



## Behavioural and physiological measures indicate subtle variations in the emotional valence of young pigs



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### HIGHLIGHTS

- Young pigs are sensitive to repeated moderate aversive and rewarding events.
- Responses in behaviour and heart rate are weak but measurable.
- A multi-disciplinary approach can detect subtle differences in emotional valence.
- Moderate, but consistent, changes in husbandry may affect animal welfare.

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### ABSTRACT

In the study of animal emotions, emotional valence has been found to be difficult to measure. Many studies of farm animals' emotions have therefore focussed on the identification of indicators of strong, mainly negative, emotions. However, subtle variations in emotional valence, such as those caused by rather moderate differences in husbandry conditions, may also affect animals' mood and welfare when such variations occur consistently. In this study, we investigated whether repeated moderate aversive or rewarding events could lead to measurable differences in emotional valence in young, weaned pigs. We conditioned 105 female pigs in a test arena to either a repeated startling procedure (sudden noises or appearances of objects) or a repeated rewarding procedure (applesauce, toy and straw) over 11 sessions. Control pigs were also regularly exposed to the same test arena but without conditioning. Before and after conditioning, we measured heart rate and its variability as well as the behavioural reactions of the subjects in the test arena, with a special focus on detailed acoustic analyses of their vocalisations. The behavioural and heart rate measures were analysed as changes compared to the baseline values before conditioning. A limited number of the putative indicators of emotional valence were affected by the conditioning. We found that the negatively conditioned pigs showed changes that were significantly different from those in control pigs, namely a decrease in locomotion and an increase in standing. The positively conditioned pigs, however, showed a stronger increase in heart rate and a smaller decrease in SDNN (a heart rate variability parameter indicating changes in autonomic regulation) compared to the controls. Compared to the negatively conditioned pigs, the positively conditioned pigs produced fewer vocalisations overall as well as fewer low-frequency grunts but more high-frequency grunts. The low-frequency grunts of the negatively conditioned pigs also showed lower frequency parameters (bandwidth, maximum frequency, 25% and 50% quartiles) compared to those of the positively conditioned pigs. In any of the statistically significant results, the conditioning accounted for 1.5–11.9% of variability in the outcome variable. Hence, we conclude that repeated moderate aversive and rewarding events have weak but measurable effects on some aspects of behaviour and physiology in young pigs, possibly indicating changes in emotional valence, which could ultimately affect their welfare. The combination of ethophysiological indicators, i.e., the concurrent examination of heart rate measures, behavioural responses and especially vocalisation patterns, as used in the current study, might be a useful way of examining subtle effects on emotional valence in further studies.

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### 1. Introduction

The study of emotions in animals is a central issue within the field of animal welfare. Indeed, the welfare of an individual has been defined as “its state as regards its attempts to cope with its environment” [1] and

its emotional assessment of the result [2]. It is now also acknowledged that good animal welfare constitutes not only the absence of negative experiences such as pain, fear and stress but also the presence of positive experiences such as joy and pleasure (e.g., [3, 4]). In accordance with this, a framework has recently been proposed to study emotions according to their core affect [5], whereby emotions are represented as locations in two-dimensional space, with arousal (low/high) and valence (positive/negative) as axes [6]. This framework provides the basis for studying not only the arousal but also the valence of emotions, which leads to better understanding of positive as well as negative emotions in animals.

The study of animal emotions is also challenging because we can only infer emotions indirectly from physiological and behavioural measurements [7]. Most of these measurements can often measure the arousal dimension quite well, but the valence dimension is more difficult to grasp [8]. Still, some measurements can provide insight into the emotional valence of a situation. One of these measurements is heart rate variability [9, 10]. Heart rate variability results from the non-additive activity that originates from the two branches of the autonomic nervous system: the sympathetic and the parasympathetic (vagal) systems (e.g., [11, 12]). The parasympathetic system is active during rest (increasing heart rate variability), while the sympathetic system is activated during stress (decreasing heart rate variability). Stress is here defined as the non-specific response of the body to any demand made upon it [13]. Indicators of parasympathetic activity (RMSSD) and the balance between parasympathetic and sympathetic activity (RMSSD/SDNN ratio) have been suggested to reflect positive emotional valence (e.g., [3, 14, 15]), although others suggest that the connection between heart rate variability and emotional valence is not so clear (see [16] for a review in humans). Behaviour may also provide insight into the emotional valence of a situation. For instance, in pigs, escape behaviour may indicate increased stress [17, 18], while exploration or play behaviour may reflect positive emotions (e.g., [3]). However, most behaviours are difficult to interpret [19] and are also specific to particular species, sex, ages and even individuals [20]. One behavioural aspect that has been shown to provide good insight into an animal's emotional state is its vocalisation [21]. For example, in pigs, negative situations are accompanied by an increased amount of high frequency calls, such as screams (e.g., [22–24]), whereas reunions between sows and piglets, a positive situation, are accompanied by 'quacking' of the piglets [25, 26]. In addition, it has recently been suggested that variations in vocal structure can be used to study both the arousal and the valence dimensions of emotion [27]. So, heart rate, behaviour and vocalisations in particular may all measure aspects of emotional valence. However, no single measure of welfare, of which emotion is an important part, is considered adequate on its own [20, 28]. Thus, we suggest the use of a combination of the different measures in a multi-disciplinary approach, which should be able to provide a more complete overview of the core affect of a certain situation.

