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Assessing emotions in pigs: determining negative and positive mental states

Eimear Murphy, Luca Melotti and Michael Mendl, Bristol Veterinary School, University of Bristol, UK

1 Introduction

1.1 Definitions and concepts

The study of animal emotion is a challenging area, not least because of the confusion that is generated by the terms used in this field (Anderson and Adolphs, 2014; Barrett, 2006; LeDoux, 1996; Mendl and Paul, 2020; Paul and Mendl, 2018). The concept of emotion ultimately stems from our own human experiences of mental states that we label with 'emotion words' such as 'enjoyment', 'fear', 'anger' and 'sadness' (Ekman, 1992). Because these are such potent features of our everyday experience, we are naturally interested in whether they also exist in other species and, if so, how we might assess and even measure them. The latter question is particularly relevant to understanding and monitoring the welfare of other animals, especially if one follows the widely held view that a key determinant of an animal's welfare is its mental state, in particular feelings such as pain, fear, anxiety, pleasure and other sensations and emotions (Davis and Cheeke, 1998; Fraser et al., 1997; Duncan, 1993; Paul et al., 2020; Yeates and Main, 2008). However, conscious experiences such as feelings are essentially private and inaccessible to direct measurement by third-parties, hence generating confusion about exactly what we mean when we talk about animal emotions and their assessment – are we referring to these conscious experiences, or to something else?

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Clear definitions are essential to avoid misunderstandings. In making such definitions, we can draw a distinction between those that are descriptive and those that are prescriptive (Paul and Mendl, 2018). Descriptive definitions are dictionary-style definitions describing, as clearly as possible, how a word is used in everyday language or in a particular context such as scientific research. Descriptive definitions of emotion terms as used in scientific/psychological literature make the following points. Emotions are short-term responses to stimuli or events that comprise changes in a range of 'components' including the subject's conscious experience (usually regarded as the key feature), and its behaviour, cognitive and neurophysiological state (Paul et al., 2005; Paul and Mendl, 2018; Scherer, 1984). These changes do not always co-occur and can even dissociate in some circumstances (Winkielman et al., 2005). Importantly for our purposes, while direct measurement of conscious experience in non-human species is not currently possible (in humans we tend to rely on language as a gold-standard metric), we are able to measure the other components and can use these as indicators of animal emotions and proxies for the feelings that they may comprise. Working out whether the species of interest has the capacity for conscious experience is a separate question with a separate and expanding literature (Paul et al., 2020).

The key feature that distinguishes emotions from other mental states is that they are *valenced* – positive or negative, pleasant or unpleasant, rewarding or punishing. Valenced states are also referred to as 'affective states', an overarching term that includes both short-term 'emotions', but also longer-term and free-floating (not directly related to any one stimulus or event) 'moods'. Events inducing short-term emotions are usually relevant to an individual's well-being and survival, and longer-term moods may reflect the cumulative experience of these shorter-term emotions (Mendl et al., 2010; Mendl and Paul, 2020).

A prominent conceptual view of human emotion is the 'core affect' model (Russell, 2003) which posits that two major underlying dimensions common to all affective states are valence (positivity or negativity) and arousal (how 'activated' the organism is). Figure 1 shows the core affect model with a few examples of discrete affective states (as described by common human emotion words) located in core affect space. This model has value in animal studies by focussing on the defining characteristic of emotion (valence) in combination with the concept of arousal which is amenable to both behavioural and physiological measurement. It also defines four fundamental affective states, one in each quadrant of core affect space (high or low arousal/positive or negative), that can be identified and measured with appropriate arousal and valence metrics. Moreover, these states do not require labelling with human emotion words, something which can be a source of much confusion and interpretational difficulty, especially in species more distantly related to us (LeDoux, 1996; Mendl and Paul, 2020).

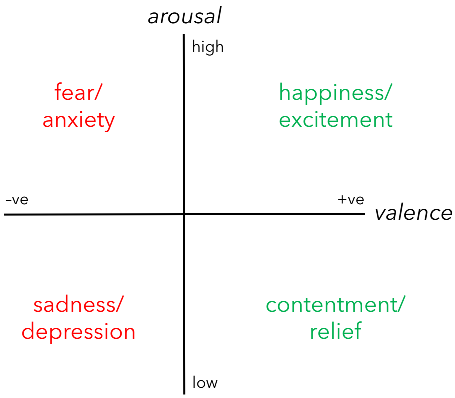

Figure 1 The core affect model with a few examples of discrete affective states (as described by common human emotion words) located in core affect space.

Most recent definitions of animal welfare stress the centrality of emotional states (e.g. Boissy et al., 2007; Dawkins, 2004; Désiré et al., 2002; Paul et al., 2005). So integral is emotion to animal welfare that Dawkins states that 'the history of animal welfare science, is, broadly speaking, the history of the search for indicators of animal emotions' (Dawkins, 2015, p. 6). However, descriptive definitions of the sort we have been considering can still leave us unsure about when an animal is in an emotional state, what that particular state might be, and even whether it is positively or negatively valenced. In seeking to develop measures of animal emotion, we thus need to have some way of providing 'ground-truth' information about what the animal's emotional state is at a particular time so that we can then identify those behavioural, neurophysiological and cognitive biomarkers that reliably co-vary with this state and hence may be valid indicators of it.

Prescriptive definitions can help here. These set out a specific set of features and conditions that precisely describe the construct that the author is referring to, 'this is what I am talking about when I use the term emotion in my research' (Izard, 2010; Paul and Mendl, 2018). A number of prescriptive definitions for animal emotion have recently been offered and three of these (Anderson and Adolphs, 2014; Dickinson and Bernard, 2010; Rolls, 2005, 2014) are reviewed in detail by Paul and Mendl (2018). Here we provide one example which builds on reinforcement learning concepts of emotion (e.g. Corr, 2008; Gray, 1975, 1987; Rolls, 2005, 2014) and can be operationalised to allow empirical investigation of the concept in animals. 'Animal affective states are elicited by rewards and punishers or their predictors. A reward is anything for which an animal will work, and a punisher is anything that it will work to escape or avoid' (Mendl and Paul, 2020; c.f. Rolls, 2005, 2014). By operationalising this prescriptive definition in terms of behaviour, it can be used to identify scenarios that, a priori, generate positively or negatively valenced affective states. 'Rewards or the absence of punishers, and associated predictions thereof, induce positive affect. Punishers or the absence of rewards, and associated predictions thereof, induce negative affect. Short-term emotion-like states follow immediately from individual rewarding or punishing events, whilst cumulative experience of events influences longer-term mood-like states' (Mendl and Paul, 2020).

Furthermore, a number of theories posit that the activity of neuro-behavioural systems involved with two fundamental processes essential to survival – Reward Acquisition (RAS) and Punishment Avoidance (PAS) – map onto core affect space as shown in Fig. 2 (Burgdorf and Panksepp, 2006; Carver, 2001; Mendl et al., 2010; Mendl and Paul, 2020; Russell and Barrett, 1999). Thus, the presence of rewards activates RAS and leads to a high-arousal positively valenced state while their absence leads to RAS deactivation and a low-arousal negative state. Likewise, punishers activate PAS resulting in high-arousal negative states while their absence leads to low-arousal positive states (Mendl and Paul, 2020). In this way, we can see how this particular prescriptive and operational definition of animal affect dovetails with the core affect concept and allows researchers to generate states lying in each quadrant of core affect space by applying/withholding behaviourally defined rewards and punishers. Behavioural, cognitive and neurophysiological biomarkers which vary reliably according to which core affect quadrant an animal is in can then be used as markers of these states. This sort of framework for inducing and 'ground-truthing' animal affective states is therefore hugely valuable for empirical research into animal emotion and, in turn, animal welfare.

