

Scotland's Rural College

Once bitten, twice shy: aggressive and defeated pigs begin agonistic encounters with more negative emotions

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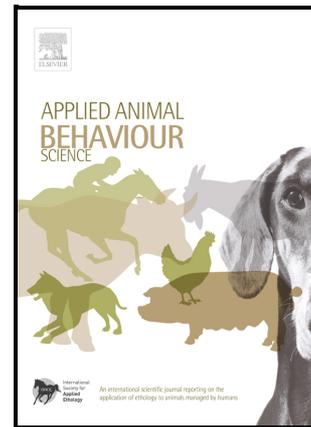
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Once bitten, twice shy: aggressive and defeated pigs begin agonistic encounters with more negative emotions

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Abstract

Aggression between unfamiliar commercial pigs is common and likely invokes strong emotions in contestants. Furthermore, contest outcomes affect subsequent aggressive behaviour, suggesting a potential lasting influence on affective state. Here we used a combination of qualitative and quantitative methods to assess the emotional expression of pigs in agonistic encounters. We investigated how recent victory or defeat influences emotions expressed in a subsequent contest, and the role of aggressiveness as a personality trait in emotional expression. We observed the pre-escalation contest behaviour (second contest; age 13 wks) in animals of different aggressiveness (categorised using two resident intruder tests as Agg+ or Agg-, age 9 wks), which had recently won or lost a contest (first contest; 10 wks). We measured gaze direction and ear position. Observers watched video clips of the

initial 30s of the second contest and evaluated the emotional expression of 57 pigs (25 contest 1 winners, 32 contest 1 losers) using qualitative behavioural assessment (QBA) with a fixed list of 20 descriptive terms.

QBA identified three principal components (PCs), accounting for 68% of the variation:

PC1 (agitated/tense to relaxed/content), PC2 (fearful/aimless to confident/enjoying) and PC3 (listless/ indifferent). Agg- pigs and males showed a more positive emotionality (PC2). PC1 and PC3 were unaffected by first contest outcome and aggressiveness. Agg+ pigs were more likely to hold their ears back ($\chi^2=7.8$, $p=0.005$) during the early contest period. Differences in attention were detected in the contest outcome \times aggressiveness interaction ($\chi^2=4.3$, $p=0.04$), whereby approaching the opponent was influenced by winning and losing in the Agg- pigs only. QBA and gaze behaviour reveal differences in emotional valence between pigs of different aggressiveness: less aggressive pigs may be more susceptible to the emotional impact of victory and defeat but overall, more aggressive pigs express more negative emotionality at the start of agonistic encounters.

Keywords:

Pig, Aggression, Emotion, QBA, Gaze

1. Introduction

Commercial pig farming is often associated with high levels of aggression due to the regrouping of unfamiliar animals (Peden et al., 2018). Negative consequences of aggression include skin injuries (due to bites), lameness and social stress (McGlone, 1985; Ruis et al., 2001; Rydhmer et al., 2006), therefore down-regulating aggressive behaviour after losing fights is adaptive (Dugatkin, 2014). In dyadic contests, losers received on average twice as many skin lesions as winners, and lesion scores

increase with fight duration and blood lactate, so provide a biologically relevant estimate of contest cost (Camerlink et al., 2017). Repeated social defeat has a lasting effect on pigs' stress reactivity and social behaviour (Otten et al., 2002). In losing fights, animals gain information about their own fighting ability and the costs of losing and so become more submissive ("loser effects"), whilst winning fights maintains their drive to engage in aggression ("winner effects") (Hsu et al., 2009). Winner and loser effects have profound and long-lasting effects in pigs (Oldham et al., 2020).

Extreme aggression on farms is usually driven by a minority of aggressive individuals engaging in high numbers of fights (Turner et al., 2006). The personality trait of "aggressiveness", analysed using the validated "resident-intruder test", shows consistent inter-individual variation over repeated testing (D'Eath, 2004; Clark and D'Eath, 2013). Aggressiveness is partially genetically determined (D'Eath et al., 2009; Stukenborg et al., 2012; Desire et al., 2015) and is not a reliable predictor of contest success (Camerlink et al., 2015). This suggests that aggressiveness is associated with individual differences in the perception of the rewards and costs of aggression (Arnott and Elwood, 2009; Goubault et al., 2019) and emotions are expected to be key to how animals manage the conflicting motivations to engage or withdraw (Rolls, 2000; Paul et al., 2020; Crump et al., 2020).

Studies with laboratory mice demonstrate that aggressive animals gain a reward from contest success. Outbred, CD-1 mice that persistently attacked a weaker opponent in resident-intruder tests developed conditioned place preference, whereas mice which did not attack the intruder developed conditioned place aversion (Golden et al., 2016). In humans, proactive aggression is reported to be cathartic (Carver & Harmon-Jones, 2009, Chester, 2017, Chester & DeWall, 2017a) and some

individuals are more genetically predisposed to find aggression rewarding, due to differences in serotonergic and dopaminergic neurohormonal pathways (Goodwin et al., 2020), as modelled in laboratory rodents (e.g. Yates et al., 2015). We do not know whether highly aggressive pigs find aggression more rewarding than low aggressive pigs, although serotonin is involved in mood regulation (Stracke et al., 2010) and aggressive female pigs were found to express fewer serotonin receptors in areas of the brain linked with processing social stimuli (medial amygdala) and rewards (lateral septum) (D'Eath et al., 2005). Dietary tryptophan supplementation (a precursor for serotonin) was shown to reduce aggressiveness (by delaying attacks) in young pigs (Poletto et al., 2010). The aim of this study is to explore the effect of individual variation in aggressiveness and experience of aggression on emotions at the start of an agonistic encounter.

Emotions in animals can be assessed indirectly via their behavioural expression. In pigs, certain postures and behaviours have been shown to be displayed across scenarios that are expected to have similar emotional valence (Murphy et al., 2014), including ear posture (Goumon and Špinka, 2016; Reimert et al., 2013), vocalisations (Manteuffel et al., 2004; Leliveld et al., 2017; Briefer et al., 2019), escape behaviours (Otten et al., 2007; Kanitz et al., 2009) and, in other species, gaze direction (orangutans: Bloomfield et al., 2015; dogs: Ogura et al., 2020). In addition, we applied qualitative behavioural assessment, which evaluates the whole animal's body language and incorporates subtle elements of emotional expression such as body tension (Rutherford et al., 2012, Cooper and Wemelsfelder, 2020). We hypothesise that aggressiveness reduces the emotional costs of losing contests and increases the reward of winning.