Traditionally, research on emotions in the domestic pig has primarily focussed on measuring emotions in strong (mainly) negative situations, such as castration (e.g., [22, 29]) and transport (e.g., [30, 31]). However, such husbandry measures causing strong emotional responses are rare in a pig's life. In a usual day, husbandry measures may only cause subtle variations in emotional valence. Negative emotional events consist of short startling events, such as when the caretaker comes in unannounced or when the pigs hear sounds that they do not recognise. Positive events take the form of food and (sometimes) straw or other materials to play with. However, these moderate emotional events may ultimately also considerably affect the animals' welfare by changing the (long-term) mood of the animal [6], leading, for instance in case of negative events, to chronic anxiety. Recently, two studies have aimed to study more subtle variations in emotional valence within the same general context to identify physiological and behavioural indicators of emotional valence in pigs [18, 32]. These two studies showed that subtle changes in emotional valence can be reflected in behavioural

parameters (including counts of low- and high-frequency vocalisations), while such effects were not found in heart rate parameters [32]. However, a multi-disciplinary approach that uses behavioural, physiological and detailed acoustic analyses may shed more light on how sensitive young pigs are to repeated moderate rewarding or aversive events that are caused by husbandry measures. Therefore, the aim of this study was to examine whether repeated moderate aversive or rewarding events can lead to measurable differences in emotional states.

To achieve this aim, we used a conditioning paradigm to induce anticipation of positive or negative events in female juvenile pigs and then studied their affective response during anticipation by measuring heart rate, behaviour and vocalisation. We focussed on young, weaned pigs because they are still relatively naïve to farm management and therefore may be more receptive to moderate aversive and rewarding events. Using this method, we expected to find subtle differences in emotional valence between positively and negatively conditioned pigs, as expressed through changes in heart rate variability, behaviour and vocalisations. According to the evidence discussed above, we expected an increase of heart rate variability (i.e. RMSSD and RMSSD/SDNN ratio), exploration, and a decrease in high frequency calls and escape behaviour, after positive conditioning. Negative conditioning was expected to lead to opposite results.

## 2. Materials and methods

### 2.1. Animals and housing

The subjects were female German Landrace pigs (age 4–7 weeks). Experiments were performed in 7 consecutive replicates. In each replicate, 15 subjects were tested, resulting in 105 subjects in total. Before weaning, the subjects had been submitted to 4 backtests to determine whether they had an active or a passive coping style (these results will be discussed in another publication) according to the method of Zebunke et al. [33]. At weaning, 20 females were selected. We limited the number of full siblings as much as possible (maximum of 4 subjects from the same mother) and tried to avoid piglets that weighed <6 kg or that appeared to be in ill health. The 20 selected females were then grouped together and housed in an enlarged weaner pen (3.75 × 1.8 m with fully slatted plastic floors and a solid heated area in the middle) for 8 days. After that, they were moved to another pen, closer to the test room, where they stayed until the end of the experiment. This pen measured 4 × 2.1 m and had fully slatted plastic floors as well as two solid concrete sections. Water and food were available ad libitum, and straw was given once per day. On the 6th day after weaning, 15 subjects were selected for further testing based on their readiness to eat applesauce (i.e. the time they spent eating) when alone. To test this, each subject was given 3 min to eat applesauce in the entrance area of the test room (see description in Section 2.2). Subjects had to be of good health and tolerate handling enough to enable the fitting of heart rate measurement belts. Active and passive copers were preferred over individuals classified as 'doubtful' based on the backtests. The selected subjects were then pseudo-randomly assigned to one of three different conditioning groups (see Section 2.3). Full siblings were balanced across the three groups, and 5 subjects were included per conditioning group per replicate.

### 2.2. Experimental setup

All sessions were performed in the same sound-attenuated test room. This room consisted of an arena of 3 × 3 × 1.25 m (L × W × H) with opaque walls and an entrance area. A camera that was connected to a digital video recorder (Everfocus EDR HD-4H4) was positioned centrally above the arena to observe the behaviour from above. Next to the camera, a microphone (Sennheiser ME64/K6) was positioned. This microphone was connected to a recorder (Marantz PMD 670; sampling rate: 44.1 kHz, accuracy: 16 bit, mono). During the tests and

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