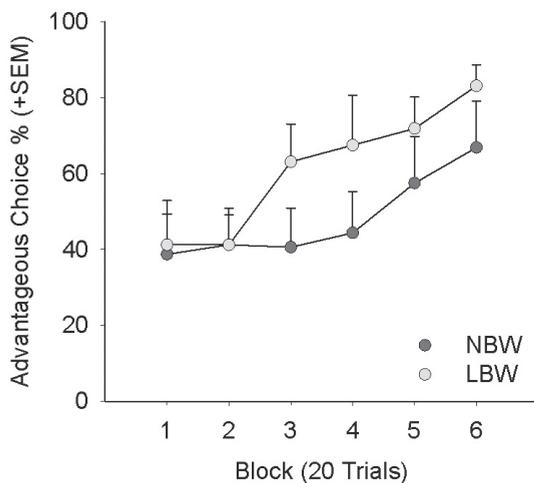
The variation in the proportion of Adv. Choice Preference across blocks was nearly exclusively covered by the linear trend component, which explained approximately 97% of this variation and thus could be taken as an adequate description of the development of advantageous choices across blocks.

### **Judgement Bias**

*Learning:* Both LBW and NBW pigs learned the conditional discrimination task at a similar rate (LBW:  $16.00 \pm 1.43$  sessions; NBW  $16.00 \pm 0.60$  sessions).



**Fig. 2** Weight development of LBW and NBW pigs.



**Fig. 3** Percentage of Advantageous Choice per block of 20 trials for LBW and NBW pigs in the PGT.

Block	Lower (5%)	Upper (95%)
Block 1	-0.558	1.143
Block 2	-0.763	1.066
Block 3	0.330*	2.205*
Block 4	0.338*	2.196*
Block 5	-0.082	1.759
Block 6	0.134*	2.020*

**Table 1.** The 95% parametric bootstrap intervals for the difference in Advantageous Choice between LBW and NBW piglets.

\*Denotes Blocks where LBW pigs had a higher Advantageous Choice than NBW pigs.

*Judgement Bias:* All pigs responded in every trial in test sessions (i.e. no omissions occurred). The mean number of Optimistic Choices in response to each cue-type is presented in Fig. 4a. The full model, the model with both main effects and the interaction, had an AIC of 192.75. The model with only the main effects, i.e. without the interaction, had a lower AIC of 190.46, indicating that the interaction is not needed in the model. Both the model without Cue-Type and the model without Birth-Weight Category had higher AICs indicating that both factors are important in the model (AIC without Cue-Type: 788.92; AIC without Birth-Weight Category: 194.84). Thus LBW pigs made fewer Optimistic Choices than NBW pigs, and both LBW and NBW pigs made increasing numbers of Optimistic Choices as the cue-type neared the positive cue (Fig. 4a). The difference in Optimistic Choice between LBW and NBW pigs occurred in response to the ambiguous tone-cues, while responses to the training tone-cues remained similar for LBW and NBW pigs (Fig. 4a).

*Latency:* The mean latency to respond (s) to each cue-type is presented in Fig. 4b. The full model, the model with both main effects and the interaction, had an AIC of -168.89. When the interaction was removed from the model, the AIC was higher (-95.53) indicating that the interaction is important in the model. Thus the difference in latency to respond to each cue-type between LBW and NBW pigs depends on the cue-type. From Fig. 4b it can be seen that both LBW and NBW pigs respond faster to cues as they near the positive cue.

*Cortisol:* There were no differences between LBW and NBW pigs in Mean Cortisol. The AIC was approximately the same when Birth-Weight was included in the model (9.89) to when it was removed (9.40). Furthermore, Mean Cortisol was not found to predict Optimistic Choice as again, the AIC was approximately the same whether it was included (196.35) or removed from the model (194.75).

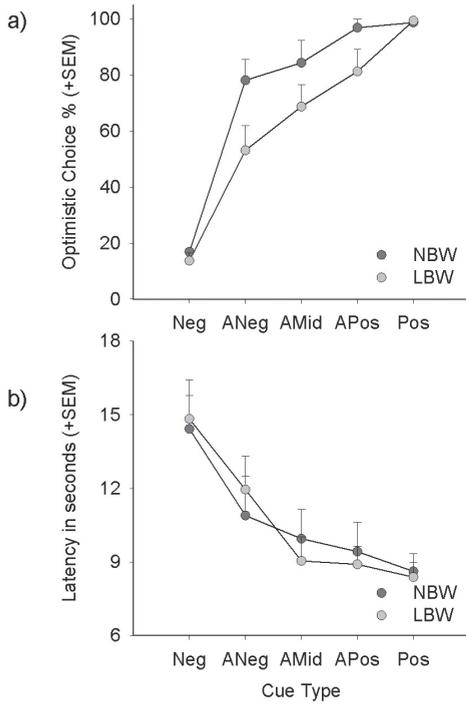
There was no relationship between Mean Cortisol and overall performance in the JBT (Mean AUC) for either LBW ( $r_s = -0.55$ ,  $P = 0.1570$ ) or NBW pigs ( $r_s = 0.33$ ,  $P = 0.4138$ ) (Fig. 5a).

### **Decision-making Under Risk & Under Ambiguity**

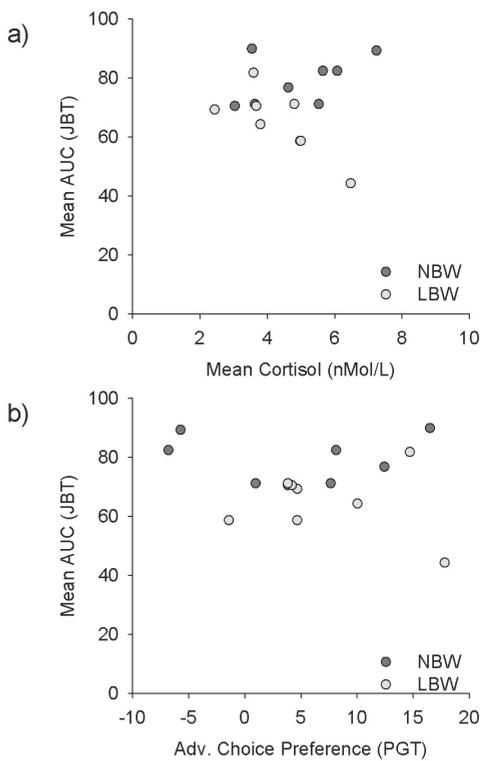
Mean AUC from the JBT was not found to predict Advantageous Choice in the PGT. The AIC was approximately the same whether Mean AUC was included (714.70) or removed from the model (715.78). Furthermore, there was no significant relationship between overall performance in the PGT (Adv. Choice Preference) and performance in the JBT (Mean AUC) for either LBW ( $r_s = -0.14$ ,  $P = 0.7435$ ) or NBW pigs ( $r_s = 0.16$ ,  $P = 0.7111$ ) (Fig. 5b).

## **DISCUSSION**

We found that low-birth-weight (LBW) piglets appear to have developed different strategies than their normal-birth-weight (NBW) siblings when making decisions under risk and ambiguity. In a Pig Gambling Task (PGT) used to look at decision-making under risk, pigs were allowed to freely choose between a low-risk, low-reward option but which overall yielded more rewards (advantageous), and a high-risk, high-reward option



**Fig. 4 a)** Mean Optimistic Choice (%), and **b)** Mean latency to respond per cue-type for LBW and NBW pigs in the JBT.



**Fig. 5 a)** Relationship between Mean Cortisol and Mean AUC in the JBT, and **b)** between Advantaged Choice Preference from the PGT and Mean AUC in the JBT. Correlation analyses revealed no relationships.

which overall provided fewer rewards (disadvantageous). While both groups appear to show an increase in choices for the advantageous option over blocks of trials (Fig. 3), we found that LBW pigs chose more often for the advantageous option in later blocks of trials than their NBW siblings.

Looking at decision-making under ambiguity using a Judgement Bias Task (JBT), we found that both LBW and NBW pigs learned the task at a similar rate, did not differ in salivary cortisol pre-testing, had similar latency responses to the different cues, and performed equally well in response to the training tone-cues. All pigs responded significantly faster to positive cues than negative cues, confirming that all animals had a preference for the larger reward as we have previously shown (Murphy et al. 2013a, 2013b). However, when presented with unfamiliar, ambiguous, cues, LBW pigs were more likely to choose the negative goal-box, i.e. demonstrated a more pessimistic bias than their NBW siblings. Interestingly, there was no relationship between individual pig's responses in the two tasks, suggesting that the two tasks are indeed measuring different facets of decision-making under uncertainty – risk and ambiguity.

Our results lend support to the theory that different personalities can arise from initial differences in state, in this case, physical size, leading to the development of different behavioural strategies (Dingemans and Wolf 2010). As predicted, our LBW pigs demonstrated a greater preference for the advantageous option compared with NBW pigs. This preference for the advantageous option in LBW pigs may reflect a better rational ('cold') decision-making or be a product of more affective ('hot') decision-making processes. LBW pigs may have been better at weighing the associated probabilities of each option in order to maximise their gain compared with NBW pigs or they may be more averse to risk as has been shown in studies of LBW in humans (Hack et al. 2002, Hack et al. 2004, Schmidt et al. 2008).

An important distinction between LBW in humans and LBW in pigs in the current study is the level of post natal care provided in human infants. We defined LBW in piglets according to the criterion used by Gieling et al. (2011, 2013), and the mean weight of LBW piglets in the present experiment also corresponds with the definition of LBW used by other authors (Gondret et al. 2006, Attig et al. 2008, Baxter et al. 2008). However, in farming practice, piglets weighing less than 1kg at birth are said to have a poor chance of survival (BPEX 2010), and more strict cut-off points for defining LBW have been used (Quiniou et al. 2002), which may reflect better the definitions used in human studies. Often the very LBW piglets do not survive without the level of postnatal care provided for human infants. Thus, the LBW piglets selected in the present study may represent only a subsection of 'viable' LBW pigs which have developed a successful behavioural strategy to compete against their NBW siblings.

Looking at decision-making under ambiguity in the JBT we found that, as predicted, the LBW piglets showed a more 'pessimistic' bias i.e. were more likely to choose the goal-box offering the lower reward in response to ambiguous tone-cues. While it is possible that differences in feeding motivation between LBW and NBW pigs contributed to this difference, some behaviours which may indicate different levels of motivation (rate of learning, speed of responding) did not differ between LBW and NBW pigs. In humans,

judgements about the likelihood of future events are influenced by mood (Blanchette and Richards 2010), and these mood-congruent biases in judgement have been proposed as a means of studying emotional valence in animals (Mendl et al. 2009). Within this framework, our findings suggest that LBW may also contribute to negative affect in pigs. Complementary to the evidence of alterations in HPA axis activity in LBW pigs (reviewed by Rutherford et al. 2013), the present study provides potential behavioural indicators of altered emotionality in LBW pigs.

Few studies of decision-making have previously been performed in pigs. In social decision-making settings, Held et al. (2000) showed that uninformed dominant (larger) pigs will choose to follow a pig which they know is informed in order to find food rewards more quickly, while subordinate (smaller) pigs may alter their foraging decisions based on whether they are foraging with a scrounging or a non-scrounging (larger) pig (Held et al. 2010). Thus, in social decision-making situations, smaller pigs may compete by altering their behaviour in the presence of a more dominant (larger) pig. Our findings suggest that LBW may alter decision-making in pigs in non-social situations too, perhaps through emotional biases in the decision-making processes. The fact that individual behaviour on each of our tests was not related, suggests that these alterations occur across different types of decision-making.

While we did not find differences in salivary cortisol, previous studies have found that cortisol differences occur in response to a challenge in LBW and NBW pigs (Poore and Fowden 2003). Since the mean weight of our LBW piglets falls well below that used by Poore and Fowden (2003), it may be that had we taken cortisol after performance of the test, we may have found a difference corresponding to the difference in behaviour of the LBW and NBW piglets.

We have demonstrated that LBW pigs develop different strategies when making decisions under uncertain conditions than NBW pigs. We do not identify the mechanisms by which these differences in behaviour come about, and our LBW piglets may not entirely reflect LBW in humans. In terms of welfare, however, understanding the cognitive and emotional functioning of these viable LBW pigs is of high interest for the very reason that they survive and have to compete with stronger siblings. The altered decision-making patterns may reflect an adaptive strategy used by LBW piglets in response to constant competition with larger pigs. We propose that this altered decision-making is guided by changes in emotionality in LBW piglets and we have provided behavioural evidence of increased negative affect in LBW piglets.



# CHAPTER 06

## JUDGEMENT BIAS IN LOW- AND NORMAL- BIRTH-WEIGHT PIGS, *SUS SCROFA*

*Manuscript in Preparation*

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## **ABSTRACT**

The numbers of Low-birth-weight (LBW) pigs is on the increase due to selective breeding for larger litter sizes. LBW in pigs is a strong predictor of post-natal mortality, but there is little information about the consequences for surviving LBW piglets. In humans, LBW is associated with a number of cognitive and emotional problems in later life. In pigs too, there is evidence of increased stress reactivity in LBW piglets, and we have previously provided evidence that LBW piglets show more pessimistic responses to ambiguity in a judgement bias task, indicative of increased negative mood. Allopurinol has been proposed as a candidate drug to reduce the negative outcomes associated with the principal cause of LBW, namely chronic placental insufficiency. In this experiment we aimed to investigate the effect of prenatal treatment with allopurinol on the behaviour of LBW pigs in a judgement bias task. LBW and NBW (normal birth weight) piglets from 11 sows, 5 of which were treated with allopurinol in the last trimester of gestation, were trained and tested in an active-choice judgement bias task using tone-cues. Optimistic responses to ambiguous tone cues were taken to reflect positive emotional states. A number of physiological measures of acute and chronic stress were also recorded. We did not find any effect of allopurinol treatment on judgement bias, nor did we confirm previous findings of a more pessimistic bias in LBW pigs. Also, measures of acute and chronic stress did not differ between the groups. Possible reasons for the lack of differences between weight groups and allopurinol treatment are discussed.

## INTRODUCTION

Intrauterine growth restriction or retardation (IUGR) has been defined as '*impaired growth and development of the mammalian embryo/foetus or its organs during pregnancy*' (Wu et al., 2006). Birth-weight is a common read-out measure used to detect IUGR in animal studies (Vuguin, 2007). In pigs, low-birth-weight (hereafter: LBW) is a strong predictor of postnatal mortality (Baxter et al., 2008; Milligan et al., 2002; Quiniou et al., 2002). Selective breeding has resulted in increasing litter sizes in pigs (Quiniou et al., 2002; Rutherford et al., 2013). With increasing litter sizes there is also an increased risk of LBW piglets (Beaulieu et al., 2010; Milligan et al., 2002). While surviving LBW piglets can show a relatively normal growth curve (Milligan et al., 2002) and some degree of compensatory growth (Douglas et al., 2013), they take longer to reach slaughter weight (Gondret et al., 2006; Quiniou et al., 2002). Aside from production outcomes, however, there is little information about the welfare consequences for these surviving LBW piglets.

In humans, LBW is associated with later cognitive and emotional problems, e.g. problems in learning and attention (O'Keeffe et al., 2003), and executive function (Anderson and Doyle, 2004), as well as increased risk of attention and hyperactivity problems, anxiety (Hayes and Sharif, 2009; Lahti et al., 2010) and depression (Raikkonen et al., 2008). Some evidence of delayed reversal learning has been found in LBW piglets in a spatial holeboard task (Gielsing et al., 2011), although this observation was not replicated in a recent study (Antonides et al., submitted article). LBW piglets have been found to show deficits in acquisition learning in a T-maze task, but not reversal learning, when tested at the very young age of 14 d (Radlowski et al., 2014).

An increased HPA reactivity (Poore and Fowden, 2003) and adrenalcortical function (Klemcke et al., 1993) in LBW piglets is suggestive of a heightened stress reactivity when measured shortly after birth and at later ages (up to 3 months) (Rutherford et al., 2013). Previously, we have demonstrated that LBW pigs show a more pessimistic interpretation of ambiguous stimuli in a cognitive bias paradigm (Chapter 05, Murphy et al., In Press), while Gielsing et al. (2014) found that LBW piglets vocalise more in an Open-Field test, providing the first behavioural evidence of altered emotionality in LBW piglets.

Various environmental and genetic factors may lead to abnormal foetal growth, but the two main proximate causes are inadequate maternal nutrition and insufficient uterine/placental capacity (Wu et al., 2006). Insufficient uterine/placental capacity, likely due to poor perfusion (Blomberg et al., 2010), leads to a reduction in the amount of nutrients and oxygen that reaches the foetus resulting in lower weight and slowed growth (Baschat, 2004). Placental insufficiency may be one of the main preventable causes of IUGR (Sankaran and Kyle, 2009). Chronic placental insufficiency and foetal hypoxia are associated with neurological alterations in humans (Maršál and Ley, 1992) and animals (Mallard et al., 1998), likely leading to the alterations in cognitive, behavioural and emotional functioning discussed above. Brain cell death and damage occurs during the period of hypoxia, as well as during the period of reoxygenation where the free-radicals built up during the period of hypoxia react with the returning oxygen (Palmer et al., 1990). Allopurinol has been proposed as a candidate drug to

reduce the damage caused during the period of reoxygenation due to its antioxidative properties. The enzyme Xanthine Oxidase (XO) is involved in various types of ischemic injuries, particularly during reoxygenation. Within a few hours after oral administration, allopurinol is almost completely metabolized to oxypurinol. Both allopurinol and its active metabolite oxypurinol are inhibitors of XO (Pacher et al., 2006).

While evidence suggests that early postnatal treatment with allopurinol is not always successful in alleviating the effects of acute hypoxia at birth in humans, particularly in severe cases (Benders et al., 2006; Chaudhari and McGuire, 2008), treatment with allopurinol both pre-and post- hypoxia has been shown to protect against reperfusion injury in rats (Palmer et al., 1990; Palmer et al., 1993), and pigs (Marro et al., 1999; Peeters-Scholte et al., 2003) suffering from acute hypoxia. Allopurinol crosses the human placenta (Boda et al., 1999), and has been found to protect against the effects of acute hypoxia when given to human mothers during labour (Torrance et al., 2009) and to pregnant ewes during late gestation (Kaandorp et al., 2014). Allopurinol, but not oxypurinol, readily crosses the placenta of pigs (Gieling et al., 2014; Van Dijk et al., 2008). In the present study, long term treatment with allopurinol – during the last trimester of pregnancy – was investigated as a means to reduce the impact of chronic hypoxia through placental insufficiency in pigs.

The present experiment was performed as part of a larger study in which the following behavioural tests and physiological and neurochemical determinations were performed: Open Field Test, Novel Object Test, Spatial Holeboard test (Gieling et al., 2014), Judgement Bias test (present study), measurements of acute and long-term stress (present study), and measurement of brain-derived neurotrophic factor (BDNF), an important regulator of neuronal plasticity and cognitive function, in the dorsal hippocampus (Prickaerts et al., 2014). Results from these studies reveal that the LBW offspring of sows which were administered allopurinol in the third trimester of pregnancy showed greater postnatal compensatory growth than LBW controls (Gieling et al., 2014). However no effects on reference memory or working memory performance in a complex spatial orientation task in a holeboard were found (Gieling et al., 2014). Maternal allopurinol treatment also had a protective effect on neuronal plasticity markers regardless of birth weight (Prickaerts et al., 2014).

Here we report the results of the Judgement Bias test and physiological measures of stress in the same animals as used by Gieling et al. (2014) and Prickaerts et al. (2014). To investigate whether prenatal treatment with Allopurinol had an effect on emotional outcomes in LBW piglets, we subjected animals to our pig version of a judgement bias task (Chapters 03 and 04, Murphy et al., 2013a; 2013b).

While numerous methods to study emotional behaviour in pigs have been used, many lack ethological validity and few are specifically designed to distinguish emotional responses by valence (i.e. positive/negative) (Chapter 02, Murphy et al., 2014). The cognitive bias paradigm, first put forward for the study of emotion in animals by Harding et al. (2004), proposes that emotion induced alterations to cognitive processes may provide more reliable measures of emotional valence than more traditional behavioural or physiological measures (Mendl et al., 2009). In the human literature, judgement, the

estimation of the likelihood of future events, has been found to vary most consistently with the valence of an emotional state (Blanchette and Richards, 2010). That is, people in a negative state are more likely to pessimistic judgements of ambiguous stimuli and vice versa. As mentioned above, we have previously demonstrated that LBW pigs do show a more pessimistic bias, as predicted, in a judgement bias task (Chapter 05, Murphy et al., In Press). The cognitive bias paradigm has also been used with pigs by Douglas et al. (2012), showing that, alterations in housing environment (enriched to barren; barren to enriched) resulted in temporary alterations in judgement in the direction predicted (increased pessimistic responses if moved from an enriched to a barren environment; increased optimistic responses if moved from a barren to an enriched environment). Although not all studies using the cognitive bias paradigm have found biases as predicted, a number of studies using a variety of species have (Mendl et al., 2009). For example, dogs showing separation anxiety exhibit a more pessimistic judgement compared to controls (Mendl et al., 2010), while rats which produce 'positive' vocalisations in response to manual tickling by an experimenter show a more optimistic judgement compared with rats which do not produce 'positive' vocalisations when tickled (Rygula et al., 2012). These findings suggest that judgement biases may provide a reliable measure of emotional valence in animals.

In previous studies (Chapters 03 and 04, Murphy et al., 2013a; 2013b), we developed an active-choice judgement bias task based on a conditional discrimination paradigm. Animals are trained to discriminate two tone cues by performing an active response (uncovering a food bowl) in two different goal-boxes, each associated with a specific cue, to receive different quantities of reward. A 'positive' cue signals the presence of a large reward in the 'positive' goal-box, while a 'negative' cue signals the presence of a small reward in a 'negative' goal-box. Once this discrimination has been learned, animals are then subjected to the judgement bias test whereby novel tone-cues, with frequencies intermediate between the positive and negative cues, are played and responses recorded. The proportion of active responses in the positive goal-box is taken as a measure of optimism, and is used as a proxy measure of the emotional state of the animal. Besides our work with pigs, active-choice paradigms have been used in a number of previous studies, e.g. with starlings (Brilot et al., 2010), with rats (Brydges et al., 2011; Enkel et al., 2010), and with bears (Keen et al., 2014).

Physiological indices of acute and chronic stress were also measured. As indicators of acute stress, cortisol measures from saliva and blood were taken before transport to the slaughterhouse. As indicators of chronic stress, hair cortisol was sampled. The neutrophil/lymphocyte ratio (N/L ratio) in the blood was determined (Puppe et al., 1997), and brain, hippocampus and spleen weights were recorded after slaughter. Hair cortisol has been proposed as a non-invasive biomarker for more chronic stress in humans and animals (Davenport et al., 2006; Macbeth et al., 2010; Russell et al., 2012) as it reflects total cortisol production/release over a long time (Laudenslager et al., 2011). Hair cortisol has been found to correlate with measures of salivary cortisol collected around the same time points throughout pregnancy (D'Anna-Hernandez et al., 2011). The N/L ratio has been found to correlate with behavioural measures of chronic

stress in rats (Swan and Hickman, 2014) and increase with increasing dietary cortisol concentrations in pigs (Widowski et al., 1989).

We hypothesize that, along with previous findings, LBW piglets should display a more pessimistic judgement bias than NBW controls. Furthermore, if allopurinol can prevent some of the prenatal neurological damage that can occur during IUGR, we expect that LBW pigs from sows treated with allopurinol in the third trimester of pregnancy should not show a pessimistic bias to the same extent as untreated LBW pigs. Finally, we expect that measures of judgement bias should correlate with measures of chronic stress in individual pigs.

## **METHODS**

### **Ethical Note**

The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

### **Subjects and Housing**

Animals used in this study were the same as in Gieling et al. (2014); see that publication for the full details of allopurinol treatment, the selection criterion, and the spatial holeboard test of learning and memory. In short, a total of 39 pigs selected from 11 sows ((Terra x Finnish landrace) x Duroc mix), bred at the farm of the Faculty of Veterinary Medicine of Utrecht University were used in this study. The experiment was performed in two batches separated by two months, with 22 pigs (from 5 sows) tested in batch 1, and 17 pigs (from 6 sows) tested in batch 2. Three of the sows from each batch had been treated with Allopurinol (15 mg.kg<sup>-1</sup>) for the 30 days ( $\pm 2$  days) leading up to farrowing, the other 5 sows were untreated controls (for full details, see Gieling et al., 2014).

Selection of LBW and NBW piglets was performed similarly to previous studies (Gieling et al., 2011; 2014; Murphy et al., 2013a). All piglets were weighed at birth, and the mean weight was calculated per litter. Pigs within each litter weighing at least 1 SD below the litter mean were considered LBW animals. A new mean weight per litter was calculated by excluding LBW piglets, and NBW piglets were selected as those nearest to the new litter mean. Up to three LBW and NBW piglets were selected per litter depending on availability. In total we used 18 LBW pigs and 21 NBW pigs.

At approximately 17 weeks of age, all animals were moved within the same research stable to two (per batch) new straw-bedded pens (5 x 3 m) alongside the Judgement Bias apparatus. Pen compositions were counterbalanced as much as possible for litter, birth weight grouping and allopurinol treatment. Pens contained a covered nest area and were provided with enrichment materials. During judgement bias testing, pigs were fed twice per day, 25% before testing and the remaining 75% after all pigs had been tested. In test-free periods, the pigs were fed 50% of their allowance in the morning, and 50% in the afternoon. Water was always available *ad libitum* (except in the judgment bias apparatus).

## **Apparatus**

The testing apparatus used was the same as that used in previous studies (see Chapters 02, 03 and 04 Murphy et al., 2013a; 2013b; In Press). The test arena (3.6 m x 2.4 m) was connected to the start box (1.2 m<sup>2</sup>) via a small antechamber (1.2 m<sup>2</sup>). Two goal-boxes were attached at the back of the test arena. Inside each goal box was a plastic food bowl, secured to the floor, which had a false base. Each food bowl was covered by a large hard-plastic ball which pigs could lift off the bowl with their snout. Guide rails prevented the ball from being knocked off the food bowl. Access to the test arena and to each goal-box could be controlled remotely by the experimenter using a series of guillotine doors.

Our active-choice JBT followed a typical conditional discrimination paradigm, whereby one tone-cue signalled the presence of reward in one goal-box and a second tone-cue signalled the presence of reward in the second goal-box. Rewards used were chocolate M&Ms<sup>®</sup> (Mars Nederland b.v., Veghel, The Netherlands). In a 'positive' trial the 'positive' cue predicted the presence of a large reward (4 M&Ms) in the 'positive' goal-box, while in a 'negative' trial a 'negative' cue predicted the presence of a small reward (1 M&M) in the 'negative' goal-box. Positive and negative tone-cues and goal-boxes were counterbalanced across animals. The open source software Audacity (<http://audacity.sourceforge.net/>) was used to generate tone-cues. The training tone-cues (positive/negative) used were a 200 Hz and a 1000 Hz pure tone (Waveform: Sine; Amplitude: 1). 'Ambiguous' tone-cues at intermediate intervals between the training tone-cues were also generated (299.07 Hz, 447.21 Hz, and 668.74 Hz pure tone; Waveform: Sine; Amplitude: 1). The tone-cues were played on MP3 player (Archos 18 Vision, 4 GB, Archos GmbH, Grevenbroich, Germany) through speakers (Logitech z-313, Logitech Europe S.A., Morges, Switzerland) attached at the back of the test arena.

The same habituation, training and testing protocol was followed for each batch and is similar to our previous studies (Chapters 04 and 05, Murphy et al., 2013b; In Press).

## **Judgement Bias Task: Habituation**

After 1 week of settling into their new pens, pigs were habituated to the apparatus at the age of 18 weeks. As all pigs had previous experience of the food rewards (chocolate M&Ms), as well as how to raise the ball from the food bowl in order to obtain the rewards (Gieling et al., 2014), the habituation period was relatively short. Pigs were first brought to the waiting area in their pen-groups and allowed to explore. Smaller groups of three to four pigs were then let into the apparatus, where rewards were available on the floor in front of each goal-box. Both goal-boxes were closed during group habituation. Groups of pigs were left to explore the apparatus for 3 minutes, and this was performed for two sessions for each group. Next, the same habituation was repeated for pairs of pigs over two sessions. Finally, pigs were trained to individually enter the apparatus and to perform trials. Each pig was let into the start box, and then released into the test arena where only one goal-box was open. One M&M reward was available in the food bowl in the open goal box. If a pig did not immediately go for the open goal-box, their attention was drawn to it by the experimenter. After the reward was obtained and consumed, the pig was gently encouraged back to the start box for another trial. Each pig received three

sessions of individual habituation with eight trials in each (equal presentations of left and right goal-boxes).

### **Judgement Bias Task: Pre-training**

Pigs received one training session per day. The first stage of pre-training was to introduce the training tone-cues and associated amounts of reward. Pigs were given four sessions of 'forced' trials, where only the correct goal-box was open, and the associated amount of reward available per trial. Each session consisted of 5 positive and 5 negative trials presented in a pseudorandom order, differing daily, with no more than two consecutive presentations of the same tone-cue. Pigs were individually brought into the start box and, after a brief delay, the tone-cue was played. The guillotine door between start box and antechamber was raised after 1 s of the tone-cue, giving the pig access to the test arena. The tone-cue continued until the pig had raised the ball off the food bowl in the goal-box to access the reward, or for a maximum of 60 s if the pig failed to do so. Once the reward was consumed pigs were gently encouraged back to the start box for the next trial.

The second stage of pre-training was to teach the pigs that a reward was available in every trial, albeit differing in quantity. Pigs were given three sessions of 'open-choice' trials, where both goal-boxes were open and pigs could sample both but only the correct, as signalled by the tone-cue, contained rewards. Each session consisted of 13 trials; three forced trials (1 positive; 2 negative), and 10 open-choice trials (5 positive; 5 negative). Tone-cues were played until the pig chose the correct goal-box, i.e. raised the ball off the food bowl, or for a maximum of 60 s.

### **Judgement Bias Task: Conditional Discrimination Training**

In order to test responses to ambiguity, we had to first ensure that pigs could discriminate consistently the positive and negative tone-cues. In this stage, pigs were given sessions of 'free' trials where both goal-boxes were open but only a choice for the correct goal-box, as signalled by the tone cue, contained rewards. The tone-cue was played until a choice was made, regardless of whether the choice was correct, or for a maximum of 30 s if a pig failed to choose within this time. If a correct choice was made, the pig received the associated reward, both goal-boxes remained open, and after the reward was consumed, the pig was returned to the start box for the next trial. If an incorrect choice was made, or the pig omitted to respond within 30 s, both goal boxes were closed and the pig remained in the test arena for a 90 s time-out penalty. Sessions consisted of 13 trials; three forced (1 positive; 2 negative), and 10 free trials (5 positive; 5 negative). In every fifth discrimination training session, the first 3 positive and 3 negative trials were treated like 'open choice' trials (see above) to serve as short reminders that rewards were available in every trial. Pigs were trained until they reached the criterion level of responding correctly in four out of five negative and positive free trials in three consecutive sessions.

### **Judgement Bias Task: Responses to Ambiguous Tone-Cues**

As soon as a pig had reached the learning criterion set, responses to the ambiguous tone-cues were tested over four daily sessions. Test sessions consisted of 16 trials; three forced trials (1 positive; 2 negative), ten free trials (5 positive; 5 negative), and three ambiguous trials (Trial nos. 6, 11, and 16). In an ambiguous trial, one of the three ambiguous tone-cues ('AmbigNeg', 'AmbigMid', and 'AmbigPos') was played instead of a training tone-cue for 30 s, and both positive and negative goal-boxes contained their associated quantity of rewards. Once a pig made a choice and had consumed the reward, it was gently returned to the start box for the next trial. In test sessions, each ambiguous cue was presented once per session, i.e. across the test sessions pigs received four presentations of each ambiguous cue. The AmbigMid cue was always presented first, while presentations of the AmbigNeg and AmbigPos cues were counterbalanced. To avoid any effect of the previous trial on responses to ambiguous tones, each ambiguous trial was preceded by equal numbers of positive and negative trials.

### **Physiological Procedures**

Blood and hair samples were taken a few days after all pigs had completed the Judgement Bias test. Pigs in the first batch were sampled at 5 months of age while pigs in the second batch were sampled at 5.5 months of age due to discrepancies in the time taken to complete behavioural testing.