2. Methods

The study used video images previously collected for earlier work (Camerlink et al., 2017) which was approved by the UK Home Office and by the SRUC animal ethical review committee (ED AE-40 2014). The contests we analysed involved 304 pigs aged 13 weeks. The use of human observers for QBA was approved by SRUC and University of Edinburgh human ethical review committees (HERC_305_19).

2.1. Experimental procedures

The experimental methods are explained fully in Camerlink et al., 2017 and Oldham et al., 2020 and are briefly described here. The experimental procedures carried out prior to the current study on agonistic behaviour and included two resident-intruder tests, two contests and regrouping. The current study analysed behaviour in the second contest only.



Figure 1 Overview of the experimental procedures of the overarching study. The current study focussed on the initial phase of the second contest only, while using the information that was obtained in the previous tests (e.g. Resident-Intruder aggressiveness).

Two resident-intruder tests (e.g. Clark and D'Eath, 2013) to assay aggressiveness were carried out at 9 weeks of age, on consecutive days, in which an unfamiliar smaller “intruder” pig (weighing ca. 65% of the body weight of the resident) entered the home pen of the resident (i.e. the focal animal). The tests were terminated as soon as an attack was initiated. If the resident attacked the intruder in both tests,

their aggressive personality was categorised as Agg+, if they attacked the intruder in one or neither test they were categorised as Agg-. If the tests reached premature endpoints (due to mounting or intruder attacks), the focal pig was “unclassified” and not included in the current study. At 10 weeks of age, all residents experienced a dyadic contest (first contest) in a novel and neutral arena. Based on the outcome of this first contest they were assigned a winner or loser status, (or undecided if no winner emerged before the endpoint). Prior to and immediately after the contest, the number of skin lesions (indicative of the receipt of bites from aggression) on all of the head and body were counted manually and summed for each individual. Pigs gained between 0 and 426 skin lesions during the first contest and above/below the median number of skin lesions (29 lesions) was used as a simple measure of the cost of fighting (see Oldham *et al.*, 2020). On average (median, IQR), lesion scores varied by treatment as follows: Agg+ first contest losers received 64 (23-90), Agg+ winners: 21 (2-49), Agg- losers: 44 (14-88) and Agg- winners: 16 (1-48) skin lesions. At 12 weeks of age, 55% of the pigs experienced regrouping to develop fighting experience, whilst the other 45% served as controls by remaining grouped with their siblings. Three weeks after the first contest, at 13 weeks of age, pigs were paired with a new opponent for a second contest.

The contests took place in a 2.9 × 3.8 m rectangular arena with a start box on each of two sides to allow simultaneous entry of both contestants. A Canon Legria HF M52 digital video camera with a wide-angle lens was mounted ca. 5 meters perpendicular above the test arena and was recording continuously. In the current study, we analysed footage of the second contest.

Table 1. The number of pigs in the second contest evaluated for quantitative behaviour (described in section 2.3) and qualitative behaviour (described in section 2.2), categorised based on the results of the resident intruder tests, outcome of the first dyadic contest and number of skin lesions accrued during the first contest (above or below the median number of skin lesions, median = 29 lesions). Individuals with “unclassified” aggressiveness and/or undecided first contest outcome were excluded.

Behaviour in resident-intruder tests	First contest outcome	Skin lesions from first contest	Number of pigs evaluated for quantitative behaviour	Number of pigs watched for QBA
Attacked intruder in both tests (Agg+)	WINNER	≤ 29	55	10
		>29	31	5
	LOSER	≤ 29	23	8
		>29	53	16
Attacked intruder in one or neither test (Agg-)	WINNER	≤ 29	25	8
		>29	15	2
	LOSER	≤ 29	21	2
		>29	32	6

Total**255****57**

2.2. Qualitative Behavioural Assessment

2.2.1. Selection of video clips

Our focus in the second contest was on the pig's response to re-exposure to the contest arena and the presence of a contestant, not its response to aggressive behaviour per se. Hence a 30 s time frame from both pigs entering the arena was set as the standard video clip length, and we included only clips which did not contain damaging aggression (biting) in this period. Of 128 second contest dyads in which both contestants had a winning or losing experience in the first contest, 98 video clips contained no biting within the first 30 s. We selected 29 clips (containing 58 individuals, one of which had "unclassified" RI aggressiveness and was excluded), which approximately represented the total variation in RI aggressiveness, first contest outcome and first contest skin lesions (Table 1). This sample contained equal numbers of males and females and the smaller pig in each dyad weighed on average $93 \pm \text{SD } 7\%$ the body weight of their opponent. Qualitative behaviour assessment (QBA) (Rutherford et al., 2012; Clarke et al., 2017) was carried out with two observation sessions per video. Contestants within a dyad were randomly selected to be the focal pig either in a first or second observation session.

Differences in the number of skin lesions between pigs at the start of the contest were not apparent on the footage (Supplementary material, Figure S1).

2.2.2. Observers and observation sessions

Four observers were recruited on a voluntary basis from PhD students in the Animal and Veterinary Sciences department of SRUC. In several livestock species, QBA

has been validated as an on-farm measure that can be effectively applied by a single or small number of trained observers (e.g. Grosso et al., 2016; Phythian et al., 2016; de Boyer des Roches et al., 2018; Muri et al., 2019). The observers did not have extensive prior experience of working with pigs and were blinded to the aggressiveness and first contest outcome of the pigs. The aim of the study was briefly introduced as an investigation into the emotional expressivity of pigs upon introduction to an unfamiliar conspecific.

A fixed list of terms had previously been developed for QBA of pigs for the Welfare Quality Protocol, and we adapted this list (including additional terms generated in studies by Rutherford et al., 2012 and Duijvesteijn et al., 2014), to reflect the likely range of emotional expression anticipated in contests. Since definitions of QBA terms for pigs had not previously been published, we produced a description of each term (Table 2), adapted from work in sheep (AWIN, 2015a), goats (Grosso et al., 2016) and donkeys (AWIN 2015b). Observers discussed these terms to address any uncertainty about their meaning and to ensure there were no substantial contrasts between the observers in their understanding of the terms. After observing the animal, each observer recorded a score for each of the 20 terms on visual analogue scales of length 125mm length, with the extremes marked “Min” and “Max”. The participants were instructed to consider minimum as “completely absent” and maximum as “completely overriding”, the animal could not be more (e.g. relaxed).

