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Emotionality in growing pigs: Is the open field a valid test?

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ABSTRACT

The ability to assess emotionality is important within animal welfare research. Yet, for farm animals, few tests of emotionality have been well validated. Here we investigated the construct validity of behavioural measures of pig emotionality in an open-field test by manipulating the experiences of pigs in three ways. In Experiment One (pharmacological manipulation), pigs pre-treated with Azaperone, a drug used to reduce stress in commercial pigs, were more active, spent more time exploring and vocalised less than control pigs. In Experiment Two (social manipulation), pigs that experienced the open-field arena with a familiar companion were also more exploratory, spent less time behaviourally idle, and were less vocal than controls although to a lesser degree than in Experiment One. In Experiment Three (novelty manipulation), pigs experiencing the open field for a second time were less active, explored less and vocalised less than they had done in the first exposure to the arena. A principal component analysis was conducted on data from all three trials. The first two components could be interpreted as relating to the form (cautious to exploratory) and magnitude (low to high arousal) of the emotional response to open-field testing. Based on these dimensions, in Experiment One, Azaperone pigs appeared to be less fearful than saline-treated controls. However, in Experiment Two, exposure to the arena with a conspecific did not affect the first two dimensions but did affect a third behavioural dimension, relating to oro-nasal exploration of the arena floor. In Experiment Three, repeat exposure altered the form but not the magnitude of emotional response: pigs were less exploratory in the second test. In conclusion, behavioural measures taken from pigs in an open-field test are sensitive to manipulations of their prior experience in a manner that suggests they reflect underlying emotionality. Behavioural measures taken during open-field exposure can be useful for making assessments of both pig emotionality and of their welfare.

1. Introduction

On commercial farms, pigs may be exposed to a range of potentially aversive experiences, such as poor handling [1], social stress from mixing aggression and social instability [2,3], various environmental challenges (e.g. elevated ammonia, noise [4]) and barren or crowded housing conditions [5]. These experiences could interact with individual genotype and early life experience to create chronic negative states of emotionality such as fear, anxiety or depression, which have implications for welfare. As such, the ability to accurately assess and interpret emotionality in pigs is important.

Although the open-field test is widely used to assess pig emotionality, Forkman et al. [6] concluded that there was insufficient evidence to back its validity as a test of fear in pigs. This is partly because, in addition to the variety of experimental designs and methodologies used, there is no clear consensus on what behavioural measures are most valuable for pig open field testing. Difficulties can also arise with the interpretation of pig open-field behaviour because a measured behaviour may reflect a component of emotionality such as fear or anxiety *per se* or something else such as exploration or activity. Indeed, a variety of underlying factors, such as fear of novelty [7], exploration [5], social reinstatement [8], and general activity level [9] may affect a pig's behaviour in the open field and some separation of these possible contributory factors is necessary for proper interpretation.

One common method of validation is to assess the impact of anxiolytic drugs on behavioural parameters. In one such study, Diazepam had no effect on pig activity (lines crossed) or number of entries into the centre of an open field [10]. However, parameters commonly used in rodent fear studies [11] may not be relevant to pigs. For example, the basis for the use of centre or periphery time as a measure of fear in the open-field is a behavioural strategy used by rodents to avoid predation in open spaces [11]. In order to capture specific aspects of pig open-field emotionality, a broader range of behaviours need to be measured.

In the present study, we tested the validity of the open-field in 6- week-old pigs using three different approaches. Firstly, we used Azaperone, a butyrophenone neuroleptic drug currently licenced for

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pigs (to prevent aggression and stress, e.g. [12]), as a pharmacological manipulation of emotionality. Secondly, we manipulated the social isolation component of the open field by observing pigs with or without a familiar companion (e.g. [8]). Finally, we manipulated the novelty component of the test by observing the impact of repeat test exposure on pig behaviour (e.g. [8]). We hypothesised, that these manipulations of test experience would alter pig emotional state and that behavioural measures in the open field might reflect underlying pig emotionality. We predicted that, compared to appropriate controls, pigs would be less fearful of the open-field when pre-treated with Azaperone, when tested in a pair, or upon their second exposure to the test.

2. Materials and methods

All experimental work was carried out under UK Home Office licence, following ethical approval by the Animal Experiments Committee at SAC.

2.1. Description of open-field arena and basic testing procedure

The open-field arena (Fig. 1) measured 1.84 m × 1.89 m and had 0.90 m high solid walls. In it were placed two unfamiliar objects, an orange ball (circumference = 65 cm) and a feeder (21.5 cm × 9.5 cm × 9.0 cm: different size and design from the one provided in the home pen) containing 150 g of the standard home pen feed. The presence of the two objects was intended to provide the pigs with an outlet for a broader range of behavioural expression. The arena was washed down with water between tests to reduce odour from the pig in the preceding test. For the open-field testing, each pig was picked up and carried to a different room where they were unable to hear other pigs. The pig was placed in the open field arena at the start point in one corner and the experimenters immediately left the room.