### **Saliva Sampling**

Salivary cortisol samples were taken from pigs in their home pens between 11h and 14.00h. Pigs chewed on cotton swabs (Heinz Herenz, Hamburg, Germany, Cotton Swabs 150x4mm WA 2PL) for 60 s until the swabs were wet through. Two samples were taken per pig and placed in centrifuge tubes (Salivette, Sarstedt, Germany). Samples were then placed on ice and transported to the laboratory and centrifuged at 3000g for 15 minutes at room temperature. The saliva was pipetted into Eppendorf tubes and stored at -20°C. Cortisol concentrations were measured using a Coat-a-Count radioimmunoassay, according to the manufacturer's procedure (Coat-a-Count Cortisol PITKCO, Siemens Healthcare Diagnostics Inc. Los Angeles, USA). All samples from both batches were assayed on the same day in duplicate, to avoid inter-assay differences.

### **Blood and Hair Cortisol Sampling**

A few days after saliva sampling, all pigs were weighed in a weigh crate. Then the pig was restrained using a snare and blood and hair samples were taken, after which the pig was immediately released. Blood and hair samples for batch one were taken between 13.30h and 16.00h, while samples from the second batch were taken between 14.00h and 15.30h.

Blood samples were collected from the superior vena cava using S-Monovette tubes (9.5 ml Sarstedt B.V., Etten-Leur, The Netherlands) and immediately after collection samples were inverted 5 times and divided into two. They were kept at room temperature for the first 30 minutes to allow clotting to occur so that blood plasma could be obtained,

then placed on ice until all samples were ready to transport to the laboratory. One sample from each animal was used for cortisol analysis. Samples were centrifuged at 1300 g for 10 min. The serum was transferred to a 2 ml Eppendorf tube and stored at  $-80^{\circ}\text{C}$ . Analysis of blood cortisol was also performed using a Coat-a-Count radioimmunoassay and carried out at the same time as the salivary cortisol analysis.

Hair samples were obtained according to the method of Davenport et al. (2006) and to a protocol provided by the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands). Separate hair samples (500 mg – 1 g) were taken from the neck and flank region of the pigs. Samples were labelled and stored separately in aluminium foil at room temperature until cortisol was measured.

Hair samples were placed in 50 ml tube (250 mg per tube) and washed twice – 5ml of isopropanol was added to the tube which was gently swirled by hand for 3 min. The isopropanol was then decanted, and the process repeated. Once washed, the hair samples were left to dry, then ground with a Beadbeater (Biospec products; Lab services BV, The Netherlands,) with stainless steel beads (Biospec products, 3.2 mm; Lab services BV, The Netherlands, catalogue no. 11079132) in micro vials of stainless steel that could be closed with rubber caps (Lab services) for a maximum of 6.5 min. Any hairs that were not pulverised at this point were removed from the samples. 50 mg of the powdered hair sample was transferred to a micro-centrifuge tube with 1 ml methanol and incubated at room temperature for 24 h with a slow rotation to extract the steroids. Next, 0.6 ml of the extract was transferred to a new tube and dried for 5 hours at  $45^{\circ}\text{C}$  in a heating block. The dried extracts were dissolved in 0.4 ml phosphate buffer, and cortisol concentrations were measured with a High Sensitivity Salivary Cortisol EIA kit (Salimetrics Europe Ltd., Newmarket, United Kingdom).

### **Blood N/L Ratio**

One of the ~5ml blood samples taken from each pig was used for the analysis of blood N/L ratio. Smears were placed on a glass slide and left to air dry. Three slides were made per animal. Once dry, smears were stained using a Hemacolor® Stain kit (Merck, Darmstadt, Germany). The slides were analysed under a microscope (20x enlargement; Olympus BX40) to determine the relative amount of lymphocytes. From each slide, three different images where the cells were separately visible (fields from 10x10 squares) were analysed. The nine observations per individual were used to calculate the final mean N/L ratio.

### **Organ Weights**

Pigs were slaughtered the morning after transportation to the slaughterhouse; pigs were electrically stunned before being bled, then immediately decapitated. The brain was dissected and weighed (scale:  $\Delta 0,01\text{g}$ , Tefal). The hippocampus was dissected from the brain, the spleen was removed from the carcass, and weights recorded.

## Data Analysis

Analyses were carried out using R version 3.0.2. The statistical approach used in the present paper is based on information theory and uses Akaike's Information Criterion (AIC) (Burnham and Anderson, 2002). Unlike more traditional stepwise variate selection, the AIC approach compares a number of contending models at the same time allowing for inferences to be made that are not conditional on a particular model (Symonds and Moussalli, 2011). In order to account for small sample sizes in the present study, a correction was applied (AICc). The relative likelihood of each model is provided by the Akaike Weight ( $w_i$ ), while the Evidence Ratio (ER) provides an estimate of how likely the best model is when compared to the next best model. Table 1 summarises the best model, according to the Akaike Weight, for each outcome variable. For all fixed effects that were important according to the AIC, confidence intervals were calculated using the parametric bootstrap with 1000 bootstrap samples. The inherent assumptions of the models used were checked graphically with a normal probability plot on the residuals, and where necessary transformations were applied to the data. Due to missing data for the physiological measures, the number of subjects in these analyses deviates from the total number of animals in the study ( $n = 39$ ): NLRatio ( $n = 35$ ), SalivaCort ( $n = 38$ ), HairCortN ( $n = 38$ ), HairCortF ( $n = 37$ ), BrainWeight ( $n = 38$ ), HippoWeight ( $n = 38$ ), SpleenWeight ( $n = 38$ ).

*Weight development:* To monitor weight development of the pigs, birth-weight was compared with weight before performance of the JBT as well as weight at slaughter. A linear model for the weight with random litter and pigs-within-litter effects was used. Treatment (Control, Allopurinol), Weight-Group (NBW, LBW), Time-Point (Birth, Pre-JBT, Slaughter) as well as the interactions between Treatment and Weight-Group, Treatment and Time-Point, and Weight-Group and Time-Point were included as fixed effects in the model.

*Judgement Bias Test (JBT):* The mean latency to respond and the mean percentage of 'Optimistic Choices', i.e. choices for the positive goal-box, were recorded per pig for each cue-type across the four test sessions. A linear model for the Log(Latency) with random litter and random pig-within-litter effects was used. Treatment (Control, Allopurinol), Weight-Group (NBW, LBW), Batch (First, Second), Cue-Type (Negative, AmbigNeg, AmbigMid, AmbigPos, Positive), as well as the interaction between Treatment and Weight-Group were included as fixed effects in the model. For Optimistic Choice, a logistic regression model using the same random and fixed effects as for Latency was performed.

To get an overall measure of judgement bias per pig, the mean Area Under the Curve ('MeanAUC') of optimistic choices in response to each cue-type was calculated as

$$\text{Mean} \left[ \left( \frac{\text{Neg}+\text{AmbigNeg}}{2} + \frac{\text{AmbigNeg}+\text{AmbigMid}}{2} + \frac{\text{AmbigMid}+\text{AmbigPos}}{2} + \frac{\text{AmbigPos}+\text{Pos}}{2} \right) \right]$$

A linear model for the Log(MeanAUC) with random litter effects was used. As with Latency and Optimistic Choice, the fixed effects were Treatment, Weight-Group, Cue-Type, Batch, and the interaction between Treatment and Wight-Group.

*Conditional discrimination learning:* The number of sessions taken to reach criterion on the task, i.e. to discriminate between the positive and negative training tone-cues, was recorded for each animal. A Poisson model with random litter effects was used. Treatment (Control, Allopurinol), Weight-Group (NBW, LBW), Batch (First, Second) as well as the interaction between Treatment and Weight-Group were included as fixed effects in the model. To see whether learning was related to any of the physiological measures, NLRatio, SalivaCort, log(BloodCort), log(HairCortN), log(HairCortF), log(BrainWeight), log(HippoWeight), log(SpleenWeight), were also included as fixed effects. Furthermore to ensure that emotion as measured by optimism in the JBT was distinct from cognition, log(MeanAUC) was included as a fixed effect.

*Organ weights and physiological measures:* Organ weights were corrected for body size by dividing the weight of the organ (g) by the weight at slaughter of the animals (kg). For each of the physiological measures (N/L Ratio, Saliva Cortisol, Blood Cortisol, Neck Hair Cortisol, Flank Hair Cortisol) and organ weights sampled (Corrected Brain Weight, Corrected Hippocampus Weight and Corrected Spleen Weight), a series of linear models for using the Log of each measure with random litter effects were used. For each model, the fixed effects were Treatment (Control, Allopurinol), Weight-Group (NBW, LBW), and the interaction between Treatment and Weight-Group. Further, to investigate if measures of emotion in the JBT related to physiological stress measures, the Log(MeanAUC) was included as a fixed effect in each analysis.

## RESULTS

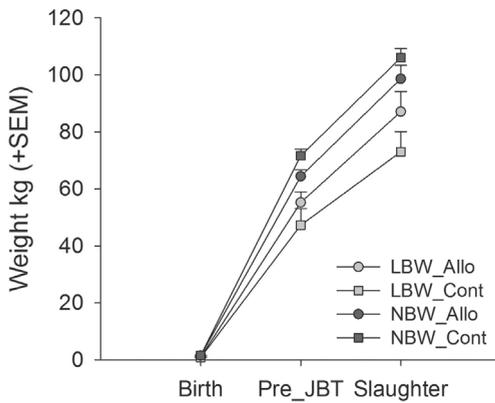
Full information on the piglets selected for this experiment is provided by Gieling et al. (2014). While only 37 pigs were used by in the study by Gieling (2014), extra piglets were selected at birth and trained in the same manner. Thus 39 animals were used in this study. Table 1 lists the factors important in the optimal model, according to the Akaike weight ( $w_i$ ), for each outcome variable. All values are presented as Mean $\pm$ SEM.

*Weight development:* At birth, piglets from the LBW group weighed 931 $\pm$ 0.04g, while NBW piglets weighed 1525 $\pm$ 0.06g. LBW pigs were still lighter than NBW pigs when weighed before commencement of the JBT and at slaughter (Fig. 1). Treatment with allopurinol in late gestation appeared to have differing effects on pigs classed as LBW or NBW. Whereas LBW pigs from allopurinol-treated sows were heavier than those from untreated sows, the opposite was found for NBW pigs (95% Confidence Intervals for allopurinol-treated pigs compared to untreated pigs; LBW: 0.82, 13.93; NBW: -8.65, -1.46).

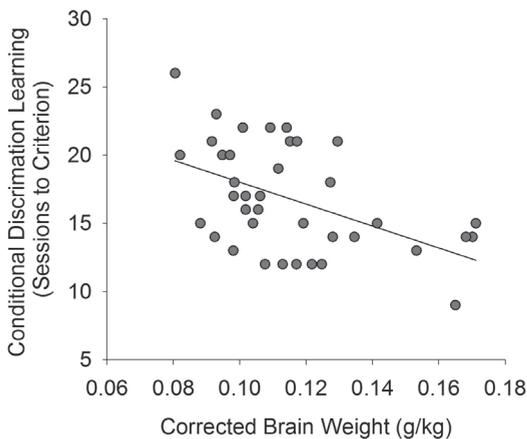
*Conditional Discrimination Learning:* All pigs learned the conditional discrimination task in 16.69 $\pm$ 0.63 sessions. There was no effect of weight group or allopurinol treatment on learning of the task. However, brain weight (corrected for differences in body weight) was important with respect to learning to distinguish negative and positive tone-cues (Estimate and 95% Confidence Interval for Log(Brain Weight): -0.66 [-0.96, -0.14]). Pigs with smaller brains, corrected for differences in body weight, took longer to learn the conditional discrimination task (Fig. 2).

**Table 1** Summary of best fitting models for each outcome variables.

Outcome Variable	Fixed effects included in selected models	AIC <sub>c</sub>	w <sub>i</sub>	ER
Weight (kg)	Time Point × Weight Group, Treatment × Weight Group, Weight Group, Treatment, Time Point	908.0	0.57	2.01
Learning (Sessions)	Brain Weight	161.5	0.36	1.64
Latency (s)	Batch, Cue-Type	-143.5	0.27	1.43
Optimistic Choice (%)	Cue-Type	516.9	0.44	2.49
Mean AUC	-	-	-	-
Neck Hair Cortisol	Weight Group	38.0	0.34	1.60
Brain Weight	Weight Group	-19.2	0.54	3.56
Hippocampus Weight	Weight Group	-10.1	0.43	2.07
Spleen Weight	Weight Group	-29.9	0.40	1.47
N/L Ratio, Saliva Cortisol, Blood Cortisol, Flank Hair Cortisol	-	-	-	-



**Fig. 1** Weight development of birth-weight and treatment groups array of best fitting models for each outcome variables.



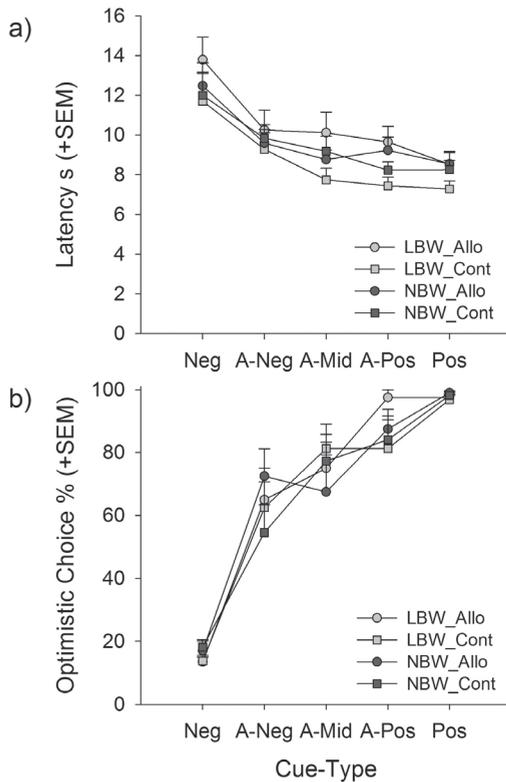
**Fig. 2** Inverse relationship between corrected brain weight and the number of sessions to reach criterion on the conditional discrimination task.

*Judgement Bias Test (JBT)*: No omissions occurred during test sessions. Mean latency to respond and the proportion of optimistic choices in response to each cue-type are presented for each weight group (NBW, LBW) and treatment group (Control, Allopurinol) in Table 2. For both Latency and Optimistic Choice, neither Weight Group nor Treatment was important for the model. Instead, the model including Cue-Type best described the data. Increasing estimates for Optimistic Choice and decreasing estimates for Latency (Table 3) indicate that pigs increasingly chose the positive goal-box and responded faster as cue-types became more similar to the positive cue (Fig. 3). Mean AUC was not affected by Weight Group nor Treatment. Interestingly, pigs in Batch 2 took longer to make a choice than pigs in Batch 1, but neither Optimistic Choice nor Mean AUC were influenced by batch. Mean AUC was not related to learning of the initial conditional discrimination, nor were any of the physiological measures of acute and chronic stress important for Mean AUC.

*Physiological Measures*: See Tables 1 and 4. Short-term cortisol levels did not differ between LBW and NBW pigs when measured in saliva or blood. Neither did long-term measures differ with samples of hair taken from the flank of the pigs. However, LBW pigs had lower levels of cortisol than NBW pigs when hair was sampled from the neck of the animal. Weight group differences were also found in organ weights. While absolute brain weight was greater in NBW pigs, LBW pigs had larger brain, hippocampus and spleen weights relative to body size than NBW pigs. No differences were found in N/L ratio.

**Table 2** Latency (s  $\pm$ SEM) and Optimistic Choice (%  $\pm$ SEM) responses to each cue type for LBW and NBW pigs in the Judgement Bias Test.

			Cue-Type					
			Negative	AmbigNeg	AmbigMid	AmbigPos	Positive	
Latency (s)	LBW	Allo	13.79 $\pm$ 1.14	10.25 $\pm$ 1.01	10.11 $\pm$ 1.04	9.65 $\pm$ 0.79	8.53 $\pm$ 0.64	
		Control	11.70 $\pm$ 1.41	9.28 $\pm$ 0.84	7.75 $\pm$ 0.57	7.44 $\pm$ 0.44	7.28 $\pm$ 0.41	
		Total	12.86 $\pm$ 0.90	9.82 $\pm$ 0.66	9.06 $\pm$ 0.68	8.67 $\pm$ 0.54	7.97 $\pm$ 0.42	
	NBW	Allo	12.48 $\pm$ 1.16	9.59 $\pm$ 0.69	8.76 $\pm$ 0.47	9.23 $\pm$ 0.66	8.55 $\pm$ 0.56	
		Control	12.00 $\pm$ 1.19	9.84 $\pm$ 0.69	9.18 $\pm$ 0.76	8.23 $\pm$ 0.41	8.25 $\pm$ 0.48	
		Total	12.23 $\pm$ 0.81	9.72 $\pm$ 0.48	8.98 $\pm$ 0.45	8.71 $\pm$ 0.39	8.39 $\pm$ 0.36	
	Optimistic Choice (%)	LBW	Allo	13.50 $\pm$ 1.50	65.00 $\pm$ 10.00	75.00 $\pm$ 8.33	97.50 $\pm$ 2.50	97.50 $\pm$ 0.83
			Control	13.75 $\pm$ 1.83	62.50 $\pm$ 8.18	81.25 $\pm$ 7.83	81.25 $\pm$ 9.15	96.88 $\pm$ 1.88
			Total	13.61 $\pm$ 1.13	63.89 $\pm$ 6.46	77.78 $\pm$ 5.68	90.28 $\pm$ 4.58	97.22 $\pm$ 0.92
NBW		Allo	17.00 $\pm$ 3.09	72.50 $\pm$ 8.70	67.50 $\pm$ 11.81	87.50 $\pm$ 4.17	99.00 $\pm$ 0.67	
		Control	18.18 $\pm$ 2.36	54.55 $\pm$ 8.80	77.27 $\pm$ 8.56	84.09 $\pm$ 9.70	98.18 $\pm$ 1.02	
		Total	17.62 $\pm$ 1.88	63.10 $\pm$ 6.37	72.62 $\pm$ 7.09	85.71 $\pm$ 5.34	98.57 $\pm$ 0.61	



**Fig. 3 a)** Mean latency to respond and **b)** Mean Optimistic Choice (%), per cue-type for birth-weight and treatment groups in the JBT.

**Table 3** Estimates and 95% confidence intervals for the effect of Cue-Type on the percentage of optimistic choices and latency to respond\*.

	AmbigNeg	AmbigMid	AmbigPos	Positive
Optimistic Choice (%)	2.84 [2.38; 3.15]	3.41 [2.87; 3.83]	4.31 [3.56; 5.00]	6.22 [5.59; 6.69]
Latency (s)	-0.23 [-0.35; -0.12]	-0.32 [-0.44; -0.20]	-0.35 [-0.45; -0.23]	-0.40 [-0.51; -0.29]

\*Each cue-type is compared with responses to the Negative Cue.

**Table 4.** Estimates and 95% confidence intervals for the effect of birth weight category on physiological measures.

Outcome Variable	LBW compared to NBW
Neck Hair Cortisol	-0.19 [-0.42; -0.00]
Brain Weight	0.18 [0.07; 0.31]
Hippocampus Weight	0.20 [0.06; 0.36]
Spleen Weight	0.11 [0.01; 0.20]

## DISCUSSION

In the present study we wanted to investigate whether treatment of sows with allopurinol in late gestation would alleviate some of the negative emotional and cognitive deficits expected to occur as a consequence of low birth weight in piglets. In order to test this, we used a judgement bias task which required the animals to first learn a conditional discrimination task, then judge whether ambiguous cues were predicting a large or a small reward. Using the same pigs as in the current study, Gieling et al. (2014) found indications of altered emotionality in the LBW group who vocalised more in an open-field test when tested in the fifth week after birth. However, in the present study, we did not find evidence that this altered emotionality persists as LBW and NBW pigs did not differ in levels of optimism as measured by responses to ambiguity in a judgement bias task when tested at ~5 months of age. This contrasts with our previous findings (Chapter 05, Murphy et al., In Press), where we found persistent effects of birth weight; LBW pigs, again tested at ~5 months of age, were more pessimistic, i.e. more likely to interpret ambiguous cues as predicting a small reward, than NBW pigs in the same judgement bias task. In the present experiment, we cannot support previous behavioural evidence for long-term altered emotionality in LBW pigs (Rutherford et al., 2013).

The other experiments performed using the same subjects as in the present experiment found that allopurinol-treated LBW pigs showed greater compensatory growth (Gieling et al., 2014). Prenatal allopurinol treatment, regardless of birth weight category, was also associated with improved neuronal plasticity (Prickaerts et al., 2014). By contrast, we did not find any effect of allopurinol on conditional discrimination learning or responses to ambiguity in our judgement bias task. Similarly, Gieling et al. (2014) did not find any effect of allopurinol treatment on learning and memory performance in a cognitive holeboard task.

Since untreated LBW pigs did not demonstrate the more pessimistic bias previously seen (Chapter 05, Murphy et al., In Press), and did not show the deficits in learning and memory previously observed (Gieling et al., 2011), perhaps no behavioural effect of allopurinol treatment was seen as the LBW group were not deficient to begin with. Alternatively, it is conceivable that the effect of birth-weight and prenatal allopurinol treatment, in these particular piglets, was not long-lasting or too subtle to be detected with the judgement bias test. Since we have previously shown that the test is sensitive enough to identify differences in responses of LBW and NBW piglets, we suggest that in the present study LBW and NBW pigs did not differ, a view supported by the lack of consistent differences in physiological measures (see below).

In humans, increased hair cortisol has been found to reflect periods of chronic stress and pain (Russell et al., 2012; Van Uum et al., 2008) and has been proposed as a non-invasive measure of stress in human adults and in neonates (Gow et al., 2010; Yamada et al., 2007). Hair cortisol has also been proposed as a measure of chronic stress in other mammals (Davenport et al., 2006; Macbeth et al., 2010), but has not yet been validated as a measure of chronic stress in pigs. Samples of hair were taken from both the neck and flank area of the pigs, and cortisol measures from each of these areas were correlated

(data not shown,  $r_s=0.63$ ,  $p<0.0001$ ). However, observations of the hair samples taken from each batch showed differences, possibly due to seasonal effects. Hair from Batch 1 (sampled in early springtime) was thicker and longer than samples from Batch 2 (sampled in early summer), particularly for neck hair; higher cortisol levels were found in animals sampled in early summer (data not shown,  $p<0.001$ ). Thus it is suggested that flank hair cortisol, being less susceptible to seasonal influences may provide a better measure of chronic stress in this experiment. In the present experiment, contrary to expectation, the level of neck hair cortisol was lower in LBW pigs, but no differences in, the possibly more reliable, flank hair cortisol were found. Another putative measure for chronic stress, the N/L ratio (Swan and Hickman, 2014) was also not affected by birth weight or allopurinol treatment.

While overall spleen weights did not differ between LBW and NBW pigs, spleen weights corrected for body weight were higher in LBW pigs. Larger relative spleen size may be indicative of chronic stress in LBW animals, but it cannot be excluded that this is an artefact due to selecting animals based on their smaller body size as there is not a 1:1 relationship between organ weight and spleen weight (Deland, 1970). This may also be a likely explanation for the larger relative brain and relative hippocampus weights found in LBW pigs (Table 4). In terms of measures of more acute stress, LBW and NBW pigs did not differ at time of sampling. Thus in the present experiment, we cannot support previous physiological evidence for long-term altered stress reactivity in LBW pigs (Rutherford et al., 2013). While previous studies have found that HPA reactivity, but not baseline levels were higher in LBW pigs tested at three months of age (Poore and Fowden, 2003), our measures of chronic stress, hair cortisol and the N/L ratio, failed to show such a difference.

No relationship was found between overall optimistic responses in the judgement bias task (Mean AUC) and any of the physiological measures of acute and chronic stress regardless of weight group or treatment with allopurinol. Chronic stress (social and environmental) treatments have previously been found to be associated with more pessimistic judgment biases in rats (Chaby et al., 2013; Papciak et al., 2013). Rats scored as more pessimistic in a judgement bias task also show a stronger anhedonic response to stress (Rygula et al., 2013). It is possible that the LBW pigs in the present experiment did not experience increased or longer-lasting chronic stress than the NBW pigs as the physiological measures we recorded do not unequivocally support a difference between the groups.

Individual performance on the judgement bias task was not found to be related to measures of chronic or acute stress. Previously we have found that salivary cortisol samples taken on the morning before testing did not predict behaviour on the judgement bias task (Chapter 05, Murphy et al., In Press). There may not be a direct relationship between physiological stress measures and emotional responses. Common physiological measures of stress, such as cortisol, may be influenced by a variety of factors, including time of day, season, environmental conditions etc. (Mormède et al., 2007). Furthermore, while physiological measures may reflect activation of the system, they may not reflect directly the emotional state.

In terms of conditional discrimination learning, while there was no effect of birth weight grouping or prenatal treatment with allopurinol, we did find that brain weight (corrected for differences in body weight) was related to the speed of learning of the initial conditional discrimination task. Regardless of group, pigs with larger brains relative to their body size needed fewer sessions to learn the discrimination. Without knowing the specific differences between larger and smaller brains, it is difficult to speculate on how a larger brain-to-body weight ratio is related to learning.

In line with our previous work (Chapters 04 and 05, Murphy et al., 2013b; Chapters 05 and 06, In Press), we did find that pigs show an increasing optimistic responses and decreasing latency to respond as cue-type becomes more similar to the positive cue. This suggests that the ambiguous cues are being interpreted within the context of the learned positive and negative cues. Furthermore, while pigs tested in the second batch took longer to make choices than pigs in the first batch, their actual choices did not differ. This difference may reflect differences in the ambient temperature as Batch 2 were tested in much warmer conditions. The robustness of the active-choice test design is indicated by the fact that judgement bias was not influenced by this environmental difference, while latency responses were. Judgement bias tasks where latency is the sole outcome measure may be more susceptible to changes in environmental conditions. Furthermore, as in previous studies, performance on the judgement bias task was found to be independent of measures of learning in the task.

Some methodological differences exist between the present study and the previous study where LBW pigs were found to show a more pessimistic bias (Chapter 05, Murphy et al., In Press). In the previous study, subjects were weighed within 12 hours of birth as opposed to immediately after birth. While the piglets selected were therefore slightly heavier, the difference in mean weights and range of LBW and NBW pigs was similar between the two studies. In both studies, animals were tested around the same age (~5 months) but the experience prior to testing differed.

While in the previous study pigs were housed in the same environment and were trained in the same apparatus on a decision-making task, pigs in the present study were housed in a different area of the research stable and trained there on a spatial holeboard task. This prior experience may influence levels of optimism; the decision-making task requires animals to accept smaller short-term gains in order to obtain larger long-term gains. Melotti et al. (2013) have previously shown that some pigs will prefer to stop responding than switch their choice to an immediate but smaller reward with increasing delays to the larger reward. The spatial holeboard task, on the other hand, potentially provides extensive cognitive enrichment for the pigs in the form of a manageable challenge as there is little cost to making mistakes. Successful coping with challenge can lead to future positive judgements (Puppe et al., 2007) and activation of reward-evaluating mechanisms in the brain (Spruijt et al., 2001) leading to positive emotions. Enrichment has been shown to induce positive judgement bias in pigs and rats (Douglas et al., 2012; Richter et al., 2012; Brydges et al., 2011), the extensive holeboard training may have had the same effect in the present study.

Furthermore, in the present study, piglets which met the criterion of LBW and NBW were used, regardless of sex, whereas only males were selected previously. While the number of gilts in the present study was low, testing occurred around the onset of puberty. As the males were not castrated, the presence of females within the group could have altered the mood state within the groups, and perhaps reduced any differences between the birth weight groups.

We did not find evidence to support altered emotionality using a judgement bias task or physiological stress reactivity in LBW piglets compared to NBW controls. Neither did we find any effect of prenatal treatment with allopurinol on behavioural or physiological outcomes. However, the choices in the judgement bias test suggest that pigs were interpreting the ambiguous cues within the context of previously learned positive and negative cues, and responses on the test were independent of learning and changes in environmental conditions.



# CHAPTER 07

## RELATIONSHIP BETWEEN PIGS' BEHAVIOUR IN A SPATIAL HOLEBOARD TASK AND RESPONSES IN A JUDGEMENT BIAS TASK

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## **ABSTRACT**

Judgement bias has been proposed as a method for establishing the valence of background emotional states in animals. Test of emotion in animals should not be confounded by differences in learning capacity, activity levels, or motivation. In order to assess the value of judgement bias tasks with respect to the degree in which such tasks specifically target emotional responses above other confounding variables, in two studies we compared behaviour of pigs in a spatial holeboard task, designed to assess learning capacity through reference memory and working memory performance, and a judgement bias task, designed to assess conditional discrimination learning and responses to ambiguity as a proxy measure of emotion. In Study 1, we found that as predicted, Göttingen minipigs (n = 8) with better reference memory performance learned the conditional discrimination faster. In contrast to expectation, better reference memory performance in conventional pigs (n = 7) was correlated to optimistic responses to ambiguous cues. However, due to small sample sizes in results may not be generalizable. In Study 2 with conventional farm pigs (n=39), we found a moderate negative correlation between working memory in acquisition of the holeboard task and conditional discrimination learning, but no relationship between working memory in reversal learning. Reference memory in acquisition and reversal phases in Study 2 was unrelated to conditional discrimination learning and responses to ambiguous cues. The spatial holeboard task and the JBT consequently measures different behavioural domains in the same animal.

## INTRODUCTION

Biases in judgement of ambiguous stimuli has recently been proposed as a method for establishing the valence of background emotional states in animals; optimistic judgements are said to be indicative of positively-valenced emotions, while pessimistic judgements indicate negative emotions (Harding et al., 2004; Mendl et al., 2009; Paul et al., 2005). This approach is receiving increasing attention in animal welfare science as suggested by the number of papers and variety of species to which it has been applied. In Chapter 02, we proposed a list of criteria for behavioural tests of emotion, one of which is that a task should *'specifically and unambiguously capture emotion-related behaviours'* (Murphy et al., 2014), by which we mean that responses on the task should not be confounded by differences in learning capacity, activity levels, or motivation.

We have developed an active-choice task to assess judgement bias (hereafter, JBT) in pigs, see Chapters 03-05 (Murphy et al., 2013a; 2013b; In Press). In this task, we first require pigs to learn an audio-spatial conditional discrimination task using tone-cues to predict the presence of large or small rewards in a left or right goal-box. Put simply, in this task pigs need to learn: if 'positive-cue' (predicting large reward), go to left goal-box; if 'negative-cue' (predicting small reward'), go to right goal-box. Once animals reach a predefined criterion level of performance on this conditional discrimination task, we then present a series of ambiguous cues, intermediate between the previously trained cues, and record 'optimistic' responses (i.e. responses indicating expectation of the large reward) as a measure of judgement bias. In a series of studies, we have repeatedly demonstrated that all pigs were able to learn this initial discrimination, and more importantly, that the measure of judgement bias used is unrelated to the rate of learning, i.e. the number of trials needed to reach the predefined criterion in the conditional discrimination training, (Chapters 04-06, Murphy et al., 2013b; In Press).

Spatial holeboard-type tasks have been established as valid instruments in neuroscience research as they have the ability to measure multiple facets of behaviour simultaneously in one test (van der Staay et al., 2012). The holeboard is a free choice maze for assessing spatial learning and memory. The holeboard contains a number of 'holes', potential reward locations, of which only a subset is baited. An animal can search freely for the baited holes within a certain time period, and in order to successfully learn the task must *'process the temporal context associated with an event'*, i.e. what happened/when, (Gielsing et al., 2013). The holeboard allows for the independent assessment of both reference memory, *'the general rules of a task'*, and working memory, *'information that is relevant only within a specific trial'* (van der Staay et al., 2012). In the conditional discrimination aspect of the JBT, it is likely that working memory is not less important for learning the task as cues are present until a response is made. By contrast, reference memory is important for successful performance since learning is based on remembering the rules, "if cue A, response A; if cue B, response B".

Arts et al. (2009) found that the spatial holeboard task was suitable for measuring cognitive capacity and spatial discrimination learning in pigs. A series of studies by Gielsing et al (2011; 2013; 2014) supported the suitability of the holeboard for measuring task-specific reference memory and trial-specific working memory in pigs. The spatial

holeboard task appears to provide more subtle and thorough measures of learning than our conditional discrimination task.

In order to assess whether spatial learning ability was related to choices on an audio-spatial judgement bias task, we used the results of two studies with pigs, both involving testing in the holeboard task first (Gieling et al., 2013; 2014), followed by the JBT (Chapters 04 and 06; Murphy et al., 2013b). Measures of spatial working and reference memory from the holeboard task were compared with conditional discrimination learning and responses to ambiguity in a JBT. Firstly we expect that spatial reference memory is important for learning the conditional discrimination task. Secondly, if the measure of judgement bias is truly independent from learning ability, then we predict no relationship between holeboard behaviour and responses to ambiguity.