Table 2. Adapted qualitative behaviour assessment (QBA) list of terms for pigs, with descriptions of the terms in the context of emotional expression in pigs.

	Term	Explanation
1	Relaxed	At ease, free from anxiety, agitation or tension. The animal appears to be

		unthreatened. May be active or resting.
2	Fearful	Startled, afraid, hesitant, timid. Attention may be focussed on a real or perceived threat or may be unrelated to something going on in the environment.
3	Agitated	Restless, not at ease, highly reactive. Whether the animal is active or lying, they make twitchy movements.
4	Content	Satisfied and at peace. The animal's needs are met, or the animal is successfully working towards their completion.
5	Tense	Uneasy and on-edge. Unable to relax.
6	Enjoying	Showing or expressing pleasure. Choosing to be engaged in a task and not distracted by others or the environment.
7	Frustrated	Annoyed and impatient because prevented from achieving goal or fulfilling satisfaction.
8	Sociable	Seeking and interacting with other pig. Appears to be enjoying/taking comfort from their contact.
9	Bored	Wearied, dull, feeling tired of something that has continued for too long; lacking in stimulation or looking for something to do. May be very active but aimlessly so.
10	Playful	Engaging in lively movements, frolicking, performing ritualized non-aggressive fights or playing with objects. Expressing pleasure, happiness and amusement.
11	Positively Occupied	Carrying out activities in a focused, directed and constructive manner.
12	Listless	Lack of vigour and energy. Animal appears lacklustre.
13	Lively	Animal is energetic. Engaged in task e.g. exploring, walking, or fighting, in a vigorous or excited manner.
14	Indifferent	Shows limited interest in or reaction to novelty, behaviour of other pig or exploring new environment. Movements are steady, not excited.
15	Irritable	Bothered or annoyed by something that can disturb, upset, trouble or exasperate.
16	Aimless	Attention is not directed to a particular activity, may change focus or direction often or without apparent purpose.
17	Distressed	Much troubled, upset, distraught, worried.
18	Curious	Explorative, intrigued by something, wishing to investigate surrounding environment (e.g. other pig, substrate, pen walls).
19	Angry	Hostile, disruptive, wants to fight/attack other pig.
20	Confident	Moves without hesitation, with purpose, and without showing fear

2.3. Quantifying behaviour prior to aggression in the second contest

Quantitative measures of behavioural expression were recorded to complement the qualitative data from the QBA. All second contest dyads were observed quantitatively for their behaviour by a single observer using the Observer XT12 software (Noldus Information Technology, Wageningen, The Netherlands). We analysed the data for all 255 pigs with a conclusive measure of RI aggressiveness and first contest outcome (see table 1). The aim of this behavioural analysis was to capture proxies of pigs' emotional valence: ear posture, vocalization and the attention each animal paid towards their opponent, the pen and the gate (Bloomfield et al., 2015; Winters et al., 2015; Crump et al., 2018; Ogura et al., 2020), during the early (pre-aggression) stage of the contest. Behaviour coding started when the shoulders of both animals were inside the arena and continued until 30 seconds had elapsed, or until the occurrence of the first aggressive behaviour (pushing, head-knocks or biting) or display behaviour involving contact (i.e. parallel walking and "heads up" postures, where pigs align themselves in a parallel position). Due to the camera angle not being set up to accurately compare differences in the eyes and snout, we scored only "ears back" posture (in common with Goumon and Špinka, 2016); the change in ear posture from its default position to being pulled back caudally towards the body. Gaze direction (direction in which the snout was pointing) was measured as a proxy for attention. A simple ethogram (Table 3) detailed three categories of gaze direction: towards opponent, towards gate or towards pen. Simultaneously it was recorded whether the animal was standing, walking, exploring (snout contact or within 10cm) or running.

Table 3. Ethogram of the behaviour of the focal pig in the early second contest. The first occurrence of pushing, head-knock, display or biting signalled the endpoint of each observation.

Behaviour	Description
States	
Gaze to opponent	Snout is pointing towards and/or in contact with opponent. *
Gaze to gate	Snout is pointing towards and/or in contact with gate. *
Gaze to pen	Snout is pointing towards and/or in contact with the walls of the pen. *
Point events	
Display	Pig has nose lifted high in the air, parallel to or facing the opponent's head <i>or</i> both opponents walk with their shoulders aligned.
Push	Pig leans onto its opponent, putting weight through its shoulder.
Squeal	Vocalizes with high amplitude and high frequency with mouth open.
Ears back	Long axis of pinnae pointing greater than 90 degrees from nose, may be pressed against body.
Bite	Rapid open mouth movement with teeth contacting any part of the opponent
Head-knock	A rapid thrust of the head against any part of the opponent.

*on horizontal plane. Head can be up, down or in line with spine.

All behaviours directed towards the gate were grouped, as were all behaviours directed towards the pen. Gazing towards the opponent while stationary was evaluated separately from approaching behaviour (explore, walk, run), resulting in four categories: “approach opponent, gaze towards opponent while stationary, attention towards pen, attention towards gate”. The “observation time” was up to the first agonistic encounter or a maximum of 30s.

2.4. Statistical analysis

All statistical analysis was carried out in “R” version 3.6.1. (R, 2017). Individuals were the experimental unit, and each model was tested for normality and heterogeneity of variance assumptions by visual inspection of residual plots.

2.4.1. Principal component analysis of Qualitative Behavioural Assessment scores

The scores for each term for all observers were analysed together using principal component analysis (PCA) with a correlation matrix, but no rotation (“prcomp” function). PCA identified three main dimensions, with eigenvalues: 6.4, 4.0 and 3.1. PC1 accounted for 32% of the variance, PC2, 20% and PC3, 16%. The coefficients for each term are detailed in the supplementary material (Table S1). Labels for dimensions were generated by selecting the highest loading descriptors for the positive and negative poles of each dimension. Where a large cluster of qualitatively similar terms loaded on the same pole, the two highest terms were selected for clarity, if this did not affect the overall meaning (Rutherford et al., 2012, Grosso et al., 2016). Therefore, we labelled the PCs: PC1: agitated/ tense to relaxed/content , PC2: fearful/aimless to enjoying/confident, and PC3 was identified only by the negative terms: listless/indifferent. Inter-observer reliability was calculated using Kendall’s rank correlation coefficient, “desctools” package (Signorell, 2017) and was

substantial for PC1 ($\tau = 0.80$, $p < 0.001$) and PC2 $\tau = 0.61$ ($p < 0.001$) and weak for PC3 ($\tau = 0.35$, $p = 0.027$). Pairs of observers were compared using Spearman's rank tests, which did not identify any of the four observers as an outlier.