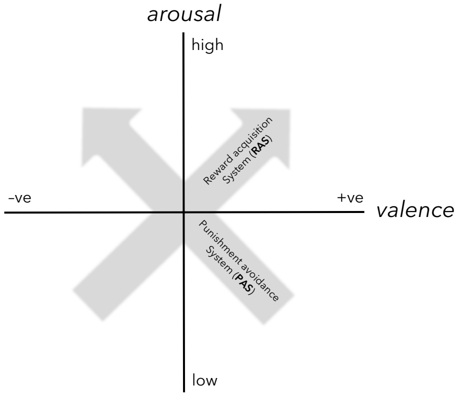

Figure 2 Reward acquisition (RAS) and punishment avoidance (PAS) processes mapped onto core affect space.

2 Behavioural indicators of emotion

2.1 Behavioural tests

Emotional responses in pigs, particularly negative emotions such as fear or anxiety, have frequently been measured using behavioural tests. Many of these tests have been adapted from research with other species, particularly rodents, and typically measure the animal's spontaneous reaction to situations involving novelty and/or social isolation. There is evidence that pigs are cautious when entering an unfamiliar space (Špinka, 2006) or demonstrate initial neophobic responses when confronted with novel stimuli. Social isolation is also considered a stressor for pigs as indicated 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). The advantages of such tests is that they rarely require extensive habituation or training, and require little in the way of infrastructure, making them popular on farm as quick measures of emotional responses in pigs. The disadvantage of such paradigms is that emotional responses can often be confounded by other factors, such as arousal, activity, and exploration, and thus the measures taken may lack the sensitivity to distinguish between positively and negatively valenced states. However, the inclusion of more subtle measures, specifically aimed at differentiating positive and negative states, may be a useful step forward in using such measures.

The most frequently performed of these behavioural tests with pigs is the Open Field test. Initially designed as a test for emotionality in rodents (Hall, 1934), it was later adapted for use with pigs (Beilharz and Cox, 1967). While the specific Open Field test designs used with pigs differ in a number of ways (e.g. size and shape of the test arena, the duration of the test), many features are common across tests. Typically, an animal is separated from its conspecifics and placed in an unfamiliar arena for a specified period of time, and the behavioural response is recorded. The most common behaviours recorded in studies with pigs include measures of movement/posture, vocalisation, location within the arena, explorative behaviour and elimination (Murphy et al., 2014), while less common behaviours include measures of exploration and escape attempts. Measures of location within the arena are one of the main indicators of anxiety in rodents, but there is little evidence that such measures are ethologically relevant measures of emotion in pigs (as reviewed by Forkman et al., 2007; Lind et al., 2007; Murphy et al., 2014). With respect to measures of movement/posture, there is conflicting evidence as to whether this reflects anxiety or purely activity or both. Crude measurements of physical activity by themselves are not sufficient to gauge emotional responses in the Open Field test (Rutherford et al., 2012). Instead, more subtle measures of behavioural postures and facial expressions may be better suited to tease apart emotional responses from activity and exploration, but these are rarely used. Vocalisations may be useful indicators of emotional responses (see later), but many open field studies have not distinguished between the types of vocalisations produced during the test. So while the Open Field test is likely to induce an emotional response in pigs, the measures commonly recorded are often not subtle enough to distinguish this emotional response from general arousal or activity. Few systematic approaches have been taken to validate this test and the different measures relevant to emotional responses in pigs (for an exception, see Donald et al., 2011).

Two further commonly used rodent models of anxiety have been adapted for use with pigs. The Elevated Plus Maze test involves placing the animal on an elevated four-armed platform where two arms have raised sides ('closed' arms), while two arms are more exposed ('open' arms) (Andersen et al., 2000a,b; Donald et al., 2010; Janczak et al., 2000, 2002; Rutherford et al., 2012). However, the assumption that animals should show unconditioned avoidance of the open arms does not appear to be true for pigs (Donald et al., 2010; Janczak et al., 2002), questioning the utility of this task in measuring emotional responses in pigs. Similarly the Light/Dark test, where the animal is placed in a dark compartment in a test arena and latency to enter a brighter compartment is measured (Andersen et al., 2000a,b) relies on the assumption that pigs show an unconditioned aversion to brightly lit spaces which is not the case (see Tanida et al., 1996). While both situations may provoke an emotional response due to novelty of the environment and the social isolation required to test an animal, the inherent assumptions of the tests do not appear to hold true for pigs (Murphy et al., 2014).

Another test frequently used to measure fear or anxiety responses in pigs is the Novel Object test where pigs are presented with an unfamiliar object and their latency to explore the object is measured, often along with the duration and frequency of object exploration. This test is based on the assumption that when animals encounter an unfamiliar object, they experience a conflict between their motivation to explore and a motivation to avoid novelty. Pigs are highly explorative animals, and are said to display both intrinsic exploration (i.e. motivated by curiosity) and extrinsic exploration (i.e. motivated to seek resources) (Day et al., 1995; Studnitz et al., 2007; Wood-Gush and Vestergaard, 1991). Pigs prefer novel objects to familiar items (Wood-Gush and Vestergaard, 1991), will work to gain access to exploratory material (Pedersen et al., 2005), and prefer environments where they have to forage for food rather where food is freely available (de Jonge et al., 2008). In the Novel Object test, avoidance of the stimulus (higher latencies to approach/investigate the object and lower durations of investigation) is taken to indicate higher levels of anxiety or fear.

Although the measures recorded in the Novel Object test have been relatively similar across studies, the method of presentation of the object itself has varied widely. In some studies the object was already in place in the arena before the animal/s entered, was placed by a person after the animal had entered, was slowly introduced (e.g. lowered via a rope), or was introduced suddenly into the testing environment (e.g. dropped or thrown; see Murphy et al., 2014). Further, tests have been conducted on individual animals, on groups, in the home pen, or in a novel arena with or without prior habituation. These different methods of testing and object introduction are likely to have a large effect on the emotional response of the animal. Appraisal theories pose that specific emotional responses to an event depend on characteristics including suddenness, familiarity and predictability (Veissier et al., 2009). Subtle differences in emotional responses may be masked by the suddenness with which objects are introduced into an unfamiliar arena, or by presenting items to group-housed pigs in their home pen such that they do not elicit a strong sense of neophobia. Systematic assessment of how these factors can alter the response would be a useful step towards developing a more standardised task design to facilitate comparison across studies. Further, the behavioural responses typically recorded may not be subtle enough to differentiate between differently valenced emotional responses; current research on defence cascade responses may provide a useful basis for future research.

Research into emotional responding in pigs has also looked at pigs' willingness to voluntary explore a, generally, unfamiliar human – the voluntary Human Approach test. Alternatively, studies have looked at the behavioural reaction of a pig in response to an approach by an experimenter – the Withdrawal Response test. These human interaction tests are assumed to induce an approach-avoidance conflict in the animal similar to the previous tests described above. The motivation to approach may be more dominant in the voluntary Human Approach test, while the motivation to avoid may be more dominant in the Withdrawal Response test (Waiblinger et al., 2006). While evidence suggests that pigs are motivated to approach humans (Terlouw and Porcher, 2005), the underlying motivation may be governed by exploration or aggression. Pigs with prior positive experience with humans have shorter latencies to approach (e.g. Hemsworth et al., 1994; Hemsworth et al., 1996a,b) as do pigs which have previously displayed more human-directed aggression (Marchant Forde, 2002). Further, approach latencies are less repeatable over time than withdrawal responses (Scott et al., 2009). The Withdrawal Response test therefore may provide a better measure of fear responses since it is based strongly on avoidance responses and is relatively stable over repeated testing (Murphy et al., 2014).