## **MATERIAL AND METHODS**

The animals and methods used in this study have been previously described in detail; Study 1: by Gieling et al. (2013) and Murphy et al. (Chapter 03, 2013a; Chapter 04, 2013b), and Study 2 by Gieling et al. (2014) and Murphy et al. (Chapter 06).

### **Ethical Note**

The studies were reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and were conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

### **Subjects and Housing**

*Study 1:* Eight female Göttingen minipigs (hereafter minipigs) obtained from Ellegaard, Denmark (<http://www.minipigs.dk/>) and 7 female conventional farm pigs (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) obtained from the breeding farm of Utrecht University were transported into our research facility at 4-6 weeks of age. Subjects were group-housed, separated by breed, in straw-bedded pens (20m<sup>2</sup>) in naturally lit and ventilated stables. Each pen contained a covered nest area, and was provided with toys for enrichment. Pigs had access to water ad libitum, and were fed twice per day (33% morning, 66% evening), amounts according to the recommendations of their breeders. At 5 months of age, subjects were moved and rehoused in adjacent stables in the same research facility under similar conditions (pen size: 15m<sup>2</sup>; feeding schedule: 25% morning, 75% evening).

*Study 2:* In total 37 piglets, selected from 10 sows ((Terra x Finnish landrace) x Duroc mix) from the breeding farm of Utrecht University were used in this study. This experiment was performed in two batches (batch 1: 22 piglets from 5 sows; batch 2: 15 piglets from 5 sows) separated in time by two months. Within each batch, 3 sows had been treated with Allopurinol (15 mg.kg<sup>-1</sup>) for the 30 days ( $\pm 2$  days) before farrowing. Furthermore, piglets were specifically selected based on birth weight; 18 low-birth-weight (LBW) piglets and 19 normal-birth-weight (NBW) siblings were selected according to the criteria of Gieling et al. (2014). Any piglet whose birth weight was at

least 1SD below the litter mean at birth was considered LBW. NBW piglets were those with birth-weights closest to the litter mean once all LBW piglets had been removed from the calculation. At 4 weeks-of age, subjects were transported into our research facility and group housed in similar conditions as described for Study 1. At 17 weeks-of age, subjects were moved and rehoused in adjacent stables as in Study 1.

## **Apparatus**

The same spatial holeboard apparatus, hereafter holeboard, and judgement bias apparatus were used in both Study 1 and 2, and have been previously described in detail by Gieling et al. (2013; 2014; 2011) and Murphy et al. (Chapters 03-05, 2013a; 2013b; In Press). In short, the holeboard consisted of a square arena (5.3 x 5.3 m) surrounded by 1 m high walls. The entire arena was surrounded by a narrow corridor (40cm width) which led to four entrances into the arena, one in the middle of each side, which could be opened by the experimenter using pulley-operated guillotine doors. Within the arena was a 4x4 matrix of food bowls, the 'holes' of the holeboard, which had a false base underneath which rewards could be placed to avoid odour cues (Road Refresher, Jolly Pet). To avoid visual cues, a large hard-plastic ball (24cm diameter) covered each food bowl which a pig could easily raise off the bowl using their snout to gain access to rewards underneath. Guide rails ensured that the ball could not be knocked off bowl and that it returned to cover the bowl once the pig had retracted its head.

The judgement bias apparatus consisted of a start box (1.2 m<sup>2</sup>) connected, via a small antechamber (1.2 m<sup>2</sup>), to a rectangular test arena (3.6 m x 2.4 m). Two goal-boxes were attached to the back wall of the test arena each of which contained a food bowl system as described above for the holeboard apparatus. Entrance to the test arena from the start box and access to each goal-box were regulated by the experimenter using pulley-operated guillotine doors. Tone was used to cue the location of rewards in the JBT. Tones were generated using the open source software Audacity (<http://audacity.sourceforge.net/>) and played on an MP3 player (Archos 18 Vision, 4 GB, Archos GmbH, Grevenbroich, Germany) through speakers (Logitech z-313, Logitech Europe S.A., Morges, Switzerland) attached at the back of the testing area. The training tone-cues used were a 30 s long 200 Hz and 1000 Hz pure tone (Waveform: Sine; Amplitude: 1). Ambiguous tone-cues were generated at equal intervals between the training tone-cues on a logarithmic scale: 299.07 Hz, 447.21 Hz, and 668.74 Hz. Rewards used in both studies were chocolate M&Ms<sup>®</sup> (Mars Nederland b.v., Veghel, The Netherlands).

## **Spatial Holeboard Task**

Full details on habituation and training of animals for both Study 1 and 2 can be found in Gieling et al. (2013; 2014).

*Study 1:* After habituation to the experimenter, rewards and apparatus, formal training in the holeboard began when the animals were approximately 13 weeks-of-age. Each animal was assigned to one of four configurations of four rewarded holes (containing 1 M&M). The configuration of rewarded holes did not change for an animal throughout the study. In each trial, a pig was let into the corridor surrounding the holeboard and

walked the perimeter of the arena until it found an open entrance door into the arena, the location of which was chosen randomly per trial. Pigs could then search the arena for the rewarded holes. Trials ended after all four rewards were obtained or a maximum trial duration of 10 minutes had elapsed. Four trials were conducted per day per animal.

Task acquisition was assessed over 104 trials. In each trial working memory (WM) and reference memory (RM) were recorded. WM involves 'information that is relevant only within a specific trial' (van der Staay et al., 2012) and was measured as the number of visits to holes which resulted in a reward (maximum of four) divided by the total number of visits to these same holes, i.e. a score of 1 would imply that a pig had not revisited any of the baited holes after obtaining a reward. RM, on the other hand, 'stores the general rules of a task' (van der Staay et al., 2012). RM was measured as the number of visits to the baited holes, divided by the total number of visits to all holes. To get an overall measure of WM and RM performance over time, data from the four trials conducted each day were averaged so that the 104 trials became 26 blocks of 4 trials. Although the pigs received extended training, we only used the first ten trial blocks for the present analysis because pigs approached ceiling performance levels after 10 trial blocks i.e. performance in later blocks reveals no information about the acquisition of the task.

*Study 2:* After habituation, pigs began formal training in the holeboard at approximately 8 weeks-of-age. Training in the acquisition phase was performed similarly to the method described for Study 1 and each animal received a minimum of 40 trials. Reversal learning was assessed once animals reached a criterion of reference memory performance greater than 0.7 in two consecutive sessions. A new configuration of four rewarded holes was assigned to each animal that reached this criterion, and training continued as before for 44 trials. Any animal which did not reach this criterion after 60 trials did not proceed to the reversal phase. Both WM and RM were assessed and, as before, the mean of each successive four blocks of trials were calculated.

### **Judgement Bias Task (JBT)**

Full details on habituation and training of animals for both Study 1 and 2 can be found in Murphy et al. (Chapters 03, 04 and 06, 2013a; 2013b).

*Study 1:* After habituation to the experimenter, rewards and apparatus, pigs (approx. 27 weeks-of-age) were trained in a conditional discrimination task to distinguish between a 'positive' and a 'negative' tone-cue. Tone-cues were played and the pig was then released from the start box into the test arena where it could freely choose between the two goal-boxes. A choice was defined as any lift/push of the ball covering a food bowl in a goal-box with enough force to cause the ball to move. A positive tone-cue predicted the presence of a large reward consisting of 4 M&Ms, in the associated 'positive' goal-box, while a negative tone-cue predicted the presence of a small reward, consisting of only 1 M&M, in the associated 'negative' goal-box. Cue and goal-box were counterbalanced for meaning across animals. Each daily session consisted of 13 trials; 3 'forced' trials where only the correct goal-box, as predicted by the tone cue, was available, and 10 'free' trials (5 positive, 5 negative), where both goal boxes were open. In this training phase, the

choice of the correct goal-box in response to a cue within 30 s in free trials resulted in the associated amount of reward, while an incorrect choice or an omission resulted in a time-out penalty where both goal-boxes were closed and the pig remained in the test arena for 90 s. Pigs were trained until they responded correctly four out of five times to both positive and negative tone-cues (free trials) in three consecutive training sessions. The number of sessions needed to reach this criterion of performance was taken as a measure of conditional discrimination learning.

Judgement bias was then assessed over four further sessions. Each daily session consisted of 16 trials; 3 forced and 10 free trials, as before, and 3 ambiguous trials where one of the three previously unheard ambiguous tone-cues was played in lieu of one of the training tone-cues. In ambiguous trials, both goal-boxes were open but neither contained a reward. Once a pig had chosen a goal-box, the trial was ended and the pig returned to the start box for the next trial. Each of the three ambiguous cues was presented once per day. The order of trials was counterbalanced so that each ambiguous trial occurred after equal numbers of positive and negative trials. The percentage of 'optimistic' choices, i.e. choice for the positive goal-box, in response to each of the five cue-types [Negative (CS-), AmbigNeg (cue near CS -), AmbigMid (cue intermediate between CS+ and CS-), AmbigPos (cue near CS+), and Positive (CS+)] was calculated per pig across the four test sessions. To get an overall measure of 'optimism' in the JBT, optimistic choices in response to the individual cue-types were used to calculate the mean Area Under the Curve (Mean AUC) as:

$$\text{Mean} \left[ \left( \frac{\text{Neg} + \text{AmbigNeg}}{2} + \frac{\text{AmbigNeg} + \text{AmbigMid}}{2} + \frac{\text{AmbigMid} + \text{AmbigPos}}{2} + \frac{\text{AmbigPos} + \text{Pos}}{2} \right) \right]$$

*Study 2:* After habituation, pigs began conditional discrimination training with the positive and negative tone-cues at approximately 20 weeks-of-age. Training was performed as for Study 1 except that in every fifth session, the first 3 positive and 3 negative trials were 'open choice' trials, where pigs could sample both goal-boxes, but only the correct, as signalled by the tone-cue, contained rewards. This was used to remind pigs that rewards were available in every trial. Again, the number of sessions needed to reach this criterion of performance was taken as a measure of conditional discrimination learning.

Once pigs had learned to discriminate positive and negative tone-cues, judgement bias was assessed over four sessions as described for Study 1. As we found that the number of optimistic choices in response to ambiguous cues decreased with repeated testing (Chapter 04, Murphy et al., 2013b), we chose to reward both goal-boxes as expected in ambiguous trials i.e. 4 M&Ms in the positive goal-box and 1 M&M in the negative goal-box. Optimism, as Mean AUC, was calculated per pig as in Study 1.

### **Statistical analysis**

A set of variables was selected for both the holeboard task and the JBT for two studies, in which pigs were tested in both tasks. The variables were taken as indices of the average

performance level and the speed of learning and subjected to correlation analysis to evaluate whether the measures between the two tasks are independent measures; that is, whether they cover different aspects of different cognitive processes. If the alternative measures for the performance level and/or learning curves cover the same trait(s) they are expected to be highly correlated. In this case, one may well dispense with one of the alternative measures (van der Staay et al., 1990; Walsh and Cummins, 1976).

*Holeboard:* In the holeboard, the slope and intercept of working memory (WM) and reference memory (RM) were estimated across successive trial blocks per pig, using the SAS PROC REG (SAS 9.4; SAS Institute Inc., Cary, NC, USA). As they reduce the bias induced by incomplete trials, the ratio-measures and not the error measures for WM and RM were used (van der Staay et al., 2012). In addition, the mean performance level per animal was calculated as arithmetic mean across the successive trial blocks.

For each of the two studies, repeated measures analysis with successive blocks as within subjects factor were performed, supplemented by polynomial contrasts (SAS PROC GLM). We determined the percent variation explained by the linear, quadratic and cubic trend component of the holeboard task over the successive trial-blocks (Cotton, 1998; Winer, 1971).

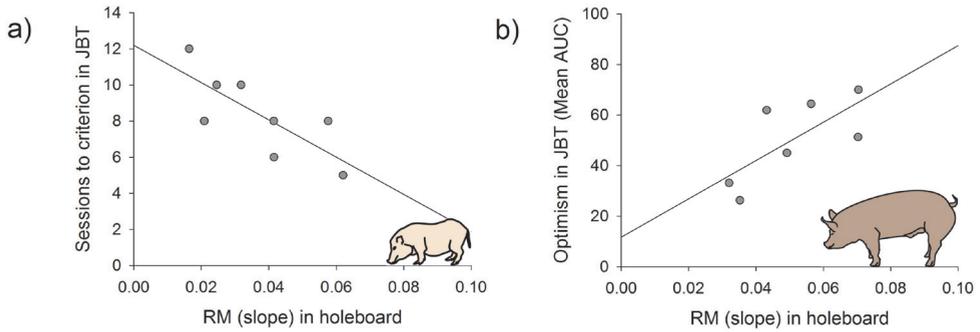
In addition, we calculated orthogonal trend components of the changes over trial blocks per pig using appropriate sets of trend coefficients (from SAS PROC IML).

The percent variation in the learning curves, explained by the linear trend components, was determined, expressed as the percentage of the sum of squares of the linear component of the total within subjects sum of squares. The slopes of both WM and RM calculated over the same blocks per animal, another index for the linear change over blocks, were used as in the subsequent correlation analysis. Note that the correlation between the linear trend component and the slope is 1.

*JBT:* Two measures per pig were used for the correlation analysis: the number of trials needed to reach the learning criterion after which the pig was subjected to judgment bias testing, and optimism, as defined as the Mean AUC across the five tone-cues used in the JBT task.

## **Correlation analysis**

For each study, the variables derived from the holeboard (slopes of WM, RM) and the JBT (sessions to criterion, optimism) were subjected to correlation analysis (SAS PROC CORR). As not all data met assumptions of normality, Spearman's rank correlation coefficient  $\rho$  was calculated. For Study 1, since Göttingen minipigs tended to show poorer RM performance, but reached criterion in the conditional discrimination task (JBT) sooner than conventional pigs (Gieling et al., 2013; Murphy et al., 2013b), correlation analysis was carried out separately for both breeds. In Study 2, no differences between birth-weight category (LBW/NBW) and/or prenatal treatment with allopurinol were found for WM and RM performance in the holeboard, nor for sessions to criterion and Mean AUC in the JBT (Gieling et al., 2014; Chapter 06). Therefore the correlation analysis was carried out across all treatment groups separately for task acquisition and task reversal phases. The correlation analyses were performed per study, because in



**Fig. 1** Correlations between learning the Reference Memory component of the Spatial Holeboard task (expressed as slope of the increase in RM performance across 10 successive blocks of 4 trials) with a) the number of sessions to reach criterion in a conditional discrimination task in Göttingen Minipigs ( $n = 8$ ), and b) optimism in a JBT in conventional farm pigs ( $n = 7$ ).

**Table 1.** Spearman's rank correlation coefficients and p-values for the correlational analysis between working memory and reference memory<sup>a</sup> in the holeboard task and conditional discrimination learning<sup>b</sup> and judgement bias<sup>c</sup> in the JBT for Studies 1 and 2. Significant relationships printed bold.

		Conditional Discrimination	Judgement Bias
<b>Study 1</b>			
Göttingen Minipigs ( $n = 8$ )	Working Memory	$r_s = -0.061$ $P = 0.885$	$r_s = 0.072$ $P = 0.866$
	Reference Memory	<b><math>r_s = -0.798</math></b> <b><math>P = 0.018</math></b>	$r_s = 0.287$ $P = 0.490$
Conventional Farm Pigs ( $n = 7$ )	Working Memory	$r_s = 0.655$ $P = 0.111$	$r_s = 0.357$ $P = 0.432$
	Reference Memory	$r_s = -0.036$ $P = 0.938$	<b><math>r_s = 0.786</math></b> <b><math>P = 0.036</math></b>
<b>Study 2</b>			
Acquisition Phase	Working Memory	<b><math>r_s = 0.414</math></b> <b><math>P = 0.011</math></b>	$r_s = 0.047$ $P = 0.783$
	Reference Memory	$r_s = 0.163$ $P = 0.335$	$r_s = -0.096$ $P = 0.570$
Reversal Phase	Working Memory	$r_s = 0.230$ $P = 0.176$	$r_s = 0.167$ $P = 0.330$
	Reference Memory	$r_s = 0.139$ $P = 0.418$	$r_s = 0.176$ $P = 0.305$

<sup>a</sup> The slope of the WM and RM performance in 10 successive blocks of 4 trials.

<sup>b</sup> The number of sessions needed to reach criterion of discrimination in the JBT

<sup>c</sup> The mean Area under the Curve across the five tone-cues

pooled data, the difference in age and breed of the animals between both studies may artificially increase the correlations (van der Staay et al., 1990).

## RESULTS

### Study 1

A considerable percentage of the variation in the increase in WM (59.14%) and nearly the entire variation in the increase of the RM (98.34%) in the holeboard task were covered by the linear trend components. Therefore, the linear components were considered as the measures that best reflect the increase in performance across the first 10 trial blocks, i.e. the improvement can adequately be described as a linear regression line of the form:  $y=ax+b$  (a: slope, b: intercept). Thus the slopes of both WM and RM were considered as representative of overall learning and memory performance in the holeboard task. The faster the acquisition of the WM and RM components of the holeboard task, the steeper the slope, and the better the WM or RM performance.

Slopes of WM and RM performance were not correlated for either minipigs ( $r_s = 0.095$ ,  $P = 0.823$ ,  $n = 8$ ) or conventional farm pigs ( $r_s = 0.357$ ,  $P = 0.432$ ,  $n = 8$ ). Furthermore, conditional discrimination learning in the JBT was unrelated to measures of optimism for both minipigs ( $r_s = -0.278$ ,  $P = 0.505$ ,  $n = 8$ ) and conventional pigs ( $r_s = 0.309$ ,  $P = 0.500$ ,  $n = 7$ ).

We found that for minipigs, WM performance in the holeboard was not related to sessions to criterion on the conditional discrimination task nor to optimism in the JBT. RM performance in the holeboard, however, while unrelated to optimism, was highly negatively correlated with sessions to criterion in the JBT (see Table 1), i.e. the better the RM performance in the holeboard, the fewer sessions were required to learn the conditional discrimination task (Fig. 1a).

For conventional pigs, we also found that WM was unrelated to both sessions to criterion on the conditional discrimination task and optimism in the JBT. RM performance was unrelated to sessions to criterion, but highly correlated with optimism, (see Table 1). Conventional pigs which showed better reference memory performance in the holeboard task made more optimistic choices in the JBT, (Fig. 1b).

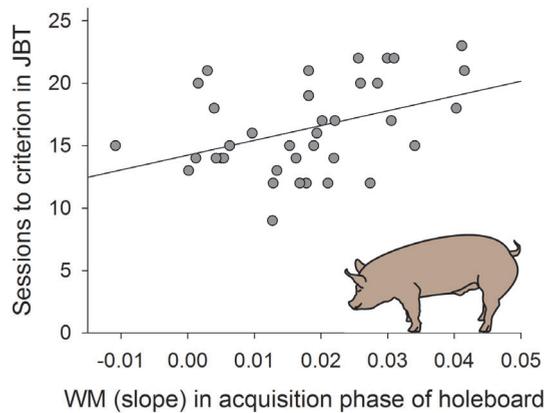
### Study 2

As with Study 1, nearly all the percentage of the variation in the increase in WM (acquisition: 82.98%; reversal: 82.02) and RM (acquisition: 99.04%; reversal: 96.63) in the holeboard task were covered by the linear trend components. Therefore, the linear components were considered as the measures that best reflect the increase in performance across the 10 blocks of the acquisition, and the 6 blocks of the reversal phase. Consequently, the slopes of both WM and RM were considered as representative of overall learning and memory performance in the holeboard task.

In the holeboard task, 37 pigs were tested in the acquisition phase, but only 36 pigs completed the reversal phase. In the JBT, all 37 pigs successfully learned the conditional discrimination task and went on to testing. Slopes of WM

and RM performance were not correlated for the acquisition phase ( $r_s = 0.223$ ,  $P = 0.184$ ,  $n = 37$ ) but there was a tendency for a correlation in the reversal phase ( $r_s = 0.301$ ,  $P = 0.074$ ,  $n = 36$ ). Furthermore, conditional discrimination learning in the JBT was unrelated to measures of optimism ( $r_s = 0.004$ ,  $P = 0.983$ ,  $n = 37$ ).

In the acquisition phase, we found that working memory performance in the holeboard moderately correlated with sessions to criterion on the conditional discrimination in the JBT, i.e. pigs with better working memory performance in the holeboard took longer to reach criterion on the conditional discrimination task (Fig. 2). Working memory was unrelated to optimism. Reference memory performance was unrelated to either sessions to criterion and optimism in the JBT (see Table 1). In the reversal phase, neither working memory nor reference memory in the holeboard task was related to sessions to criterion or optimism in the JBT (see Table 1).



**Fig. 2** Faster acquisition of the working memory component in a Spatial Holeboard task (expressed as slope of the increase in WM performance across 10 successive blocks of 4 trials) was correlated with poorer conditional discrimination learning in a JBT (as expressed by the number of sessions to criterion) in conventional farm pigs ( $n = 37$ ).

## DISCUSSION

In the present study we aimed to investigate whether ‘optimism’ in a JBT, reflected by expectation of positive outcomes, was affected by subtle differences in learning ability as measured by reference and working memory performance in a spatial holeboard task in two studies. In both studies, we found that working memory and reference memory were best represented by the linear increase or decrease in performance across successive blocks of trials over time as measured by the slope. Furthermore, we have demonstrated the independence of these two measures in pigs; reference memory and working memory in a spatial holeboard task were unrelated in both experiments. This

confirms previous findings from spatial holeboard-type studies with pigs (Gieling et al., 2011) and rats (Prickaerts et al., 1999; van der Staay et al., 1990).

In the first study we compared learning and memory in the holeboard with learning and responses to ambiguity in a JBT in two breeds of pig, Göttingen minipigs and conventional farm pigs. In this study, we found differing relationships between the variables in the two different breeds. In minipigs, better reference memory performance in the holeboard was associated with better conditional discrimination learning. However, in conventional pigs, we found that better reference memory performance in the holeboard was associated with greater optimistic responses in the JBT. Thus, while the ability to retain the rules of a task was, as predicted, related to faster discrimination learning for minipigs, it was, contrary to expectation, related to higher optimism in conventional pigs. In this experiment, we did not find any relationship between working memory and learning or performance in the JBT. However, in this first study, because of breed differences in learning (Murphy et al., 2013a), the analyses had to be performed separately per breed group. Without replication of these findings using a larger sample size, it is difficult to generalise these findings to the population of minipigs or conventional pigs.

In a second study, reference memory performance in acquisition of a spatial task was, contrary to prediction, unrelated to conditional discrimination learning, but also, as predicted, unrelated to optimism as measured by the JBT. Working memory during acquisition was inversely related to learning in the JBT, i.e. pigs with better working memory performance in the holeboard needed more sessions to reach criterion on the conditional discrimination task, but was unrelated to optimism. Both reference and working memory in reversal learning in the holeboard were unrelated to behaviour in the JBT.

Thus with a larger sample size we failed to replicate the results from conventional pigs found in Study 1. However, Studies 1 and 2 were not exact replicates and therefore some methodological variations may have contributed to the different findings. Most notably, the outcome of ambiguous trials in the JBT differed between the two studies; in Study 1, ambiguous cues were unrewarded while in Study 2 ambiguous cues were rewarded as expected. We have previously argued (Chapter 04, Murphy et al., 2013b) that by not rewarding ambiguous cues pigs may learn faster about the nature of these cues, and tentatively demonstrated that optimistic responses to ambiguity across repeated presentations reduce when ambiguous cues are unrewarded but do not change if ambiguous cues are rewarded as expected (Appendix D). Studies in a variety of mammal species have found that the behaviour and physiological responses which occur after a signalled positive reinforcer is not given can be termed '*aversive and emotional*' (Papini, 2002). For example, increased plasma corticosteroid levels and aggression have been demonstrated in response to unexpected omission of reward in pigs (Dantzer et al., 1980). One may expect, therefore, that faster learning would be related to fewer optimistic responses. In contrast to this prediction, conventional pigs with better reference memory performance in Study 1 showed more optimistic responses to non-

rewarded ambiguous cues. Further studies and larger sample sizes would be necessary to tease apart the links between reference memory and responses to unexpected omission of reward.

Only with minipigs did we find the expected relationship between reference memory and conditional discrimination learning. In Study 2, instead we found an inverse relationship between working memory and conditional discrimination learning in conventional farm pigs. However, from observations it was clear that some pigs already demonstrated a high level of working memory at the start of the holeboard task and thus appear to show little improvement. The working memory component of the spatial holeboard task is based on natural foraging behaviour using a win-shift foraging strategy; pigs have previously been shown to acquire a win-shift task faster and perform them more accurately than win-stay task (Laughlin and Mendl, 2000). Thus pigs with an initial low working memory performance may have been recorded as overall performing better on working memory due to the difference between early and later trials.

Due to the lack of relationships found when using a larger sample size between judgement bias and learning and memory in a spatial holeboard task in pigs in Study 2, we would conclude that optimistic responses to ambiguity indicating expectation of a large reward appears to be unrelated to both working memory and reference memory in acquisition and reversal learning in a spatial task. Thus biases in judgement as measured by this task appear to be independent from cognitive capacity. The spatial holeboard task and the JBT consequently measure different behavioural domains in the same animal.



# **CHAPTER 08**

## **GENERAL DISCUSSION**

The aim of this thesis was to develop and validate appropriate behavioural tasks to study emotion in pigs using cognitive paradigms. In order to accurately assess animal welfare, these tasks ought to directly address the question of what an animal wants and feels in the different contexts within which the pig is used. We aimed to validate the tasks using a variety of different approaches and demonstrate the specificity with which they target emotional responses. This discussion will focus on the three steps which were taken to achieve the above aim, namely: an evaluation of current approaches, task design and development, and finally, task validation. Our tasks will be evaluated with respect to theoretical expectations as well as in practical applications, i.e. experiments addressing the effects of experimental manipulations on pigs' emotion.

## **EVALUATION OF CURRENT APPROACHES**

In Chapter 02, we reviewed the variety of behavioural measures which have been applied to the study of emotion in pigs. We provided a list of criteria by which the relevance and validity of such measures can be assessed. In summary, we suggest that behavioural tests of emotion in pigs should (i) rely on ethologically-relevant responses, (ii) allow for the display of natural emotion-related behaviours, (iii) specifically and unambiguously target emotional behavioural responses, (iv) be sensitive enough to measure subtle differences, (v) be suitable for repeated testing, (vi) be familiar to the animal to avoid effects of novelty or stressful procedures influencing results, (vii) standardised, and ultimately (viii) automated (Murphy et al., 2014).

It was clear that while a wide variety of behavioural approaches to study emotion has been undertaken and proposed, many of the above criteria are not met. In particular, a large number of the behavioural tests commonly used to assess emotion or fear responses in pigs were based on assumptions which were not always ethologically-relevant or appropriate for pigs. Also, within-tests, tasks relied on broad measures making the identification of emotion-specific responses difficult. For example, activity levels or interference from the lack of habituation to test procedures may have contributed to the findings. In fact, across studies there was little standardisation of task design and outcome measures; outcome measures were interpreted in different ways or in the same way despite widely differing task designs. While other reviews have come to similar findings with regard to the more common behavioural tests (see Forkman et al., 2007; Lind et al., 2007), we expanded our review to incorporate specific behaviours and behavioural patterns which may provide avenues for future research in studying emotion behaviours in pigs.

As discussed in Chapter 01, few common behavioural tests are designed to specifically assess positive emotions, a key issue for animal welfare evaluation (Yeates and Main, 2008). One approach which purports to do so is the study of emotion through influences on cognition. Furthermore, such approaches also meet the first criterion proposed in Chapter 02; cognitive biases have an adaptive value across species, through guiding behaviour (Peters et al., 2006), and should, therefore, be based on ethologically-valid assumptions. However, the lack of standardised test designs for studying emotional influences on different cognitive processes meant that first we had to develop a suitable task.

## **EMOTION AND DECISION-MAKING UNDER UNCERTAINTY**

In natural situations, decision-making occurs under conditions of uncertainty (Kacelnik and Bateson, 1997) where the outcome is not definite. The outcome may be uncertain because of different probabilities associated with each choice (decision-making under risk), or because the outcome of each choice is unknown (decision-making under ambiguity) (Bechara et al., 2005; Krain et al., 2006). The role of emotion in these decision-making processes under uncertainty is to guide behaviour by giving different value to the options, or shifting attention to salient information, or even motivating the decision-making process (Peters et al., 2006). For example, negative mood may signal an unstable environment, and facilitate recall of negative events, and thus guide towards more cautious decision-making strategies (Schwarz, 2000). Emotional disorders can lead to biases in decision-making processes, where the choice made is no longer adaptive, for example individuals with dispositional anxiety show extreme risk avoidance behaviour (Maner et al., 2007). Where biases result in consistent mood-congruent effects on decision-making under uncertain conditions, these effects have been proposed as a proxy measure for the valence of emotional states in animals (Harding et al., 2004; Mendl et al., 2009; Paul et al., 2005). These cognitive paradigms represent a promising approach to the study of emotion in animals.

## **DECISION-MAKING UNDER AMBIGUITY**

### **Judgement Bias Task (JBT) Development**

The first step in developing a suitable judgement bias task (JBT) for pigs is choosing an appropriate task design. A variety of designs have been used with different species, which differ in a number of key features as discussed in Chapter 01. The main features which differ between test design, i.e. cue-type, outcome measures, and the reinforcement of cues, are discussed here with respect to developing a task for pigs.

### *Cue-Type*

One of the essential criteria of behaviour tests of emotion is that the task is ethologically valid (Chapter 02). Thus the task design ought to take into account the sensory and cognitive capabilities of the animal under study. To develop a suitable JBT for pigs, the discriminative capabilities of the pig ought to be considered, since the measurement of responses to ambiguity is contingent on the ability of the animal to first learn, and then discriminate, a 'positive' and a 'negative' cue. Positive and negative most commonly fall along the same physical axis (Gygax, 2014), e.g. colour gradient, tone frequency, or spatial location, such that ambiguous cues also fit along this axis, intermediate between positive and negative cues.

Göttingen minipigs were easily able to learn a simple black/white discrimination task where incorrect responses were punished with a time-out (Moustgaard et al., 2004), but required up to 520 trials to learn a conditional discrimination task using the same cues (Moustgaard et al., 2005). Pigs' ability to learn a visual discrimination using abstract geometric shapes is also limited. Gieling et al. (2012) found that it took piglets 337

trials on average to learn a simple discrimination task using a visual stimulus, and a further 98 trials on average to learn a simultaneous discrimination of simple geometric shapes. Zonderland et al. (2008) found that pigs have much poorer visual acuity for details and shapes compared with humans. For example, both wild boar and piglets have been shown to be able to recognise blue colours, but not to be able to distinguish between other primary colours (Eguchi et al., 1997; Tanida et al., 1991). While pigs can discriminate based on colour or shape, such discrimination learning appears to be time consuming and subject to high individual variation; they may first have to learn to pay attention to the cue. It is likely that pigs rely more on non-visual cues compared with other domestic species such as cattle (Lomas et al., 1998).

Pigs have a well-developed spatial ability; they can learn to avoid revisits to unrewarded locations between trials (spatial reference memory) and revisits to locations where rewards have already been consumed within trials (spatial working memory), while searching for food in a free choice holeboard maze (Arts et al., 2009; Gieling et al., 2011), and can discriminate between locations based on differing amounts of reward (Held et al., 2005). Olfaction is also well-developed in pigs; they can learn to discriminate odour cues predicting positive and negative outcomes (Meese et al., 1975), and will learn discrimination tasks based on odour cues faster than visual discrimination tasks (Croney et al., 2003).

Auditory range in pigs, from 42 Hz to 40.5 kHz (Heffner and Heffner, 1990), is greater than that of humans. Pigs can successfully discriminate between auditory signals predicting positive and negative situations (Imfeld-Mueller et al., 2011), and can learn to distinguish personalised tone-sequence-signals indicating the availability of reward (Ernst et al., 2005). Thus auditory, spatial or olfactory cues may be most appropriate for pigs in a judgement bias task than visual cues. Indeed, we found that pigs could, relatively easily, learn to discriminate between positive and negative pure tones which differed in frequency (Chapters 04-06). Furthermore, the selection of tone-cues allowed us to choose training (positive, negative) and ambiguous cues which fall on the same physical axis (Gygax, 2014), and to choose ambiguous cues where the location on this axis with respect to the training cues could be objectively quantified.