2.4.2 Mixed model analysis

Mixed models evaluated the fixed effects of aggressiveness (Agg+ or Agg-), first contest outcome (win or lose) and their interaction, first contest lesions and regrouping treatment between contests (regrouped or control) on the outcome variables: QBA (PC1, PC2 and PC3), ear position and attention (approach opponent, gaze towards opponent while stationary, attention towards gate, attention towards pen). Additional fixed effects considered were related to attributes of the focal individual (sex and body weight) and the second contest opponent (sex, relative body weight difference between opponents, first contest outcome and aggressiveness), which may have influenced the focal pig's emotions in the second contest. Random effects were second contest ID, nested within batch (birth cohort). The QBA models also contained observer ID (fixed effect) and pig ID (random effect). Since the observation interval ended at the first agonistic behaviour, the fixed effect "interval duration" was included in models of binary outcomes of attention. This accounted for the increase in likelihood of observing each behaviour with increasing duration.

An iterative approach was used to analyse measures of attention, due to a large number of zeroes for some behavioural outcomes (see results). Firstly, we analysed the tendency for the animal to perform each behaviour (approach opponent, gaze towards opponent while stationary, attention towards gate and attention towards pen) at all during the observation time. When the main effects did not influence the

occurrence of the behaviour (yes/no), we analysed the proportion of the observation time spent performing the behaviour, for individuals with a non-zero outcome. PC scores were analysed using linear mixed models (“lmerTest” package, Kuznetsova et al., 2017), ear position and binary measures of attention were analysed using GLMMs with a binomial distribution and a logit link (“lme4” package, Bates et al., 2007), and for proportion measures of attention, GLMMs with a beta-distribution were used (“glmmTMB” package, Brooks, Mollie et al., 2017). Ear position was analysed as a binary outcome as the majority of pigs performed “ears back” either once (77%) or not at all (16%).

Initial filtering of fixed effects was carried out, whereby global models were formed by including all fixed and random effects, then each categorical variable was tested as a single term using ANOVA and only those found to have a significant effect at $p < 0.05$ were retained in the mixed models. For these, the variance inflation factor was calculated and variables with high collinearity (values > 5) to others were removed. The final models providing the best fit with the minimum number of fixed and random effects were determined using likelihood ratio tests and by applying Akaike’s Information Criterion (AIC), and also RMSE and R^2 as model fit statistics. For linear models (lm) Wald tests were applied to check for statistical significance of the fixed effects (see supplementary information, table S2). Least squared means (“lsmeans”, Lenth, 2018) were extracted, with Tukey-adjusted pairwise comparisons of factors involved in interaction effects. LS means and 95% confidence intervals were back transformed from the logit scale.

2.4.2. Observation time for quantitative measurements

The latency to first agonistic contact (parallel walk, “heads up” position, push or bite) ranged from 1.1 to 29.4s. Forty-three dyads reached 30s without any agonistic contact. The latency distribution was multimodal, with peaks at 8s, 23s and the endpoint of 30s. Therefore, observation time was categorised as: short (1.1-15s, mean 8.8 +/- SD 3.5s), intermediate (15-29.4s, mean 23 +/-SD 3.3s) and no agonistic contact (30s).

2.4.3. Correlation between qualitative and quantitative measures

Spearman’s rank tests of correlation (cor.test) were used to assess the association between QBA dimensions and attention. To obtain a single PCA score per dimension per pig, we took the mean value of all four observers. To determine whether “ears back” posture (yes/no) was associated with QBA PCA scores and attention measures, we used Mann-Whitney U tests.

3.0. Results

3.1. Qualitative behavioural assessment

The Principal Components (PC) PC1 (describing where the pig scored on the axis of agitated/ tense to relaxed/ content) and PC3 (describing listless/ indifferent) were not affected by aggressiveness, first contest outcome (win or lose) or their interaction (full results are shown in supplementary table S2). PC1 was affected by the sex of the contest opponent; focal pigs were more agitated and tense if their opponent was male ($X^2=8.4$, $p=0.004$). Aggressiveness and sex affected PC2 (fearful/aimless to enjoying/ confident); Agg- individuals were more confident and enjoying than Agg+ individuals ($X^2=6.9$, $p=0.009$) and males were more confident and enjoying than females ($X^2=9.5$, $p=0.002$).