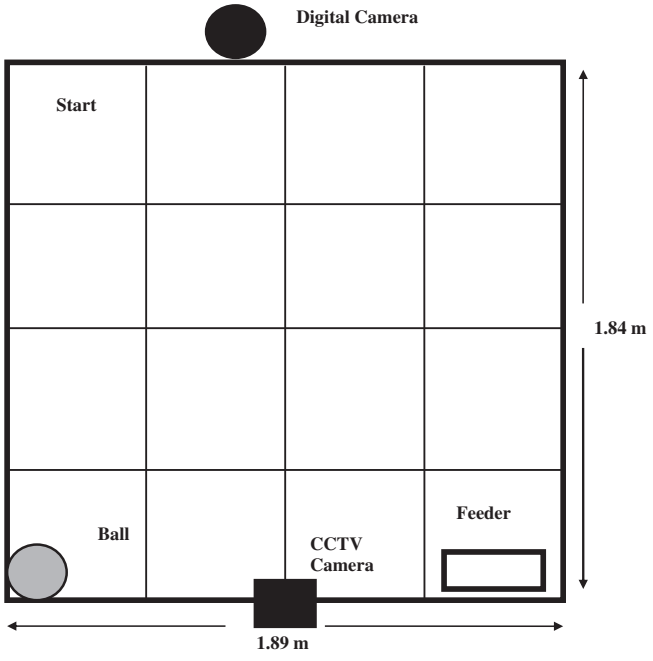

Fig. 1. Schematic diagram of the open field arena dimensions. The pig was placed at the corner start point and was observed for 10 min. For the purpose of analysis, the arena was divided into 16 equal squares.

2.2. Experiment 1: Open field with and without Azaperone

In Experiment 1, the subjects were twenty-four 5–6 week-old Large White × Landrace pigs (13 males, 11 females). Eight pigs were tested from each of three litters. Each of the litters was split at weaning (4 weeks old) into groups of four to six (two pens housing each litter). Housing fewer pigs in each pen (and balancing for test order) was intended to minimise disturbance (by entering the pen) to pigs, especially those drug-treated, while open field testing. Four pigs were tested from each of six straw bedded pens (2.85 m × 1.85 m). Pigs on a light: dark schedule of 12 h:12 h fed ad libitum on a dry commercial diet appropriate for their age.

Each pig was observed twice for 10 min in a cross-over design, once with 1 mg/kg Azaperone (A) (Stresnil®: Janssen Animal Health LTD) and once with an equivalent volume of saline (S). The first and second tests were three days apart and pigs were tested in the same order (balanced across sex, pen, litter, and treatment condition). Open field testing was carried out between 11:30 and 16:00 each day. One experimenter restrained the pig while the other gave a standardised injection intramuscularly to the pig in the home pen. The side of the neck in which the injection was made was balanced across tests, and pigs received a similar amount of handling between tests. Half of the pigs from each treatment (n = 12) were observed immediately after they were injected (injection time: T0), and half (n = 12) were observed 20 min after injection (injection time: T20). Onset of Azaperone is usually straightforward in pigs but if pigs are disturbed (e.g. by handling) within the first 20-min post injection the drug can cause them to behave unpredictably [13,14]. Consequently, T20-pigs were returned to their littermates in the home pen for 20 min before being exposed to the arena.

2.3. Experiment 2. Open field with and without companion

In Experiment 2, the subjects were twenty 5–6 week-old (10 males: 10 females) Large White × Landrace pigs from three litters. Eight pigs were tested from one litter and six pigs from each of two litters. The three litter groups were split at weaning into six pens, each housing four to six littermates. Housing and husbandry, pre- and post-weaning, closely replicated the conditions described in Experiment 1. Half of the pigs were tested in an open field alone (AL: n = 10) and half were tested in pairs (P: n = 10). Sibling pen mates (n = 10) were used as companions for pigs allocated to the paired treatments. Pairs were balanced for sex and weight. Companion pigs were given two 15-minute exposures to the open-field either one or two days prior to testing. Companions were used once and the companion from each pair was placed into the arena first on each occasion. The behaviour of the companion was not formally scored.

2.4. Experiment 3: Open field twice

In Experiment 3, the subjects were twelve 5–6-week-old (6 males: 6 females) Large White × Pietrain pigs from four litters. Four pigs were tested from one litter, three pigs from each of two litters, and two pigs from a single litter. Pigs housed in their litter groups throughout the period of testing were tested twice over two consecutive days. Test order (balanced for sex, weight and litter) was the same for both tests.