These behavioural tests have been, until more recently, the standard tests of emotional responses in pigs, and have focused overwhelmingly on negative emotions, such as fear or anxiety. In many cases, these tests were first developed for other species, and the inherent assumptions of the test may not be relevant for pigs (Murphy et al., 2014). While some aspects of the task designs are likely to induce an emotional response (novelty, social isolation), the common measures used do not allow for the differentiation of affective responses from levels of activity, and are not able to capture the subtle differences in behaviour which may be more indicative of emotional valence. Furthermore, there has been little systematic investigation into how aspects of task design, which can vary widely between studies, influence the responses of pigs in the test. These behavioural tests in their current form are not the best indicators of affective state in pigs without incorporating other measures to help differentiate emotion from other possible causes of the behaviour observed (Krugmann et al., 2019).

2.2 Qualitative behaviour assessment

Qualitative behaviour assessment (QBA) as proposed by Wemelsfelder et al. (2000, 2001) is an approach that sees the behaviour of the whole animal as an expression of its inner experience. This approach takes into account the behaviour of the animal as it interacts with its environment in a dynamic way, and uses it to gauge how the animal is experiencing its own situation. Human observers make qualitative assessments of the animal's behaviour and the assumption is that these qualitative judgements actually reflect the emotional experience of the animal.

QBA methodology generally follows one of two approaches. First, a free-choice profiling approach (Francoise Wemelsfelder et al., 2001) can be used, where observers rate the animal's behavioural expression according to a series of one-word descriptors (e.g. calm, distressed, agitated) they themselves generate from watching sample clips. The subsequent analysis looks for consistency across observers in the semantic meaning of the descriptors, and creates a (number of) dimensions upon which an individual animal's behavioural expression can be scored. In the second approach, often used in on-farm welfare quality protocols, instead of free-choice profiling, assessors score animals according to a fixed list of descriptors. The QBA approach has been used to study human assessment of emotional states in a variety of animal species (e.g. horses, Hintze et al., 2017; dairy cows, de Boyer des Roches et al., 2018; dogs, Arena et al., 2017) including pigs (e.g. Camerlink et al., 2016; Rutherford et al., 2012).

Aspects of reliability and validity of the QBA paradigm have been addressed in a number of studies. Studies with pigs have demonstrated that observers show a high degree of intra- and inter-observer reliability in their judgements of pigs' behavioural expression in terms of the semantic descriptors used (Czycholl et al., 2017; Francoise Wemelsfelder et al., 2001), despite potential bias due to observer background (e.g. farmer, veterinarian, animal rights activist; Francoise Wemelsfelder et al., 2012). However, assessments based on a fixed list of descriptors, as used in welfare quality protocols, were less reliable within and between observers (Czycholl et al., 2017; Temple et al., 2013) and were subject to bias due to the background/experience of observers (Duijvesteijn et al., 2014). Clarke et al. (2016) suggest that the differences in reliability may not be due to the application of free-choice profiling or fixed lists of descriptors, but how the data are analysed. While the environment in which a pig is filmed can influence observers' judgements (e.g. indoor vs. outdoor backgrounds), the effect is relatively small (Françoise Wemelsfelder et al., 2009). Observers rate pigs in extensive or enriched housing as expressing more positively valenced emotions than pigs in more intensive/barren conditions (Carreras et al., 2016b; Temple et al., 2011). Pharmacological validation of the paradigm has also been studied; observers rated pigs treated with an anxiolytic drug, Azperone, more positive on a dimension ranging from 'unsure/nervous' to 'confident/curious' than saline treated pigs (Rutherford et al., 2012). While observers did not distinguish between pigs that had either just won or just lost a fight, they did score pigs with more skin lesions (not visible on the video clips) as experiencing more negatively valenced states (Camerlink et al., 2016), indicating that observers could pick up on differences in emotional expression possibly related to pain.

The QBA approach to studying subjective states in animals has received criticism for being at risk of anthropomorphism (Würbel, 2009) and is likely to be affected by our level of similarity with the species under study. One has to accept the philosophical assumption that observers' interpretation of an emotional state in animals reflects the actual emotional state experienced by the animal. However, accepting this, the methodology is quite reliable and robust in the face of potential observer- and environmental-bias. Further, increasing evidence with different species of convergent and predictive validity of the QBA approach (e.g. Minero et al., 2018; Rutherford et al., 2012; Stockman et al., 2011) is helping to assuage critics. Application of the QBA method to assess emotion in pigs has the potential not only to discriminate between positively and negatively valenced states, but also between states of the same valence (e.g. Hintze et al., 2017). Further work aimed at specifically and systematically validating the paradigm can help establish if this is the case in research with pigs.

2.3 Vocalisations

Analysis of vocalisations has been suggested as a non-invasive method for studying the emotional state of an animal (Manteuffel et al., 2004). Vocalisations, however, can convey different messages (alarm calls, contact calls, pain) or be a reflection of arousal. They can also express an emotional state, or their nature may be altered by physical changes in the vocal apparatus due to an emotional state (Briefer, 2012). The value of specific vocalisations as measures of emotion in pigs is dependent on a number of factors – they must be honest signals (i.e. truly reflecting the animal's state), they should be specific to emotional responses, and they should allow for the distinction between negative and positive states. For example, greater hunger in piglets is signalled by a higher calling rate and calls that differ in frequency and duration (Weary and Fraser, 1995), and painful experiences, such as castration, are signalled by increased high-frequency vocalisations in piglets (Marx et al., 2003; Puppe et al., 2005; Taylor and Weary, 2000; Weary et al., 1998) suggesting some level of honest signalling.

Pigs make a wide range of calls from high-pitched 'squeals' or 'screams' to low-pitched and short 'barks', as well as low-pitched 'grunts'. High-pitched squeals and screams reflect high arousal (Held et al., 2009; Manteuffel et al., 2004) and are associated to situations of distress like social isolation, restraint, castration and food deprivation (reviewed in Held et al., 2009). Further, 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). The negative valence of this type of vocalisations has been further demonstrated through pharmacological validation. Stimulation of the amygdala in pigs using acetylcholine produced high-pitched vocalisations similar to the screams associated with distress (Manteuffel et al., 2007). Conversely, pigs treated with an anxiolytic, Azaperone, vocalised less in an Open Field test than non-treated pigs (Donald et al., 2011).

Barks (low-pitched atonal calls of short duration; Chan et al., 2011) occur both during potential positive experiences, such as play, but are also used to express alarm (Newberry et al., 1988). Subtle acoustic differences in peak frequencies between barks used in contexts of play and alarm may be useful to distinguish differently valenced states (Chan, 2011; Poletto et al., 2006).