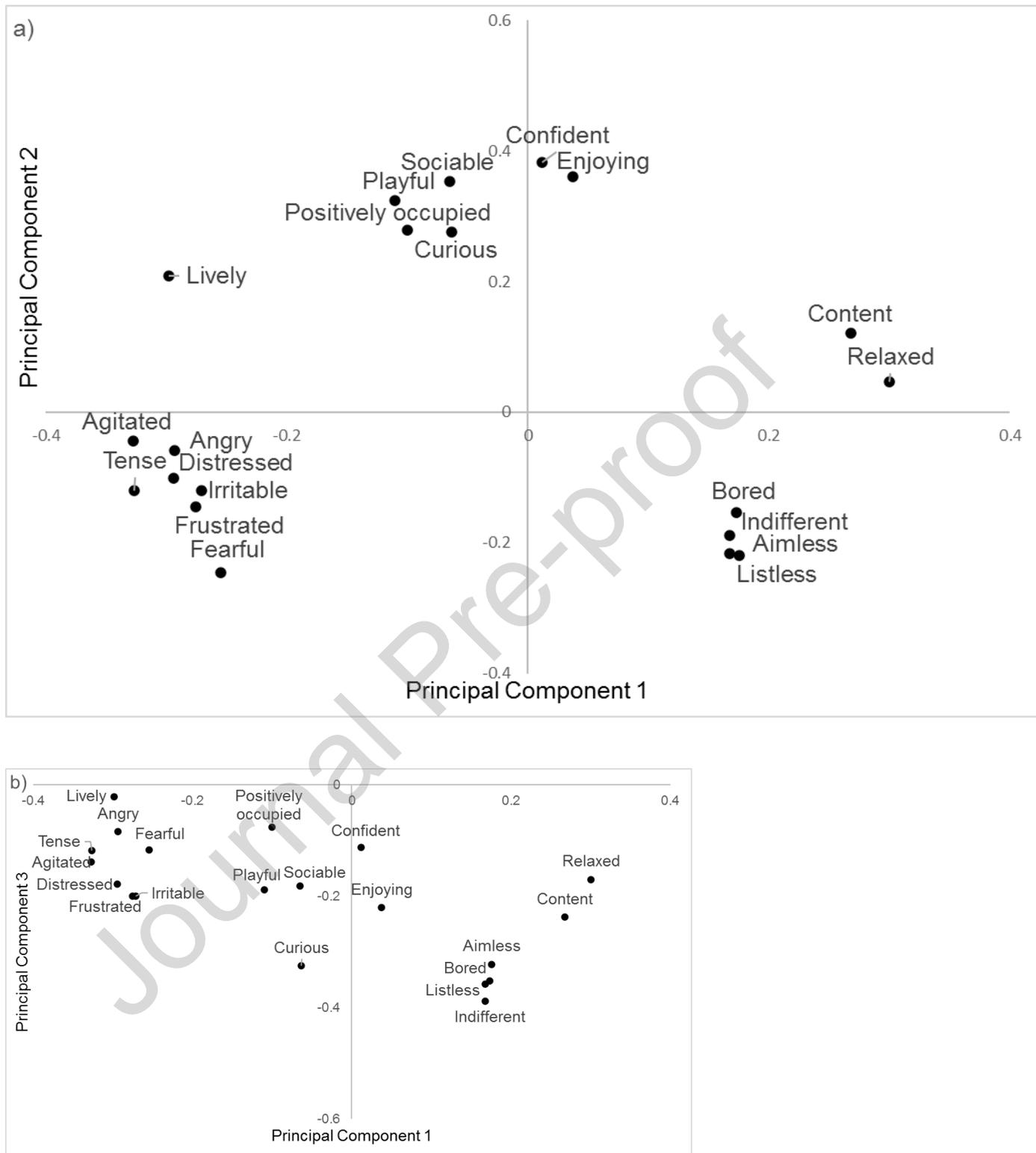


Figure 2. a) Distribution of QBA descriptors along PC1 and PC2 and b) for the QBA descriptors along PC1 and PC3.

There was variation between observers in the absolute values of the PCA scores, as indicated by the significant effect of observer on PC1 and PC3 (supplementary table S2). However, as stated in section 2.4.1., inter-observer agreement on the ranking of individuals was strong for PC1 and PC2.

3.2. Quantitative behaviour measures

Agg+ pigs were twice as likely to be observed with their ears back than Agg- pigs ($X^2=7.8$, $p=0.005$), but first contest outcome did not influence ear position ($X^2= 0.52$, $p=0.5$). Only one single high-pitched vocalisation (squeal) occurred in total, and therefore we did not include vocalisation in further analyses.

With respect to gaze direction, pigs dedicated most of their attention towards the opponent, spending over half of the time approaching the opponent (median 53%, IQR 34-75 %) and 0% (0-7%) of the time gazing towards the opponent while stationary. On average 12% (0-29%) of their attention was directed towards the gate and 15% (0-32%) towards the pen. Pigs which spent a greater proportion of the observed time approaching the opponent spent less time gazing towards the opponent whilst stationary ($r_s= -0.30$, $p<0.001$). The influence of aggressiveness, first contest outcome and their interaction on attention are summarised in Table 4. Where the main effects (aggressiveness, first contest outcome and/ or their interaction) did not influence the tendency to perform a behaviour (yes or no), we include the outcome measure “proportion of time” spent performing the behaviour, for individuals with a non-zero outcome.

There was an interaction effect between aggressiveness and first contest outcome on how much attention pigs paid to their opponent in the second contest. Between Agg- individuals only, first contest winners spent a greater proportion of the observed

time approaching the opponent than first contest losers ($p=0.04$). Agg+ first contest winners were significantly ($p=0.008$) more likely than Agg- first contest losers to gaze towards the opponent while stationary. Pigs which had lost the first contest spent more of the interval looking towards the gate than first contest winners ($p=0.02$). The proportion of pigs paying any attention towards the gate was not affected by regrouping, proportions were 0.73 (0.60-0.82) of regrouped pigs and 0.77 (0.63-0.87) of controls ($\chi^2=0.40$, $p=0.5$), but regrouped pigs paid less attention to the gate for less of the observed time: 25% (20-30%), compared with 31% (25-36%) for the controls ($\chi^2=4.9$, $p=0.03$).

The body weight of the focal pig influenced whether or not they paid any attention to the pen, with lighter individuals being more likely to gaze towards the pen, logit (standard error) = -0.08 (0.03), $\chi^2=6.2$, $p=0.01$), but body weight did not affect the proportion of time gazing towards the pen: logit (SE) = 0.02, (0,1), $\chi^2=2.3$, $p=0.1$. The full model outputs are included in supplementary table S3. First contest lesions did not significantly explain variance in any outcome measure.

Table 4. Summary of the effects of aggressiveness and first contest outcome (win/lose), on attention in the second contest. Section a) shows individual treatment effects, section b) shows interaction effects. Tendencies are derived from GLMMS with binomial link function. All values are least squared means (LSM) with their 95% confidence intervals (CI) with the Chi-square (χ^2) and P-value.* tendency not analysed, as 247/255 pigs approached the opponent

a)	Agg+	Agg-	χ^2	P	Winner	Loser	χ^2	P
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Ears back	0.28 (0.18- 0.39)	0.13 (0.08- 0.27)	7.8	0.005	0.20 (0.12- 0.33)	0.18 (0.10- 0.29)	0.52	0.5
Approaching opponent*	0.51 (0.47- 0.56)	0.53 (0.47- 0.58)	0.03	0.9	0.48 (0.43- 0.53)	0.56 (0.50- 0.61)	2.1	0.2
Gaze	0.53 (0.44- 0.61)	0.35 (0.24- 0.47)	-4.5	0.03	0.37 (0.27- 0.49)	0.50 (0.41- 0.60)	-0.8	0.4
Attention gate: Tendency	0.71 (0.59- 0.80)	0.78 (0.64- 0.88)	1.1	0.3	0.76 (0.63- 0.85)	0.73 (0.60- 0.83)	0.09	0.8
Attention gate: Prop. time	0.29 (0.24- 0.34)	0.26 (0.21- 0.32)	0.7	0.4	0.25 (0.20- 0.30)	0.31 (0.26- 0.36)	5.4	0.02
Attention pen: Tendency	0.85 (0.79- 0.91)	0.84 (0.76- 0.92)	- 0.04	0.8	0.95 (0.89- 0.98)	0.91 (0.79- 0.96)	2.8	0.09
Attention pen: Prop. time	0.27 (0.23- 0.31)	0.32 (0.27- 0.37)	2.7	0.1	0.28 (0.24- 0.33)	0.31 (0.26- 0.35)	0.3	0.6
b)	Winner		Loser					
	Agg+	Agg-	Agg+	Agg-	χ^2	P		
Ears back	0.30 (0.19- 0.45)	0.13 (0.06- 0.29)	0.25 (0.15- 0.39)	0.12 (0.05- 0.25)	0.05	0.8		