2.5. Behavioural observations

Behavioural observations were recorded from a camera mounted above the arena using GeoVision Digital Surveillance System© (ezCCTV Ltd, Herts, UK). Vocalisations were separately recorded from a digital camera positioned at the end of the arena (Fig. 1). Noldus Observer 5.0 (Wageningen, The Netherlands) was used for behavioural analysis. Locomotor activity was scored as the number of times the pig entered (midpoint of head between the ears) one of 16

equal squares (0.46×0.47 m) (Fig. 1). The proportion of time the pig spent in the squares near the novel objects was calculated. Level of exploration was measured from latency to make contact and time interacting with the ball and feeder (object directed), and time nosing the floor and walls of the arena (arena directed). For descriptions of postures, vocalisations, and other behavioural events refer to the ethogram (Table 1). In Experiment 2, social interactions between the focal and companion pigs were scored in addition to the ethogram of behaviours that were recorded in Experiments 1 and 3. In addition, combined vocalisations were scored from pairs in Experiment 2 because there was no way to distinguish between them.

2.6. Statistical methods

Data were analysed in Genstat 10 or 11 (Genstat Release 10/11, VSN International Ltd, Hemel Hempstead, U.K.). Histograms and residuals plots were used to check assumptions of normality and variance. If the criterion could not be met, the variable was normalised using the transformation that produced the most normal distribution. Significance level was fixed at ≤0.05 for all three experiments. The untransformed means ± SD are reported for simplicity and ease of interpretation in the discussion.

Table 1
Ethogram of the behaviours recorded in the open field.

Behaviour	Definition
Activity & location	
Freq. squares entered	Number of squares (n = 16) the pig enters (midpoint of head between the ears) during 10 min test period.
Time periphery	Time pigs spent in the periphery of the arena (n = 12 squares)
Time centre	Time pigs spent in the centre of the arena (n = 4 squares)
Time next to feeder	Time in the square in which the feeder was allocated
Time next to ball	Time in the square in which the ball was allocated
Posture	
Stand	Body raised off ground, all feet in contact with floor, includes walking
Sit	Rump in contact with ground, body raised by extension of front legs
Kneel	Front legs flexed, rump raised off ground by hind legs
Lie ventral	Recumbent, sternum in contact with ground, front legs extended forward
Lie Lateral	Recumbent with shoulder and pelvis in contact with the ground
Exploration	
Nose floor	Repetitive snout contact with floor
Nose wall	Repetitive snout contact with wall
Interaction with feeder	Pig makes snout contact with feeder
Interaction with ball	Pig makes snout contact with ball
Idle	Pig does not make snout or any other deliberate contact with the fixtures of the arena
Social interaction[a]	Any social interaction (nosing, head knocks, snapping >1 s) in which the focal pig responds (actor or recipient) to the companion pig
Vocalisation	
Grunt	Low pitched vocalisation
Squeal	High pitched vocalisation
Other vocalisation	Any other vocalisation
Events	
Head shake	Pig makes repetitive (n>2) side-to-side head movements
Rear	Standing on hind legs with front legs in contact with wall
Wobble	Repositioning of feet, unsteady side-to-side movement of body

[a] Experiment 2 only.

2.6.1. Experiment 1: Open field with and without Azaperone

Unless stated otherwise, General ANOVA was used for analyses. Pigs were tested twice and so pig identity was used to block and all other explanatory variables were fitted to the treatment structure (day + pen + litter + sex + treatment.injection time + treatment.test + injection time.test). Average weight over the two tests was fitted as a covariate. Day and pen were dropped from the final models, however, pen was kept in models for latency to make contact with the feeder and ball, and time with and next to the feeder. Mann–Whitney-U test was used to compare time exploring the feeder with time exploring the ball. Time lying (lie ventral + lie lateral) and time in other postures (sit + kneel + other) were infrequent so could not be normalised to meet ANOVA assumptions. The same model was used for these responses in GLMM, fitting Poisson distribution and log function. The behaviours headshake, wobble and rear were so rare that it was more appropriate to compare the numbers of pigs under the main treatment condition observed displaying the behaviour or not as a binary trait.

2.6.2. Experiment 2: Open field with and without companion

Data were analysed using the Residual Maximum Likelihood (REML) procedure. Litter and pig identity (litter/eartag) were fitted as a random effect and day + pen + sex + treatment were fitted as fixed effects with weight fitted as a covariate. Preliminary analysis ruled out influence of day, so it was dropped from all of the models. Squeals and other vocalisations were too rare for separate analysis so were grouped with the category low vocalisation. The Mann–Whitney-U test was used to compare time with the feeder with time with the ball.