Grunts have been classified based on their duration (Marchant et al., 2001), with shorter grunts being emitted during exploration and longer grunts serving as contact calls (Leliveld et al., 2017; Marchant et al., 2001). More recent research has further investigated if emotional valence can be inferred based on the acoustic characteristics of pig grunts. Grunts emitted in contexts assumed to be positive (i.e. in an environmentally and socially enriched arena with food rewards, or in anticipation of a food reward) were significantly shorter than grunts produced in contexts assumed to be negative (i.e. during social isolation without food, or in anticipation of unpalatable food; Briefer et al., 2019; Friel et al., 2019, respectively). This finding has also been shown in wild boars (Maigrot et al., 2018). Other acoustic parameters of grunts, such as those related to the third formant (a resonance of the vocal tract that can vary based on the vocal tract length) also differ between positive and negative contexts, suggesting that pigs lower their larynx and consequently increase the length of their vocal tract when vocalising in negative situations (Briefer et al., 2019). However, acoustic parameters other than grunt duration were not found to differ between positive and negative contexts in another study, possibly due to a relatively smaller difference in emotional valence between positive and negative contexts (Friel et al., 2019). Furthermore, context (and therefore emotional valence) has been found to account for only a small part of the variation in acoustic parameters in pigs (Leliveld et al., 2016) suggesting that other factors, such as individuality (Leliveld et al., 2017) and/or motivation (Briefer, 2012), may affect pigs vocalisations.

In conclusion, 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). Further, vocalisations may even differ within the same valence allowing for more subtle differentiation between states.

2.4 Play behaviour

Play generally falls into three categories; social play between conspecifics, locomotor-rotational play and object play (Held and Špinka, 2011). Play is said to have both long-term adaptive benefits, such as providing training for future skills or unexpected situations (Pellis and Pellis, 2013; 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, 2013). 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). However, a recent review suggests that while there is evidence that play is reduced in negative situations in normally playful animals, there is not enough research on the link between play and positive experiences to draw definite conclusions (Ahloy-Dallaire et al., 2018). In fact, play may also increase as a way of coping with challenging contexts, such as, competition (Palagi et al., 2006), social tension (Antonacci et al., 2010) or a reduction in maternal care (Bateson et al., 1990). Nevertheless, while the association between play and animal welfare is context-dependent, there is evidence that play, in itself, is rewarding, and may be accompanied by positive emotions (Held and Špinka, 2011). Indeed, during play, the endogenous opioid system is activated which modulates the hedonic properties of rewards leading to the experience of hedonic pleasure (Vanderschuren, 2010; Vanderschuren et al., 1995). Play is also contagious; thus the presence of one or few playing/playful individuals in a group can induce others to play as well (reviewed in Held and Špinka, 2011).

Pigs are highly playful animals, and as with other species, their play falls into the three categories of locomotor-rotational, object and social play. Play is more common in young pigs than adults, with the peak between two and six weeks, and can be affected by sex (reviewed in Lawrence et al., 2018). To distinguish real play from other behaviours, many authors use 'play markers' (Chalmers and Locke-Haydon, 1981), that is, indicators in a behavioural sequence which signify that the behaviour is playful. Common play markers in pigs' locomotor play include waving/tossing of the head, scampering, jumping, hopping, pawing, pivots, flops and gambolling (Bolhuis et al., 2005; Brown et al., 2018; Chan et al., 2011; Dudink et al., 2006; Newberry et al., 1988; O'Connor et al., 2010; Reimert et al., 2013). Object play in pigs entails the physical manipulation of inanimate objects using the snout or forelimbs (Horback, 2014) and often involves some of the play markers used in locomotor play such as tossing of the head. However, object manipulation can also be a form of stereotypy in pigs (Mason and Mendl, 1997), so the context in which the behaviour is occurring should be taken into account. Distinguishing social play from real conflict can be more difficult as many of the behaviours observed resemble those of antagonistic behaviour. Social play often involves one pig pushing or nudging another pig with the head or snout, mouthing parts of the other pig's body, mounting or climbing on the other pig, and chasing (Brown et al., 2018; Chaloupková et al., 2007; Dobao et al., 1985; Donaldson et al., 2002; Weller et al., 2019), and can be performed between pairs of litter/pen mates or between a piglet and a sow. The presence of play markers during these bouts, such as pivoting or head tossing, can be used as an indicator that the behaviour is playful (Newberry et al., 1988).

Research on play in pigs generally supports the conclusion that environmental conditions assumed to improve welfare are associated with increased play, especially when a comparison with a control, more barren condition is made (reviewed in Lawrence et al., 2018). Both solitary and pairs of pigs displayed locomotor and object play behaviours when given access to a compartment containing straw, digging substrate and rewards, but no play was observed when the animals were exposed to a second barren compartment (Reimert et al., 2013). Similarly, young pigs play more in enriched-housing environments compared with conventional environments (Bolhuis et al., 2005; Chaloupková et al., 2007; Martin et al., 2015), and if the ammonia concentration in their room is relatively low (<5 ppm vs. 20 ppm concentration; O'Connor et al., 2010).

Taken together, while play may not be a good indicator of past positive experiences, pigs do show more play in more stimulating environments. In understanding the link between play and affective states the interpretation of results needs to be looked at in context. Further play in itself is very likely to induce positive emotions; so while it may not reflect past experiences, environments that promote play may stimulate more experiences that are positive.

2.5 Defence cascade responses

Defence cascade responses have been proposed as a potentially promising measure of emotional valence in animals (Statham et al., 2011), and some preliminary work has been carried out with pigs. Across species, animals show an adaptive response to sudden and/or unexpected stimuli involving initial detection of the stimulus (startle response), followed by an evaluation phase (freezing response), and ending with either a defensive response or the resumption of normal behaviour (Statham et al., 2020). The startle response may represent a useful measure of emotional valence (Paul et al., 2005; Statham et al., 2020). Evidence from human and rodent research indicates that the response is increased when the subject is in a negative emotional state, but reduced when the subject is in a positive state (Statham et al., 2020). As pigs display clear startle and freeze responses to, for example, sudden and loud auditory stimuli (Blackshaw et al., 1998), followed by either fleeing or resumption of normal behaviour typical of the defence cascade response (Statham et al., 2020), interest in using these behaviours to assess emotional valence in pigs has increased.

Initial investigations revealed that, in line with predictions, pigs treated with an anxiolytic, midazolam, showed smaller startle responses and shorter freeze durations to a sudden, loud noise compared with saline-treated controls (Statham et al., 2011). Experience of prior positive or negative handling treatments, however, had no differential effect on defence cascade responses in pigs (Carreras et al., 2017). However, as the handling treatments in this study failed to influence any of the outcome measures, the possibility that the treatment itself failed to induce a difference between the groups cannot be excluded. Some factors which may modulate the response have been identified (Statham et al., 2020), such as the pigs' orientation towards the stimulus, and the baseline alertness level of the pig, and must be taken into account when using this measure to assess emotional valence in pigs. As a practical measure of emotion on farm, manual scoring of defence cascade responses are time-consuming, but efforts are underway to address this using computer vision image analysis (Statham et al., 2020).

2.6 Facial expression and body posture

In recent years there has been increased interest in the potential for facial expressions and body postures to be used as indicators of emotion and emotional valence in a wide variety of animal species, including, sheep, rats, horses, dogs and mice (Boissy et al., 2011; Caeiro et al., 2017; Dolensek et al., 2020; Finlayson et al., 2016; Hintze et al., 2016). Research into facial expressions in animals is also of interest to those wishing to investigate pain, since the first 'grimace scale' was developed for mice (Langford et al., 2010); grimace scales have been developed for a variety of species including horses, pigs, sheep and rats (e.g. Dalla Costa et al., 2014; di Giminiani et al., 2016; Häger et al., 2017; Sotocina et al., 2011; for review see McLennan et al., 2019).