Approaching opponent*	0.51 (0.45- 0.58)	0.60 (0.50- 0.69)	0.52 (0.45- 0.59)	0.45 (0.37- 0.53)	4.4	0.04
Gaze	0.56 (0.41- 0.70)	0.19 (0.08- 0.40)	0.49 (0.34- 0.64)	0.51 (0.34- 0.68)	7.0	0.008
Attention gate: Tendency	0.70 (0.56- 0.82)	0.76 (0.56- 0.89)	0.71 (0.56- 0.83)	0.80 (0.63- 0.90)	0.06	0.8
Attention gate: Prop. time	0.26 (0.21- 0.32)	0.23 (0.17- 0.31)	0.32 (0.26- 0.39)	0.30 (0.24- 0.37)	0.06	0.8
Attention pen: Tendency	0.90 (0.81- 0.99)	0.85 (0.73- 0.97)	0.80 (0.71- 0.89)	0.83 (0.72- 0.93)	-	0.5 0.08
Attention pen: Prop. time	0.27 (0.22- 0.32)	0.29 (0.22- 0.38)	0.27 (0.22- 0.32)	0.35 (0.28- 0.42)	0.7	0.4

3.3. Correlations between qualitative and quantitative measures

Pigs that were more relaxed and content on PC1 spent less time approaching the opponent, more time gazing towards the opponent while stationary and more time gazing towards the gate (see table 5). PC2 (fearful/aimless to enjoying/confident) and PC3 (listless/indifferent) did not correlate significantly with any measures of attention. Expression of “ears back” posture did not correlate with the principal components of QBA scores, apart from a statistical tendency ($p=0.09$) for pigs which

expressed the behaviour “ears back” to score more negatively on PC2 (median = -1.1) than pigs which did not (median = 0.2).

Table 5. Association between the principal components of QBA (mean per pig) and quantitative measures of attention and ear posture. Spearman's test of correlation was used to compare continuous outcome variables and Mann-Whitney U tests for binary outcomes.

Outcome variable	PC1 (agitated/tense to relaxed/ content)	PC2 (fearful/aimless to enjoying/confident)	PC3 (listless/ indifferent)	Ears back (yes/no)
Approach opponent (prop.)	$r_s = -0.40, p=0.002$	$r_s = 0.09, p=0.5$	$r_s = 0.08, p=0.6$	U= 7806, p=0.4
Gaze (prop)	$r_s = 0.30, p=0.02$	$r_s = -0.17, p=0.4$	$r_s = -0.21, p=0.1$	U=7712, p=0.6
Gaze towards gate (prop)	$r_s = 0.26, p=0.048$	$r_s = -0.001, p=0.99$	$r_s = 0.06, p=0.6$	U= 6813, p=0.2
Gaze towards pen (prop)	$r_s = 0.12, p=0.4$	$r_s = 0.02, p=0.9$	$r_s = 0.02, p=0.9$	U=24971, p=0.6
Ears-back posture (yes/no)	U=501, p =0.6	U= 374, p= 0.09	U= 452, p= 0.7	

4.0. Discussion

The hypothesis that winning a dyadic contest would result in pigs expressing more positive emotional valence in the initial stages of a subsequent agonistic encounter was partly supported by measures of attention: first contest winners paid less attention towards the exit gate and, within Agg- pigs, had a greater inclination to approach an unfamiliar individual. However, first contest outcome did not affect QBA scores of emotional expressivity. Both qualitative and quantitative measures indicated that, contrary to our prediction, aggressive personality does not counteract the negative emotional costs of losing contests. Agg+ pigs were more likely to hold their ears back than Agg- pigs and were more fearful/aimless on PC2, which may reflect more negatively valenced emotions during the second contest. Aggressive personality increased the likelihood of pigs gazing towards their opponent while stationary, but only between prior contest winners. Higher aggressiveness appeared to reduce the impact of first contest outcome on gaze behaviour early in the second contest. Attention towards the second contest opponent differed in Agg- pigs according to whether they had experienced winning or losing in the first contest.

4.1. Qualitative Behavioural Assessment

Qualitative behavioural assessment provides a useful measure of emotion in the early contest setting, since it is non-invasive, and does not require animals to undergo training or social isolation, such as in tests of cognitive bias (Mendl et al., 2009; Crump et al., 2018). The most important dimension of emotional expression, PC1, distinguished between agitated, tense pigs and relaxed, content pigs. PC1 captures aspects of mood, (with negatively valenced emotions loading most negatively and strongly) and arousal, or vigour (with relaxed and content loading positively, and lively and angry loading negatively). PC2 captured additional

variation in valence not explained by PC1, contrasting fearful and aimless pigs with enjoying and confident pigs. PC3 highlighted the pigs that were indifferent, bored and listless (although in a contest setting where pigs were highly active, this dimension likely identifies pigs which were relatively less interested and energetic), however it had weak agreement between observers in both the actual scores and their relative rank. Pigs that were more relaxed/content on PC1 (agitated/ tense to relaxed/content) gazed for less time towards the gate and towards the opponent whilst stationary and spent more time approaching the second contest opponent, although these correlations were weak. Otherwise, there was little direct association between our qualitative and quantitative measures of emotion.

Winning contests is expected to inflate an animal's estimation of its own fighting ability (Fawcett and Johnstone, 2010; Fuxjager and Marler, 2010), however we did not find a significant influence of first contest outcome on emotional expressivity. In a previous study assessing the emotional dimension of contest outcome, QBA of contest winners and losers directly after a contest did not show a more positive valence in winners compared with losers (Camerlink et al., 2016). However, there was an interaction effect whereby winners were more passive when in a positively valenced state, whereas losers were more active, which may reflect the solicitation of social support following the stress of a social defeat (Otten et al., 2002; Camerlink et al., 2016). Furthermore, being removed from the contest arena and returned to the home pen may elicit positive emotions such as relief in the losers, i.e. a release effect (Doyle et al., 2010). We found that focal pigs were more tense and agitated on PC1 if their opponent was male, which may reflect a greater expectation of defeat, since in mixed- sex contests, males are more likely to win (Oldham et al., 2020). Female pigs expressed more negatively valenced emotionality on PC2, which again

could indicate a low confidence of winning (although the interaction between focal and opponent sex was not significant) or may reflect a difference in the contest dynamics, since mixed or female-only contests tend to involve less display behaviour and progress to fighting more quickly than male-male contests (Camerlink et al., 2015).