2.6.3. Experiment 3: Open field twice

Data were analysed using the Residual Maximum Likelihood (REML) procedure. Pig identity (eartag) was fitted as a random effect and all other explanatory variables were fitted as fixed effects (litter + sex + replicate) with weight fitted as a covariate.

2.6.4. Principal components analysis

PCA was used to identify underlying (latent) variables which might indicate an anxious state in the open field. Relationships between variables can reveal patterns of behaviour that may be useful for predicting future test outcomes [15]. For the PCA the transformed data were standardised. The unrotated loadings are presented here. Components with eigenvalues greater than 1 were retained and only loadings >0.3 were considered for interpretation. ANOVA or REML was used on individual pig scores from each of the three experiments as appropriate.

3. Results

Test statistics and P-values are presented alongside experimental data in the tables.

3.1. Experiment 1: Open field with and without Azaperone

3.1.1. Activity and location

Pigs treated with Azaperone (A) were more active than those receiving saline (S) (Table 2). S-pigs showed least activity in the second test. All pigs spent more time in the periphery (twelve perimeter squares) of the open field than in the centre (four central squares) but A-pigs spent more time in the periphery compared to S-pigs. A-pigs also spent more time than S-pigs next to the feeder and ball. Repeating the test reduced the time pigs spent next to the ball.

3.1.2. Arena-directed exploration

S-pigs spent more time behaviourally idle than did A-pigs. Less time was spent idle on second exposure to the open field (Table 2). Arena-directed exploration (floor + walls) accounted for 27.4%

Table 2
Effect of saline (S) or 1 mg/kg Azaperone (A) on the behaviour (mean ± S.D.) of 6-week-old pigs (n = 24) observed for 10 min in an open field. Pigs were tested twice (Test 1 or Test 2), once with Azaperone and once with saline. Half of the pigs were tested in the open field immediately (T0) and half were tested 20 min (T20) after they were injected (Inj. time).