Much of the research with pigs has focussed on the potential for ear and tail movements/postures to reflect positive and negative emotional valence. (Reimert et al., 2013, 2015) noted that pigs showed increased tail movement (wagging) during a rewarding event (exposure to substrate and treats in a test arena). Instead, during the aversive event (separation and short restraint), pigs were more likely to keep their ears in a backwards position, while tails remained in a more static position (curled). Similarly, Marcet Rius et al. (2018b) found that (mini) pigs showed higher tail movement duration and lower ear movement duration when presented with toys in their home pens than during a control situation. Straw provision alone however did not appear to have this effect on tail movement, but the frequency of ear movements was reduced compared to a control situation (Marcet Rius et al., 2019b). Playful interactions with enrichment and conspecifics have also been shown to be positively correlated with duration of tail movement (Marcet Rius et al., 2018a; Marcet Rius et al., 2019a). However, more intense tail wagging or tail flicking in pigs has been associated with negative experiences (such as pain or frustration; for review see, Camerlink and Ursinus, 2020). These results suggest that positive experiences in pigs (appetitive, explorative, playful) may be identified more by relaxed tail movements while negative experiences may be identified by ear position and movements.

In terms of more specific facial expressions, two approaches have been developed. First, Camerlink et al. (2018) investigated how facial expressions in pigs may change in response to different negative emotional experiences, namely aggression and fear. They identified three facial expressions involving the snout, the ears and the eyes and investigated how they changed over the course of aggressive interactions between pigs. While measures of the snout did not change throughout the interaction, pigs in retreat from the aggressive encounters, hypothesised to indicate fear, had their ears in a more backwards position and their eyes narrower when compared with before and during the aggressive encounter (Camerlink et al., 2018) Secondly, research has looked at how facial expressions may be used as indicators of pain. Di Giminiani et al., (2016) looked at how castration and tail docking affected facial expressions caused by contraction or relaxation of individual or groups of facial muscles in pigs. The facial action units that changed from pre- to post-treatment were identified and used to develop a piglet grimace scale (for sample of the Piglet Grimace Scale, see Fig. 3). This scale was then used by observers blind to the treatments to assess changes in facial expression in response to pain in neonatal piglets. It was found that piglets displayed orbital tightening, that is, narrowing of the eyes, after the tail docking procedure, indicating that, similar

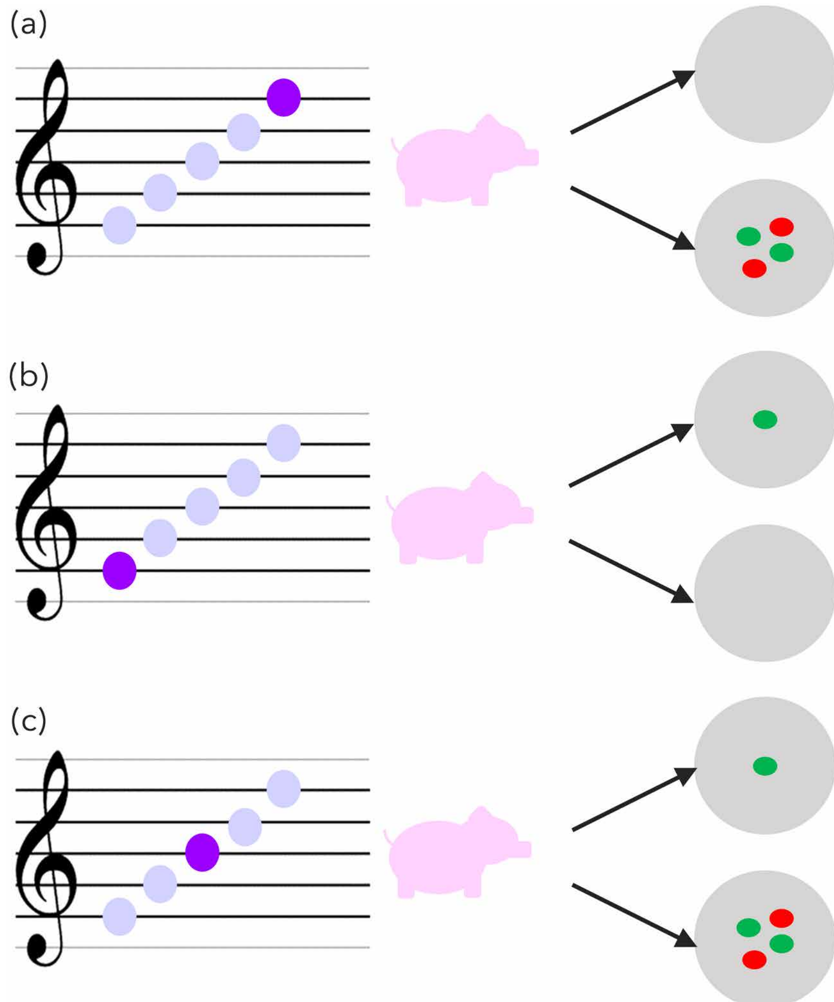

Figure 3 Example of an active choice Go-Go judgement bias task used with pigs from Murphy et al. (2013). Pigs were trained to respond to tone cues (a) predicting the presence of a large reward (positive cue) or (b) predicting the presence of a small reward (negative cue) in one of two locations. Pigs had to choose the correct location in order to obtain the reward. Once the association between cue type and reward location/quantity had been learned, (c) intermediate ambiguous tone cues were presented and the response of the pig (choice of reward location) was recorded. In response to ambiguous probe cues, a choice for the location associated with the positive cue was considered an optimistic response (i.e. expectation of a large reward), while a choice for the location associated with the negative cue was considered a pessimistic response (i.e. expectation of a small reward).

to results with other species, this may be a useful measure of painful experience in pigs (di Giminiani et al., 2016). Pigs were found to have higher scores on the grimace scale post-surgery, indicative of more pain, compared with before surgery (Vullo et al., 2020). Moreover scores were related to activity levels of piglets' post-surgery, with less-active piglets receiving higher scores (Viscardi et al., 2017). These results indicate that the pigs' grimace scales are picking up on facial expression related to pain in pigs. Such a methodology could be used to identify facial expressions related to other affective states in pigs, both positive and negative. Recent developments in automatic detection of individual pig faces using deep learning methods (Hansen et al., 2018) raise the exciting future possibility of monitoring facial expressions of identified individuals in situ on farm.

3 Cognitive indicators of emotion in pigs

3.1 Judgement bias

Affective states can lead to biases in various cognitive processes such as memory, attention and judgement. From human research, there is evidence that positive affective states lead to more positive judgements of ambiguous stimuli, while negative states lead to more negative judgements of the same stimuli (Blanchette and Richards, 2010). Harding et al. (2004) proposed that emotional valence in animals could be studied by measuring these differences in judgement of ambiguous stimuli, and developed the first animal-judgement bias task. Since this seminal paper, cognitive judgement biases have been investigated in a wide range of species, including birds, rodents, farm animals and primates. While these tasks have been performed in quite a variety of ways, most have, in principle, followed the original design of Harding et al. (2004). Animals are first trained on a discrimination task where the two cues along the same modality (e.g. tone frequencies; spatial location; texture gradients) offer differential outcomes (e.g. reward, punishment; reward, non-reward; large reward, small reward). Animals are trained to perform a different response depending on which cue is presented (e.g. latency to respond, Go/No-Go response; active choice, Go-Go response). Once this discrimination has been learned, animals are then presented with a series of ambiguous probe cues intermediate to the previously learned reference cues, interspersed among presentations of the reference cues. Their responses to these probe cues are scored in relation to their responses to the reference cues and interpreted as suggesting either positive or negative judgement biases. These biases are said to reflect a positive or negative underlying affective state (Mendl et al., 2009).