More aggressive animals had a more negatively valenced body language (effects on PC2). This suggests that avoidance of aggression is not necessarily due to overwhelming negative emotions such as fear or anxiety, associated with pessimism or negative self-assessment. The relationship between aggression and anxiety as personality traits is complex. In rodent research, low anxiety rat strains demonstrate higher aggressiveness (offensive aggression in the resident-intruder test) (Neumann et al., 2010). A hypothesised relationship between high aggressiveness and fearlessness has not been demonstrated directly in pigs (Janczak et al., 2003; D'Eath et al., 2009; Desire, 2015; Scheffler et al., 2016), although this could be because tests of fear in non-social contexts do not replicate the emotional complexity of the social environment. Most Agg- winners observed for QBA won the first contest with fewer than average skin lesions. Although first contest skin lesions were included in the mixed models, the imbalance in sample size for this effect likely limited how accurately we could evaluate it. A propensity to avoid conflict in the Agg-pigs may lead to more neutral or positive expectations of social encounters. Since Agg-pigs were less likely to seek conflict with the opponent, they could also explore the novel environment, and engage in non-aggressive social interaction.

Proactive and reactive aggression may be accompanied by different emotions, e.g. confidence/sensation-seeking versus anger/rage (Panksepp, 2011; Chester et al.,

2016, Wranghama, 2017), and our understanding of the effect of winning and defeat on emotional valence could be further developed using behavioural assays which reduce the influence of the opponent, such as observing anticipatory behaviour (e.g. facial expression; Bremhorst et al., 2019; or vocalisations: Villain et al., 2020) or creating a “mock” contest using a dummy opponent. However, attempts to replicate agonistic encounters using fake opponents, as implemented in studies of aggression in fish (Earley et al., 2000) and temperament in dogs (Svartberg et al., 2005, Banard et al, 2019) does not accurately recreate behaviour against a live opponent (Li et al., 2018; Verdon et al., 2018).

Interestingly, aggressiveness and first contest outcome did not significantly influence PC1. In an earlier QBA study of pigs in a non-social context (elevated plus maze), treatment with the sedative drug azaperone altered emotionality on PC1 from unsure/nervous to confident/curious (Rutherford et al., 2012) without affecting PC2 (calm/relaxed to agitated/angry). In a controlled social situation, such as a “mock-contest”, azaperone could be used to distinguish whether the trait of aggressiveness is associated with greater expression of anger, when the individual differences in anxiety/confidence are alleviated. To account for variation in absolute PCA scores of observers, we included “observer” as a fixed term in the model before the main effects, as in earlier studies (e.g., Grosso et al., 2016). Observers showed high agreement in how they ranked the animals on the dimensions PC1 and PC2 and we evaluate only the pigs’ positions on each dimension relative to one-another; therefore the absolute numerical scores should be considered as scaling values rather than a unit of emotional expressivity.

4.2. Ear position

We found that the majority of pigs did not adopt an ears-back position prior to agonistic contact, in agreement with findings that the pre-agonistic phase of the contest is associated with ears being orientated forwards more than during later stages (Camerlink et al., 2018). The probability of pinning the ears back against the body was higher for Agg+ pigs, conflicting with our expectation that aggressiveness and prior winning would be associated with a more forward ear-position. Pigs move their ears back during restraint stress (Goumon and Špinka, 2016) and express this behaviour more during aversive than rewarding events (Reimert et al., 2013), indicating that Agg+ pigs found the early contest period more aversive than Agg- pigs. However, the correlation between our QBA results and occurrence of “ears back” posture was weak, and functional reasons for holding the ears back against the body, for example in anticipation of delivering an attack, could also explain the greater occurrence of the ears-back posture in Agg+ pigs.

4.3. Attention

We considered gaze direction and approach behaviour as measures of where the pig was directing its attention (Goumon and Špinka, 2016). The experience of contest success or defeat affected not just how much attention a pig paid towards a subsequent opponent, but the manner in which they did so. Agg- first contest winners spent most time approaching the second contest opponent and Agg- losers spent the least time approaching the opponent, suggesting that for Agg- pigs, losing the first contest resulted in aversion towards approaching an unfamiliar pig. Gazing towards the opponent while stationary was greatest in Agg+ winners, it is less apparent in Agg- winners and therefore may reflect a trade-off between approaching and gazing whilst stationary- i.e. the Agg- winners were more likely to approach and

so did not spend as much time standing still. Pigs demonstrate vigilance-avoidance behaviour (Verbeek et al., 2021) and a reduction in looking towards the opponent has a protective function, since in agonistic encounters, a fixed gaze may signal hostility (Giersing and Andersson, 1998; Vas et al., 2005), whilst looking away signals submission (Jensen, 1982).

First contest losers paid more attention towards the exit gate, reflecting avoidance of a social encounter with an unfamiliar pig. Social defeat is stressful (Ruis et al., 2001) and can lead to conditioned place aversion, whilst winning can result in conditioned place preference (Farrell and Wilczynski, 2006; Golden et al., 2017; Kim et al., 2018), therefore this avoidance behaviour indicates a negative emotional state. Attention towards the exit gate was not affected by RI aggressiveness, in contrast with rodent studies, which have demonstrated amongst certain aggressive strains a preference to revisit places in which they have won an aggressive encounter (Martínez et al., 1995; Golden et al., 2017). Pigs that had experienced regrouping spent less time looking towards the gate than did the controls. This may suggest that regrouping reduced the negative emotional aspect of encountering unfamiliar pigs, or that after regrouping, pigs had a greater awareness that a contest was likely and unavoidable, and focussed their attention on assessing the opponent. The effect of treatment on attention towards the gate represented a difference of approx. 1.8s, which is comparable to treatment differences in the duration of attention shown by piglets towards the location of auditory threat (3.8s in piglets given probiotics compared with 2.1s in controls), (Verbeek et al., 2021). Pigs' decisions to engage in fighting in dyadic contests appears to be sensitive to small differences in behaviour during the pre-escalation phase. In a prior study, dyads which did not fight in

contests spent on average 1.7s longer parallel walking than dyads which fought (Camerlink et al., 2016).