### ***Outcome Measure: Go/No-Go vs. Active Choice***

In Chapter 03 (Murphy et al., 2013a) we looked at discrimination learning of pigs using two different task designs; go/no-go style learning and an active-choice task. The first task we developed was based on a go/no-go design, where a positive tone-cue signalled the presence of a reward in a goal-box at the end of a runway, while a negative tone-cue signalled that the goal-box was empty. The outcome measure used in this task was latency to reach the goal box based on the assumption that pigs would continue to respond to positive cues but would, with exposure over time, reduce the speed of response to the negative cue or would stop responding. To run a strict go/no-go task where the outcome measure would reflect the choice of response, go or no-go, it was expected to require extensive training as reported by Moustgaard et al. (2005) unless a strong negative reinforcer was used.

Common go/no-go JBTs in animals are often based on spatial designs where the physical location of a goal pot, e.g. left vs right side of test arena, is used as the discrimination criterion. In such designs, the latency of responses can artificially mimic the expected gradient of responses to the different cue-types if an animal develops a pattern of always heading towards the positive location, then moving towards the actual location of the goal-pot in test sessions. To avoid this potential issue, we developed a go/no-go style task using a single runway so that the route to the goal-box was the same for each cue-type. While we did find that some pigs, with time, decreased their latency to respond to negative cues, this behaviour was not consistent between and within individuals (Chapter 03, Murphy et al., 2013a). This made it difficult to decide on a moment of 'learning' after which the presentation of ambiguous cues could begin. Furthermore, responses in cognitive tasks may also be susceptible to differences in impulsivity (Bizot and Thiébot, 1996); impulsive action can occur in response to both negative and positive emotions (Cyders and Smith, 2007). Thus, impulsive responses to ambiguous cues in latency-based and go/no-go tasks can confound the measures used to distinguish between positive and negative judgement biases. Pigs have previously been shown to have different response styles of impulsivity (Melotti et al., 2013; Nawroth et al., 2013); pigs which respond too quickly in discrimination tasks may make more mistakes as they do not pay attention to salient cues (Nawroth et al., 2013; Zonderland et al., 2008).

A second test design we developed was based on active choices. Pigs had to make an active response to both positive and negative tone-cues by choosing the correct goal-box. Outcome measures of optimistic responses on this task were not based on the response latency but on which goal-box was chosen. With some adaptations to the initial design (Chapter 03), we found that all pigs were able to learn the conditional discrimination task between positive and negative cues, and furthermore that the point of 'learning' for each individual was clear, being based on a simple criterion of performance on the discrimination task. Such tasks may avoid the interference of impulsive action, and furthermore, allow animals a third possible response to ambiguous cues, an omission, which is not classed as either an optimistic or pessimistic response.

### ***Reinforcement of cues***

In both go/no-go and active choice designs, there is no standard reinforcer for the 'positive' and 'negative' cues. In active-choice designs reinforcers must be clearly different in value for the animal and must encourage an animal to perform an active response. While the responses to the positive cue most often yield a food reward, to get an animal to actively respond to the negative cue, two approaches have been used; a response can yield a less palatable reward (Brydges et al., 2011; Keen et al., 2014) or a response can prevent the occurrence of a negative event (Enkel et al., 2010). Evidence from human research suggests that learning to avoid an aversive stimulus can actually be positive. Kim et al. (2006) found that the neural activity responses when people successfully prevented an aversive outcome are similar to when they received explicit rewards. We therefore chose the former option, i.e. to reward both the positive and negative cues with a reinforcer which did not differ in quality, but in quantity. In

our design, training was facilitated by using a mild-negative outcome; incorrect choices and omissions, regardless of the trial-type (positive or negative) resulted in a time-out penalty.

A second issue about reinforcement relates to the outcomes of ambiguous trials. One of the issues with current judgement bias methods is their unsuitability for repeated testing (Doyle et al., 2010; Chapters 04-06, Murphy et al., 2013b) as animals quickly learn about outcomes in ambiguous trials, rendering them no longer ambiguous. To prevent some level of reinforcement learning, it is most common to not reward ambiguous cues in judgement bias studies. To avoid that the non-reward of ambiguous cues would come as a surprise, some studies have introduced a partial reinforcement schedule once animals had learned to discriminate positive and negative cues (Matheson et al., 2008). In our active-choice design, we have instead proposed to reward ambiguous cues 'as expected' after witnessing that pigs will quickly alter their responses to ambiguous cues across repeated presentations (Chapter 04 Murphy et al., 2013b). Unexpected omission of signalled reward has been said to induce negative affect (Papini and Dudley, 1997; Papini, 2002), for example increased stress response and aggression in pigs (Dantzer et al., 1980), and thus facilitate the storage of such events in memory (Papini, 2003). In Appendix D, we tentatively support these findings; in our studies, pigs altered responses to ambiguous cues within a test, evident of learning, when ambiguous cues were unrewarded but did not change when ambiguous cues were rewarded.

### *Theoretical expectations*

Gygax (2014) lists five theoretical expectations which ought to be fulfilled in a JBT design. These expectations are based on go/no-go designs. They have slightly been adapted to evaluate our active-choice task for the purpose of this discussion. The first four expectations relate to the task design and will be discussed here. The fifth expectation is related to the validity of the task and will be discussed below.

While in a go/no-go design animals should (i) not respond to the negative cue but (ii) respond to the positive cue, in our active-choice design, we propose that animals should (i) show pessimistic responses to the negative cue but (ii) optimistic responses to the positive cue. In testing animals should (iii) show a '*monotonic graded response*' from negative to positive cues. In testing judgement biases, groups should (iv) not differ in their responses to the training (positive and negative) cues (adapted from Gygax, 2014).

Regarding assumptions (i) and (ii), we found that pigs could, relatively easily, learn to discriminate between positive and negative tone-cues which predicted the location of large and small food rewards (Chapters 04-06). When ambiguous cues were then introduced, we found that responses to ambiguous cues fell along the gradient between responses to the positive and negative cues; indeed as ambiguous cues neared the positive cue in tone frequency, we found increasing optimistic responses as would be expected by assumption (iii). Interestingly, latency to choose almost mirrored optimistic responses, with decreasing latencies across cues from negative to positive (Chapters 04-06). Latency, however, was found to be susceptible to breed differences

(Chapter 04, Murphy et al., 2013b) and seasonal influences (Chapter 06), and did not accurately reflect group differences in optimistic responses on the JBT (Chapter 05). This corroborates findings from Mendl et al. (1997) that pigs' latency to respond in spatial tasks can be disrupted by disturbances, further corroborating the notion that latency to respond may not be a reliable measure of judgement bias. Optimistic choice in our JBT was not susceptible to such influences and we suggest therefore that this is a more robust measure. In test sessions, behavioural responses to the training cues did not differ; pigs continued to discriminate positive and negative cues at the level of performance required to reach criterion. Thus our task design meets all four relevant theoretical assumptions as described by Gygas (2014).

### **Judgement Bias Task Validation**

The fifth assumption with respect to theoretical expectations of JBTs according to Gygas (2014) is that animals expected to differ in emotional state should differ in the slope of responses across the ambiguous cues. In order to test this assumption, subjects that are expected to differ in emotional state ought to be selected.

#### *Predictive Validity*

As discussed in Chapter 01, studies have tried to show predictive validity of their JBT using a variety of experimental manipulations of short and long term emotion and mood, for example, pharmacological stimulation (Doyle et al., 2011) or environmental enrichment (Brydges et al., 2011; Douglas et al., 2012; Richter et al., 2012). Others have chosen groups based on existing differences in the population, for example, stereotypic behaviour (Brilot et al., 2010) or separation anxiety (Mendl et al., 2010).

We tried to validate the JBT in experiments where we manipulated the emotional state of pigs, using a short-term restraint stressor (Chapter 04, Murphy et al., 2013b); as well as looking for naturally occurring differences in low- and normal-birth-weight pigs (Chapters 03, 05 and 06). Furthermore, we were interested in whether effects in these naturally occurring populations could be alleviated pharmacologically (Chapter 06).

In Chapter 04 we found no effect of the short-term manipulation, restraint treatment, on responses to ambiguity in the judgement bias paradigm. However, due to methodological issues, for example the effect of repeated testing as discussed in Chapter 04, it cannot be concluded that the test is not suitable to assess the effect of short-term manipulations of emotional state.

#### *The low-birth-weight pig model*

Our selection of the low-birth-weight (LBW) pig as a model to validate our JBT was based on a number of considerations. Most importantly, evidence from human research suggests that LBW children are more at risk for developing anxiety problems and depression (Hayes and Sharif, 2009; Lahti et al., 2010; Raikkonen et al., 2008). LBW in piglets is associated with increased adrenocortical function and HPA reactivity even at three months after birth (Klemcke et al., 1993; Poore and Fowden, 2003), suggesting that these animals have a heightened stress reactivity (Rutherford et al., 2013). Thus it

was expected that LBW pigs would differ in emotion and mood compared to normal-birth-weight pigs.

Extending from this, LBW is a potential welfare issue in piglets; LBW is increasing due to selection for larger litter sizes (Milligan et al., 2002; Quiniou et al., 2002). While many LBW piglets do not survive (Baxter et al., 2008), surviving LBW piglets are at greater risk of health problems (see review by Rutherford et al., 2013). Tasks which can investigate whether these issues contribute to reduced welfare in these animals are therefore important.

Alongside the potential difference in emotionality, LBW is also associated with cognitive deficits in humans (Anderson and Doyle, 2004; O’Keeffe et al., 2003), and early research within our group suggested such deficits could also be identified in LBW pigs (Gieling et al., 2011). Choosing a model which also potentially differed in learning ability would allow us to test whether the discrimination learning necessary for our JBT was an impediment to applying our task in different contexts.

In a pilot study (Appendix A and Chapter 03, Murphy et al., 2013a), we found that LBW piglets which were able to learn the discrimination between positive and negative cues, also showed fewer optimistic responses to the ambiguous cue closest to the positive cue (see Appendix A). This finding suggested that LBW pigs did indeed differ in optimism, indicative of more negatively-valenced emotion in these animals compared with a normal-birth-weight (NBW) control group. However not all animals learned the task and thus the sample sizes available for statistical analysis were very small. A follow-up study (Chapter 05, Murphy et al., In Press) again found that LBW pigs showed in general fewer optimistic responses, but not in response to specific ambiguous cues compared with NBW controls. However, a third experiment (Chapter 06) failed to confirm the results of the previous two experiments; LBW and NBW pigs did not differ in optimistic responses to ambiguous cues. Furthermore, in this third experiment, physiological measures of chronic stress did not differ between LBW and NBW pigs, which suggests that LBW pig in this study did not experience heightened levels of stress compared to NBW pigs. Some possible reasons for this, discussed in Chapter 06, include differences in past experience and the gender composition of the groups.

If these factors do indeed alter emotionality to such a degree, then this implies that the welfare consequences of LBW may be easily overcome, or may not last into later life. Alternatively, while we did find evidence that LBW pigs show behavioural responses indicative of more negative emotion within the framework of the judgement bias paradigm, this effect was not consistent across studies suggesting that LBW in pigs does not reliably lead to higher levels of negative emotion later in life. As discussed in Chapter 05, comparisons between surviving LBW piglets and LBW in humans may not be appropriate due to the fact that these piglets survive without the level of postnatal care provided to human LBW infants.

Because of the lack of differences between groups in Chapter 06, we were further unable to show any effect of the neuroprotective drug, allopurinol, on potential negative consequences of LBW. Such effects if found, would have strongly supported the predictive validity of the task.

With the exception of a pilot study (Appendix A), we did not find any differences in conditional discrimination learning between LBW and NBW pigs which would have confirmed the existence of cognitive deficits. A recent study (Antonides et al., submitted) failed to replicate cognitive deficits in LBW pigs reported by Gieling (2011). Consequently, we cannot draw any definite conclusions about how well our task can be learned when groups do differ in cognitive ability, for example, age related deficits. However, as the task, in its final form (Chapters 04-06) was consistently learned by all animals, we can say that it is suitable for testing pigs without deficits.

***Construct validity: does our judgement bias task appear to specifically measure emotional responses?***

The judgement bias paradigm has been proposed as one of the few measures to specifically discriminate between positively- and negatively-valenced emotional states in animals (Mendl et al., 2009; Paul et al., 2005). As no standard test of emotional-valence in pigs exists, it is difficult to assess the construct validity of the JBT. One of our criteria for behavioural tests of emotions in pigs developed in Chapter 02 (Murphy et al., 2014) is that a task should '*specifically and unambiguously capture emotion-related behaviours*'. One approach to assessing the construct validity of our JBT is therefore to demonstrate the independence of responses to ambiguity from potential confounding factors such as motivation, activity levels, or learning.

As discussed previously, by not relying on latency measures, responses in our JBT are less susceptible to interference from confounding variables such as impulsivity and environmental disturbances. Optimistic choice does not depend on the speed of response and therefore should be independent of activity levels. Further, differences or changes in motivation could be assessed by consistency in response to the training cues or even by learning in the conditional discrimination task.

In each application of our JBT we were able to demonstrate that learning the conditional discrimination paradigm was unrelated to pigs' optimistic responses. In Chapter 07, we explored the relationships between learning ability, using a task designed to distinguish between learning based on trial-specific working memory and test-specific reference memory and the training phase of the JBT, where the pig had to acquire a conditional discrimination to a predetermined criterion. In a first study we found the unexpected result that reference memory, i.e. memory for the rules of the holeboard task (van der Staay et al., 2012) was related to optimistic responses in conventional pigs in the JBT (Chapter 07). However it was argued that due to the low sample size this result may not reflect relationships beyond the specific group of animals used in the study. In a second study, using a larger sample size, we did not find any relationship between working memory and reference memory in either task acquisition or task reversal learning with optimistic responses to ambiguity (Chapter 07). Based on this finding, we conclude that optimistic responses in our judgement bias test are not dependent on learning ability.

In Chapter 05 we investigated behaviour in the JBT with responses in a second task we developed, the Pig Gambling Task (PGT), see below. We found responses to ambiguity

in the JBT not to be related to choices in the PGT, confirming that the two tests are measuring different facets of decision-making; risk and ambiguity.

In Appendix A and Appendix C, we investigated the relationship between optimistic responses in our JBT and a measure of coping styles in pigs (Hessing et al., 1993). The backtest is proposed as a potential measure of personality in pigs (Forkman et al., 1995; Melotti et al., 2011; Ruis et al., 2000). In two studies (Appendix A and C) we found contrasting relationships between pigs classed as either high- or low-resistant with optimistic responses. However, due to low sample sizes, and the narrow range of responses in measures of the backtest, little can be concluded about the independence of personality and measures of emotion in our JBT.

### **Judgement Bias Task - Evaluation & Future Applications**

We have demonstrated that our JBT meets all theoretical expectations of such tasks as proposed by Gygax (2014). Furthermore, returning to our criteria for evaluating behavioural tasks of emotion in pigs proposed in Chapter 02 (Murphy et al., 2014), we have designed the task taking into account species-specific capabilities and using ethologically relevant behaviour. We have gone some way towards demonstrating both predictive and construct validity of our JBT.

In terms of practical application, we have demonstrated that animals from the two target populations of pigs with respect to welfare interests, namely laboratory (minipigs) and farm (commercial) breeds, are capable of learning our task (Chapter 04, Murphy et al., 2013b), and despite differences in learning and speed of responding, behave similarly in response to ambiguous cues.

However, some methodological problems still exist. On a theoretical level, we have not demonstrated the ability of our task to detect subtle differences in emotional responding. A major restriction on current methods of judgement bias is that only limited repeated presentations of ambiguous cues can be performed. This limitation reduces the sensitivity of the task. While we have demonstrated that rewarding as expected can slow down learning about ambiguous cues (Appendix D), it is not likely to stop some learning altogether. Thus, the design we have proposed has limited applicability for repeated measures, and therefore cannot be used to investigate changes over time. Suggestions to increase the number of ambiguous cues possible are given in (Chapter 04, Murphy et al., 2013b), such as increasing the ratio of training cues to ambiguous cues. A further option may be to introduce some level of variability to the training cues (pers. com. Prof. Hanno Würbel) so that the novelty-value of ambiguous cues is reduced due to expectation of some level of variability.

With respect to practical applications, our task has limited suitability for use outside the research setting. The length of time needed to learn the discrimination before the test can be applied means that the task, as is, has little applicability on-farm/in-lab short welfare assessments.

## **DECISION-MAKING UNDER RISK**

### **Pig Gambling Task Development**

While the relationship between decision-making under risk and emotional valence is not as straightforward as for decision-making under ambiguity (see Chapter 01) we were interested to see whether risky decision-making paradigms offer an opportunity to study emotion in animals. The Iowa Gambling Task (IGT), first developed to study risky decision-making in humans by Bechara et al. (1994), is said to measure emotion based choices in a natural setting. In the IGT, participants have a choice of four options which offer rewards or losses. Two options are primed to be long-term advantageous (offering short-term low rewards but small chance of loss) and two are primed to be long-term disadvantageous (higher short-term rewards but high chance of loss). Positive moods are said to lead to an earlier preference for the advantageous options, and negative moods are associated with more disadvantageous choices (de Vries et al., 2008; Suhr and Tsanadis, 2007). Thus we were interested in whether similar affects could be found for pigs.

In order to adapt the IGT to pigs, we looked at rodent versions of the task. To model the wins in the IGT, rodent studies have generally replaced monetary gain with food reward (de Visser et al., 2011). However, different approaches have been taken to model losses including using unpalatable food (Hombert et al., 2008; Koot et al., 2010; 2013; van den Bos et al., 2006), time-out penalties (Rivalan et al., 2009; Zeeb et al., 2009), or the probability of reward (Pais-Vieira et al., 2007; 2012). Studies on taste preferences shows that pigs tend to prefer control solutions to those containing 0.025% quinine sulphate (Nelson and Sanregret, 1997) and discriminate against diets containing concentrations of quinine sulphate above 0.05% (Leamaster and Cheeke, 1979). Thus we initially proposed to use quinine treated rewards to model the losses in our Pig Gambling Task (PGT).

Furthermore, to model short vs. long-term differences of the options in the IGT we used variable amounts of rewards and altered the probability of win and loss trials associated with each choice based on a rodent version of the IGT (Koot et al., 2010). To simplify the task for pigs we reduced the number of options from four to two as has been done in versions for children (Kerr and Zelazo, 2004) and rodents (Pais-Vieira et al., 2007).

### **Pig Gambling Task Validation & Future Directions**

The two main assumptions of the IGT are (i) that in a normal healthy population, individuals should show an increasing preference for the advantageous options over time, and (ii) in populations where decision-making is impaired, this preference will take longer or not occur within the task time-frame. In piloting our PGT with a sample of normal healthy pigs, we failed to meet the first assumption (see Appendix B). Pigs did not show an increase in preference for the advantageous option. One reason for this is that it was observed that some pigs continued to eat or increased consumption of the quinine-coated rewards. As discussed in Appendix B, it is likely that some pigs overcame any initial aversion to the quinine flavoured rewards once no negative consequences

occurred (Held et al., 2009). Thus we would conclude that using bitter flavoured rewards for pigs is not appropriate to model IGT losses.

By altering the original task design (see Chapter 05) we had more success in meeting the first assumption of the IGT. Instead of using normal food and quinine-flavoured food to model wins and losses, we manipulated the accessibility of the food rewards. So for a win trial, after a choice was made, rewards were delivered into a food bowl from which a clear Perspex perforated lid was lifted so that the pig could eat. In a loss trial, rewards were again delivered into the bowl, but the lid remained in place above the bowl so that pigs could not access the reward underneath. In this design we saw increasing advantageous choices over time.

However, one difference between pigs and rodent and human versions we found was that pigs did not appear to sample between the options in our pilot (Appendix B). Therefore we had to introduce an extra training sessions where pigs were introduced to the different quantities of reward associated with each option, meaning that at the start of the task they already had some knowledge of the potential short-term outcomes of each option. In IGT studies in humans, the pre-programmed schedules are unknown to the subject (Bechara et al., 1994). However, similar to human and rodent versions pigs were not exposed to the long-term outcomes of each option until the test itself. Further differences between animal and human studies is the training needed to get animals to perform the task in the first place.

To test if the second assumption of the IGT was met in our PGT, we attempted to validate our PGT using a sample group of pigs expected to show higher levels of negative affect; as with the JBT, we used the LBW pig model. While we did find that LBW pigs differed from NBW pigs in their performance on the PGT, contrary to prediction we found that they showed a preference for the advantageous option in later blocks of trials, not found for NBW pigs (Chapter 05). Possible reasons for this are discussed in Chapter 05, where it is hypothesised that our PGT more closely resembles traditional decision-making tasks in animal studies than accurately models the IGT. Thus responses of LBW pigs more resemble risk avoidance, and may reflect a different behavioural strategy due to size differences between LBW and NBW pigs (Dingemans and Wolf, 2010). These same animals showed more pessimistic responses in the JBT, suggestive of more negative states in LBW pigs. It is possible therefore, that negative states contributed to the risk avoidance strategy adopted in the PGT. Due to the different theoretical approaches that underlie the relationship between emotional valence and risky decision-making, we conclude that the PGT may be less suitable for assessing emotional valence in animals than the judgement bias paradigm.

## **CONCLUSIONS**

We developed two cognitive tasks to study two facets of decision-making under uncertainty in pigs; decision making under ambiguity (Judgement Bias Task), and decision making under risk (Pig Gambling Task). Both tasks were developed and adapted taking into account ethologically-relevant abilities and behavior of pigs, and both met the intrinsic theoretical assumptions of the cognitive paradigms upon which they were based.

In a series of experiments to validate the tasks, we demonstrated that the JBT can identify differences as predicted in groups of pigs expected to differ in emotional valence, whereas the PGT showed that groups differed contrary to original predictions, possibly due to divergent behavioural strategies. We further demonstrated that the behavior on the JBT was independent of learning ability, motivation, decision making under risk and environmental effects, suggestive of construct validity with respect to specifically targeting emotional responses. Therefore, we conclude that our JBT represents a promising and valuable tool for the assessment of welfare in pigs through the measurement of emotional valence.



# APPENDIX A

## PILOT STUDY I: DEVELOPING AN ACTIVE-CHOICE JUDGEMENT BIAS TASK FOR PIGS, *SUS SCROFA*: ARE LOW-BIRTH- WEIGHT PIGLETS MORE PESSIMISTIC?

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## INTRODUCTION

Evidence from human research indicates that emotion can influence a wide variety of cognitive processes such as decision making (de Vries et al., 2008), judgement (Blanchette and Richards, 2010), memory and attention (Mackay et al., 2004). Consistent mood congruent biases have been found for judgement of ambiguity (Blanchette and Richards, 2010) i.e. people in a positive mood are more likely to judge an ambiguous stimulus as potentially positive while the reverse is true for people in a negative mood. Judgement bias has therefore been proposed as a potential way to study emotional valence in animals (Mendl et al., 2009), and results of the first judgement bias study in animals study indicated that this method had promise (Harding et al., 2004). Since then, judgement biases have been studied with varying success in a variety of species.

However, no standard judgement bias test exists and the methods used vary depending on the study and the species under study. Only a few published studies of judgement bias for pigs have been performed (Douglas et al., 2012; Döpjan et al., 2013; Scollo et al., 2014), each of which used a Go/No-Go style task whereby pigs were trained to make an active response in response to the “positive” cue in order to receive a reward, and to refrain from responding in response to a negative cue to avoid a punishment (no food, negative outcome). We have previously found that the more common Go/No-Go style tasks can be difficult to interpret and do not offer an opportunity for an animal not to respond (Chapter 03, Murphy et al., 2013a).

We wanted to develop an active-choice judgement bias task for pigs whereby an active choice is required in response to both positive and negative cues. Active-choice tasks have previously been used in a number of species including starlings (Brilot et al., 2010) and rats (Brydges et al., 2011; Enkel et al., 2010). Such tasks first require the learning of a conditional discrimination whereby training cues are associated with a positive and a negative outcome. Responses to cues intermediate to these training cues are then used to assess judgement bias. In order to test our task we wanted to choose a model which may naturally differ in emotion as opposed to using a treatment to induce changes in mood. Low-birth-weight (LBW) in humans has been found to have long lasting effects on cognitive, behavioural and emotional outcomes (Hayes and Sharif, 2009; Lahti et al., 2010; O’Keeffe et al., 2003; Raikkonen et al., 2008). LBW occurs spontaneously in pig litters and is on the increase due to selection for bigger litter sizes (Beaulieu et al., 2010; Milligan et al., 2002). Thus, the LBW pig may be a useful model to assess whether firstly, possible cognitive deficits affect learning of the active-choice task, and secondly whether potential negative emotional states in pigs contribute to more pessimistic judgement of ambiguous stimuli.

Selection of LBW and NBW piglets was done shortly after birth. Then coping styles (Koolhaas J.M. et al., 1999) using the backtest (Bolhuis et al., 2004) were measured as an indication of personality in pigs (Melotti et al., 2011). After weaning, pigs were trained to discriminate ‘positive’ and ‘negative’ tone-cues, predicting large and small rewards respectively, by performing an active response to each cue. Pigs that learned this discrimination were then tested using intermediate tone cues and their responses were recorded. We predict that LBW pigs will show a more pessimistic bias than NBW pigs.

## METHODS

*Ethical Note:* The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

*Subjects and Housing:* We used 11 male piglets (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) bred at the farm of the Faculty of Veterinary Medicine of Utrecht University. Six normal-birth-weight (NBW) and 5 low-birth-weight (LBW) male piglets were selected from 3 litters according to the criteria of Gieling et al. (2011). All pigs from 3 litters were weighed within 12 hours of birth. LBW piglets were selected as those that weighed at least 1SD less than the litter mean. All LBW pigs were then excluded and the litter mean recalculated. NBW pigs were selected as those that were closest to the new litter mean. Pigs were six weeks old at the start of the study. Pigs were group-housed in one straw-bedded pen (15 m x 3 m) with a nest area and toys for enrichment. Water was available ad libitum, and animals were not food restricted. Instead, they received 25% of their normal allowance before testing in the morning and the remaining 75% in the evening after testing. Pigs were weighed weekly to monitor weight gain. When the pigs were 3 months old the pen area was increased by connecting two adjacent pens.

*Apparatus:* A detailed description of the testing apparatus can be found in Murphy et al. (Chapters 03 and 04, 2013a; 2013b). We used a two choice apparatus in which pigs could choose from a right or left “goal-box” in the test arena. The start box (1.2 m<sup>2</sup>) was connected to the test arena (3.6 m x 2.4 m) via a set of swing doors controlled by the experimenter. In each goal-box was a food bowl attached securely to the floor and surrounded by 4 plastic columns at each corner. Placed on top of each food bowl was a large red hard plastic ball, which could be raised off the bowl, but not pushed off, to gain access to rewards within the bowl. Each food bowl had a false base under which rewards were placed to avoid pigs using smell cues to locate the correct goal-box. Each goal-box could be closed by the experimenter using a set of guillotine doors operated by a pulley system.

We used tones as cues in our judgement bias task generated using the open source software Audacity (<http://audacity.sourceforge.net/>). The training tone-cues used were 200 Hz and 500 Hz (Waveform: Sine; Amplitude: 1), while the ambiguous tone-cues were at equal intervals between the training tones on a logarithmic scale (251.49 Hz, 316.23 Hz, and 397.64 Hz). Tone cues were played via an mp3 player (Archos 18 Vision 4gb, Archos GmbH, Grevenbroich, Germany) through speakers (Trust SP-2200 2.0, Trust International B.V., Dordrecht, The Netherlands) attached above the start box.

*Backtest:* The backtest was performed for all pigs at 14 days of age according to the method described by Bolhuis et al. (2004). Pigs were individually taken from their pens and held in a supine position by the experimenter for 60 s. Pigs were then returned to the home pen. Pig’s responses in the backtest were recorded for later analysis. The number of escape attempts, as well as the latency to first escape, and the number of vocalisations were recorded for each pig.

*Habituation and Training:* A detailed description of the training procedure used is given in Murphy et al. (Chapter 03, 2013a Experiment 2). Pigs were trained to associate one training tone with the availability of a large reward (4 chocolate M&Ms®) in one goal-box while the second training tone predicted the availability of only a small reward (1 chocolate M&M®) in the other goal-box. In this way, one training tone and its associated goal-box were labelled “positive”, while the second training tone and associated goal-box were labelled “negative”. Only one goal-box was rewarded per trial and the meaning (positive/negative) of training tones (200 Hz/500 Hz) and goal-boxes (right/left) was counterbalanced across animals.

Pigs were trained for up to 46 sessions, one session per day. Modifications made to the training procedure were made along the way - see Murphy et al. (Chapter 03, 2013a Experiment 2). Each session consisted of 12 trials, of which two trials were “forced”, whereby only the correct goal-box, as signalled by the tone-cue played, was available per trial, and 10 trials were “free”, whereby both goal-boxes were available and pigs had to choose the correct goal-box depending on the tone-cue. Forced trials consisted of one positive and one negative trial, presented in a different random order per session, while free trials consisted of five positive and five negative trials presented in a different pseudorandom order per session with a maximum of two consecutive positive or negative trials. Tone-cues were played until a choice was made or for a maximum of 30 s. Pigs which chose the correct goal-box within 30 s received the amount of reward predicted by the tone-cue. When a pig chose incorrectly, or failed to make a choice (omission), both goal-boxes were closed and pigs remained in the test arena for a 90 s time-out penalty before commencing the next trial. To qualify for testing, pigs had to reach a criterion of performance demonstrating that they had learned to discriminate the training tones. Pigs had to respond correctly in 80% of negative and 80% of positive free trials in three out of four consecutive sessions.

*Judgement Bias Task:* Pigs which reached this criterion were then tested over four sessions, one session per day. Test sessions consisted of 14 trials; two forced and ten free trials, as before, along with two ambiguous trials (Trials 7 and 12). In an ambiguous trial, one of the three ambiguous tone-cues (termed: “AmbigNeg”, “AmbigMid”, “AmbigPos” depending upon their relationship to the negative and positive training tone-cues) was played instead of a training tone-cue for 30 s, and neither goal-box contained a reward. The AmbigMid cue was presented four times (once per test session) while the AmbigNeg and AmbigPos cues were presented twice each. Test session trial orders were balanced such that pigs had equal numbers of presentation of positive and negative tone-cues before presentations of each ambiguous tone-cue.

*Data Recording & Analysis:* Results of the training phase are reported elsewhere (Chapter 03, Murphy et al., 2013a). All analyses were carried out using the statistical package SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Responses in the backtest were analysed for weight-group (NBW/LBW) differences using Wilcoxon’s-Mann-Whitney statistics.

In test sessions, the latency to choose and the choice of goal-box (positive, negative or omit) was recorded per animal. The mean latency (s) to respond to each cue type for

free trials during test phases was determined per pig. “Optimistic Choice” was calculated as the percentage of choices for the positive goal-box for each training (Positive/Negative) and ambiguous tone-cue (AmbigNeg, AmbigMid, AmbigPos). Latency and Optimistic choice responses were analysed for weight-group (NBW/LBW) differences using Wilcoxon’s-Mann-Whitney statistics.

To get an overall measure of ‘optimism’ in the judgement bias task, optimistic choices in response to the individual cue-types were used to calculate the mean Area Under the Curve (Mean AUC) as:

$$\text{Mean} \left[ \left( \frac{\text{Neg}+\text{AmbigNeg}}{2} + \frac{\text{AmbigNeg}+\text{AmbigMid}}{2} + \frac{\text{AmbigMid}+\text{AmbigPos}}{2} + \frac{\text{AmbigPos}+\text{Pos}}{2} \right) \right]$$

Mean AUC was analysed for weight-group (NBW/LBW) differences using Wilcoxon’s-Mann-Whitney statistics.

To compare backtest behaviour and behaviour in the judgement bias task, the variables derived from the backtest (number of and latency to escape, and the number of vocalisations) and Mean AUC from the judgement bias task were subjected to correlation analysis (SAS PROC CORR). As not all data met assumptions of normality, Spearman’s rank correlation coefficient  $\rho$  was calculated.

## RESULTS

*Learning:* As shown in Murphy et al. (Chapter 03, 2013a), eight out of the 11 pigs (LBW  $n=3$ ; NBW  $n=5$ ) learned the discrimination within 46 sessions. Of these learners, LBW pigs reached criterion in fewer sessions than NBW pigs (Chapter 03, Murphy et al., 2013a). Therefore judgement bias measured only for these 8 animals.