Two recent reviews and meta-analyses of the judgement bias research done with non-human animals have recently been published. The first has demonstrated that, based on the results from pharmacological manipulations of mood state, the paradigm demonstrates some degree of predictive validity; in general pharmacological manipulations shifted judgement bias at the probe cues in the direction predicted (Neville et al., 2020). The second demonstrated that non-pharmacological manipulations of affective state also shifted judgement bias in the direction predicted (Lagisz et al., 2020). While both of these reviews support the validity of the paradigm as a method to assess emotional valence in animals, the authors stress that some important factors need to be taken into account when using this paradigm. First, manipulations predicted to induce negative states had a larger effect than those predicted to induce positive states (Neville et al., 2020). Secondly, effects were highly variable and aspects of the task design can moderate the effects found (for details see Lagisz et al., 2020).

No systematic validation of the judgement bias paradigm has been carried out with pigs, and various task designs have been developed and implemented. Go/No-Go style tasks have been more commonly used (e.g. Carreras et al., 2015; Douglas et al., 2012; Leliveld et al., 2017) but active choice of Go/Go tasks have also been developed (e.g. Murphy et al., 2013, see also Fig. 4). Moving animals from barren to enriched housing conditions, often used to induce positive states in animals, has shown mixed effects on judgement bias in pigs; Douglas et al. (2012) found that moving pigs to enriched environment induced an optimistic bias, while Carreras et al. (2016b) found no effect. Pigs that experienced a positive-handling treatment were more likely to show an optimistic bias compared to those in negative or minimal handling treatments in one study (Brajon et al., 2015) but this result was not confirmed (Carreras et al., 2017). Social isolation was also found not to have an effect on judgement bias in piglets (Düpjan et al., 2013). Pigs classified as having a pessimistic bias also showed a longer latency to contact a novel object (Carreras et al., 2016a) demonstrating some convergent validity. Pharmacological depletion of serotonin levels in the brain was found to induce a pessimistic bias in pigs (Stracke et al., 2017a), but dietary supplementation with tryptophan to enhance brain serotonin levels also indicated a shift towards a negative bias in pigs contrary to predictions (Stracke et al., 2017b). Individual performance of pigs in repeated testing was found not to be consistent over time (Carreras et al., 2015), and pigs have shown an increasing latency to respond to unrewarded ambiguous cues with repeated testing (Murphy et al., 2013). No effects of sex on responses to ambiguous cues was found (Carreras et al., 2016a; Roelofs et al., 2017).

While the general judgement bias literature promotes the validity of the paradigm to measure emotional valence in animals (Lagisz et al., 2020; Neville et al., 2020), the results of studies with pigs are not consistent enough to promote one particular method. Much more work is needed to validate the paradigm for use with pigs and develop the optimal task design. The sensitivity of the task to pick up differences is likely affected by aspects of task design

Figure 4 Sample piglet-grimace scale as developed by di Giminiani et al., 2016.

and the relative severity of the manipulations used (Lagisz et al., 2020), as well as the relatively low numbers of presentations of ambiguous cues (in order to reduce learning about the outcomes of ambiguous cues). As a further caution, many judgement bias tasks are relatively time-consuming and are impractical to run outside of a lab setting, although attempts to design tasks which are faster to run but do not compromise much on design have been developed (e.g. Hintze et al., 2018; Jones et al., 2018) but not yet tested with pigs. With a properly validated task design, the judgement bias paradigm is a promising method to differentiate between positively and negatively valenced affective states in pigs.

4 Neurophysiological indicators of emotion in pigs

4.1 Physiology of negative emotions

When it comes to looking at the interplay between physiology and negative affective state in animals it is important to include stress. Traditionally, studies of animal welfare have used the concept of stress to measure welfare, as, until recently, the study of emotion in animals was seen as too subjective. Nonetheless 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). However, stress is a loosely defined term often used in the literature which can take a variety of forms; an aversive stimulus is generally called a ‘stressor’, the ‘stress response’ is the physiological reaction within an animal to an aversive stimulus, and ‘distress’ is sometimes used to describe the psychological state of the animal (Mcmillan, 2008). Stress can be defined as an adaptive response as a consequence of the subjective appraisal of environmental challenges by an individual (Mormède et al., 2007; von Borell and Veissier, 2007). Thus stress has an adaptive value; the function of the stress response is to allow an animal to react to and potentially adapt to a challenge. In these terms, measurement of stress responses may be useful to infer about emotional state. Generally, investigations into animal stress can be viewed as the studies of responses to conditions that are likely to be aversive (Paul et al., 2005). 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).

In relation to measurement of emotional response, 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 (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 levels of arousal (Hagemann et al., 2003). Therefore, while ANS activity may be a useful measurement of the intensity of an emotion, without other measures it may not be useful to distinguish different emotions or even simply positive from negative emotions. However, studies that used experimental treatments known to induce distress have identified a general pattern of the physiological response to negative situations. For example, subordinate pigs experiencing social defeat show a more sustained increase in heart rate compared to dominant pigs (De Jong et al., 2000; Marchant et al., 1995). Similarly, heart rate also increases after social isolation (De Jong et al., 1998) and castration (White et al., 1995). Further, both social defeat and isolation caused a more sustained cardiovascular activity in response to a novel and an aversive test (Ruis et al., 2001a, b).

Heart rate variability (HRV) in particular has been promoted as a potential measure of psycho-physiological state and emotional valence (Boissy et al., 2007; von Borell et al., 2007). Indeed, the measure of heart rate alone reflects only the net outcome of the interaction between vagal (reducing heart rate) and sympathetic (increasing heart rate) systems, and thus does not provide specific information on the activity pattern of each separate cardiac regulatory system. Instead, measures of HRV can disentangle such activity patterns (von Borell et al., 2007). HRV is a measure of the variation of the time intervals between consecutive heart beats (Malik, 1996; McCraty and Shaffer, 2015). Such variation is normally present in healthy individuals and serves to maintain the activity of the different cardiac regulatory systems within the ideal range for responding to challenges (McCraty and Shaffer, 2015). Behavioural and mental disorders, and psychological stress, are associated with a decrease in HRV (McCraty and Shaffer, 2015; von Borell et al., 2007) which suggests the experience of a negative emotional state. Research aiming to link HRV with negative emotions in pigs has been scarce. So far, both social defeat (De Jong et al., 2000) and restraint (Geverink et al., 2002) did not induce significant changes in HRV in pigs, although a decrease in HRV in the face of a stressor would be expected and has been demonstrated in other farm animals (Boissy et al., 2007). Thus, more research exploring the relationship between HRV and negative emotions in pigs is needed. Individual differences may also play an important role in how the emotional state of pigs can be affected by a stressor. Pigs with a proactive coping style show higher heart rate in feeding, resting and handling contexts compared to pigs with a reactive coping style, by means of an increased sympathetic activity and/or decreased vagal tone depending on the context considered (Krause et al., 2017).

Cortisol is the primary active hormone of the HPA axis in pigs and is often called the ‘stress hormone’. Synthesis of cortisol is controlled by adrenocorticotropic hormone, ACTH, which is itself synthesised in the anterior pituitary gland. Corticotrophin-releasing hormone, CRH, and vasopressin, AVP, two neuropeptides synthesised in the hypothalamus cause the release of ACTH which is transported to the adrenal cortex where it causes the synthesis of cortisol. The HPA axis is a negative feedback system whereby cortisol in turn influences production of the neuropeptides in the hypothalamus. The HPA axis is activated in aversive situations (Mormède et al., 2007) and therefore measures of cortisol in blood or saliva are commonly used in studies of stress in animals. However, while higher levels 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, there is not a linear relationship between the amount of cortisol and the apparent severity of the stress in animals (Mormède et al., 2007). So, while cortisol can provide an indication of stress in an animal, therefore an indication of negative affect, it may not give an indication of the intensity of the emotion, and could be influenced by a variety of other factors. In pigs, social isolation, restraint and social defeat increase plasma cortisol (isolation: Schrader and Ladewig, 1999; restraint: Jarvis et al., 2008; social defeat: Ruis et al., 2001a), and social isolation also induces a longer-term higher cortisol response to novel object tests (Ruis et al., 2001b). Further, mixing with unfamiliar conspecifics increases salivary cortisol (Ison et al., 2010). However less acute, longer-term treatments produced inconclusive and somewhat contradictory results. For instance, housing growing pigs at high or low density did not significantly affect salivary cortisol or alpha-amylase levels (Scollo et al., 2014). Furthermore and contrary to expectations, pigs housed in an enriched environment showed higher baseline levels of salivary cortisol than pigs kept in a barren environment (De Jong et al., 1998).