Measure		Test 1		Test 2		Main treatment effects			Interaction effects		
						Treat	Inj. time	Test	Treat × inj. time	Treat × test	Inj. time × test
		Saline	Azaperone	Saline	Azaperone	P ($F_{1,20}$)	P ($F_{1,20}$)	P ($F_{1,20}$)	P ($F_{1,20}$)	P ($F_{1,20}$)	P ($F_{1,20}$)
Activity											
Freq. squares entered	T0	83.3 ± 5.1	92.8 ± 13.8	28.2 ± 11.6	100.8 ± 3.7	<**0.001**	0.962	<**0.001**	0.854	0.132	0.124
	T20	81.3 ± 3.7	133.8 ± 10.5	36.3 ± 24.2	73.7 ± 43.2	(**30.62**)	(0.01)	(**29.58**)	(0.03)	(2.50)	(2.58)
Location											
Centre (s)	T0	36.1 ± 5.8	109.3 ± 76.4	174.1 ± 63.3	103.6 ± 42.1	**0.024**	**0.047**	0.798	0.784	0.439	0.259
	T20	136.7 ± 55.9	57.1 ± 21.3	87.3 ± 45.5	76.9 ± 82.5	(**5.95**)	(**4.58**)	(0.07)	(0.08)	(0.63)	(1.35)
Periphery (s)	T0	463.9 ± 45.8	490.7 ± 76.4	425.5 ± 63.3	469.4 ± 42.2	**0.01**	0.076	0.939	0.913	0.779	0.367
	T20	463.3 ± 55.9	542.9 ± 21.3	512.7 ± 82.5	523.1 ± 45.5	(**8.16**)	(3.58)	(0.01)	(0.01)	(0.08)	(0.85)
Next to feeder (s)	T0	47.7 ± 25.9	46.4 ± 32.6	31.0 ± 64.0	78.7 ± 59.9	**0.008**	0.210	0.519	0.610	0.710	0.670
	T20	23.8 ± 21.6	121.8 ± 74.8	84.0 ± 101.0	73.7 ± 75.9	(**8.68**)	(0.65)	(0.43)	(0.27)	(0.12)	(0.19)
Next to ball (s)	T0	38.2 ± 26.8	37.0 ± 27.9	6.0 ± 10.5	28.8 ± 16.7	**0.013**	0.848	<**0.001**	0.918	0.420	0.923
	T20	24.3 ± 6.5	57.8 ± 35.6	13.9 ± 21.4	22.9 ± 21.2	(**7.36**)	(0.04)	(**15.58**)	(0.01)	(0.68)	(0.01)
Exploration											
Idle (s)	T0	387.3 ± 68.1	238.2 ± 36.8	498.0 ± 53.7	278.3 ± 77.9	<**0.001**	0.455	<**0.001**	**0.049**	0.981	0.265
	T20	347.9 ± 91.8	237.6 ± 65.9	452.7 ± 112.1	391.8 ± 107.9	(**32.70**)	(0.59)	(**18.83**)	(**4.38**)	(0.00)	(1.31)
Nose floor (s)	T0	189.0 ± 66.7	301.8 ± 28.7	92.3 ± 52.9	246.1 ± 100.8	**0.003**	0.116	<**0.001**	0.740	0.207	0.480
	T20	234.6 ± 37.0	237.6 ± 37.1	104.8 ± 35.6	154.7 ± 31.5	(**11.83**)	(2.74)	(**16.50**)	(3.56)	(1.72)	(0.52)
Nose wall (s)	T0	14.2 ± 8.5	30.1 ± 24.8	3.7 ± 4.3	22.4 ± 14.2	<**0.001**	0.127	**0.007**	0.601	0.792	0.878
	T20	12.7 ± 4.6	55.5 ± 25.3	16.3 ± 19.2	26.4 ± 19.1	(**21.19**)	(2.58)	(**8.88**)	(0.28)	(0.07)	(0.02)
Interact feeder (s)	T0	6.0 ± 4.0	16.8 ± 24.5	2.4 ± 3.8	44.7 ± 38.7	<**0.001**	0.616	0.428	0.85	0.819	0.660
	T20	2.1 ± 1.2	52.9 ± 36.6	26.1 ± 50.4	21.7 ± 16.9	(**39.69**)	(0.27)	(0.67)	(0.03)	(0.05)	(0.20)
Interact ball (s)	T0	3.7 ± 1.9	13.0 ± 11.2	2.0 ± 4.8	8.5 ± 6.0	<**0.001**	0.451	**0.002**	0.616	0.851	0.392
	T20	2.7 ± 1.5	16.5 ± 10.7	0.1 ± 0.2	5.5 ± 3.9	(**30.96**)	(0.59)	(**12.72**)	(0.26)	(0.04)	(0.76)
Lat to feeder (s)	T0	201.4 ± 166.4	276.5 ± 120.5	496.8 ± 168.4	391.1 ± 127.5	0.990	0.097	**0.002**	0.523	0.137	0.301
	T20	194.8 ± 96.9	178.9 ± 150.2	362.7 ± 204.7	233.1 ± 193.1	(0.33)	(3.24)	(**12.79**)	(0.04)	(2.54)	(1.13)
Lat to ball (s)	T0	237.0 ± 71.5	249.3 ± 136.3	565.7 ± 84.7	354.6 ± 155.0	**0.007**	0.993	<**0.001**	0.851	0.058	0.938
	T20	243.8 ± 202.3	242.0 ± 202.2	566.3 ± 83.7	342.4 ± 149.8	(**9.13**)	(0.00)	(**37.20**)	(0.04)	(4.38)	(0.01)
Vocalisation											
Vocalisation freq.	T0	67.8 ± 57.4	6.5 ± 5.1	21.3 ± 13.2	3.0 ± 2.0	<**0.001**	0.171	**0.009**	0.136	0.568	0.574
	T20	47.7 ± 22.2	28.8 ± 19.3	41.0 ± 31.8	14.2 ± 16.7	(**31.51**)	(2.05)	(**8.47**)	(2.41)	(0.34)	(0.33)
Postures											
Stand (s)	T0	598.0 ± 4.9	545.3 ± 55.6	594.9 ± 8.6	576.2 ± 17.5	**0.023**	0.113	0.135	0.206	0.243	**0.026**
	T20	600.0 ± 0.0	582.7 ± 35.3	572.0 ± 68.5	417.0 ± 184.3	(**5.61**)	(2.64)	(2.33)	(1.66)	(1.41)	(**5.37**)
Lie (s)	T0	0.0 ± 0.0	50.3 ± 54.6	3.5 ± 8.5	9.6 ± 16.4	**0.035**	0.132	0.371	0.138	0.986	**0.003**
	T20	0.0 ± 0.0	22.1 ± 35.0	26.8 ± 65.6	178.9 ± 185.3	(**5.02**)	(1.13)	(0.85)	(2.37)	(0.00)	(**12.16**)
Sit + kneel + other (s)	T0	2.0 ± 4.9	4.3 ± 4.8	0.0 ± 0.0	7.6 ± 10.2	<**0.001**	0.827	0.581	0.691	0.702	**0.006**
	T20	0.0 ± 0.0	1.6 ± 3.7	1.3 ± 2.9	4.1 ± 4.1	(**16.62**)	(0.05)	(0.31)	(0.16)	(0.15)	(**8.53**)

Significant (p = <0.05) results are indicated in bold.