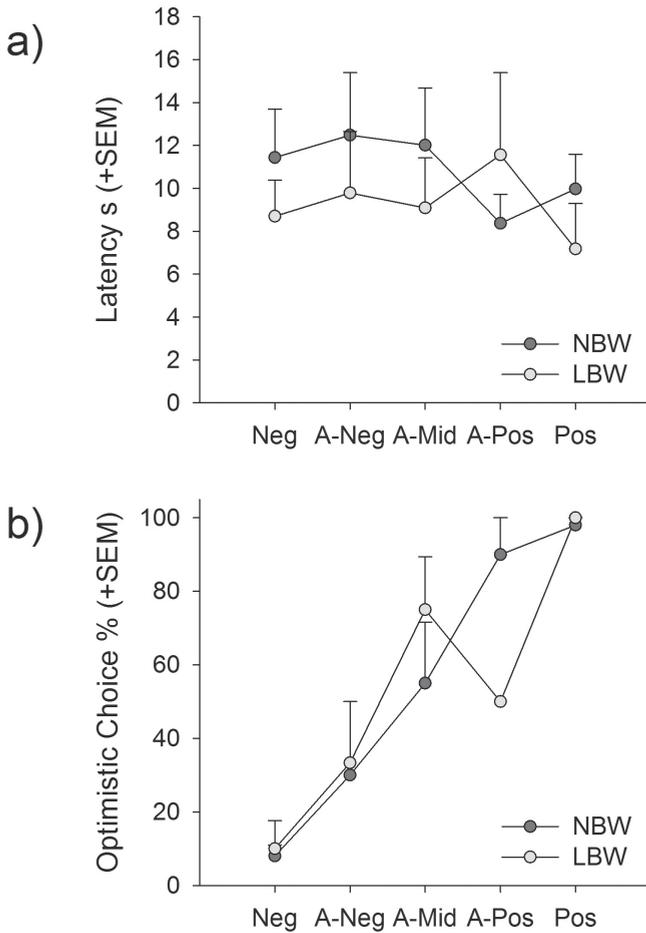
*Backtest:* LBW and NBW learner pigs did not differ on any of the back test measures (Struggle Bouts:  $W_s = 14.50$ ,  $z = 0.31$ ,  $P = 0.76$ ,  $r = 0.11$ ; Latency to First Struggle:  $W_s = 12.00$ ,  $z = -0.45$ ,  $P = 0.65$ ,  $r = -0.16$ ; Vocalisations  $W_s = 18.00$ ,  $z = 1.35$ ,  $P = 0.18$ ,  $r = 0.48$ ).

*Judgement Bias:* NBW and LBW pigs did not differ in latency responses to the five cue-types (Negative:  $W_s = 12.00$ ,  $z = -0.45$ ,  $P = 0.65$ ,  $r = -0.16$ ; AmbigNeg:  $W_s = 12.00$ ,  $z = -0.45$ ,  $P = 0.65$ ,  $r = -0.16$ ; AmbigMid:  $W_s = 12.00$ ,  $z = -0.45$ ,  $P = 0.65$ ,  $r = -0.16$ ; AmbigPos:  $W_s = 16.00$ ,  $z = 0.75$ ,  $P = 0.46$ ,  $r = 0.26$ ; Positive:  $W_s = 9.00$ ,  $z = -1.34$ ,  $P = 0.18$ ,  $r = -0.47$ ) (Fig. 1b).

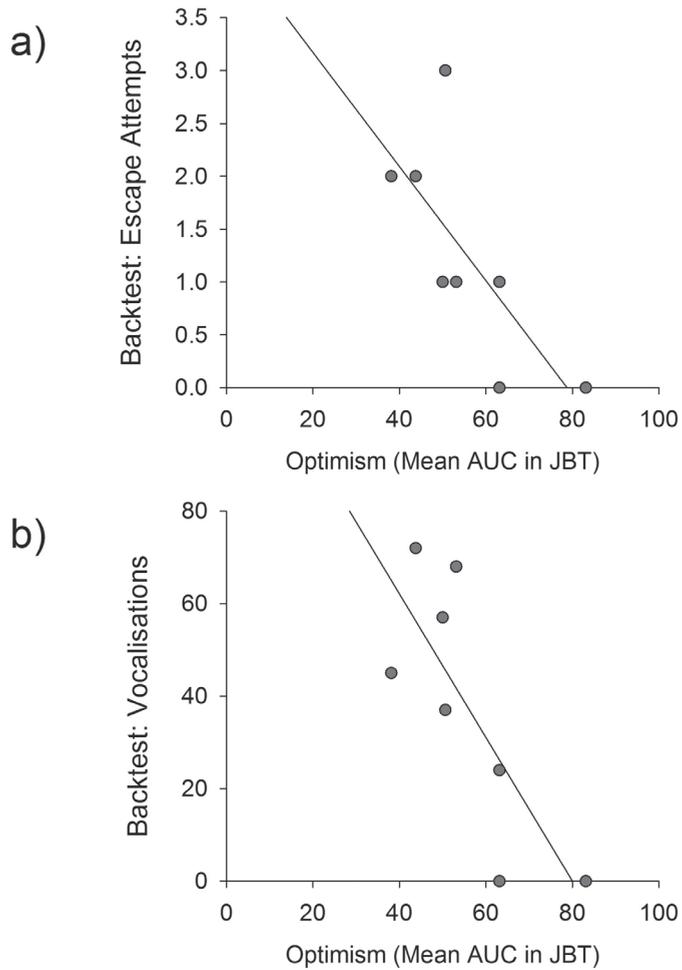
NBW and LBW piglets did not differ in their proportion of optimistic choice in response to the training cues in test sessions (Negative:  $W_s = 13.50$ ,  $z = 0.00$ ,  $P = 1.00$ ,  $r = 0.00$ ; Positive:  $W_s = 15.00$ ,  $z = 0.77$ ,  $P = 0.44$ ,  $r = 0.27$ ). There was also no difference between NBW and LBW pigs in response to the ambiguous cue near negative and intermediate ambiguous cue (AmbigNeg:  $W_s = 14.50$ ,  $z = 0.33$ ,  $P = 0.74$ ,  $r = 0.12$ ; AmbigMid:  $W_s = 16.00$ ,  $z = 0.77$ ,  $P = 0.46$ ,  $r = 0.27$ ). However, LBW piglets showed fewer optimistic choices than NBW pigs in response to the ambiguous cue near the positive cue (AmbigPos:  $W_s = 7.5$ ,  $z = -2.04$ ,  $P = 0.04$ ,  $r = -0.72$ ) (Fig. 1b).

NBW and LBW piglets did not differ in their overall measure of optimism (Mean AUC:  $W_s = 13.50$ ,  $z = 0.00$ ,  $P = 1.00$ ,  $r = 0.00$ ).

*Backtest & Judgement Bias:* As NBW and LBW pigs did not differ in backtest measures nor for the Mean AUC, data was pooled for this analysis. Overall optimistic responses on the JBT were found to be negatively related to both the number of escape attempts ( $r_s = -0.76, P = 0.03, n = 8$ ) and the number of vocalisations produced during the backtest ( $r_s = -0.74, P = 0.03, n = 8$ ) (Fig. 2a,b). No relationship was found between Mean AUC and the latency to first escape ( $r_s = 0.52, P = 0.18, n = 8$ ).



**Fig. 1** Latency and Optimistic Choice responses across the five cue-types for both NBW and LBW pigs. LBW pigs showed a more pessimistic bias in response to the AmbigPos cue-type.



**Fig. 2** Relationship between optimism on the judgement bias task and a) escape attempts and b) vocalisations in the backtest.

## DISCUSSION

In this experiment we aimed to develop an active-choice task suitable for assessing judgement bias in pigs. With some modifications to the training procedure, we succeeded in getting eight out of 11 pigs to learn the conditional discrimination of positive and negative cues necessary for performance of the judgement bias task (Chapter 03, Murphy et al., 2013a). We have since shown with that the final training method developed in this study, all pigs can learn this active choice task (Chapters 03 – 06). Furthermore, we have demonstrated in this study, that once pigs have learned the discrimination of positive and negative cues, their responses to these trained cues remains consistent, even after introduction of the ambiguous cues.

While we did not find that LBW pigs showed an overall more pessimistic bias than NBW pigs, we did show that they showed more pessimistic responses in response to the ambiguous cue closes to the positive cue. A differing response to the different ambiguous cues has been proposed to reflect differences in the expectation of negative and positive events – anxiety can lead to an increased expectation of negative events, while depression can lead to a low expectation of positive events (Burman et al., 2009; Miranda and Mennin, 2007). However, since we only gave pigs two presentations of the ambiguous cues towards the extreme, we ought to be cautious in interpreting these results.

Latency response to the five cue-types did not yield as consistent a response as optimistic choices, choices for the positive goal-box. Latency to respond, therefore, may not be an accurate measure of judgement bias.

Interestingly, while backtest measures of personality did not differ between LBW and NBW learners, we found strong relationships between back test responses and overall optimism in the judgement bias task. Pigs which made more escape attempts and vocalised more made fewer optimistic choices. Since the number of pigs used for these analyses was low, more research would have to be done to establish whether these relationships can be generalised beyond the sample used in this study.

We have developed an active-choice task capable of measuring differences in judgement bias in pigs. Further refinement of the procedure is expected to increase learning of the task. Further, we have found evidence to suggest that LBW pigs are a suitable model to use to validate our task as, consistent with human research, they show more negative emotional states as indicated by more pessimistic judgement of an ambiguous stimulus. However, to confirm these results, replication with a larger sample is necessary.





# APPENDIX B

## PILOT STUDY II: DECISION MAKING IN JUVENILE PIGS, *SUS SCROFA*, IN A GAMBLING TASK

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## INTRODUCTION

The Iowa Gambling Task (IGT) is commonly used to assess decision making in humans (Bechara et al., 1994). In this decision making task, subjects are presented with advantageous options offering low “wins” but also a low probability of “loss” and disadvantageous options offering high “wins” but even higher chance of “loss”. Normal subjects on this task start choosing randomly between the options but increasingly choose from the advantageous options. The IGT has demonstrated deficits in decision making in a number of clinical populations, such as pathological gamblers and drug addicts, and is associated with damage to the prefrontal cortex and amygdala (Buelow and Suhr, 2009). Furthermore, there is increasing evidence that the IGT may involve “hot” decision making (Buelow and Suhr, 2009); de Vries et al. (2008) found that positive mood states are associated with an earlier preference for advantageous choices in humans, while Suhr and Tsanadis (2007) found that riskier choices were associated with negative affect and, independently, fun seeking personalities. Thus the IGT may prove a suitable task for measuring emotional state in animals.

The IGT has been successfully adapted to look at decision making in rodents in a number of studies. Van den Bos et al. (2006) showed that, as with humans, rats and mice show an increasing preference for the advantageous choices as testing progresses. Various rodent versions of the IGT have been proposed and each includes a number of key features of the original task (de Visser et al., 2011) such as the occurrence of rewards and punishments, and the conflict between short- and long-term gains.

We aimed to develop a simplified two-choice IGT-style task to assess decision making in pigs. Two-choice versions have previously been used to test children (Kerr and Zelazo, 2004) and rats (Homberg et al., 2008; van den Bos et al., 2006). Knowledge about decision making in pigs is of interest to a variety of disciplines. Pigs have been proposed as a suitable model species in neuroscience research due to greater similarities in brain growth and development (Lind et al., 2007) but few validated cognitive tests for pigs exist (Gieling et al., 2011). Knowledge about cognitive abilities in pigs may directly and indirectly impact on production outcomes (Held et al., 2002) through the animal’s ability to learn and make decisions within its environment and through the effects of poor decision making upon stress and welfare. Decision making may also provide a proxy measure of emotional state.

In the present study, the task was based on win/loss probabilities used previously with rodents (Koot et al., 2010). In our task, pigs could freely choose between an advantageous option offering small wins in eight out of every ten trials, and a disadvantageous option offering high wins in only three out of ten trials. In this pilot we aimed to look at decision making in a normal population of conventional farm pigs with wins resulting in almond rewards while losses resulted in bitter tasting almonds (almonds soaked in a quinine solution). Bitter tasting rewards have previously been used as losses in rodent models of the IGT (Homberg et al., 2008; van den Bos et al., 2006) although rodents don’t always show an aversion to the quinine flavoured rewards (van den Bos et al., 2006). We expected that, similarly to a normal population in human research, as trials increase pigs will increasingly choose for the advantageous option.

## METHODS

*Ethical Note:* The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

*Apparatus:* The testing apparatus used has been previously described in Chapter 03 (Murphy et al., 2013) (see Fig. 1). A start box (1.2 m<sup>2</sup>; “antechamber”, Fig. 1) was connected directly to the test arena (3.6 m x 2.4 m). The experimenter controlled exit from the start box. Two goal-boxes were situated at the back of the test arena, each of which contained a food bowl covered by a large hard-plastic ball which could be raised by the pig in order to gain access the bowl underneath. Access to each goal-box could be controlled remotely by the experimenter using guillotine doors positioned at the front of each goal-box. While a pig was trained or tested, its group mates were kept in a straw-bedded waiting pen, adjacent to the apparatus with ad lib access to water.

*Pig Gambling Task (PGT) – Task Description:* Pigs were freely allowed to choose from two options (Fig. 2). A choice for the advantageous option yielded small wins, but at a high probability. A choice for the disadvantageous option yielded large wins, but at a low probability. Both wins and losses resulted in almonds but the quality of the almonds differed. Wins consisted of normal almonds while losses consisted of bitter tasting almonds (soaked in a quinine solution). Pigs could choose between the left a right goal-box and win and loss almonds were available directly in the food bowl of each goal-box.

*Subjects and Housing:* We used nine male piglets (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) bred at the farm of the Faculty of Veterinary Medicine of Utrecht University which were six weeks old at the start of the study. Pigs were housed together in a straw-bedded pen (5 x 3 m) with a nest area and toys for enrichment. Pigs were fed twice per day and were not food restricted. Water was available ad libitum.

*Habituation:* After arrival in their new pens, pigs were left to settle undisturbed for a day. Over the following 4 days, pigs were habituated to the experimenter and to the taste of almonds. The experimenter repeatedly entered the pen and allowed the pigs to approach. After exiting the pen, rewards were scattered on the home pen floor. Next, over 4 days, pigs were habituated to the testing apparatus, initially as a large group of 9 pigs for 12 sessions, then in smaller subgroups of 3 pigs each for 8 sessions and finally individually for 9 sessions so that all pigs were comfortable being alone in the apparatus. Each session lasted approximately 3 minutes.

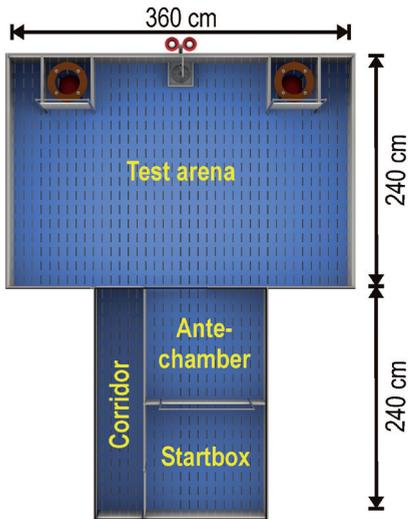
*Training:* Pigs were first trained to raise the ball in each goal-box in order to find pieces of almond in the food bowls underneath. Pieces of almonds were placed within each food bowl and pigs were shown how to access the almonds by the experimenter (1 session of 3 minutes). Next they were taught to perform trials i.e. that they had to return to the start box before they could obtain the next reward (1 session of 10 trials). Finally, pigs were taught that both goal-boxes would yield a reward. Over 3 days pigs received 3 sessions of 20 “forced” trials in which only one goal-box was available per trial. Left and right goal-boxes were available in a pseudorandom order with a maximum of two consecutive presentations of the same goal-box. Two almonds were available in each trial.

*Pig Gambling Task:* Over 12 blocks of 20 trials each, pigs could choose freely between left and right goal-boxes. For each pig, one goal-box was always advantageous and one always disadvantageous according to the probabilities shown in Fig. 2. In this experiment, almonds were used as rewards and quinine soaked almonds were used as losses. Almonds were soaked in a solution of quinine sulphate ( $20\text{g}\cdot\text{L}^{-1}$  water, i.e. 2.0%) for 5 minutes, then the excess solution was drained away and the almonds were left to dry overnight. Pigs have previously shown only a tendency to find solutions of quinine sulphate aversive at 0.025% (Nelson and Sanregret, 1997) and to show a preference for control diets over those containing quinine above 0.05% (Leamaster and Cheeke, 1979). We selected a much higher concentration of quinine sulphate for our solution to ensure that the coating on the almonds would be sufficiently aversive. However, the overall ratio of quinine to food was not likely to exceed the 0.05 used by Leamaster and Cheeke (1979) as only the outside of the almonds were coated. As most pigs were still showing a preference for the disadvantageous side in Block 6, the concentration of quinine sulphate was increased ( $30\text{g}\cdot\text{L}^{-1}$  water i.e. 3.0%) in Blocks 7 to 12. The order of wins and losses differed daily but the probability remained the same within each series of 10 trials. Since pigs showed a strong side bias irrespective of advantageous/disadvantageous goal-boxes in Block 1, Block 2 commenced with 10 forced trials where only one goal-box was available per trial, and goal-boxes were rewarded as advantageous or disadvantageous, followed by 10 free trials where both were available. Thus, pigs received a total of 230 free choice trials. The number of advantageous choices was recorded per pig for each of the 12 blocks of trials.

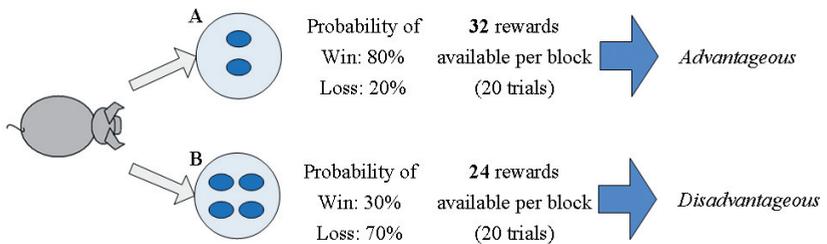
*Data Analysis:* All analyses were carried out using SAS 9.2. The proportion of advantageous choices was calculated per pig for each block (1-12). The forced trials in Block 2 were not considered in the analysis. Advantageous choice data was checked for normality using a Kolmogorov-Smirnov test, then analysed for differences across blocks of trials using a repeated measures ANOVA with the within subjects factor Block (1 – 12). In addition, the change in choice of the advantageous option was analysed by contrasts between successive blocks. To check if the proportion of advantageous choices differed from random choice, we subtracted 50% from each data point and used a series of one-sample t-tests to see if this score differed from zero for each block. To see if responses differed after the concentration of quinine was increased, advantageous choices in the final 3 blocks for each dose were compared ( $20\text{g}\cdot\text{L}^{-1}$  water: Blocks 4-6;  $30\text{g}\cdot\text{L}^{-1}$ : Blocks 10-12) using a paired t-test.

## RESULTS

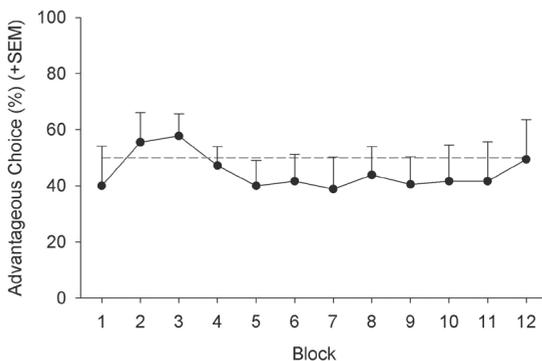
Pigs did not show an increase in the proportion of advantageous choices across blocks ( $F_{11,88} = 0.92$ ,  $P = 0.5208$ ) and advantageous choice was not found to differ from random choice (Fig. 3). There was a high variation in individual advantageous choice. Looking at individual responses shows that while some pigs showed a clear preference for the advantageous goal-box in later blocks, others showed the opposite, suggesting that the flavour of quinine was only aversive for some individuals. Only three out of nine pigs did not eat the quinine soaked almonds in loss trials, all others ate the almonds in all



**Fig. 1** Overview of apparatus. “Antechamber” was used as the start box in this pilot study.



**Fig. 2** Overview of the Pig Gambling Task (PGT). Pigs choose freely between two options. Option A yields low wins per trial (2 rewards) but has a low probability of losses while Option B yields higher wins per trial (4 rewards) but there is a greater probability of incurring a loss. Overall gain is greater in Option A and it is therefore considered “advantageous” while Option B is considered “disadvantageous”.



**Fig. 3** Proportion of advantageous choices across blocks ( $n = 9$ ). The proportion of advantageous choices did not change across blocks and did not differ from random choice (dashed line).

trials, either wholly or partially. A lack of aversiveness of the quinine was supported by the finding that there was no difference in advantageous choices in the last three blocks of each concentration of quinine ( $t_8 = -0.12$ ,  $P = 0.9062$ ), i.e. increasing the quinine concentration did not increase aversiveness.

## DISCUSSION AND CONCLUSION

In this pilot experiment we aimed to develop a decision making task for pigs (PGT) based on the principles of wins and losses of the Iowa Gambling Task (Bechara et al., 1994), a task often used in clinical research with humans. We used almonds to signify wins and quinine soaked almonds to signify losses. Contrary to expectation we found that overall, pigs did not show a preference for either the advantageous or disadvantageous option. Not all of the pigs showed the expected aversion to the quinine soaked almonds, even after increasing the concentration of quinine, thus rendering our “disadvantageous” option more advantageous in both the short and long term for these pigs.

Previous work on taste preferences in pigs has found that pigs show a tendency to prefer control solutions above those containing 0.025% quinine sulphate (Nelson and Sanregret, 1997) and discriminate against diets containing concentrations of quinine sulphate above 0.05% (Leamaster and Cheeke, 1979). We selected a concentration of quinine that was well above both of these concentrations, which evidently not all animals found sufficiently aversive. There may be a number of reasons for this. Firstly, perhaps the concentration we used, despite being high in the solution, wasn't sufficiently aversive once mixed with the almonds. It is difficult to determine the ratio of quinine to almonds with the methods we used. However, an increase of 50% in the quinine concentration in the later blocks still failed to produce the expected results.

Secondly, an important difference in our study and previous studies on taste preferences in pigs is that the quantity of the food available differed with each option. It is likely, therefore, that pigs which did not perceive the bitter taste as highly aversive, had a preference for the higher quantity of almonds. Blair and Fitzsimmons (1970, as cited in Held et al., 2009) found that pigs will show an initial aversive response to bitter substances, but can overcome this aversion with hunger and in the absence of negative consequences (Held et al., 2009). In previous versions of the IGT with rodents using quinine soaked pellets to signify losses, rats which continued to eat the pellets were excluded from the analysis (Koot et al., 2013; van den Bos et al., 2006). However, with such an exclusion criterion, we would have lost two-thirds of our sample. Thus, in future experiments, the outcomes of win and loss trials ought to be modified while maintaining the same ratio of wins to losses for both advantageous and disadvantageous choices.

We also encountered the issue that pigs did not appear to sample from the two options in the first block of trials as rats appear to do. Koot et al. (2013) taught rats to alternate in a training session prior to commencing the gambling task. While rats quickly learn a win-shift foraging strategy (Olton and Schlosberg, 1978), pigs appear to show a win-stay strategy when foraging (Mendl et al., 1997). Nielsen et al. (2009) also found that pigs did not naturally alternate in a Delayed Non-Match to Sample task. Thus, in future experiments, pigs should be given some training where they

can understand that each option can result in different outcomes which may encourage sampling behaviour.

We have developed a two-choice task to assess decision making in pigs based on rodent versions of the Iowa Gambling Task. With some modifications to the outcomes of advantageous and disadvantageous choices, we expect that this task can be used to assess decision making under risk in pigs.



# APPENDIX C

## IS JUDGEMENT BIAS IN PIGS, *SUS SCROFA*, RELATED TO MEASURES OF PERSONALITY?

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## INTRODUCTION

Some measures from the backtest (Hessing et al., 1993), a test of coping styles in which pigs are classified according to their resistance to restraint, and potentially a measure of personality (Melotti et al., 2011), appear to be highly related to choices reflecting overall optimism in a judgement bias task (see Appendix A). We found that pigs showing more low-resistant style behaviours in the backtest, fewer escape attempts and lower vocalisation rates, chose more optimistically in our active-choice judgement bias task. However, the sample size in this study was low and possibly the results more susceptible to outliers.

Bolhuis et al. (2004) found that high-resistant pigs did not show impaired learning in a T-Maze, but were less successful in reversal learning, suggesting that high resistant pigs show less behavioural flexibility. If backtest behaviour is related to judgement of ambiguous stimuli, perhaps optimism and positive mood contribute to behavioural flexibility (Dreisbach and Goschke, 2004).

## METHODS

We used 15 male piglets (cross-breeds Duroc x Yorkshire and Duroc x Danish Landrace) bred at the farm of the Faculty of Veterinary Medicine of Utrecht University. We used 8 male low birth weight (LBW) piglets and 7 male normal birth weight (NBW) piglets. Full details on subjects, housing, training and testing the in judgement bias task can be found in Chapter 05 (Murphy et al., In Press).

*Backtest:* The backtest was performed for  $n=7$  NBW pigs and  $n=8$  LBW pigs at 14 days of age. Pigs were individually removed from their pens and held in a supine position for 60s according to the method described by Bolhuis et al. (2004). Pigs were then returned to the home pen. The number and duration of escape attempts, the latency to first escape attempt, and the number of vocalisations were recorded per piglet.

*Analysis:* The variables derived from the backtest (number of, duration of, and latency to escape attempts, and the number of vocalisations) and Mean AUC from the judgement bias task (Chapter 05, Murphy et al., In Press) were subjected to correlation analysis (SAS PROC CORR). As not all data met assumptions of normality, Spearman's rank correlation coefficient  $\rho$  was calculated. Analyses were carried out separately for LBW and NBW piglets as groups were found to differ in optimism.

## RESULTS & DISCUSSION

*LBW ( $n=8$ ):* Mean AUC in the judgement bias task was unrelated to any of the backtest measures (Number of escape attempts:  $r_s = 0.16$   $P = 0.70$ ; duration of escape struggles:  $r_s = -0.05$   $P = 0.91$ ; latency to first escape attempt:  $r_s = -0.27$   $P = 0.52$ ; vocalisations:  $r_s = -0.01$   $P = 0.97$ ).

*NBW ( $n=7$ ):* Mean AUC in the judgement bias task was not related to the number of escape attempts ( $r_s = 0.27$   $P = 0.56$ ) nor to the number of vocalisations ( $r_s = -0.17$   $P = 0.71$ ). However there was a strong tendency for a relationship between Mean AUC and the latency to first escape attempt ( $r_s = -0.72$   $P = 0.06$ ) and the duration of escape struggles ( $r_s = 0.74$   $P = 0.05$ ). Thus NBW pigs who score higher on measures of optimism

in the judgement bias task, have a tendency to start struggling sooner and struggle for longer in the backtest i.e. behaviours characteristic of more high-resistant coping styles.

The above results fail to confirm, and even contradict our previous findings (Appendix A) that behaviour characteristic of low-resistant coping styles in pigs is related to more optimistic judgement bias. However in the present study and the previous study, the sample size was relatively low and the ranges of behaviours recorded in the backtest, particularly the numbers of escape attempts was low. Without increasing the sample size, it could be possible to select subjects based on backtest behaviour, i.e. the most high- and low- resistant, and then look at performance in the judgement bias task.



# APPENDIX D

## REWARDING AS EXPECTED: WHAT DO PIGS LEARN ABOUT THE OUTCOME OF AMBIGUOUS TRIALS?

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## INTRODUCTION

One of the problems associated with judgement bias tasks in animals is that animals may learn the outcomes of ambiguous trials with repeated testing, rendering them no longer ambiguous. In this thesis we showed that pigs quickly learn that ambiguous cues are not rewarded (Chapter 04, Murphy et al., 2013) and change their behavioural response. Similar results have been found in sheep (Doyle et al., 2010). Different suggestions have been given to slow down learning about the outcomes of ambiguous trials. A number of studies introduce a partial reinforcement schedule before testing so that so that animals are used to not receiving a reward in every trial (Bateson and Matheson, 2007; Brilot et al., 2010; Brydges et al., 2011; Matheson et al., 2008; Richter et al., 2012). Papini et al. (1997) suggest that unexpected omissions of signalled reward result in a physiological stress-type response leading to frustration. In consequences of experiencing such an unexpected event, an animal will update its knowledge about the environment (Papini, 2003) thus making it more likely that an unexpected event is remembered. This may explain why there was a drop in optimistic responses to unrewarded ambiguous cues in Chapter 04 (Murphy et al., 2013). In later studies (Chapters 05 and 06, Murphy et al., In Press; in prep.), we chose instead to reward ambiguous cues as expected so that in ambiguous trials, both the goal-boxes contained their associated amount of reward.

## METHODS

Using the data from Chapters 04 – 06, we divided responses in the four judgement bias test sessions into two and calculated the Mean AUC for sessions 1 and 2 ('first' sessions) and for sessions 3 and 4 ('last' sessions). The first and last two sessions were combined because, in each combination, ambiguous trials occurred after both after a negative and after a positive trial.

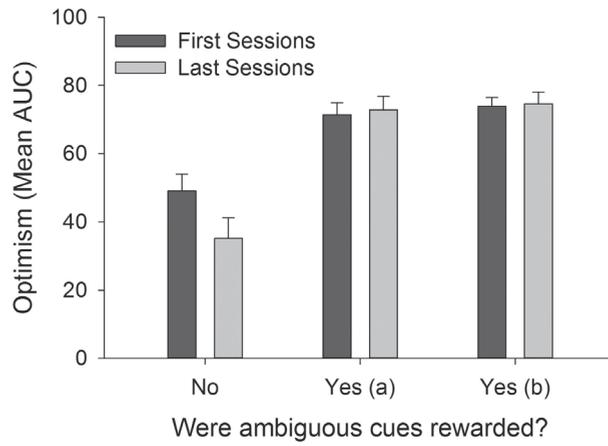
We then calculated the difference score between the first two sessions and last two sessions and performed some exploratory t-tests to see if this difference in Mean AUC from first to last session deviated from zero.

## RESULTS AND DISCUSSION

When ambiguous cues were not rewarded (Chapter 04, Murphy et al., 2013 Phase I) overall "Optimism" as measured by Mean AUC across the four test sessions appears to be lower than studies where ambiguous cues were rewarded as expected (Chapters 05 and 06) (fig. 1).

The exploratory analysis revealed that difference scores between optimistic responses in first and last sessions were significantly different from zero when ambiguous cues were unrewarded ( $t_{15} = 3.10, P = 0.008$ ), but not when cues were rewarded as expected ( $t_{16} = -0.27, P = 0.794$ ;  $t_{39} = -0.31, P = 0.777$ ).

Thus, when ambiguous cues are unrewarded in our active-choice judgement bias task, these results tentatively suggest that not only do optimistic choices reduce across different testing phases, but also within one test phase (fig. 1). When ambiguous cues are rewarded as expected, this alteration in responses does not occur.



**Fig. 1** Mean AUC responses in an active-choice judgement bias task to first and last test sessions when ambiguous cues are not rewarded ('No', n = 15) or rewarded as expected ('Yes (a)', n = 16; 'Yes (b)', n = 39).