Although both elevated HPA and sympathetic activity may occur during putatively negative situations, they are also expressed in high-arousal positive situations. For example, Buwalda et al. (2012) demonstrate that male rats show almost identical corticosterone responses to the presence of an avoided aggressive-conspecific and a desired female partner. These and similar findings suggest that HPA activity and simple measures of sympathetic activation such as heart rate may primarily reflect affective arousal in preparation for action in both positively and negatively valenced situations, rather than affective valence per se. More comprehensive approaches combining multiple physiological (and behavioural) measures appear to be more promising in identifying and quantifying negative emotional states. In particular, the combined assessment of the activity of both sympathetic adrenomedullary (SAM) system and HPA axis can help quantify the stress response to unpredictable and uncontrollable situations (Koolhaas et al., 2011). For example, pigs show a rise in both cortisol and adrenaline levels after a social isolation treatment but, if such treatment is repeated several times, cortisol levels decrease over time indicating habituation to the stressor, while adrenaline levels do not consistently vary, suggesting a longer-term negative effect of social isolation (Schrader and Ladewig, 1999).

4.2 Physiology of positive emotions

Only in recent years, have physiological parameters been used to assess the positively valenced states in animals (Boissy et al., 2007). Although research in this direction is still scarce, cardiovascular activity, particularly heart rate variability (HRV) as a proxy measure of vagal tone, has been promoted as a way to assess positive affective state (Boissy et al., 2007). A good indicator of positive affective state is the relative increase in the variance between inter-beat intervals, which can be considered a proxy measure of vagal activity (reviewed in Boissy et al., 2007). Pigs that received cognitive enrichment through a cognitive paradigm involving discrimination of acoustic cues to obtain food showed both arousal, as measured by an increase in heart rate, and positive affective state as indicated by a specific pattern of the HRV parameters in response to the acoustic signals and during consumption of the food (Zebunke et al., 2011). However, pigs that were positively conditioned by receiving rewarding items (food, toy, straw) in repeated sessions mainly showed sympathetic activation (indicating arousal) in anticipation of the rewards instead of vagal activation (indicating positive valence; Leliveld et al., 2016), thus calling for further research to assess the measurability of positive affective state using HRV.

Another promising physiological indicator of positive emotional state in pigs is represented by the activity of the opioid system (Boissy et al., 2007). The endogenous opioid system acts on specific subcortical brain areas (i.e. nucleus accumbens, ventral pallidum and amygdala) and modulates the subjective hedonic experience of rewards such as play, sex and food (Vanderschuren, 2010). It differentiates from the dopamine system, which mediates the motivation towards obtaining a reward ('wanting'), and therefore can be used as a proxy measure of consummatory, hedonic pleasure ('liking': Berridge et al., 2009; Boissy et al., 2007; Vanderschuren, 2010). Using the same auditory cognitive paradigm for pigs described above (i.e. study from Zebunke et al., 2011), Kalbe and Puppe (2010) demonstrated that frequently occurring positive events in the form of cognitive enrichment modified the expression of specific brain opioid receptors in the amygdala, which is responsible for the processing of reward-sensitive stimuli (Kalbe and Puppe, 2010). This preliminary study sets the basis for further research in this area to be conducted.

The hormone oxytocin has also been proposed as a measure of improved welfare (Boissy et al., 2007; Broom and Zanella, 2004). Brain oxytocin is involved in the regulation of positive social bonding, both between mates and between mother and offspring (Winslow and Insel, 2002); thus its assessment in the context of positive social interactions could be a marker of positive emotional valence (Rault, 2016). In pigs, positive interaction with a human induced a sustained increase in oxytocin levels in the cerebrospinal fluid (Rault, 2016), supporting the above hypothesis. Another study in minipigs, which investigated peripheral levels of oxytocin in response to repeated provision of toys in the home pen, showed unclear results as whether oxytocin levels could indicate a positive emotional state or a response to challenge (i.e. blood collection; Marcet Rius et al., 2018a). However, there is mounting evidence of no consistent relationship between central and peripheral changes in oxytocin levels, since oxytocin does not pass easily through the blood-brain barrier (reviewed in Rault, 2016); thus oxytocin may be a better biomarker of positive emotional state if measured centrally. Finally, pigs which were conditioned to expect an aversive event were less likely to defecate in the presence of naïve pen mates which had received oxytocin intranasally than in the presence of control pen mates, suggesting that relatively higher levels of oxytocin in one individual can alleviate a negative state in other familiar individuals (Reimert et al., 2015).

5 Summary and future trends

The study of animal emotion is a challenging area, not only because of the difficulty in defining what we mean when we talk about terms such as emotion, affect or mental states, but also because of the variety of approaches proposed to measure these states in animals. However, in order to assess animal welfare, the development of reliable and valid measures of affective states is essential. While the key component of emotion is considered to be the conscious experience, this is not yet possible to study directly. Instead, approaches in animal research have focussed on the other components, namely, behavioural, cognitive and neurophysiological components (Paul et al., 2005; Paul and Mendl, 2018; Scherer, 1984). As with research in other species, research aimed at assessing affective states in pigs has also focussed on these three components.

In terms of behaviour, the standard approach until relatively recently was to look at unconditioned responses of pigs in a series of behaviour tests designed to provoke fear or anxiety. While some aspects of these tasks are likely to induce an emotional response in pigs, the measures used to record this are not always ethologically relevant for pigs or specific enough to differentiate between measures of emotion and activity (reviewed in Murphy et al., 2014). Further, some tasks are performed in such a variety of ways and there is little systematic study on how the different approaches may, in themselves, influence the outcome measures.

Aside from these behavioural tests, more recently, a variety of spontaneous behaviours have been proposed as potential measures of affective states in animals, including in pigs. Moving on from fear and anxiety, these approaches have also broadened the field into the study of both positive and negative states. QBA is based on the premise that people can infer an animal's emotional state from observing the behavioural expression of an animal as it interacts with its environment. Once you accept this philosophical premise, this approach is one of the few which allows for the differentiation of states of the same valence.

Pigs are quite vocal animals and make a variety of calls from squeals to barks and grunts. Squeals are assumed to be indicative of high arousal negative states, while barks can occur in positive and negative contexts. Recent work has focussed on differentiating grunts produced in positive and negative contexts based on their acoustic parameters and has identified some potential parameters for future research. Systematic study of vocalisation types and parameters could be useful to determine if the call type or acoustic structure of the call can be used to differentiate between states within the same valence. Pigs are also highly playful animals, particularly as juveniles. While the occurrence of play in itself does not necessarily indicate past positive experiences, it is likely to induce positive affect, and environments that stimulate play could potentially increase positive experiences.