(SD = 16.4%) and 44.7% (SD = 15.3%) of behaviours observed in S- and A-pigs over 10 min, respectively. A-pigs spent longer nosing the floor and walls of the arena than did S-pigs. Time nosing the floor and walls reduced from the first to second test.

3.1.3. Object-directed exploration

Object-directed exploration (ball + feeder) accounted for 1.8% (SD = 25%) and 7.5% (SD = 35%) of behaviour in S- and A-pigs, respectively. Over both tests, 50% of S-, and 83% of A-pigs made contact with both the feeder and ball. Azaperone did not affect latency to touch the feeder but did reduce latency to make contact with the ball (Table 2). Pigs treated with Azaperone first took longer to touch the ball when they received saline in the second test. A-pigs explored the objects for longer than S-pigs and both groups took longer to touch the feeder and ball in the second test. Both drug groups tended to spend more time with the feeder than with the ball ($U_1 = 912.5$, P = 0.08) (Table 2). Prior test experience reduced time exploring the ball.

3.1.4. Vocalisations

A-pigs vocalised less than did S-pigs and vocalisation frequency reduced on second exposure to the test (Table 2).

3.1.5. Postures

A-pigs spent more time lying down than did S-pigs even though A-pigs were more active overall (Table 2). Lying down occurred in 75% of A-pigs compared to 8.3% of S-pigs. T20 pigs were more likely to lie down in the second test. Postures (sit + kneel + other) were grouped for analyses. Sitting and kneeling were rare and mainly observed in A-pigs. AT20-pigs spent more time in postures other than standing in the second test.

3.1.6. Events

A-pigs were more likely to headshake (S = 0, A = 8; P = 0.008), wobble (S = 2, A = 16; P < 0.001) and rear (S = 0, A = 8; P = 0.008) than S-pigs.

3.2. Experiment 2: Open field with and without companion

3.2.1. Activity and location

Pigs tested alone (AL) or in a pair (P) did not differ in their activity or in the time they spent in each location of the arena (Table 3).

3.2.2. Arena-directed exploration

AL-pigs spent more time idle than did P-pigs (Table 3). Arena-directed exploration (floor + walls) accounted for 38.2% (SD = 14.4%) and 57.4% (SD = 19.5%) of behaviours observed in 10 min in AL and P-pigs, respectively. P-pigs spent longer nosing the floor than did AL-pigs. AL-pigs and P-pigs did not differ in the time they spent nosing the walls.

3.2.3. Object-directed exploration

Object-directed exploration accounted for 3.9% (SD = 2.5%) and 6.6% (SD = 5.2%) of the behaviours observed in ten minute observations in AL

Table 3
Behaviours (mean ± SD) observed in pigs tested in a 10-minute open field alone (Alone: n = 10) or with a familiar companion (Paired: n = 10).

Measure	Alone	Paired	$Wald_{1,19}$	P-value
Activity				
Freq. squares entered	135.1 ± 42.4	115.7 ± 28.8	0.70	0.417
Location				
Time centre (s)	139.5 ± 41.7	154.0 ± 97.0	0.15	0.704
Time periphery (s)	460.5 ± 41.7	446.0 ± 97.0	0.70	0.415
Time next to feeder (s)	8.4 ± 4.3	5.5 ± 3.3	2.90	0.109
Time next to ball (s)	5.1 ± 3.3	4.9 ± 1.8	0.31	0.589
Exploration				
Time idle (s)	347.0 ± 91.0	202.0 ± 118.0	9.92	0.007
Time nose floor (s)	192.6 ± 74.7	319.0 ± 121.0	7.82	0.013
Time nose wall (s)	36.8 ± 21.3	25.3 ± 21.5	1.75	0.206
Time interact feeder (s)	13.8 ± 8.4	25.0 ± 26.5	0.07	0.799
Time interact ball (s)	9.5 ± 10.1	14.5 ± 14.2	0.68	0.421
Latency to feeder (s)	135.0 ± 167.0	136.0 ± 178.0	0.51	0.487
Latency to ball (s)	185.0 ± 161.0	114.4 ± 83.2	1.85	0.194
Vocalisations				
Total vocalisations[a] (n)	130.4 ± 89.6	81.2 ± 69.0	2.59	0.133
Postures				
Time stand (s)	576.0 ± 51.0	544.0 ± 98.0	0.28	0.609
Time lie (s)	22.6 ± 49.2	51.0 ± 93.0	0.00	0.960
Time sit + kneel + other (s)	1.3 ± 2.4	5.1 ± 9.8	0.55	0.475

[a] Combined vocalisation scores from pairs.