# REFERENCE LIST

- Andersen IL, Bøe KE, Førevik G, Janczak AM, Bakken M (2000a) Behavioural evaluation of methods for assessing fear responses in weaned pigs. *Appl Anim Behav Sci* 69:227-40
- Andersen IL, Førevik G, Bøe KE, Janczak AM, Bakken M (2000b) Effects of diazepam on the behaviour of weaned pigs in three putative models of anxiety. *Appl Anim Behav Sci* 68:121-30
- Anderson MH, Hardcastle C, Munafò MR, Robinson ES (2012) Evaluation of a novel translational task for assessing emotional biases in different species. *Cogn Affect Behav Neurosci* 12:373-81
- Anderson PJ, Doyle LW (2004) Executive functioning in school-aged children who were born very preterm or with extremely low birth weight in the 1990s. *Pediatrics* 114:50-7
- Anderson EE (1938) The Interrelationship of drives in the male albino rat: III. Interrelations among measures of emotional, sexual, and exploratory behavior. *J Genet Psychol* 53:335-52
- Archer J (1973) Tests for emotionality in rats and mice: A review. *Anim Behav* 21:205-35.
- Arnold MB (1960) Emotion and personality. New York: Columbia University Press
- Arts JWM, van der Staay FJ, Ekkel ED (2009) Working and reference memory of pigs in the spatial holeboard discrimination task. *Behav Brain Res* 205:303-6
- Attig L, Djiane J, Gertler A, Rampin O, Larcher T, Boukthir S, Anton PM, Madec JY, Gourdou I, Abdennebi-Najar L (2008) Study of hypothalamic leptin receptor expression in low-birth-weight piglets and effects of leptin supplementation on neonatal growth and development. *Am J Physiol Endocrinol Metab* 295:E1117-25
- Bachorowski J, Owren MJ (2008) Vocal expressions of emotion. In: Lewis M, Haviland-Jones JM and Feldman Barrett L (eds) *Handbook of Emotions*, Third Edition edn. The Guilford Press, New York, pp 196-210
- Balcombe J (2009) Animal pleasure and its moral significance. *Appl Anim Behav Sci* 118:208-16
- Baldwin BA, Meese GB (1977) Sensory reinforcement and illumination preference in the domesticated pig. *Anim Behav* 25:497-507
- Baldwin BA, Start IB (1985) Illumination preferences of pigs. *Appl Anim Behav Sci* 14:233-43
- Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ, Van Ijzendoorn MH (2007) Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychol Bull* 133:1
- Barnes RH, Levitsky DA, Pond WG, Moore U (1976) Effect of postnatal dietary protein and energy restriction on exploratory behavior in young pigs. *Dev Psychobiol* 9:425-35
- Barrett LF (2006) Are emotions natural kinds? *Perspect Psychol Sci* 1:28-58
- Baschat DAA (2004) Fetal responses to placental insufficiency: an update. *BJOG-Int J Obstet Gy* 111:1031-41
- Bassett L, Buchanan-Smith HM (2007) Effects of predictability on the welfare of captive animals. *Appl Anim Behav Sci* 102:223-45
- Bateson M, Desire S, Gartside S, Wright G (2011) Agitated honeybees exhibit pessimistic cognitive biases. *Curr Biol* 21:1070-3
- Bates D, Maechler M, Bolker B (2012) lme4: Linear mixed-effects models using Eigen and R package version 0.99875-6.
- Bateson M, Matheson SM (2007) Performance on a categorisation task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). *Anim Welfare* 16:33-6
- Baxter EM, Jarvis S, D'Eath RB, Ross DW, Robson SK, Farish M, Nevison IM, Lawrence AB, Edwards SA (2008) Investigating the behavioural and physiological indicators of neonatal survival in pigs. *Theriogenology* 69:773-83

- Beattie VE, Walker N, Sneddon IA (1995) Effect of rearing environment and change of environment on the behaviour of gilts. *Appl Anim Behav Sci* 46:57-65
- Beaulieu AD, Aalhus JL, Williams NH, Patience JF (2010) Impact of piglet birth weight, birth order, and litter size on subsequent growth performance, carcass quality, muscle composition, and eating quality of pork. *J Anim Sci* 88:2767-78
- Bechara A, Damasio H, Tranel D, Damasio AR (2005) The Iowa Gambling Task and the somatic marker hypothesis: some questions and answers. *Trends Cogn Sci (Regul Ed)* 9:159-62
- Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition* 50:7-15. 3
- Beilharz RG, Cox DF (1967) Genetic analysis of open field behaviour in swine. *J Anim Sci* 26:988-90
- Benders MJ, Bos AF, Rademaker CM, Rijken M, Torrance HL, Groenendaal F, van Bel F (2006) Early postnatal allopurinol does not improve short term outcome after severe birth asphyxia. *Arch Dis Child Fetal Neonatal Ed* 91:F163-5
- Bethell E, Holmes A, Maclarnon A, Semple S (2012) Cognitive bias in a non-human primate: husbandry procedures influence cognitive indicators of psychological well-being in captive rhesus macaques. *Anim Welfare* 21:185
- Bizot JC, Thiébot MH (1996) Impulsivity as a confounding factor in certain animal tests of cognitive function. *Cognitive Brain Res* 3:243-50
- Blache D, Terlouw C, Maloney SK (2011) Chapter 8: Physiology. In: Appleby MC, Mench JA, Olsson IAS and Hughes BO (eds) *Animal Welfare*, 2nd edn. CABI, Wallingford, U.K., pp 155-182
- Blackshaw JK, Blackshaw AW, McGlone JJ (1998) Startle-Freeze behaviour in weaned pigs. *J Comp Psychol* 11:30-9
- Blanchette I, Richards A (2010) The influence of affect on higher level cognition: a review of research on interpretation, judgement, decision making and reasoning. *Cogn Emot* 24:561-95
- Blomberg LA, Schreier LL, David Guthrie H, Sample GL, Vallet J, Caperna T, Ramsay T (2010) The effect of intrauterine growth retardation on the expression of developmental factors in porcine placenta subsequent to the initiation of placentation. *Placenta* 31:549-52
- Boda D, Nemeth I, Kiss P, Orvos H (1999) Treatment of mothers with allopurinol to produce therapeutic blood levels in newborns. *Prenat Neonat Med* 4:130-4
- Boissy A (1995) Fear and fearfulness in animals. *Q Rev Biol* 70:165-91
- Boissy A, Arnould C, Chaillou E, Désiré L, Duvaux-Ponter C, Greiveldinger L, Leterrier C, Richard S, Roussel S, Saint-Dizier H, Meunier-Salaün MC, Valance D, Veissier I (2007a) Emotions and cognition: a new approach to animal welfare. *Anim Welfare* 16(s):37-43
- Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C, Forkman B, Dimitrov I, Langbein J, Bakken M, Veissier I, Aubert A (2007b) Assessment of positive emotions in animals to improve their welfare. *Physiol Behav* 92:375-97
- Boleij H, Klooster Jv, Lavrijsen M, Kirchoff S, Arndt SS, Ohl F (2012) A test to identify judgement bias in mice. *Behav Brain Res* 233:45-54
- Bolhuis EJ, Schouten WGP, Schrama JW, Wiegant VM (2005a) Individual coping characteristics, aggressiveness and fighting strategies in pigs. *Anim Behav* 69:1085-91
- Bolhuis JE, Schouten WGP, Leeuw JAd, Schrama JW, Wiegant VM (2004) Individual coping characteristics, rearing conditions and behavioural flexibility in pigs. *Behav Brain Res* 152:351-60

- Bolhuis JE, Schouten WGP, Schrama JW, Wiegant VM (2005b) Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. *Appl Anim Behav Sci* 93:213-28
- Bollen P, Ritskes-Hoitinga M (2007) The welfare of pigs and minipigs. In: Eila Kaliste (Ed.) *The welfare of laboratory animals*. Springer, Netherlands, pp 275-289
- Bourin M, Hascoët M (2003) The mouse light/dark box test. *Eur J Pharmacol* 463:55-65
- BPEX (2010) Knowledge Transfer bulletin 2: Low birth weight piglets. <http://www.bpex.org/publications/2TS/KTBulletins.aspx> Downloaded in: 2014
- Bracke MBM, Spoolder HAM (2008) Novel object test can detect marginal differences in environmental enrichment in pigs. *Appl Anim Behav Sci* 109:39-48
- Brambell Report (1965) Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems: Presented to Parliament by the Secretary of State for Scotland and the Minister of Agriculture, Fisheries and Food by Command of Her Majesty December, 1965. HM Stationery Office, 1965
- Briefer EF (2012) Vocal expression of emotions in mammals: mechanisms of production and evidence. *J Zool* 288:1-20
- Brilot BO, Asher L, Bateson M (2010) Stereotyping starlings are more 'pessimistic'. *Anim Cogn* 13:721-31
- Brilot BO, Normandale CL, Parkin A, Bateson M (2009) Can we use starlings' aversion to eyespots as the basis for a novel 'cognitive bias' task? *Appl Anim Behav Sci* 118:182-90
- Brown JA, Dewey C, Delange CFM, Mandell IB, Purslow PP, Robinson JA, Squires EJ, Widowski TM (2009) Reliability of temperament tests on finishing pigs in group-housing and comparison to social tests. *Appl Anim Behav Sci* 118:28-35
- Brydges NM, Leach M, Nicol K, Wright R, Bateson M (2011) Environmental enrichment induces optimistic cognitive bias in rats. *Anim Behav* 81:169-75
- Buelow MT, Suhr JA (2009) Construct validity of the Iowa gambling task. *Neuropsychol Rev* 19:102-14
- Burgdorf J, Panksepp J, Beinfeld MC, Kroes RA, Moskal JR (2006) Regional brain cholecystokinin changes as a function of rough-and-tumble play behavior in adolescent rats. *Peptides* 27:172-7
- Burghardt GM (2005) *The genesis of animal play: Testing the limits*. MIT Press
- Burman OHP, Parker RMA, Paul ES, Mendl MT (2009) Anxiety-induced cognitive bias in non-human animals. *Physiol Behav* 98:345-50
- Burman OHP, Parker R, Paul ES, Mendl M (2008) A spatial judgement task to determine background emotional state in laboratory rats, *Rattus norvegicus*. *Anim Behav* 76:801-9
- Burman O, McGowan R, Mendl M, Norling Y, Paul E, Rehn T, Keeling L (2011) Using judgement bias to measure positive affective state in dogs. *Appl Anim Behav Sci* 132:160-8
- Burne THJ, Murfitt PJE, Gilbert CL (2001) Effects of ovariectomy on prostaglandin F2 $\alpha$ -induced nesting behaviour in pigs. *Physiol Behav* 74:145-52
- Burnham KP, Anderson DR (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. Springer-Verlag, New York
- Cabanac M (2002) What is emotion? *Behav Process* 60:69-83
- Carey MP, Fry JP (1995) Evaluation of animal welfare by the self-expression of an anxiety state. *Lab Anim-UK* 29:370-9
- Carey MP, Fry JP (1993) A behavioural and pharmacological evaluation of the discriminative stimulus induced by pentylentetrazole in the pig. *Psychopharmacology* 111:244-50

- Carey MP, Fry JP, White DG (1992) The detection of changes in psychological state using a novel pharmacological conditioning procedure. *J Neurosci Methods* 43:69-76
- Carobrez AP, Bertoglio LJ (2005) Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neurosci Biobehav R* 29:1193-205
- Cavigelli SA (2005) Animal personality and health. *Behaviour* 142:1223-44
- Chaby LE, Cavigelli SA, White A, Wang K, Braithwaite VA (2013) Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence. *Front Hum Neurosci* 7
- Chalmers NR, Locke-Haydon J (1981) Temporal patterns of play bouts in captive common marmosets (*Callithrix jacchus*). *Anim Behav* 29:1229-38
- Chaloupková H, Illmann G, Bartoš L, Špinka M (2007) The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Appl Anim Behav Sci* 103:25-34
- Chan WY, Cloutier S, Newberry RC (2011a) Chapter 03: It's all in the bark: affective qualities of juvenile domestic pig barks. From: The meaning of barks: vocal communication of fearful and playful affective states in pigs. Ph.D. dissertation, Washington State University
- Chan WY, Newberry RC (2011) Chapter 04: The role of acoustic morphology and context on the behavioral responses of juvenile domestic pigs to playbacks of bark vocalizations. From: The meaning of barks: vocal communication of fearful and playful affective states in pigs. Ph.D. dissertation, Washington State University
- Chan WY, Cloutier S, Newberry RC (2011b) Barking pigs: differences in acoustic morphology predict juvenile responses to alarm calls. *Anim Behav* 82:767-74
- Chaudhari T, McGuire W (2008) Allopurinol for preventing mortality and morbidity in newborn infants with suspected hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev* 2
- Clouard C, Meunier-Salaün M, Devillers N (2011) Development of approach and handling tests for the assessment of reactivity to humans of sows housed in stall or in group. *Appl Anim Behav Sci* 33:26-39
- Colpaert FC (1999) Drug discrimination in neurobiology. *Pharmacol Biochem Be* 64:337-45
- Colson V, Martin E, Orgeur P, Prunier A (2012) Influence of housing and social changes on growth, behaviour and cortisol in piglets at weaning. *Physiol Behav* 107:59-64
- Cotton JW (1998) Analyzing within-subjects experiments. London: Lawrence Erlbaum Associates
- Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Be* 13:167-70
- Cronley CC, Adams KM, Washington CG, Stricklin WR (2003) A note on visual, olfactory and spatial cue use in foraging behavior of pigs: indirectly assessing cognitive abilities. *Appl Anim Behav Sci* 83:303-8
- Cyders MA, Smith GT (2007) Mood-based rash action and its components: Positive and negative urgency. *Pers Individ Differ* 43:839-50
- Dalmau A, Fabrega E, Velarde A (2009) Fear assessment in pigs exposed to a novel object test. *Appl Anim Behav Sci* 117:173-80
- D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML (2011) Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. *Physiol Behav* 104:348-53
- Dantzer R (2002) Can farm animal welfare be understood without taking into account the issues of emotion and cognition? *J Anim Sci* 80:E1-9

- Dantzer R, Arnone M, Mormède P (1980) Effects of frustration on behaviour and plasma corticosteroid levels in pigs. *Physiol Behav* 24:1-4
- Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS (2006) Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol* 147:255-61
- Dawkins M (2004) Using behaviour to assess animal welfare. *Anim Welfare* 13:S3-S7
- Day JEL, Kyriazakis I, Lawrence AB (1995) The effect of food deprivation on the expression of foraging and exploratory behaviour in the growing pig. *Appl Anim Behav Sci* 42:193-206
- de Jong IC, Ekkel ED, van de Burgwal JA, Lambooij E, Korte SM, Ruis MAW, Koolhaas JM, Blokhuis HJ (1998) Effects of strawbedding on physiological responses to stressors and behavior in growing pigs. *Physiol Behav* 64:303-10
- de Jong IC, Prelle IT, van de Burgwal JA, Lambooij E, Korte SM, Blokhuis HJ, Koolhaas JM (2000) Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs. *Physiol Behav* 68:571-8
- de Jonge FH, Boleij H, Baars AM, Dudink S, Spruijt BM (2008a) Music during play-time: Using context conditioning as a tool to improve welfare in piglets. *Appl Anim Behav Sci* 115:138-48
- de Jonge FH, Tilly S, Baars AM, Spruijt BM (2008b) On the rewarding nature of appetitive feeding behaviour in pigs (*Sus scrofa*): Do domesticated pigs contrafreeload? *Appl Anim Behav Sci* 114:359-72
- de Sevilla XF, Casellas J, Tibau J, Fàbrega E (2009) Consistency and influence on performance of behavioural differences in Large White and Landrace purebred pigs. *Appl Anim Behav Sci* 117:13-94
- de Visser L, Baars A, Lavrijsen M, Van der Weerd C, Van Den Bos R (2011) Decision-making performance is related to levels of anxiety and differential recruitment of frontostriatal areas in male rats. *Neuroscience* 184:97-106
- de Visser L, Van Der Knaap L, van de Loo A, van der Weerd C, Ohl F, Van Den Bos R (2010) Trait anxiety affects decision-making differently in healthy men and women: towards gender-specific endophenotypes of anxiety. *Neuropsychologia* 48:1598-606
- de Visser L, Homberg JR, Mitsogiannis M, Zeeb FD, Rivalan M, Fitoussi A, Galhardo V, van den Bos R, Winstanley CA, Dellu-Hagedorn F (2011) Rodent versions of the iowa gambling task: opportunities and challenges for the understanding of decision-making. *Front Neurosci* 5
- de Vries M, Holland RW, Witteman CLM (2008) In the winning mood: Affect in the Iowa gambling task. *J Neurosci Meth* 3:42-50
- de Waal FBM (2011) What is an animal emotion? *Ann NY Acad Sci* 1224:191-206
- Deland FH (1970) Normal Spleen Size 1. *Radiology* 97:589-92
- Dellmeier GR, Friend TH (1991) Behavior and extensive management of domestic sows (*Sus scrofa*) and litters. *Appl Anim Behav Sci* 29:327-41
- Désautés C, Bidanel J, Mormède P (1997) Genetic study of behavioral and pituitary-adrenocortical reactivity in response to an environmental challenge in pigs. *Physiol Behav* 62:337-45
- Désautés C, Sarrieau A, Caritez J, Mormède P (1999) Behavior and pituitary-adrenal function in large white and Meishan pigs. *Domest Anim Endocrinol* 16:193-205
- Désiré L, Boissy A, Veissier I (2002) Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behav Process* 60:165-80

- Désiré L, Veissier I, Després G, Boissy A (2004) On the way to assess emotions in animals: Do lambs (*Ovis aries*) evaluate an event through its suddenness, novelty, or unpredictability? *J Comp Psychol* 118:363-74
- Désiré L, Veissier I, Després G, Delval E, Toporenko G, Boissy A (2006) Appraisal process in sheep (*Ovis aries*): Interactive effect of suddenness and unfamiliarity on cardiac and behavioral responses. *J Comp Psychol* 120:280-7
- Dingemanse NJ, Wolf M (2010) Recent models for adaptive personality differences: a review. *Philos Trans R Soc Lond B Biol Sci* 365:3947-58
- Dobao MT, Rodríguez J, Silio L (1985) Choice of companions in social play in piglets. *Appl Anim Behav Sci* 13:259-66
- Dolan RJ (2002) Emotion, cognition, and behavior. *Science* 298:1191-4
- Donald RD, Rutherford KMD, Lawrence AB (2010) Effect of Azaperone on the behaviour of pigs in an elevated-plus-maze. *Proceedings of the UFAW Conference, 30th June 2010*
- Donald RD, Healy SD, Lawrence AB, Rutherford KMD (2011) Emotionality in growing pigs: Is the open field a valid test? *Physiol Behav* 104:906-13
- Donaldson TM, Newberry RC, Špinková M, Cloutier S (2002) Effects of early play experience on play behaviour of piglets after weaning. *Appl Anim Behav Sci* 79:221-31
- Douglas C, Bateson M, Walsh C, Bédoué A, Edwards SA (2012) Environmental enrichment induces optimistic cognitive biases in pigs. *Appl Anim Behav Sci* 139:65-73
- Douglas SL, Edwards SA, Sutcliffe E, Knap PW, Kyriazakis I (2013) Identification of risk factors associated with poor lifetime growth performance in pigs. *J Anim Sci* 91:4123-32
- Doyle RE, Fisher AD, Hinch GN, Boissy A, Lee C (2010a) Release from restraint generates a positive judgement bias in sheep. *Appl Anim Behav Sci* 122:28-34
- Doyle RE, Hinch GN, Fisher AD, Boissy A, Henshall JM, Lee C (2011a) Administration of serotonin inhibitor p-Chlorophenylalanine induces pessimistic-like judgement bias in sheep. *Psychoneuroendocrinology* 36:279-88
- Doyle RE, Lee C, Deiss V, Fisher AD, Hinch GN, Boissy A (2011b) Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiol Behav* 102:503-10
- Doyle RE, Vidal S, Hinch GN, Fisher AD, Boissy A, Lee C (2010b) The effect of repeated testing on judgement biases in sheep. *Behav Process* 83:349-52
- Dreisbach G, Goschke T (2004) How positive affect modulates cognitive control: reduced perseveration at the cost of increased distractibility. *J Exp Psychol Learn* 30:343-53
- Dudink S, Simonse H, Marks I, de Jonge FH, Spruijt BM (2006) Announcing the arrival of enrichment increases play behaviour and reduces weaning-stress-induced behaviours of piglets directly after weaning. *Appl Anim Behav Sci* 101:86-101
- Duncan IJ (1996) Animal welfare defined in terms of feelings. *Acta Agr Scand A-AN Supplementum* (Denmark)
- Duncan S, Feldman Barrett L (2007) Affect is a form of cognition: A neurobiological analysis. *Cogn Emot* 21:1184-211
- Döpjan S, Ramp C, Kanitz E, Tuchscherer A, Puppe B (2013) A design for studies on cognitive bias in the domestic pig. *J Vet Behav* 8:485-9
- Döpjan S, Schön P, Puppe B, Tuchscherer A, Manteuffel G (2008) Differential vocal responses to physical and mental stressors in domestic pigs (*Sus scrofa*). *Appl Anim Behav Sci* 114:105-15

- Döpjan S, Tuchscherer A, Langbein J, Schön P, Manteuffel G, Puppe B (2011) Behavioural and cardiac responses towards conspecific distress calls in domestic pigs (*Sus scrofa*). *Physiol Behav* 103:445-52
- Eagle DM, Bari A, Robbins TW (2008) The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology* 199:439-56
- Edgar JL, Nicol CJ, Clark CCA, Paul ES (2012) Measuring empathic responses in animals. *Appl Anim Behav Sci* 138:182-93
- Eguchi Y, Tanida H, Tanaka T, Yoshimoto T (1997) Color discrimination in wild boars. *J Ethol* 15:1-7
- Ellegaard L, Cunningham A, Edwards S, Grand N, Nevalainen T, Prescott M, Schuurman T (2010) Welfare of the minipig with special reference to use in regulatory toxicology studies. *J Pharmacol Toxicol Methods* 62:167-83
- Enkel T, Gholizadeh D, von Bohlen und Halbach O, Sanchis-Segura C, Hurlmann R, Spanagel R, Gass P, Vollmayr B (2010) Ambiguous-cue interpretation is biased under stress- and depression-like states in rats. *Neuropsychopharmacol* 35:1008-15
- Erhard HW, Mendl M (1999) Tonic immobility and emergence time in pigs - more evidence for behavioural strategies. *Appl Anim Behav Sci* 61:227-37
- Ernst K, Tuchscherer M, Kanitz E, Puppe B, Manteuffel G (2006) Effects of attention and rewarded activity on immune parameters and wound healing in pigs. *Physiol Behav* 89:448-56
- Ernst K, Puppe B, Schön PC, Manteuffel G (2005) A complex automatic feeding system for pigs aimed to induce successful behavioural coping by cognitive adaptation. *Appl Anim Behav Sci* 91:205-18
- Faul F, Erdfelder E, Lang A, Buchner A (2007) G\* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Meth* 39:175-91
- Forkman B, Boissy A, Meunier-Salaün M, Canali E, Jones RB (2007) A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol Behav* 92:340-74
- Forkman B, Furuhaug IL, Jensen P (1995) Personality, coping patterns, and aggression in piglets. *Appl Anim Behav Sci* 45:31-42
- Fraser D, Weary DM, Pajor EA, Milligan BN (1997) A scientific conception of animal welfare that reflects ethical concerns. *Anim Welfare* 6:187-205
- Fraser D, Nicol CJ (2011) Chapter 11: Preference and Motivation Research. In: Appleby MC, Mench JA, Olsson IAS and Hughes BO (eds) *Animal Welfare*, 2nd edn. CABI, Wallingford, U.K., pp 183-199
- Fraser D (1974) The vocalizations and other behaviour of growing pigs in an "open field" test. *Appl Anim Ethol* 1:3-16-0
- Fraser D, Duncan IJH (1998) 'Pleasures', 'pains' and animal welfare: Toward a natural history of affect. *Anim Welfare* 7:383-96
- Fraser D (2009) Animal behaviour, animal welfare and the scientific study of affect. *Appl Anim Behav Sci* 118:108-17
- Frijda NH (2009) Emotion experience and its varieties. *Emot Rev* 1:264-71
- Gieling ET, Antonides A, Fink-Gremmels J, ter Haar K, Kuller WI, Meijer E, Nordquist RE, Stouten JM, Zeinstra E, van der Staay, Franz Josef (2014) Chronic allopurinol treatment during the last trimester of pregnancy in sows: effects on low and normal birth weight offspring. *PloS one* 9:e86396
- Gieling E (2013) Dissertation: Pigs as animal model for low-birth-weight babies. Developing cognitive tests and examining neuroprotection. Ph.D., Utrecht University

- Gieling E, Wehkamp W, Willigenburg R, Nordquist RE, Ganderup N, van der Staay FJ (2013) Performance of conventional pigs and Göttingen miniature pigs in a spatial holeboard task: effects of the putative muscarinic cognition impairer Biperiden. *Behav Brain Funct* 9:4
- Gieling ET, Musschenga MA, Nordquist RE, van der Staay FJ (2012) Juvenile pigs use simple geometric 2D shapes but not portrait photographs of conspecifics as visual discriminative stimuli. *Appl Anim Behav Sci* 142:142-53
- Gieling ET, Nordquist RE, van der Staay FJ (2011a) Assessing learning and memory in pigs. *Anim Cogn* 14:151-73
- Gieling ET, Park SY, Nordquist RE, van der Staay FJ (2011b) Cognitive performance of low- and normal-birth-weight piglets in a spatial holeboard discrimination task. *Pediatr Res* 71:71-6
- Gieling ET, Schuurman T, Nordquist RE, van der Staay FJ (2011c) The Pig as a Model Animal for Studying Cognition and Neurobehavioral Disorders. In: Hagan JJ (ed) *Molecular and Functional Models in Neuropsychiatry* Springer, Heidelberg, pp 359-383
- Giroux S, Martineau G, Robert S (2000) Relationships between individual behavioural traits and post-weaning growth in segregated early-weaned piglets. *Appl Anim Behav Sci* 70:41-8
- Giuffra E, Kijas JM, Amarger V, Carlborg O, Jeon JT, Andersson L (2000) The origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* 154:1785-91
- Glennon RA, Rosecrans JA, Young R (1983) Drug-induced discrimination: A description of the paradigm and a review of its specific application to the study of hallucinogenic agents. *Med Res Rev* 3:289-340
- Gondret F, Lefaucheur L, Juin H, Louveau I, Lebreton B (2006) Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. *J Anim Sci* 84:93-103
- Gould TD, Dao DT, Kovacsics CE (2009) The Open Field Test. In: Gould TD (ed) *Mood and Anxiety Related Phenotypes in Mice*. *Neuromethods* 42
- Gow R, Thomson S, Rieder M, Van Uum S, Koren G (2010) An assessment of cortisol analysis in hair and its clinical applications. *Forensic Sci Int* 196:32-7
- Greiveldinger L, Veissier I, Boissy A (2009) Behavioural and physiological responses of lambs to controllable vs. uncontrollable aversive events. *Psychoneuroendocrinology* 34:805-14
- Greiveldinger L, Veissier I, Boissy A (2007) Emotional experience in sheep: Predictability of a sudden event lowers subsequent emotional responses. *Physiol Behav* 92:675-83
- Gygax L (2014) The A to Z of statistics for testing cognitive judgement bias. *Anim Behav* 95:59-69
- Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N (2002) Outcomes in young adulthood for very-low-birth-weight infants. *N Engl J Med* 346:149-57
- Hack M, Youngstrom EA, Cartar L, Schluchter M, Taylor HG, Flannery D, Klein N, Borawski E (2004) Behavioral outcomes and evidence of psychopathology among very low birth weight infants at age 20 years. *Pediatrics* 114:932-40
- Hagemann D, Waldstein SR, Thayer JF (2003) Central and autonomic nervous system integration in emotion. *Brain Cogn* 52:79-87
- Hall CS (1934) Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *J Comp Psychol* 18:385-403
- Handley SL, Mithani S (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *N-S Arch Pharmacol* 327:1-5

- Harding EJ, Paul ES, Mendl M (2004) Cognitive bias and affective state. *Nature* 427:312
- Harrison J (1984) The functional analysis of auditory discrimination. *J Acoust Soc Am* 75:1848
- Hartley CA, Phelps EA (2012) Anxiety and decision-making. *Biol Psychiatry* 72:113-8
- Hascoët M, Bourin M, Nic Dhonnchadha BÁ (2001) The mouse light-dark paradigm: A review. *Prog Neuro-Psychopharmacol Biol Psychiatry* 25:141-66
- Hayes B, Sharif F (2009) Behavioural and emotional outcome of very low birth weight infants - literature review. *J Matern Fetal Neonatal Med* 22:849-56
- Hayne SM, Gonyou HW (2006) Behavioural uniformity or diversity? Effects on behaviour and performance following regrouping in pigs. *Appl Anim Behav Sci* 98:28-44
- Hayne SM, Gonyou HW (2003) Effects of regrouping on the individual behavioural characteristics of pigs. *Appl Anim Behav Sci* 82:267-78
- Heffner RS, Heffner HE (1990) Hearing in domestic pigs (*Sus scrofa*) and goats (*Capra hircus*). *Hear Res* 48:231-40
- Held SDE, Špinko M (2011) Animal play and animal welfare. *Anim Behav* 81:891-9
- Held SDE, Byrne RW, Jones S, Murphy E, Friel M, Mendl MT (2010) Domestic pigs, *Sus scrofa*, adjust their foraging behaviour to whom they are foraging with. *Anim Behav* 79:857-62
- Held S, Cooper J, Mendl MT (2009) Advances in the study of cognition, behavioural priorities and emotions. In: Marchant Forde JN (ed) *The Welfare of Pigs*. Springer: Heidelberg, pp 47-94
- Held S, Baumgartner J, Kilbride A, Byrne R, Mendl M (2005) Foraging behaviour in domestic pigs (*Sus scrofa*): remembering and prioritizing food sites of different value. *Anim Cogn* 8:114-21
- Held S, Mendl M, Laughlin K, Byrne RW (2002) Cognition studies with pigs: Livestock cognition and its implication for production. *J Anim Sci* 80:E10-7
- Held S, Mendl M, Devereux C, Byrne RW (2000) Social tactics of pigs in a competitive foraging task: the 'informed forager' paradigm. *Anim Behav* 59:569-76
- Hemsworth PH, Brand A, Willems P (1981a) The behavioural response of sows to the presence of human beings and its relation to productivity. *Livest Prod Sci* 8:67-74
- Hemsworth PH (2003) Human-animal interactions in livestock production. *Appl Anim Behav Sci* 81:185-98
- Hemsworth PH, Barnett JL, Hansen C (1981b) The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. *Horm Behav* 15:396-403
- Hemsworth PH, Coleman GJ, Cox M, Barnett JL (1994) Stimulus generalization: the inability of pigs to discriminate between humans on the basis of their previous handling experience. *Appl Anim Behav Sci* 40:129-42
- Hemsworth PH, Price EO, Borgwardt R (1996a) Behavioural responses of domestic pigs and cattle to humans and novel stimuli. *Appl Anim Behav Sci* 50:43-56
- Hemsworth PH, Verge J, Coleman GJ (1996b) Conditioned approach-avoidance responses to humans: the ability of pigs to associate feeding and aversive social experiences in the presence of humans with humans. *Appl Anim Behav Sci* 50:71-82
- Herskin MS, Jensen KH (2000) Effects of different degrees of social isolation on the behaviour of weaned piglets kept for experimental purposes. *Anim Welfare* 9:237-49
- Hessing MJC, Hagelsø AM, van Beek JAM, Wiepkema RP, Schouten WGP, Krukow R (1993) Individual behavioural characteristics in pigs. *Appl Anim Behav Sci* 37:285-95

- Hessing MJC, Hagelso AM, Schouten WGP, Wiepkema PR, Van Beek JAM (1994) Individual behavioral and physiological strategies in pigs. *Physiol Behav* 55:39-46
- Hillmann E, Mayer C, Schön P, Puppe B, Schrader L (2004) Vocalisation of domestic pigs (*Sus scrofa domestica*) as an indicator for their adaptation towards ambient temperatures. *Appl Anim Behav Sci* 89:195-206
- Hodes GE, Shors TJ (2005) Distinctive stress effects on learning during puberty. *Horm Behav* 48:163-71
- Hogg S (1996) A review of the validity and variability of the Elevated Plus-Maze as an animal model of anxiety. *Pharmacol Biochem Be* 54:21-30
- Holtz W (2010) Pigs and minipigs. In: Hubrecht R and Kirkwood J (eds) *The UFAW Handbook on: The Care and Management of Laboratory and Other Research Animals*, 8th edn. Wiley-Blackwell, United Kingdom, pp 473-494
- Homberg JR, van den Bos R, den Heijer E, Suer R, Cuppen E (2008) Serotonin transporter dosage modulates long-term decision-making in rat and human. *Neuropharmacology* 55:80-4
- Imfeld-Mueller S, Van Wezemael L, Stauffacher M, Gygax L, Hillmann E (2011) Do pigs distinguish between situations of different emotional valences during anticipation? *Appl Anim Behav Sci* 131:86-93
- Isen AM (2008) Some ways in which positive affect influences decision making and problem solving. In: Lewis M, Haviland-Jones JM and Feldman Barrett L (eds) *Handbook of Emotions*, Third Edition edn. The Guilford Press, New York, pp 548
- Izard CE (2007) Basic emotions, natural kinds, emotion schemas, and a new paradigm. *Perspect Psychol Sci* 2:260-80
- James W (1884) What is an emotion? *Mind* 9:188-205
- Jamieson RK, Crump MJC, Hannah SD (2012) An instance theory of associative learning. *Learn Behav* 40:61-82
- Janczak AM, Andersen IL, Bøe KE, Færevik G, Bakken M (2002a) Factor analysis of behaviour in the porcine and murine elevated plus-maze models of anxiety. *Appl Anim Behav Sci* 77:155-66
- Janczak AM, Pedersen LJ, Bakken M (2003a) Aggression, fearfulness and coping styles in female pigs. *Appl Anim Behav Sci* 81:13-28
- Janczak AM, Pedersen LJ, Bakken M (2002b) Effects of variation in pre-test transport duration and animal age on behaviour in the porcine elevated plus-maze - a brief report. *Appl Anim Behav Sci* 77:233-8
- Janczak AM, Pedersen LJ, Jensen KH, Andersen IL, Bøe KE, Bakken M (2000) No effect of variation in handling on behaviour in a porcine elevated plus-maze - a brief report. *Appl Anim Behav Sci* 69:169-73
- Janczak AM, Pedersen LJ, Rydhmer L, Bakken M (2003b) Relation between early fear- and anxiety-related behaviour and maternal ability in sows. *Appl Anim Behav Sci* 82:121-35
- Jarvis S, Moinard C, Robson SK, Baxter E, Ormandy E, Douglas AJ, Seckl JR, Russell JA, Lawrence AB (2006) Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. *Horm Behav* 49:68-80
- Jensen J, McIntosh AR, Crawley AP, Mikulis DJ, Remington G, Kapur S (2003) Direct activation of the ventral striatum in anticipation of aversive stimuli. *Neuron* 40:1251-7
- Jensen KH, Oksbjerg N, Jørgensen E (1994) Dietary salbutamol and level of protein: Effects on the acute stress response in pigs. *Physiol Behav* 55:375-9
- Jensen P (1994) Fighting between unacquainted pigs - effects of age and of individual reaction pattern. *Appl Anim Behav Sci* 41:37-52