In summary, social defeat led pigs to be more hesitant about approaching, and/or looking at an unfamiliar opponent, and this may reflect differences in their emotional state. Social anxiety in humans is associated with visual bias towards, and difficulty in disengaging attention from social threats (for example angry facial expressions) (Roy et al., 2008; Buckner et al., 2010; Schofield et al., 2012), which has led to the evaluation of “attention bias” in animals as an indicator of affective state (a combination of current emotional response and background mood (Mendl et al., 2010; Bethell et al., 2012; Lee et al., 2016; Crump et al., 2018)). The varied results of these studies suggest attention bias is species and situation specific. For example, there is evidence in pigs and sheep that long-term negative experiences result in less attention paid towards a threat (Luo et al., 2019; Verbeek et al., 2021). The current study reinforces the findings of Luo et al., 2019, that attention bias towards a potential threat in pigs is influenced by a combination of personality and recent positive or negative experience, and furthermore, pigs facing a potential social threat may reduce their attention in degrees, from approaching the opponent, to gazing whilst standing, to looking away, to attempting to escape. Using a social stimulus to assess attention adds complexity to the interpretation since, for some animals, there may be a conflict between assessing a threat and a desire to submit without provoking the opponent by looking at them.

We found some evidence that an aggressive personality reduces the emotional impact of winning and losing; Agg- individuals were less likely to approach the opponent if they had experienced defeat in the first contest, whereas Agg+ losers approached the opponent just as much as Agg+ winners. Aggressiveness could

relate to reduced behavioural flexibility (Bolhuis et al., 2004), which may be more relevant at the beginning of a contest, where pigs have less information about their opponent and are more likely to rely on a heuristic rule.

4.4. Associations between QBA and gaze direction

Pigs with a low PC1 score (i.e. that were less tense and agitated) paid more attention to the gate, spent more time gazing towards the opponent while stationary, and less time approaching the opponent, although the correlations were modest. As described above, PC1 captures aspects of both mood and arousal. High tension when approaching the opponent may reflect the role of emotion in action readiness (Ridderinkhof, 2017); pigs are highly alert in anticipation of interacting with the opponent. This correlation between approaching and PC1 suggests that pigs which directed more attention towards the opponent rather than the exit gate expressed more negative emotionality. Therefore, negatively valenced emotions may be expressed when approaching the opponent and when approaching the gate. Approach-related, negatively valenced emotions, including anger and frustration (Carver and Harmon-Jones, 2009; Panksepp, 2011; Zahn et al., 2018), loaded relatively highly on PC1. Two-dimensional models of emotion (i.e. valence and arousal) may fail to distinguish between fear and anger, and the “PAD” (pleasure, arousal, dominance) emotional state model (Mehrabian, 1996) may be more effective in understanding individual differences in aggressiveness.

Conclusion and future directions

The emotional background to aggression has been overlooked in animals, with aggression often viewed as a functional and inflexible response (Adolphs and Anderson, 2018). However, our results illustrate a range of emotions expressed by

pigs in agonistic encounters, influenced by sex, aggressive personality and contest experience. Pigs with a highly resident-intruder aggressiveness tended to express more negative emotions in the early stage of an aggressive contest (more frequent ears-back posture, more negative score on PC2), and aggressiveness may reduce the emotional impact of winning and losing contests in pigs. Attention bias shows promise as a measure of emotionality in pigs, however the role of gaze in social signalling must be taken into account if using attention to understand social emotions. Understanding of the effect of winning and defeat on emotional valence, and separating the affective components of ear posture and gaze behaviour from their protective and signalling functions in aggression, could be further developed using behavioural assays which reduce the influence of the opponent. The comparison of behaviour in the contest arena (an uncontrollable situation) with a controllable situation, such as in conditioned place preference paradigms, may help to describe how negative social emotions, such as anger, fear and frustration shape individual differences in aggression.

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Figures for manuscript: “Once bitten, twice shy: aggressive and defeated pigs begin agonistic encounters with more negative emotions”

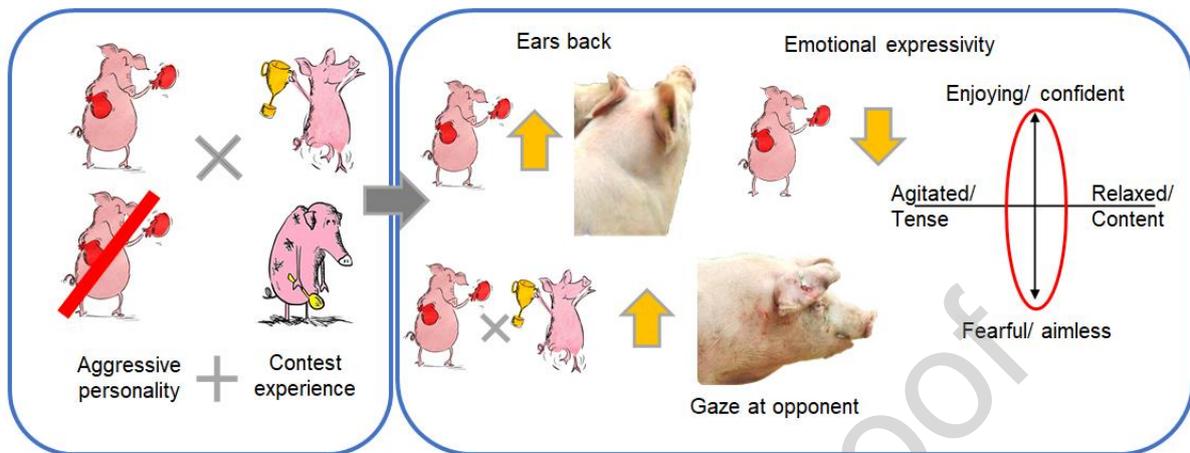


Figure 1 Overview of the experimental procedures of the overarching study. The current study focussed on the initial phase of the second contest only, while using the information that was obtained in the previous tests (e.g. Resident-Intruder aggressiveness).



Figure 2. a) Distribution of QBA descriptors along PC1 and PC2 and b) for the QBA descriptors along PC1 and PC3.

Graphical abstract



Highlights

- The emotional expression of pigs meeting a new opponent is influenced by past contest experience.
- Prior losers gazed towards the exit gate more than winners.
- Aggressive personality influenced ear posture, gaze direction and emotional expressivity.