Table 4
Behaviours (mean ± SD) observed in pigs (n = 12) exposed to an open field twice.

Measure	Test 1	Test 2	$Wald_{1,11}$	P-value
Activity				
Freq. squares entered	125.3 ± 36.6	62.3 ± 25.4	44.73	<0.001
Location				
Time centre (s)	114.6 ± 39.2	168.9 ± 92.1	3.56	0.086
Time periphery (s)	482.4 ± 39.9	431.1 ± 92.1	3.36	0.094
Time next to feeder (s)	105.9 ± 46.7	69.6 ± 84.7	3.80	0.077
Time next to ball (s)	49.9 ± 25.3	20.2 ± 28.2	24.03	<0.001
Exploration				
Time Idle (s)	298.0 ± 82.4	480.7 ± 64.7	25.02	<0.001
Time nose floor (s)	238.7 ± 78.8	108.4 ± 58.3	30.95	<0.001
Time nose wall (s)	26.23 ± 15.3	7.6 ± 12.2	20.45	<0.001
Time interact feeder (s)	26.5 ± 20.0	3.0 ± 7.6	40.48	<0.001
Time interact ball (s)	7.2 ± 7.0	0.2 ± 0.8	25.02	<0.001
Latency to feeder (s)	189.1 ± 109.9	484.8 ± 158.2	28.96	<0.001
Latency to ball (s)	251.3 ± 183.2	574.0 ± 70.3	29.16	<0.001
Vocalisations				
Total vocalisations (n)	114.5 ± 72.4	33.4 ± 34.7	12.15	0.005
Postures				
Time stand (s)[a]	596.8 ± 7.4	600.0 ± 0.0	–	–
Time lie (s)	0.0 ± 0.0	0.0 ± 0.0	–	–
Time sit + kneel + other (s)	0.0 ± 0.0	0.0 ± 0.0	–	–

[a] The behavioural analysis of two pigs in the first test was of a shorter duration than the test duration of 10 min.

and P-pigs, respectively. Over the ten minute test, 80% of AL-pigs, and 100% of P-pigs touched both the feeder and the ball. Overall, time interacting with the feeder did not differ to time interacting with the ball. P-pigs tended to spend more time with the ball and feeder than did AL-pigs, but this difference was not significant (Table 3).

3.2.4. Vocalisations

Pairs vocalised less on average than did AL-pigs but not significantly so (Table 3). The mean number (±SD) of squeals was 12.4 (±27.4) and 0.4 (±0.9) in pigs tested alone or in a pair, respectively. Other vocalisations were rare (AL: 2.3 ± 2.5, P: 0.4 ± 0.8).

3.2.5. Postures

AL-pigs and P-pigs did not differ in the time spent standing, lying down or in any other postures (Table 3).

3.2.6. Events

Headshaking, wobbling, rearing and other events occurred too infrequently for analysis.

3.3. Experiment 3: Open field twice

Pigs were less active, spent more time idle, and vocalised less in the second test compared to the first (Table 4). Latency to touch the feeder or ball was also longer in the second test and pigs spent less time next to, or interacting with, the feeder or ball. The time spent nosing the arena (floor or walls) was also lower in the second test compared to the first. In the second test, pigs tended to spend more of their time in the centre than the periphery of the arena.

3.4. Principal components analyses (PCA)

3.4.1. PCA components

Six components were extracted from an initial analysis. Only the first three components were considered for interpretation, since these three explained 67.04% of the total variation (Table 5), with further components only accumulating a few percentage points thereafter. The first component (PC1) accounting for 39.44% of the total variation in behaviour showed high independent loadings for behaviours associated with exploration (squares entered, object and wall directed exploration) or avoidance (latency to the feeder and ball) of novelty (Table 5). A second component (PC2) accounted for 15.12% of the total variation in behaviour and showed high positive loadings on vocalisations and time standing, and high negative loadings for time lying and time sitting and kneeling. A third component (PC3), explaining 12.48% of the total variation, had a strong positive loading for time nosing the floor and strong negative loadings for time spent idle and frequency of rearing and vocalising.

3.4.2. Experiment 1: Open field with and without Azaperone

ANOVA of individual pig scores from the PCA showed an effect of Azaperone on PC1 and PC2 (Table 6; Fig. 2a). A-pigs had lower PC1 (reflecting high levels of activity, wall and object exploration) and PC2 (reflecting fewer vocalisations, and more time lying and sitting and kneeling) scores than did the S-pigs. Scores also differed between the first and second tests on PC1 and PC2. On PC1, pigs had lower scores in

Table 5
Factor loadings of each variable that was included in a PCA of the data from pigs tested under different open field test manipulations. Loadings of variables >0.3 were considered for interpretation.