- Jensen P, Forkman B, Thodberg K, Köster E (1995) Individual variation and consistency in piglet behaviour. *Appl Anim Behav Sci* 45:43-52
- Jones JB, Wathes CM, White RP, Jones RB (2000) Do pigs find a familiar odourant attractive in novel surroundings? *Appl Anim Behav Sci* 70:115-26
- Jones R, Nicol CJ (1998) A note on the effect of control of the thermal environment on the well-being of growing pigs. *Appl Anim Behav Sci* 60:1-9
- Kaandorp JJ, Derks JB, Oudijk MA, Torrance HL, Harmsen MG, Nikkels PG, van Bel F, Visser GH, Giussani DA (2014) Antenatal allopurinol reduces hippocampal brain damage after acute birth asphyxia in late gestation fetal sheep. *Reprod Sci* 21:251-9
- Kacelnik A, Bateson M (1997) Risk-sensitivity: crossroads for theories of decision-making. *Trends Cogn Sci (Regul Ed)* 1:304-9
- Kanitz E, Puppe B, Tuchscherer M, Heberer M, Viergutz T, Tuchscherer A (2009) A single exposure to social isolation in domestic piglets activates behavioural arousal, neuroendocrine stress hormones, and stress-related gene expression in the brain. *Physiol Behav* 98:176-85
- Kanitz E, Otten W, Tuchscherer M (2005) Central and peripheral effects of repeated noise stress on hypothalamic–pituitary–adrenocortical axis in pigs. *Livest Prod Sci* 94:213-24
- Kanitz E, Tuchscherer M, Puppe B, Tuchscherer A, Stabenow B (2004) Consequences of repeated early isolation in domestic piglets (*Sus scrofa*) on their behavioural, neuroendocrine, and immunological responses. *Brain Behav Immun* 18:35-45
- Keen HA, Nelson OL, Robbins CT, Evans M, Shepherdson DJ, Newberry RC (2014) Validation of a novel cognitive bias task based on difference in quantity of reinforcement for assessing environmental enrichment. *Anim Cogn* 17:529-41
- Kelly HRC, Bruce JM, English PR, Fowler VR, Edwards SA (2000) Behaviour of 3-week weaned pigs in Straw-Flow®, deep straw and flatdeck housing systems. *Appl Anim Behav Sci* 68:269-80
- Kerr A, Zelazo PD (2004) Development of “hot” executive function: The children’s gambling task. *Brain Cogn* 55:148-57
- Kim H, Shimojo S, O’Doherty JP (2006) Is avoiding an aversive outcome rewarding? Neural substrates of avoidance learning in the human brain. *PLoS Biol* 4:e233
- Kirkden RD, Pajor EA (2006) Using preference, motivation and aversion tests to ask scientific questions about animals’ feelings. *Appl Anim Behav Sci* 100:29-47
- Klemcke HG, Lunstra DD, Brown-Borg HM, Borg KE, Christenson RK (1993) Association between low birth weight and increased adrenocortical function in neonatal pigs. *J Anim Sci* 71:1010-8
- Knutson B, Burgdorf J, Panksepp J (2002) Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull* 128:961-77
- Knutson B, Burgdorf J, Panksepp J (1998) Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *J Comp Psychol* 112:65-73
- Koba Y, Tanida H (1999) How do miniature pigs discriminate between people? The effect of exchanging cues between a non-handler and their familiar handler on discrimination. *Appl Anim Behav Sci* 61:239-52
- Koolhaas J.M., Korte S.M., De Boer S.F., Van Der Vegt B.J., Van Reenen C.G., Hopster H., De Jong I.C., Ruis M.A.W., Blokhuis H.J. (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neurosci Biobehav R* 23:925-35
- Koolhaas JM, de Boer SF, Buwalda B (2006) Stress and adaptation toward ecologically relevant animal models. *Curr Dir Psychol Sci* 15:109-12

- Koot S, van den Bos R, Adriani W, Laviola G (2010, August 24-27) Home cage testing of decision-making. In Eds. Spink, AJ, Grieco, F, Krips, OE, Loijens, LWS, Noldus, LPJJ, and Zimmerman, PH, Proceedings of Measuring Behavior, vol. 25. Eindhoven, The Netherlands
- Koot S, Baars A, Hesselings P, van den Bos R, Joëls M (2013) Time-dependent effects of corticosterone on reward-based decision-making in a rodent model of the Iowa Gambling Task. *Neuropharmacology* 70:306-15
- Korte SM, Olivier B, Koolhaas JM (2007) A new animal welfare concept based on allostasis. *Physiol Behav* 92:422-8
- Krain AL, Wilson AM, Arbuckle R, Castellanos FX, Milham MP (2006) Distinct neural mechanisms of risk and ambiguity: A meta-analysis of decision-making. *Neuroimage* 32:477-84
- Kranendonk G, Hopster H, Fillerup M, Ekkel ED, Mulder EJH, Taverne MAM (2006) Cortisol administration to pregnant sows affects novelty-induced locomotion, aggressive behaviour, and blunts gender differences in their offspring. *Horm Behav* 49:663-72
- Kreibitz SD (2010) Autonomic nervous system activity in emotion: A review. *Biol Psychol* 84:394-421
- Lahti J, Räikkönen K, Pesonen A, Heinonen K, Kajantie E, Forsen T, Osmond C, Barker D, Eriksson J (2010) Prenatal growth, postnatal growth and trait anxiety in late adulthood—the Helsinki Birth Cohort Study. *Acta Psychiatr Scand* 121:227-35
- Laudenslager ML, Jorgensen MJ, Grzywa R, Fairbanks LA (2011) A novelty seeking phenotype is related to chronic hypothalamic-pituitary-adrenal activity reflected by hair cortisol. *Physiol Behav* 104:291-5
- Laughlin K, Mendl M (2000) Pigs shift too: foraging strategies and spatial memory in the domestic pig. *Anim Behav* 60:403-10
- Lawrence AB, Terlouw EM (1993) A review of behavioral factors involved in the development and continued performance of stereotypic behaviors in pigs. *J Anim Sci* 71:2815-25
- Lawrence AB, Terlouw EMC, Illius AW (1991) Individual differences in behavioural responses of pigs exposed to non-social and social challenges. *Appl Anim Behav Sci* 30:73-86
- Lazarus RS (1991) Cognition and motivation in emotion. *Am Psychol* 46:352-67
- Leamaster B, Cheeke P (1979) Feed preferences of swine: Alfalfa meal, high and low saponin alfalfa, and quinine sulfate. *Can J Anim Sci* 59:467-9
- Levine LJ, Edelstein RS (2009) Emotion and memory narrowing: A review and goal-relevance approach. *Cogn Emot* 23:833
- Lind NM, Moustgaard A (2005) Response to novelty correlates with learning rate in a Go/No-go task in Göttingen minipigs. *Neural Plast* 12:341-5
- Lind NM, Gjedde A, Moustgaard A, Olsen AK, Jensen SB, Jakobsen S, Arnfred SM, Hansen AK, Hemmingsen RP, Cumming P (2005) Behavioral response to novelty correlates with dopamine receptor availability in striatum of Göttingen minipigs. *Behav Brain Res* 164:172-7
- Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK (2007) The use of pigs in neuroscience: Modeling brain disorders. *Neurosci Biobehav R* 31:728-51
- Loijens LWS, Schouten WGP, Wiepkema PR, Wiegant VM (2002) Brain opioid receptor density reflects behavioral and heart rate responses in pigs. *Physiol Behav* 76:579-87
- Lomas CA, Piggins D, Phillips CJC (1998) Visual awareness. *Appl Anim Behav Sci* 57:247-57
- Lunney JK (2007) Advances in swine biomedical model genomics. *Int J Biol Sci* 3:179-84

- Macbeth B, Cattet M, Stenhouse G, Gibeau M, Janz D (2010) Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (*Ursus arctos*): considerations with implications for other wildlife. *Can J Zool* 88:935-49
- Mackay DG, Shafto M, Taylor JK, Marian DE, Abrams L, Dyer JR (2004) Relations between emotion, memory, and attention: Evidence from taboo Stroop, lexical decision, and immediate memory tasks. *Mem Cognition* 32:474-88
- Magnani D, Cafazzo S, Calà P, Costa LN (2012) Searching for differences in the behavioural response of piglet groups subjected to novel situations. *Behav Process* 89:68-73
- Mallard EC, Rees S, Stringer M, Cock ML, Harding R (1998) Effects of chronic placental insufficiency on brain development in fetal sheep. *Pediatr Res* 43:262-70
- Maner JK, Richey JA, Cromer K, Mallott M, Lejuez CW, Joiner TE, Schmidt NB (2007) Dispositional anxiety and risk-avoidant decision-making. *Pers Individ Differ* 42:665-75
- Manteuffel G, Schön PC (2004) STREMODO, ein innovatives Verfahren zur kontinuierlichen Erfassung der Stressbelastung von Schweinen bei Haltung und Transport. *Arch Tierzucht* 47:173-81
- Manteuffel G, Puppe B, Schön PC (2004) Vocalization of farm animals as a measure of welfare. *Appl Anim Behav Sci* 88:163-82
- Manteuffel G, Schön PC, Döpjan S, Tuchscherer A, Bellmann O (2007) Acetylcholine injection into the amygdala elicits vocalization in domestic pigs (*Sus scrofa*). *Behav Brain Res* 178:177-80
- Mar AC, Dalley JW (2010) Cognition: Attention and Impulsivity. In: Koob GF, Le Moal M and Thompson RF (eds) *Encyclopedia of Behavioral Neuroscience*. Academic Press, Oxford, pp 262-271
- Marchant JN, Whittaker X, Broom DM (2001) Vocalisations of the adult female domestic pig during a standard human approach test and their relationships with behavioural and heart rate measures. *Appl Anim Behav Sci* 72:23-39
- Marchant-Forde JN (2009) Introduction to the Welfare of Pigs. In: Marchant-Forde JN (ed) *The Welfare of Pigs*. Springer, Heidelberg, pp 1-12
- Marchant-Forde JN (2002) Piglet- and stockperson-directed sow aggression after farrowing and the relationship with a pre-farrowing, human approach test. *Appl Anim Behav Sci* 75:115-32
- Marchant-Forde JN, Bradshaw RH, Marchant-Forde RM, Broom DM (2003) A note on the effect of gestation housing environment on approach test measures in gilts. *Appl Anim Behav Sci* 80:287-96.
- Marro PJ, Andersen CB, Mishra OP, Delivoria-Papadopoulos M (1999) Effect of allopurinol on hypoxia-induced modification of the NMDA receptor in newborn piglets. *Neurochem Res* 24:1301-6
- Maršál K, Ley D (1992) Intrauterine blood flow and postnatal neurological development in growth-retarded fetuses. *Neonatology* 62:258-64
- Martínez L, Marí-Beffa P, Roldán-Tapia D, Ramos-Lizana J, Fuentes LJ, Estévez AF (2012) Training with differential outcomes enhances discriminative learning and visuospatial recognition memory in children born prematurely. *Res Dev Disabil* 33:76-84
- Marx G, Horn T, Thielebein J, Knubel B, von Borell E (2003) Analysis of pain-related vocalization in young pigs. *J Sound Vibrat* 266:687-98
- Matheson SM, Asher L, Bateson M (2008) Larger, enriched cages are associated with 'optimistic' response biases in captive European starlings (*Sturnus vulgaris*). *Appl Anim Behav Sci* 109:374-83

- Matthews LR, Ladewig J (1994) Environmental requirements of pigs measured by behavioural demand functions. *Anim Behav* 47:713-9
- Mauss IB, Robinson MD (2009) Measures of emotion: A review. *Cogn Emot* 23:209-37
- Mazur JE (1988) Choice between small certain and large uncertain reinforcers. *Anim Learn Behav* 16:199-205
- McElreath R, Strimling P (2006) How noisy information and individual asymmetries can make 'personality' an adaptation: a simple model. *Anim Behav* 72:1135-9
- McMillan FD (2008) Stress, Distress, and Emotion: Distinctions and Implications for Mental Well-Being. In: McMillan FD (ed) *Mental Health and Well-Being in Animals* Blackwell Publishing Ltd, Oxford, UK, pp 93-111
- Meese GB, Conner DJ, Baldwin BA (1975) Ability of the pig to distinguish between conspecific urine samples using olfaction. *Physiol Behav* 15:121-5
- Melotti L, Oostindjer M, Bolhuis EJ, Held S, Mendl M (2011) Coping personality type and environmental enrichment affect aggression at weaning in pigs. *Appl Anim Behav Sci* 133:144-53
- Melotti L, Thomsen LR, Toscano MJ, Mendl M, Held S (2013) Delay discounting task in pigs reveals response strategies related to dopamine metabolite. *Physiol Behav* 120:182-92
- Mendl M (1999) Performing under pressure: stress and cognitive function. *Appl Anim Behav Sci* 65:221-44
- Mendl M, Laughlin K, Hitchcock D (1997) Pigs in space: spatial memory and its susceptibility to interference. *Anim Behav* 54:1491-508
- Mendl MT, Burman OHP, Paul ES (2010a) An integrative and functional framework for the study of animal emotion and mood. *Proc R Soc B: Biol Sci* 277:2895-904
- Mendl M, Brooks J, Basse C, Burman O, Paul E, Blackwell E, Casey R (2010b) Dogs showing separation-related behaviour exhibit a 'pessimistic' cognitive bias. *Curr Biol* 20:R839-40
- Mendl M, Burman OHP, Parker RMA, Paul ES (2009) Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Appl Anim Behav Sci* 118:161-81
- Meunier-Salaün MC, Edwards SA, Robert S (2001) Effect of dietary fibre on the behaviour and health of the restricted fed sow. *Anim Feed Sci Technol* 90:53-69
- Mick E, Biederman J, Prince J, Fischer MJ, Faraone SV (2002) Impact of low birth weight on attention-deficit hyperactivity disorder. *J Dev Behav Pediatr* 23:16-22
- Milligan BN, Fraser D, Kramer DL (2002) Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. *Livest Prod Sci* 76:181-91
- Miranda R, Mennin DS (2007) Depression, generalized anxiety disorder, and certainty in pessimistic predictions about the future. *Cognitive therapy and research* 31:71-82
- Misslin R, Cigrang M (1986) Does neophobia necessarily imply fear or anxiety? *Behav Process* 12:45-50
- Miu AC, Heilman RM, Houser D (2008) Anxiety impairs decision-making: psychophysiological evidence from an Iowa Gambling Task. *Biol Psychol* 77:353-8
- Modo M, Sowinski P, Hodges H (2000) Conditional discrimination learning in rats with global ischaemic brain damage. *Behav Brain Res* 111:213-21
- Montgomery KC (1955) The relation between fear induced by novel stimulation and exploratory drive. *J Comp Physiol Psychol* 48:254-60

- Moors A (2009) Theories of emotion causation: A review. *Cogn Emot* 23:625-62
- Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S, Veissier I (2007) Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiol Behav* 92:317-39
- Mormède P, García-Belenguier S, Dulluc J, Oliver C (1994) Independent segregation of a hyperactive hypothalamic-hypophyso-adrenal axis and a reduced behavioural reactivity in pigs. *Psychoneuroendocrinology* 19:305-11
- Morrison RS, Johnston LJ, Hilbrands AM (2007) The behaviour, welfare, growth performance and meat quality of pigs housed in a deep-litter, large group housing system compared to a conventional confinement system. *Appl Anim Behav Sci* 103:12-24
- Moura DJ, Silva WT, Naas IA, Tolón YA, Lima KAO, Vale MM (2008) Real time computer stress monitoring of piglets using vocalization analysis. *Comput Electron Agric* 64:11-8
- Moustgaard A, Arnfred SM, Lind NM, Hansen AK, Hemmingsen R (2004) Discriminations, reversals, and extra-dimensional shifts in the Göttingen minipig. *Behav Process* 67:27-37
- Moustgaard A, Arnfred SM, Lind NM, Hemmingsen R, Hansen AK (2005) Acquisition of visually guided conditional associative tasks in Göttingen minipigs. *Behav Process* 68:97-102
- Murphy E, Kraak L, Nordquist RE, van der Staay FJ (2013a) Successive and conditional discrimination learning in pigs. *Anim Cogn* 63:883-93
- Murphy E, Kraak L, van den Broek J, Nordquist RE, van der Staay FJ (In Press) Decision-making under risk and ambiguity in low-birth-weight pigs. *Anim Cogn*
- Murphy E, Nordquist RE, van der Staay FJ (2013b) Responses of conventional pigs and Göttingen miniature pigs in an active choice judgement bias task. *Appl Anim Behav Sci* 148:64-76
- Murphy E, Scholman L, Gieling ET, Nordquist RE, van der Staay, Franz Josef (in prep.) Judgement bias in low- and normal-birth-weight pigs, *Sus scrofa*.
- Murphy E, Nordquist RE, van der Staay, Franz Josef (2014) A review of behavioural methods to study emotion and mood in pigs, *Sus scrofa*. *Appl Anim Behav Sci* 159:9-28
- Nawroth C, Ebersbach M, von Borell E (2013) Are juvenile domestic pigs (*Sus scrofa domestica*) sensitive to the attentive states of humans? The impact of impulsivity on choice behaviour. *Behav Process* 96:53-8
- Neave HW, Daros RR, Costa JH, von Keyserlingk MA, Weary DM (2013) Pain and pessimism: dairy calves exhibit negative judgement bias following hot-iron disbudding. *PLoS One* 8:e80556
- Neill JC, Harrison J (1987) Auditory discrimination: the Konorski quality-location effect. *J Exp Anal Behav* 48:81-95
- Nelson SL, Sanregret JD (1997) Response of pigs to bitter-tasting compounds. *Chem Senses* 22:129-32
- Newberry RC, Wood-Gush DGM, Hall JW (1988) Playful behaviour of piglets. *Behav Process* 17:205-16
- Nielsen TR, Kornum BR, Moustgaard A, Gade A, Lind NM, Knudsen GM (2009) A novel spatial delayed non-match to sample (DNMS) task in the Göttingen minipig. *Behav Brain Res* 196:93-8

- O'Connor EA, Parker MO, McLeman MA, Demmers TG, Lowe JC, Cui L, Davey EL, Owen RC, Wathes CM, Abeyesinghe SM (2010) The impact of chronic environmental stressors on growing pigs, *Sus scrofa* (Part 1): stress physiology, production and play behaviour. *Animal* 4:1899-909
- O'Keefe MJ, O'Callaghan M, Williams GM, Najman JM, Bor W (2003) Learning, cognitive, and attentional problems in adolescents born small for gestational age. *Pediatrics* 112:301-7
- Ohl F, van der Staay FJ (2012) Animal welfare: At the interface between science and society. *Vet J* 192:13-9
- Ohl F (2003) Testing for anxiety. *Clin Neurosci Res* 3:233-8
- Olsson IAS, de Jonge FH, Schuurman T, Helmond FA (1999) Poor rearing conditions and social stress in pigs: repeated social challenge and the effect on behavioural and physiological responses to stressors. *Behav Process* 46:201-15
- Olsson IAS, Würbel H, Mench JA (2011) Chapter 9: Behaviour. In: Appleby MC, Mench JA, Olsson IAS and Hughes BO (eds) *Animal Welfare*, 2nd edn. CABI, Wallingford, U.K., pp 138-154
- Olton DS, Schlosberg P (1978) Food-searching strategies in young rats: Win-shift predominates over win-stay. *J Comp Physiol Psychol* 92:609
- Otten W, Kanitz E, Tuchscherer M, Puppe B, Nürnberg G (2007) Repeated administrations of adrenocorticotrophic hormone during gestation in gilts: Effects on growth, behaviour and immune responses of their piglets. *Livest Sci* 106:261-70
- Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 58:87-114
- Pais-Vieira M, Aguiar P, Lima D, Galhardo V (2012) Inflammatory pain disrupts the orbitofrontal neuronal activity and risk-assessment performance in a rodent decision-making task. *Pain* 153:1625-35
- Pais-Vieira M, Lima D, Galhardo V (2007) Orbitofrontal cortex lesions disrupt risk assessment in a novel serial decision-making task for rats. *Neuroscience* 145:225-31
- Palagi E, Cordoni G, Borgognini Tarli S (2004) Immediate and delayed benefits of play behaviour: new evidence from chimpanzees (*Pan troglodytes*). *Ethology* 110:949-62
- Palmer C, Towfighi J, Roberts RL, Heitjan DF (1993) Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatr Res* 33:405-11
- Palmer C, Vannucci RC, Towfighi J (1990) Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. *Pediatr Res* 27:332-6
- Panksepp J (1982) Toward a general psychobiological theory of emotions. *Behav Brain Sci* 5:407-22
- Papciak J, Popik P, Fuchs E, Rygula R (2013) Chronic psychosocial stress makes rats more 'pessimistic' in the ambiguous-cue interpretation paradigm. *Behav Brain Res* 256:305-10
- Papini MR (2003) Comparative psychology of surprising nonreward. *Brain Behav Evol* 62:83-95
- Papini MR (2002) Pattern and process in the evolution of learning. *Psychol Rev* 109:186-201
- Papini MR, Dudley RT (1997) Consequences of surprising reward omissions. *Rev Gen Psychol* 1:175-97
- Paré WP, Tejani-Butt S, Kluczynski J (2001) The emergence test: effects of psychotropic drugs on neophobic disposition in Wistar Kyoto (WKY) and Sprague Dawley Rats. *Prog Neuro-Psychoph* 25:1615-28

- Parrott RF, Vellucci SV, Goode JA (2000) Behavioral and hormonal effects of centrally injected “anxiogenic” neuropeptides in growing pigs. *Pharmacol Biochem Be* 65:123-9
- Paul ES, Harding EJ, Mendl M (2005) Measuring emotional processes in animals: the utility of a cognitive approach. *Neurosci Biobehav R* 29:469-91
- Pearce GP, Paterson AM (1993) The effect of space restriction and provision of toys during rearing on the behaviour, productivity and physiology of male pigs. *Appl Anim Behav Sci* 36:11-28
- Pechtel P, Pizzagalli DA (2011) Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology* 214:55-70
- Pedersen LJ, Holm L, Jensen MB, Jørgensen E (2005) The strength of pigs’ preferences for different rooting materials measured using concurrent schedules of reinforcement. *Appl Anim Behav Sci* 94:31-48
- Pedersen LJ, Jensen MB, Hansen SW, Munksgaard L, Ladewig J, Matthews L (2002) Social isolation affects the motivation to work for food and straw in pigs as measured by operant conditioning techniques. *Appl Anim Behav Sci* 77:295-309
- Peters E, Västfjäll D, Gärling T, Slovic P (2006) Affect and decision making: A “hot” topic. *J Behav Decis Making* 19:79-85
- Peeters-Scholte C, Braun K, Koster J, Kops N, Blomgren K, Buonocore G, van Buul-Offers S, Hagberg H, Nicolay K, van Bel F (2003) Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. *Pediatr Res* 54:516-22
- Pellis S, Pellis V (2009) *The playful brain: venturing to the limits of neuroscience.* Oneworld Oxford, UK
- Pessoa L (2008) On the relationship between emotion and cognition. *Nat Rev Neurosci* 9:148-58
- Peters E, Västfjäll D, Gärling T, Slovic P (2006) Affect and decision making: A “hot” topic. *J Behav Decis Making* 19:79-85
- Phillips C (2009) Definitions and Concepts of Animal Welfare. In: Phillips C (Ed) *The Welfare of Animals.* Springer, Netherlands, pp 1-12
- Phillips PA, Thompson BK, Fraser D (1988) Preference tests of ramp designs for young pigs. *Can J Anim Sci* 68:41-8
- Phillips P, Fraser D, Thompson B (1996) Sow preference for types of flooring in farrowing crates. *Can J Anim Sci* 76:485-9
- Poletto R, Steibel JP, Siegford JM, Zanella AJ (2006) Effects of early weaning and social isolation on the expression of glucocorticoid and mineralocorticoid receptor and 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 mRNAs in the frontal cortex and hippocampus of piglets. *Brain Res* 1067:36-42
- Pomerantz O, Terkel J, Suomi SJ, Paukner A (2012) Stereotypic head twirls, but not pacing, are related to a ‘pessimistic’-like judgment bias among captive tufted capuchins (*Cebus apella*). *Anim Cogn* 15:689-98
- Poore KR, Fowden AL (2003) The effect of birth weight on hypothalamo-pituitary-adrenal axis function in juvenile and adult pigs. *J Physiol* 547:107-16
- Prickaerts J, Honig W, Schmidt BH, Blokland A (1999) Mefenazine improves working but not reference memory performance in a spatial cone field task. *Eur J Pharmacol* 380:61-5
- Prickaerts J, Gieling ET, Bruder AK, van der Staay, Franz J, Vanmierlo T (2014) Long-term effects of prenatal allopurinol treatment on brain plasticity markers in low and normal birth weight piglets. *Int J Dev Neurosci* 33:29-32

- Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 463:3-33
- Puppe B, Tuchscherer M, Tuchscherer A (1997) The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livest Prod Sci* 48:157-64
- Puppe B, Ernst K, Schön PC, Manteuffel G (2007) Cognitive enrichment affects behavioural reactivity in domestic pigs. *Appl Anim Behav Sci* 105:75-86
- Puppe B, Schön PC, Tuchscherer A, Manteuffel G (2005) Castration-induced vocalisation in domestic piglets, *Sus scrofa*: Complex and specific alterations of the vocal quality. *Appl Anim Behav Sci* 95:67-78
- Purslow PP, Mandell IB, Widowski TM, Brown J, deLange CFM, Robinson JAB, Squires EJ, Cha MC, VanderVoort G (2008) Modelling quality variations in commercial Ontario pork production. *Meat Sci* 80:123-31
- Quiniou N, Dagorn J, Gaudré D (2002) Variation of piglets' birth weight and consequences on subsequent performance. *Livest Prod Sci* 78:63-70
- Radlowski EC, Conrad MS, Lezmi S, Dilger RN, Sutton B, Larsen R, Johnson RW (2014) A neonatal piglet model for investigating brain and cognitive development in small for gestational age human infants. *PloS one* 9:e91951
- Raikkonen K, Pesonen A, Heinonen K, Kajantie E, Hovi P, Jarvenpaa A, Eriksson JG, Andersson S (2008) Depression in young adults with very low birth weight: the Helsinki study of very low-birth-weight adults. *Arch Gen Psychiatry* 65:290
- Ramos A, Pereira E, Martins GC, Wehrmeister TD, Izídio GS (2008) Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. *Behav Brain Res* 193:277-88
- Reimert I, Bolhuis JE, Kemp B, Rodenburg TB (2013) Indicators of positive and negative emotions and emotional contagion in pigs. *Physiol Behav* 109:42-50
- Reynolds LP (2009) Perspectives: The decline of domestic animal research in agriculture and biomedicine. *J Anim Sci* 87:4181-2
- Richter SH, Schick A, Hoyer C, Lankisch K, Gass P, Vollmayr B (2012) A glass full of optimism: Enrichment effects on cognitive bias in a rat model of depression. *Cogn Affect Behav Ne* 12:527-42
- Rivalan M, Ahmed SH, Dellu-Hagedorn F (2009) Risk-prone individuals prefer the wrong options on a rat version of the Iowa Gambling Task. *Biol Psychiatry* 66:743-9
- Rolls ET (2000) Précis of the brain and emotion. *Behav Brain Sci* 23:177-234
- Ruis MAW, de Groot J, te Brake JHA, Dinand Ekkel E, van de Burgwal JA, Erkens JHF, Engel B, Buist WG, Blokhuis HJ, Koolhaas JM (2001a) Behavioural and physiological consequences of acute social defeat in growing gilts: effects of the social environment. *Appl Anim Behav Sci* 70:201-25
- Ruis MAW, te Brake JHA, Engel B, Buist WG, Blokhuis HJ, Koolhaas JM (2002) Implications of coping characteristics and social status for welfare and production of paired growing gilts. *Appl Anim Behav Sci* 75:207-31
- Ruis MAW, te Brake JHA, Engel B, Buist WG, Blokhuis HJ, Koolhaas JM (2001b) Adaptation to social isolation: Acute and long-term stress responses of growing gilts with different coping characteristics. *Physiol Behav* 73:541-51
- Ruis MAW, te Brake JHA, van de Burgwal JA, de Jong IC, Blokhuis HJ, Koolhaas JM (2000) Personalities in female domesticated pigs: behavioural and physiological indications. *Appl Anim Behav Sci* 66:31-47

- Rushen J (2003) Changing concepts of farm animal welfare: bridging the gap between applied and basic research. *Appl Anim Behav Sci* 81:199-214-2
- Russell E, Koren G, Rieder M, Van Uum S (2012) Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. *Psychoneuroendocrinology* 37:589-601
- Russell JA (2003) Core affect and the psychological construction of emotion. *Psychol Rev* 110:145-72
- Rutherford KMD, Baxter EM, D'Eath RB, Turner SP, Arnott G, Roehe R, Ask B, Sandøe P, Moustsen VA, Thorup F (2013) The welfare implications of large litter size in the domestic pig I: biological factors. *Anim Welfare* 22:199-218
- Rutherford KMD, Donald RD, Lawrence AB, Wemelsfelder F (2012) Qualitative Behavioural Assessment of emotionality in pigs. *Appl Anim Behav Sci* 139:218-24
- Rutherford KMD, Haskell MJ, Glasbey C, Lawrence AB (2006) The responses of growing pigs to a chronic-intermittent stress treatment. *Physiol Behav* 89:670-80
- Rygula R, Papciak J, Popik P (2014) The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on the cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm. *Eur Neuropsychopharmacol* 24:1103-1111
- Rygula R, Papciak J, Popik P (2013) Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology* 38:2188-96
- Rygula R, Pluta H, Popik P (2012) Laughing rats are optimistic. *PloS one* 7:e51959
- Salmeto AL, Hymel KA, Carpenter EC, Brilot BO, Bateson M, Sufka KJ (2011) Cognitive bias in the chick anxiety–depression model. *Brain Res* 1373:124-30
- Sanger ME, Doyle RE, Hinch GN, Lee C (2011) Sheep exhibit a positive judgement bias and stress-induced hyperthermia following shearing. *Appl Anim Behav Sci* 131:94-103
- Sankaran S, Kyle PM (2009) Aetiology and pathogenesis of IUGR. *Best Pract Res Cl OB* 23:765-77
- Schachar R, Logan GD, Robaey P, Chen S, Ickowicz A, Barr C (2007) Restraint and cancellation: Multiple inhibition deficits in attention deficit hyperactivity disorder. *J Abnorm Child Psychol* 35:229-38
- Scherer KR (1999) Appraisal Theory. In: Dalglish T and Power M (eds) *Handbook of cognition and Emotion*. John Wiley & Sons Ltd., Chichester, UK, pp 637
- Scherer KR (2005) What are emotions? And how can they be measured? *Soc Sci Inform* 44:695-729
- Schmidt LA, Miskovic V, Boyle MH, Saigal S (2008) Shyness and timidity in young adults who were born at extremely low birth weight. *Pediatrics* 122:e181-7
- Schnall S (2010) Affect, mood and emotions. In: Penelope Peterson, Eva Baker and Barry McGaw (eds) *International encyclopedia of education*. Elsevier, Oxford, UK, pp 544-548
- Schön PC, Puppe B, Manteuffel G (2004) Automated recording of stress vocalisations as a tool to document impaired welfare in pigs. *Anim Welfare* 13:105-10
- Schwarz N (2000) Emotion, cognition, and decision making. *Cogn Emot* 14:433-40
- Scollo A, Gottardo F, Contiero B, Edwards SA (2014) Does stocking density modify affective state in pigs as assessed by cognitive bias, behavioural and physiological parameters? *Appl Anim Behav Sci* 153:26-35
- Scott SK, Sauter D, McGettigan C (2009a) Brain mechanisms for processing perceived emotional vocalizations in humans. In: Brudzynski SM (ed) *Handbook of Mammalian Vocalization*. Academic Press, Oxford, U.K., pp 187-198