Startle behaviour in response to sudden loud stimuli, part of the defence cascade, has been proposed as one method to distinguish between positive and negative affective states. Research on this behavioural response in pigs is still in the early stages. However, evidence that pharmacological manipulation of state does affect startle responses in the direction predicted makes this a promising area for future research. Furthermore, defence cascade responses could be incorporated into typical behavioural tests, such as a novel object task, as they provide a priori expectations of how affective state should influence the behavioural response.

Another growing area of study is how emotions are expressed through changes in body posture and facial expressions in pigs. Research has identified relaxed tail movements as potential indicators of positive affective states and changes in ear postures as potential indicators of negative affective states. Furthermore, some changes in facial expression (e.g. narrowing of the eyes and ears angled backwards) may even be useful for distinguishing between affective states of the same valence (e.g. fear and pain vs. aggression).

Another component of affective states is the cognitive component. Affective states can potentially be studied through their impact on cognitive processes. The most commonly studied in animal research is how biases in judgement of ambiguous stimuli can be used to distinguish between positively and negatively valenced states. There is growing evidence that, across species, both pharmacological and non-pharmacological manipulations of emotional states shifted the bias in the direction predicted (Lagisz et al., 2020; Neville et al., 2020). There is also some debate about whether the shift in bias at different probe cues may reflect differences in states of the same valence (Salmeto et al., 2011; reviewed in Roelofs et al., 2016). While the judgement bias paradigm is a promising approach to the measurement of emotion in animals, the research with pigs is patchy and a systematic approach in validating the paradigm is lacking.

There are several neurophysiological measures that can be used to infer on the affective state of an animal. Confounds in the interpretation of these measures exist, as it is sometimes difficult to disentangle emotional valence from arousal, and the assessment of one physiological parameter alone may not be sufficient to pinpoint a specific emotion or even valence. However, combining multiple physiological measures and using validated methods can provide a more comprehensive picture on emotional responses. Promising indicators of negative affective state deriving from stress physiology are the combined evaluation of the activity of both sympathetic adrenomedullary system and HPA axis (e.g. adrenalin and cortisol levels), and the assessment of cardiovascular responses, especially heart-rate variability. The latter is of particular interest as it potentially allows us to make inferences about positive affective states. Furthermore, the activity of the endogenous opioid system as a measure of hedonic pleasure, and brain oxytocin levels as a proxy measure of affective state deriving from positive social interactions, are other promising indicators of positive affect. Research in pigs, however, especially with respect to positive affective state, is still scarce and sometimes with contrasting results; thus these measures still need further validation and standardisation in methodology (e.g. for oxytocin measurement, see Rault et al., 2017).

Finally, it is worth considering that pigs, being highly social animals, can influence each other's affective state through their social and sensory interactions. Through emotional contagion, that is, a change in emotional state of one individual as a direct consequence of perceiving and sharing the emotional state of another (Hatfield et al., 1994; Preston and De Waal, 2012), the emotional response of just one or few individuals can alter the affective state of the whole social group (Held and Špinka, 2011; Špinka, 2012). In pigs, research on emotional contagion, especially on the positive spectrum, is only at an early stage, and faces the challenge of demonstrating that contagion occurs not only at the behavioural level but also internally (i.e. affecting emotional valence; Špinka, 2012). Further, some studies have failed to show contagion (e.g. through playback of distress calls; (Düpjan et al., 2011). However, this is a field calling for further investigation and several ways of assessing emotional contagion have been suggested, ranging from the use of olfactory cues, of the playback of vocalisations, the change of housing conditions, to the transfer of locomotor-rotational play (Held and Špinka, 2011; Lawrence et al., 2018; Špinka, 2012). While preliminary research suggests that particularly tail movements, defecation and play may be indicative of emotional contagion in pigs (Reimert et al., 2013, 2015, 2017), these measures first have to be properly validated as reliable indicators of affective state.

In conclusion, in this chapter we have tried to provide a critical overview of the various methods that have been proposed and applied as measures of affective states in pigs. To fully evaluate the behavioural, cognitive and neurophysiological measures proposed, it is fundamental that these measures are evaluated in a systematic way. Finding measures that vary reliably according to which core affect quadrant a pig is in is essential in establishing valid and reliable markers of affective states.

6 Where to look for further information

For some interesting discussion on the nature of emotions and their implications for animal welfare, the following articles are of relevance:

- Paul, E. S. and Mendl, M. T. (2018). Animal emotion: descriptive and prescriptive definitions and their implications for a comparative perspective. *Applied Animal Behaviour Science* 205, 202–209. https://doi.org/10.1016/j.applanim.2018.01.008.
- Paul, E. S., Sher, S., Tamietto, M., Winkielman, P. and Mendl, M. T. (2020). Towards a comparative science of emotion: affect and consciousness in humans and animals. *Neuroscience and Biobehavioral Reviews* 108, 749–770. https://doi.org/10.1016/j.neubiorev.2019.11.014.
- Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I. and Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology and Behavior* 92(3), 375–397. https://doi.org/10.1016/j.physbeh.2007.02.003.
- Lawrence, A. B., Vigors, B. and Sandøe, P. (2019). What is so positive about positive animal welfare?—a critical review of the literature. *Animals* 9(10) 783. https://doi.org/10.3390/ani9100783.

Here are relevant readings more specific to emotion in pigs:

- Held, S. D. E., Cooper, J. J. and Mendl, M. T. (2009). Advances in the study of cognition, behavioural priorities and emotions. In: Marchant-Forde, J. N. (Ed.), *The Welfare of Pigs*. Springer. https://doi.org/10.1007/978-1-4020-8909-1_3.
- Murphy, E., Nordquist, R. E. and van der Staay, F. J. (2014). A review of behavioural methods to study emotion and mood in pigs, Sus scrofa.

Applied Animal Behaviour Science 159, 9–28. https://doi.org/10.1016/j.applanim.2014.08.002.
- Lawrence, A. B., Newberry, R. C. and Špinka, M. (2018). Positive welfare: what does it add to the debate over pig welfare? *Advances in Pig Welfare*. https://doi.org/10.1016/B978-0-08-101012-9.00014-9.

Key international meetings in which the topic of emotions in animals, including pigs, is discussed:

- ISAE (International Society for Applied Ethology) annual meetings often focus on the applied assessment of animal emotions within settings involving human-animal interaction, such as farming and keeping of companion and laboratory animals.
- The biannual meeting "Behaviour", which hosts the IEC (International Ethological Conference) and ASAB (Association for the Study of Animal Behaviour) / ABS (Animal Behavior Society) conferences, also deals with the study of animal emotion from a more fundamental and theoretical perspective.
- UFAW (Universities Federation for Animal Welfare) annual meetings touch upon the assessment of animal emotions from an ethical perspective and with the aim of improving animal welfare.

7 References

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Andersen, I. L., Bøe, K. E., Fœrevik, G., Janczak, A. M. and Bakken, M. (2000a). Behavioural evaluation of methods for assessing fear responses in weaned pigs. *Applied Animal Behaviour Science* 69(3), 227–240. https://doi.org/10.1016/S0168-1591(00)00133-7.

Andersen, I. L., Færevik, G., Bøe, K. E., Janczak, A. M. and Bakken, M. (2000b). Effects of diazepam on the behaviour of weaned pigs in three putative models of anxiety. *Applied Animal Behaviour Science* 68(2), 121–130. https://doi.org/10.1016/S0168-1591(00)00098-8.

Anderson, D. J. and Adolphs, R. (2014). A framework for studying emotions across species. *Cell Press* 157(1), 187–200. https://doi.org/10.1016/j.cell.2014.03.003.

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