- Scott K, Laws DM, Courboulay V, Meunier-Salaün M, Edwards SA (2009b) Comparison of methods to assess fear of humans in sows. *Appl Anim Behav Sci* 118:36-41
- Selye H (1973) The evolution of the stress concept: The originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. *Am Sci* 61:692-9
- Seyfarth RM, Cheney DL (2003) Signallers and receivers in animal communication. *Annu Rev Psychol* 54:145-73
- Siegfurd JM, Rucker G, Zanella AJ (2008) Effects of pre-weaning exposure to a maze on stress responses in pigs at weaning and on subsequent performance in spatial and fear-related tests. *Appl Anim Behav Sci* 110:189-202
- Silva A, Metha Z, O'Callaghan FJ (2006) The relative effect of size at birth, postnatal growth and social factors on cognitive function in late childhood. *Ann Epidemiol* 16:469-76
- Smulders D, Verbeke G, Mormède P, Geers R (2006) Validation of a behavioral observation tool to assess pig welfare. *Physiol Behav* 89:438-47
- Spake JR, Gray KA, Cassady JP (2012) Relationship between backtest and coping styles in pigs. *Appl Anim Behav Sci* 140:146-53
- Špinka M, Newberry RC, Bekoff M (2001) Mammalian play: training for the unexpected. *Q Rev Biol* 76:141-68
- Špinka M (2012) Social dimension of emotions and its implication for animal welfare. *Appl Anim Behav Sci* 138:170-81
- Špinka M (2009) Behaviour of Pigs. In: Jensen P (ed) *The Ethology of Domestic Animals: An Introductory Text*, 2nd Edition edn. CAB International, Wallingford, pp 177
- Špinka M (2006) How important is natural behaviour in animal farming systems? *Appl Anim Behav Sci* 100:117-28
- Spoolder HA, Burbidge JA, Edwards SA, Howard Simmins P, Lawrence AB (1995) Provision of straw as a foraging substrate reduces the development of excessive chain and bar manipulation in food restricted sows. *Appl Anim Behav Sci* 43:249-62
- Spoolder HAM, Burbidge JA, Lawrence AB, Simmins PH, Edwards SA (1996) Individual behavioural differences in pigs: intra-and inter-test consistency. *Appl Anim Behav Sci* 49:185-98
- Spruijt BM, van den Bos R, Pijlman FTA (2001) A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Appl Anim Behav Sci* 72:145-71
- Stockman CA, Collins T, Barnes AL, Miller D, Wickham SL, Beatty DT, Blache D, Wemelsfelder E, Fleming PA (2011) Qualitative behavioural assessment and quantitative physiological measurement of cattle naïve and habituated to road transport. *Anim Prod Sci* 51:240-9
- Strauss RS (2000) Adult functional outcome of those born small for gestational age. *JAMA-J Am Med Assoc* 283:625-32
- Studnitz M, Jensen MB, Pedersen LJ (2007) Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environmental enrichment. *Appl Anim Behav Sci* 107:183-97
- Sugrue LP, Corrado GS, Newsome WT (2005) Choosing the greater of two goods: neural currencies for valuation and decision making. *Nat Rev Neurosci* 6:363-75
- Suhr JA, Tsanadis J (2007) Affect and personality correlates of the Iowa Gambling Task. *Pers Individ Differ* 43:27-36
- Sumner BEH, D'Eath RB, Farnworth MJ, Robson S, Russell JA, Lawrence AB, Jarvis S (2008) Early weaning results in less active behaviour, accompanied by lower 5-HT1A and higher 5-HT2A receptor mRNA expression in specific brain regions of female pigs. *Psychoneuroendocrinology* 33:1077-92

- Swan MP, Hickman DL (2014) Evaluation of the neutrophil-lymphocyte ratio as a measure of distress in rats. *Lab Anim (NY)* 43:276-82
- Swindle M, Smith AC (2008) Swine in biomedical research. In: Conn PM (ed) *Sourcebook of Models for Biomedical Research* Humana Press, Totowa, New Jersey, pp 233
- Swindle MM, Makin A, Herron AJ, Clubb FJ, Jr, Frazier KS (2012) Swine as models in biomedical research and toxicology testing. *Vet Pathol* 49:344-56
- Symonds MR, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav Ecol Sociobiol* 65:13-21
- Talling JC, Waran NK, Wathes CM, Lines JA (1996) Behavioural and physiological responses of pigs to sound. *Appl Anim Behav Sci* 48:187-201
- Tanida H, Miura A, Tanaka T, Yoshimoto T (1996) Behavioral responses of piglets to darkness and shadows. *Appl Anim Behav Sci* 49:173-83
- Tanida H, Miura A, Tanaka T, Yoshimoto T (1995) Behavioral response to humans in individually handled weanling pigs. *Appl Anim Behav Sci* 42:249-59
- Tanida H, Senda K, Suzuki S, Tanaka T, Yoshimoto T (1991) Color discrimination in weanling pigs. *Anim Sci Technol (Japan)* 62:1029-34
- Taylor AA, Weary DM (2000) Vocal responses of piglets to castration: identifying procedural sources of pain. *Appl Anim Behav Sci* 70:17-26
- Taylor L, Friend TH (1986) Open-field test behavior of growing swine maintained on a concrete floor and a pasture. *Appl Anim Behav Sci* 16:143-8
- Temple D, Manteca X, Velarde A, Dalmau A (2011) Assessment of animal welfare through behavioural parameters in Iberian pigs in intensive and extensive conditions. *Appl Anim Behav Sci* 131:29-39
- Terlouw EMC, Porcher J (2005) Repeated handling of pigs during rearing. I. Refusal of contact by the handler and reactivity to familiar and unfamiliar humans. *J Anim Sci* 83:1653-63
- Terlouw E, Lawrence AB, Illius AW (1991) Influences of feeding level and physical restriction on development of stereotypies in sows. *Anim Behav* 42:981-91
- Thodberg K, Jensen KH, Herskin MS (1999) A general reaction pattern across situations in prepubertal gilts. *Appl Anim Behav Sci* 63:103-19
- Thurffjell H, Ball JP, Åhlén P, Kornacher P, Dettki H, Sjöberg K (2009) Habitat use and spatial patterns of wild boar *Sus scrofa* (L.): agricultural fields and edges. *Eur J Wildlife Res* 55:517-23
- Tönepöhl B, Appel AK, Welp S, Voß B, König von Borstel U, Gauly M (2012) Effect of marginal environmental and social enrichment during rearing on pigs' reactions to novelty, conspecifics and handling. *Appl Anim Behav Sci* 140:137-45
- Torrance HL, Benders MJ, Derks JB, Rademaker CM, Bos AF, Van Den Berg P, Longini M, Buonocore G, Venegas M, Baquero H, Visser GH, Van Bel F (2009) Maternal allopurinol during fetal hypoxia lowers cord blood levels of the brain injury marker S-100B. *Pediatrics* 124:350-7
- Trapold MA (1970) Are expectancies based upon different positive reinforcing events discriminably different? *Learn Motiv* 1:129-40
- Treit D, Menard J, Royan C (1993) Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Be* 44:463-9
- Tsutsumi H, Morikawa N, Niki R, Tanigawa M (2001) Acclimatization and response of minipigs toward humans. *Lab Anim* 35:236-42

- Tuchscherer M, Kanitz E, Puppe B, Tuchscherer A, Stabenow B (2004) Effects of postnatal social isolation on hormonal and immune responses of pigs to an acute endotoxin challenge. *Physiol Behav* 82:503-11
- Turnbull OH, Evans CE, Bunce A, Carzolio B, O'Connor J (2005) Emotion-based learning and central executive resources: An investigation of intuition and the Iowa Gambling Task. *Brain Cogn* 57:244-7
- Tuytens FAM (2005) The importance of straw for pig and cattle welfare: A review. *Appl Anim Behav Sci* 92:261-82
- Urquioli P (2005) Behavioral and associative effects of differential learning outcomes in discrimination testing. *Learn Behav* 33:1-21
- van den Bos R, Lasthuis W, den Heijer E, van der Harst J, Spruijt B (2006) Toward a rodent model of the Iowa gambling task. *Behav Res Meth* 38:470-8
- van der Staay FJ, Pouzet B, Mahieu M, Nordquist RE, Schuurman T (2009a) The *d*-amphetamine-treated Göttingen miniature pig: an animal model for assessing behavioral effects of antipsychotics. *Psychopharmacology* 206:715-29
- van der Staay FJ, van Nies J, Raaijmakers W (1990) The effects of aging in rats on working and reference memory performance in a spatial holeboard discrimination task. *Behav Neural Biol* 53:356-70
- van der Staay FJ, Arndt S, Nordquist RE (2009b) Evaluation of animal models of neurobehavioral disorders. *Behav Brain Funct* 5:11
- van der Staay FJ, Arndt SS, Nordquist RE (2010) The standardization-generalization dilemma: a way out. *Genes Brain Behav* 9:849-55
- van der Staay FJ, Gieling ET, Pinzón NE, Nordquist RE, Ohl F (2012) The appetitively motivated "cognitive" holeboard: A family of complex spatial discrimination tasks for assessing learning and memory. *Neurosci Biobehav R* 36:379-403
- Van Dijk AJ, Parvizi N, Taverne MAM, Fink-Gremmels J (2008) Placental transfer and pharmacokinetics of allopurinol in late pregnant sows and their fetuses. *J Vet Pharmacol Ther* 31:489-95
- van Erp-van der Kooij E, Kuijpers AH, Schrama JW, van Eerdenburg FJCM, Schouten WGP, Tielen MJM (2002) Can we predict behaviour in pigs?: Searching for consistency in behaviour over time and across situations. *Appl Anim Behav Sci* 75:293-305
- van Putten G, Elshof WJ (1978) Observations on the effect of transport on the well being and lean quality of slaughter pigs. *Anim Regulat Stud* 1:247-71
- Van Uum S, Sauve B, Fraser L, Morley-Forster P, Paul T, Koren G (2008) Elevated content of cortisol in hair of patients with severe chronic pain: A novel biomarker for stress: Short communication. *Stress* 11:483-8
- van Zutphen L, Baumans V, Beynen A (Eds.) (2001) *Principles of Laboratory Animal Science: A Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Results*. Amsterdam: Elsevier
- Vanderschuren L (2010) How the brain makes play fun. *Am J Play* 2:315-37
- Veissier I, Boissy A, Désiré L, Greiveldinger L (2009) Animals' emotions: studies in sheep using appraisal theories. *Anim Welfare* 18:347-54
- Vodicka P, Smetana K, Dvoránková B, Emerick T, Xu YZ, Ourednik J, Ourednik V, Motlík J (2005) The miniature pig as an animal model in biomedical research. *Ann N Y Acad Sci* 1049:161-71
- von Borell E, Schäffer D (2005) Legal requirements and assessment of stress and welfare during transportation and pre-slaughter handling of pigs. *Livest Prod Sci* 97:81-7

- von Borell E, Ladewig J (1992) Relationship between behaviour and adrenocortical response pattern in domestic pigs. *Appl Anim Behav Sci* 34:195-206
- Vuguin PM (2007) Animal models for small for gestational age and fetal programming of adult disease. *Horm Res* 68:113-23
- Waiblinger S, Boivin X, Pedersen V, Tosi M, Janczak AM, Visser EK, Jones RB (2006) Assessing the human-animal relationship in farmed species: A critical review. *Appl Anim Behav Sci* 101:185-242
- Walf AA, Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2:322-328
- Walk RD, Gibson EJ (1961) A comparative and analytical study of visual depth perception. *Psychol Monogr-Gen A* 75:1-44
- Walsh RN, Cummins RA (1976) The open-field test: A critical review. *Psychol Bull* 83:482-504
- Weary DM, Braithwaite LA, Fraser D (1998) Vocal response to pain in piglets. *Appl Anim Behav Sci* 56:161-72
- Weary DM, Fraser D (1995) Calling by domestic piglets: reliable signals of need? *Anim Behav* 50:1047-55
- Wemelsfelder F, Haskell M, Mendl MT, Calvert S, Lawrence AB (2000) Diversity of behaviour during novel object tests is reduced in pigs housed in substrate-impooverished conditions. *Anim Behav* 60:385-94
- Wemelsfelder F, Hunter TEA, Mendl MT, Lawrence AB (2001) Assessing the 'whole animal': a free choice profiling approach. *Anim Behav* 62:209-20
- Wemelsfelder F, Nevison I, Lawrence AB (2009) The effect of perceived environmental background on qualitative assessments of pig behaviour. *Anim Behav* 78:477-84
- Widowski T, Curtis S, Graves C (1989) The neutrophil: lymphocyte ratio in pigs fed cortisol. *Can J Anim Sci* 69:501-4
- Winer BJ (1971) *Statistical principles in experimental design*. New York: McGraw-Hill
- Wood-Gush DGM, Jensen P, Algers B (1990) Behaviour of pigs in a novel semi-natural environment. *Biol Behav* 15:62-73
- Wood-Gush DGM, Vestergaard K (1991) The seeking of novelty and its relation to play. *Anim Behav* 42:599-606
- Wu G, Bazer FW, Wallace JM, Spencer TE (2006) Board-invited review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci* 84:2316-37
- Würbel H (2009a) The state of ethological approaches to the assessment of animal suffering and welfare. *Appl Anim Behav Sci* 118:105-7
- Würbel H (2009b) Ethology applied to animal ethics. *Appl Anim Behav Sci* 118:118-27
- Xin H, DeShazer J, Leger DW (1989) Pig vocalizations under selected husbandry practices. *Trans ASAE* 32:2181-4
- Yamada J, Stevens B, de Silva N, Gibbins S, Beyene J, Taddio A, Newman C, Koren G (2007) Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. *Neonatology* 92:42-9
- Yeates JW, Main DCJ (2008) Assessment of positive welfare: A review. *Vet J* 175:293-300
- Zeeb FD, Robbins, T.W., Winstanley CA (2009) Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. *Neuropsychopharmacology* 34:2329-43
- Zonderland JJ, Cornelissen L, Wolthuis-Fillerup M, Spoolder HA (2008) Visual acuity of pigs at different light intensities. *Appl Anim Behav Sci* 111:28-37
- Zupan M, Janczak AM, Framstad T, Zanella AJ (2012) The effect of biting tails and having tails bitten in pigs. *Physiol Behav* 106:638-44



## NEDERLANDSE SAMENVATTING

Varkens werden duizenden jaren geleden gedomesticeerd en de varkenshouderij is uitgegroeid tot een van de meest waardevolle agrarische bedrijfstakken. In de EU werden vorig jaar meer dan 146 miljoen varkens geproduceerd, en de gemiddelde consumptie van varkensvlees in 2013 bedroeg 41 kg per inwoner. Meer dan de helft van het varkensvlees over de hele wereld is afkomstig uit de intensieve veehouderij. Varkens worden ook ingezet in biomedisch onderzoek, omdat er sterkere anatomische en fysiologische overeenkomsten tussen mens en varken bestaan dan tussen mens en knaagdier. Knaagdieren worden het meest als proefdier gebruikt.

Het welzijn van varkens op boerderijen en in laboratoria is van belang, en wetten en regels van de EU moeten er zorg voor dragen dat aan bepaalde normen wordt voldaan. Er zijn nog veel potentiële problemen voor varkens in deze leefomgevingen, ondanks deze regels. Om te begrijpen hoe belangrijk deze problemen voor de varkens zijn, hebben we een goede manier nodig om hun welzijn te meten.

De eerste vraag is: wat is welzijn? Sommige algemene definities van welzijn behelzen dat het dier gezond moet zijn, dat het een natuurlijk leven moet kunnen leiden, of dat het problemen aan moet kunnen waarmee het wordt geconfronteerd. Meer recent staat centraal te begrijpen hoe het dier zich voelt, of het gelukkig is. Inderdaad leggen de wetten ter bescherming van dieren aan ons op dat we lijden en misère bij dieren moeten voorkomen. Welzijn houdt echter meer in dan alleen het voorkomen van adverse gebeurtenissen. Het houdt ook in dat we randvoorwaarden scheppen om een dier de kans te geven zich goed te voelen.

Een belangrijke vervolgvraag in het onderzoek naar verbetering van het welzijn van varkens is: wat maakt een varken gelukkig?

Maar hoe zien we dan of een dier blij of verdrietig is? En kan een droevig dier soms ook vrolijk zijn? Om deze tweede vraag te beantwoorden moeten we het verschil tussen emoties en stemming begrijpen. Een emotie is een korte reactie op iets wat gebeurd is, terwijl de stemming een meer algemene toestand is die langer duurt, en geen reactie is op een specifieke gebeurtenis. Stemmingen en emoties beïnvloeden elkaar: een in het algemeen ongelukkig dier zal niet vaak geluk tonen (maar natuurlijk kan het gebeuren). Maar een dier dat vaak goede ervaringen opdoet is waarschijnlijk meestal tevredener.

Aan mensen kun je eenvoudig vragen hoe zij zich voelen. Dit is bij dieren niet mogelijk; in plaats daarvan zijn we op andere indicatoren aangewezen om te achterhalen hoe een dier zich voelt. Dit kan op verschillende manieren. Wetenschappers hebben geprobeerd om emoties met behulp van veranderingen in de fysiologie te meten bijvoorbeeld veranderingen in hartslag en lichaamstemperatuur, of veranderingen in hormoonspiegels die optreden in reactie op veranderingen in de omgeving. Sommige van deze veranderingen weerspiegelen de “stressrespons” van een dier. Maar deze veranderingen kunnen optreden als reactie op zowel goede als slechte situaties en zijn een index voor de sterkte van de reactie. Ze zijn geen index voor de ‘valentie’, d.w.z. of het dier zich goed of slecht voelt.

Je kunt ook kijken naar hoe een dier zich gedraagt. Emoties hebben een functie; een blij of verdrietig gevoel kan ons gedrag beïnvloeden, en hoe we ons gedragen kan dan

weer aangeven hoe we ons voelen. In **hoofdstuk 02** bespreken we de verschillende methoden die worden toegepast om via het gedrag emoties bij varkens te meten. Een reeks verschillende gedragstesten, veelal afgeleid uit onderzoek met knaagdieren, werd gebruikt om emoties bij varkens meten. In veel van deze testen wordt het dier in een onbekende situatie geplaatst – confrontatie met nieuwigheid is een van de sterkste manieren om een negatieve emotionele reactie op te roepen. Hoewel deze testen de standaard methoden zijn voor het meten van emotionele reacties bij varkens, is niet altijd duidelijk wat ze meten. Ten eerste zijn vele van de testen voor ratten ontwikkeld en op veronderstellingen gebaseerd die bij andere soorten niet opgaan. Zo vermijden ratten open ruimten en verblijven in de buurt van de randen in een onbekende plaats. Varkens, anderzijds, vertonen dit gedrag niet, en dus is dit geen relevante maat voor emoties bij varkens. Met behulp van deze traditionele testen is het moeilijk te bepalen of een gedraging emoties weerspiegelt, of iets anders, bv. hoe actief een dier is: een dier dat veel beweegt wanneer geplaatst in een onbekende omgeving kan deze omgeving verkennen, maar het kan ook pogingen doen aan deze omgeving te ontsnappen. Beide gedragingen worden gekenmerkt door een verhoogde activiteit.

Een recentere test om emotie bij dieren te bestuderen kijkt naar hoe emoties en stemmingen cognitieve processen beïnvloeden. Zoals we al eerder zagen, kan emotie gedrag sturen. Dit kan op een aantal manieren gebeuren. Emotie kan ons geheugen voor gebeurtenissen beïnvloeden: de herinnering aan gebeurtenissen die zeer emotioneel waren is sterker dan die voor gebeurtenissen die niet met sterke gevoelens geassocieerd waren. Bij mensen is de beoordeling van de waarschijnlijkheid van toekomstige gebeurtenissen vaak gebaseerd op onze stemming of emotie op het moment van de beslissing. Iedereen kent wel het voorbeeld waarin mensen in een goed humeur een glas als half vol beschouwen (de optimisten), terwijl mensen in een slecht humeur zullen zeggen dat hetzelfde glas half leeg is (de pessimisten). Wanneer een persoon consequent een optimist of pessimist is, en hij zijn keuze niet aan de realiteit toetst, dan noemen we dit een beoordelingsbias. Het bestuderen van deze bias in de beoordeling kan ons zeggen of een dier een optimist is of een pessimist, d.w.z. of het over het algemeen tevreden is of ontevreden.

Deze theorie werd voor het eerst in 2004 met ratten getest. De ratten leerden dat als ze een bepaald toon-signaal hoorden, de druk op een hendel een beloning opleverde. Ze leerden ook dat het drukken op een hendel gevolgd wordt door een milde straf als ze een ander toon-signaal hoorden. Zodra de ratten hadden geleerd dat de ene toon een beloning voorspelt, en de ander een straf, presenteerden onderzoekers nieuwe “ambigue” toonsignalen die tussen de positieve en negatieve toon in lagen en registreerden of de rat op de hendel drukte of niet. Drukte de rat op de hendel, dan werd dit als volgt geïnterpreteerd: de rat verwachtte een beloning (optimistisch). Drukte de rat niet op de hendel, dan verwachtte de rat blijkbaar een straf (pessimistisch). De onderzoekers ontdekten dat ratten die in een onvoorspelbare stressvolle omgeving leefden pessimistischer waren dan ratten in een voorspelbare stressarme omgeving.

We wilden onderzoeken of dezelfde benadering bij varkens ons in staat zou stellen een indicatie voor hun emotie te verkrijgen. Deze indicatie moest ons niet alleen vertellen

of een dier ongelukkig is of niet, maar specifiek ook aangeven of een dier gelukkig is. De eerste stap was de ontwikkeling van een geschikte test. In **hoofdstuk 03** ontwierpen we een ‘beoordelingsbias’ test die kan worden gebruikt om optimisme en pessimisme bij varkens te bestuderen. Varkens leerden dat ze een grote beloning zouden krijgen als ze rechts kozen, terwijl ze rechts geen beloning zouden krijgen na het horen van de ‘negatieve toon’, of een kleine beloning als ze links lozen. Zodra ze correct op elke toon reageerden, dat wil zeggen rechts of links kozen afhankelijk van de toon, werden ambigue tonen aangeboden, zoals eerder beschreven bij de rattenstudie. We registreerden welke zijde het varken koos nadat we een ambigue toon aanboden. Optimistische varkens kozen de zijde waar ze de grote beloning verwachtten, terwijl pessimistische varkens de zijde kozen waar ze de kleine beloning verwachtten.

In **hoofdstuk 03** hebben we getest of de varkens correct op de tonen reageerden, daarna onderzochten we in **hoofdstuk 04** hoe varkens uit de commerciële houderij en varkens die specifiek voor laboratoriumonderzoek worden gefokt (het Göttingen minizwijn) in deze test presteren. Na een aantal procedurele aanpassingen vonden we dat alle varkens in staat bleken deze taak (**hoofdstuk 03**) te leren. De varkens uit de commerciële houderij en de minizwijnen reageerden op soortgelijke wijze op de ambigue tonen (**hoofdstuk 04**). Dit laatste resultaat is belangrijk; het toont aan dat de test gebruikt kan worden om emotie bij verschillende lijnen te meten.

Om te onderzoeken of onze test in staat is om te detecteren dat gelukkige varkens optimistisch zijn, kozen we groepen varkens waarvan we verwachtten dat ze voor de mate van geluk verschilden. Laag geboortegewicht bij varkens komt vaak door grotere nesten. In deze nesten komt het vaak voor dat een of twee biggen een veel lager geboortegewicht hebben dan hun broertjes en zusjes. Kinderen geboren met een laag geboortegewicht lopen een groter risico op het ontwikkelen van emotionele problemen op latere leeftijd, en in studies bij varkens met een laag geboortegewicht werd gevonden dat ze sterker reageerden op stress. We verwachtten dan ook dat een varken met een laag geboortegewicht waarschijnlijk een negatievere stemming heeft. Dit konden we in onze beoordelingsbias-taak testen. In twee experimenten, beschreven in **Bijlage A** en **hoofdstuk 05**, vonden we inderdaad dat een varken met een laag geboortegewicht vaker pessimistisch op ambigue tonen reageert.

In een derde experiment (**hoofdstuk 06**) vonden we echter dat varkens met een laag geboortegewicht in deze test niet anders reageerden dan varkens met een normaal geboortegewicht. In **hoofdstuk 06** hebben we ook naar de oorzaken gekeken voor een laag geboortegewicht bij varkens. Laag geboortegewicht kan worden veroorzaakt door perioden van slechte prenatale verzorging met nutriënten, waarschijnlijk door een slechter functionerende placenta. Ook wordt de foetus van minder zuurstof voorzien. Dat kan leiden tot enig hersenletsel. Behandeling met Allopurinol zou de schade kunnen verminderen die ontstaat door zuurstoftekort en vrije radicalen. Wij onderzochten de effecten van prenatale behandeling met Allopurinol op biggen met een laag en biggen met een normaal geboortegewicht. Maar omdat de biggen met een laag geboortegewicht niet van de broertjes en zusjes met een normaal geboortegewicht verschilden met betrekking tot emoties in deze studie, konden we de effecten van Allopurinol niet verder onderzoeken.

In onze experimenten hebben we ook geprobeerd alle factoren buiten de emotie uit te sluiten die ertoe zouden kunnen bijdragen dat varkens de grote dan wel de kleine beloning kiezen. Misschien koos een varken de kleinere beloning omdat het minder honger had, of minder gemotiveerd was door voedsel dan andere varkens. Om motivatie te meten hebben we gekeken naar het leren van deze taak omdat gemotiveerdere dieren sneller zullen leren; we keken naar latentie tot een keuze, omdat een varken dat meer gemotiveerd is sneller een keuze zal maken. We keken ook naar het aantal fouten dat een varken maakte in reactie op de positieve en negatieve tonen, omdat meer gemotiveerde varkens minder fouten zullen maken. We vonden dat de pessimistischere varkens tekenen van lagere motivatie lieten zien, **hoofdstuk 05**.

Het kan ook zijn dat onze test beïnvloed werd door de geheugenprestatie van het varken, en niet door de emotionele toestand van het dier. Het is denkbaar dat varkens met een goed geheugen anders reageren dan varkens met een slecht geheugen. In **hoofdstuk 07** vergeleken we de optimistische en pessimistische reacties van varkens in onze beoordelingsbias-taak met hun prestatie in een complexe spatiële geheugentaak. We vonden geen sterke aanwijzingen voor deze hypotheses.

Tot slot hebben we gekeken of onze test niet de reacties op ambiguïteit mat, maar een ander type van de besluitvorming, namelijk beslissen onder risico, **hoofdstuk 06**. We spreken van ambiguïteit wanneer de uitkomsten van een keuze onbekend zijn, en we spreken van risico wanneer de uitkomsten bekend zijn, maar de kans dat ze zich voordoen verschilt. We testten onze varkens in een 'gok' taak waarbij naar de vraag werd gekeken of de varkens liever een kleine kans op een grote beloning kozen, dan met een grote kans op een kleine beloning. Vervolgens werden dezelfde varkens in onze beoordelingsbias-taak getest. We vonden dat de varkens met een laag geboortegewicht die de optie met de kleine maar frequente beloningen prefereerden ook pessimistischer waren. Belangrijker is echter dat de keuze van een varken op de ene taak niet was gerelateerd aan keuzes op de andere taak. Dit betekent dat de twee testen waarschijnlijk niet hetzelfde meten.

## **Conclusies**

De meest voorkomende gedragstesten die worden gebruikt om emotie bij varkens te meten zijn problematisch, en mogelijk niet geschikt voor varkens.

Optimistische en pessimistische reacties bij varkens kunnen een bruikbare aanwijzing opleveren voor de emotie van het dier.

We hebben laten zien dat de taak die we ontwierpen geschikt is voor varkens. Zowel de varkens uit de commerciële houderij als de minizwijnen leerden de discriminatie taak die ten grondslag ligt aan de test, en ze vertoonden soortgelijk gedrag bij het uitvoeren van de taak.

Terwijl onze verwachtingen m.b.t. varkens met een laag geboortegewicht eerst werd bevestigd – ze waren pessimistischer dan varkens met een normaal geboortegewicht – leidde een laag geboortegewicht in een vervolgstudie niet tot negatieve emoties en stemming bij varkens.

Tot op zekere hoogte zijn we erin geslaagd om aan te tonen dat de pessimistische reacties niet door verschillen in motivatie, geheugen of risicoaversie worden bepaald.

Onze test is momenteel alleen geschikt voor laboratoriumonderzoek. Het duurt zeer lang om de varkens te trainen; het is daarom tijdrovend om emoties bij varkens op boerderijen of in laboratoria te meten.

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## **ABOUT THE AUTHOR**

Eimear Murphy was born on 19<sup>th</sup> September, 1980, in Dublin, Ireland. Having completed her Leaving Certificate at St. Dominic's High School, Sutton in 1999, she went on to study Science at University College Dublin, specialising in Psychology, where she received her BSc. In 2005 she moved to Scotland to study for an MSc. in Applied Animal Behaviour and Animal Welfare at the University of Edinburgh. As part of this MSc. she participated in a five-month research project looking at the effect of increasing urbanization on European Badgers (*Meles meles*). In this period she also volunteered with the Education Department at Edinburgh Zoo. After her MSc., Eimear was employed as a Research Assistant on a six-month project at the University of Edinburgh in 2007, under the supervision of Prof. Michael Cockram. The aim of this project was to identify potential behavioural and physiological indicators of fatigue in sheep. Following this, she went on to take up another Research Assistant position in the Animal Welfare and Behaviour Group at the University of Bristol, England in 2008. Under the supervision of Prof. Michael Mendl and Dr. Suzanne Held, she got her first taste of working with pigs on a 15-month project investigating social learning in pigs. In May 2009, she moved to the Netherlands to start her PhD studies at the Emotion and Cognition Group within the Department of Farm Animal Health at Utrecht University, from which the results are presented in this thesis. Eimear is currently working as a research technician at the Animal Welfare Division of the University of Bern, Switzerland.

## LIST OF PUBLICATIONS

- **Murphy E**, Kraak L, van den Broek J, Nordquist RE, van der Staay FJ (In Press) Decision-making under risk and ambiguity in low-birth-weight pigs. *Anim Cogn*
- **Murphy E**, Nordquist RE, van der Staay, Franz Josef (2014) A review of behavioural methods to study emotion and mood in pigs, *Sus scrofa*. *Appl Anim Behav Sci* 159:9-28
- **Murphy E**, Kraak L, Nordquist RE, van der Staay FJ (2013) Successive and conditional discrimination learning in pigs. *Anim Cogn* 63:883-93
- **Murphy E**, Nordquist RE, van der Staay FJ (2013) Responses of conventional pigs and Göttingen miniature pigs in an active choice judgement bias task. *Appl Anim Behav Sci* 148:64-76
- Melotti L, Bailoo JD, **Murphy E**, Burman O, Würbel H (2014) Play in rats: Association across contexts and types, and analysis of structure. *Anim Behav Cogn* 1(4):489-501
- Cockram MS, **Murphy E**, Ringrose S, Wemelsfelder F, Miedema HM, Sandercock DA (2012) Behavioural and physiological measures following treadmill exercise as potential indicators to evaluate fatigue in sheep. *Animal* 6(09):1491-1502
- Held SDE, Byrne RW, Jones S, **Murphy E**, Friel M, Mendl MT (2010) Domestic pigs, *Sus scrofa*, adjust their foraging behaviour to whom they are foraging with. *Anim Behav* 79:857-62

## CONTRIBUTIONS TO CONFERENCES AND SYMPOSIA

- **Murphy E**, Nordquist, RE, van der Staay FJ (2013) Oral presentation: Decision Making and Judgement Bias in low and normal birth weight pigs. Behaviour 2013, Newcastle, U.K.
- **Murphy E**, (2012) Studying emotion in pigs (using M&Ms!). Oral presentation: De Beschaving Festival, Utrecht. (Oral Presentation)
- **Murphy E**, Nordquist RE, van der Staay FJ (2012) Oral presentation: An active-choice judgement bias task for pigs: a comparison between Göttingen minipigs and conventional farm pigs. Proceedings of the 46<sup>th</sup> Congress of the International Society for Applied Ethology (ISAE), Vienna, Austria.
- **Murphy E**, Nordquist RE, and van der Staay FJ (2010) Poster Presentation: Toward developing a Cognitive Bias task for pigs. Annual Meeting of the Netherlands Society for Behavioural Biology (NVG), Soesterberg, Netherlands.