Behaviour measure	PC1	PC2	PC3
Variance explained	39.44	15.12	12.48
Eigen value	4.902	1.879	1.551
Activity			
Squares entered crossings	**−0.3987**	0.0785	−0.0758
Exploration			
Time nose floor	−0.2868	0.0579	**0.4834**
Time nose wall	**−0.3396**	−0.0813	−0.1097
Time interact feeder	**−0.3351**	−0.0513	−0.0405
Time interact ball	**−0.3523**	−0.1806	0.0681
Time idle	**0.3467**	0.0119	**−0.4119**
Latency feeder	**0.3232**	−0.1209	0.1166
Latency ball	**0.3550**	0.0318	0.0821
Vocalisation			
Vocalisation freq	−0.1057	**0.4429**	**−0.3849**
Events			
Frequency rear	−0.1874	−0.0497	**−0.6147**
Postures			
Time standing	−0.0624	**0.5112**	0.1020
Time lying	0.0026	**−0.5370**	−0.0723
Time kneel + sit	−0.0790	**−0.4296**	−0.1037

Significant (p = <0.05) results are indicated in bold.

Table 6
Analysis of pig scores on three Principal Component dimensions indentified from a PCA of data from all three experiments.

Experiment *Variable*	PC1 P (test stat.)	PC2 P (test stat.)	PC3 P (test stat.)
Experiment one: effect of Azaperone			
Treatment	<**0.001** ($F_{1,20}=40.83$)	<**0.001** ($F_{1,20}=61.60$)	0.194 ($F_{1,20}=1.81$)
Litter	0.142 ($F_{1,20}=2.19$)	0.281 ($F_{1,20}=1.37$)	0.090 ($F_{1,20}=2.78$)
Sex	0.684 ($F_{1,20}=0.17$)	0.316 ($F_{1,20}=1.07$)	0.882 ($F_{1,20}=0.02$)
Weight	0.501 ($F_{1,20}=0.47$)	0.298 ($F_{1,20}=1.15$)	0.408 ($F_{1,20}=0.72$)
Inj. Time	0.785 ($F_{1,20}=0.10$)	0.623 ($F_{1,20}=0.25$)	**0.023** ($F_{1,20}=6.24$)
Test	<**0.001** ($F_{1,20}=33.00$)	**0.019** ($F_{1,20}=6.56$)	0.127 ($F_{1,20}=2.53$)
Experiment two: effect of paired exposure			
Treatment	0.333 (W = 0.29)	0.477 (W = 0.53)	**0.020** (W = 6.91)
Litter	0.149 (W = 4.38)	**0.035** (W = 8.59)	0.505 (W = 1.43)
Sex	0.970 (W = 0.00)	0.369 (W = 0.86)	0.138 (W = 2.47)
Weight	0.600 (W = 0.29)	0.082 (W = 3.52)	0.665 (W = 0.20)
Experiment three: effect of repeat exposure			
Treatment	<**0.001** (W = 68.59)	0.720 (W = 0.14)	0.896 (W = 0.02)
Litter	0.888 (W = 0.62)	0.831 (W = 0.87)	0.319 (W = 4.35)
Sex	0.470 (W = 0.59)	0.722 (W = 0.14)	**0.021** (W = 9.63)
Weight	0.377 (W = 0.91)	0.299 (W = 1.29)	0.839 (W = 0.05)

Significant ($p = <0.05$) results are indicated in bold.

the first test (more exploration and activity) than in the second test (Test $1 = -1.06 \pm 1.40$; Test $2 = 1.13 \pm 2.45$). On PC2, pigs scored higher (increased vocalisations and time standing) in the first test than in the second test (Test $1 = 0.32 \pm 1.26$; Test $2 = -0.34 \pm 1.64$). Test and injection time interacted ($F_{1,20} = 6.22$, $p = 0.022$) such that T20 pigs showed reduced scores on PC2 (fewer vocalisations and more time lying, sitting and kneeling) in the second test compared to the first (Test 1, $T0 = 0.01 \pm 1.44$, $T20 = 0.63 \pm 1.03$; Test 2: $T0 = -0.00 \pm 1.04$, $T20 = -0.68 \pm 2.07$). Although there was no main effect of Azaperone on PC3, there was an interaction between treatment and injection time ($F_{1,20} = 6.47$; $P = 0.019$). A-pigs at T0 had higher scores (AT0: 0.92 ± 0.98, ST0: -0.19 ± 0.66, AT20: -0.44 ± 1.46, ST20: -0.10 ± 0.74) (more time nosing the floor, increased rearing and vocalising, and less time idle) than did S-pigs at T0.

3.4.3. Experiment 2: OF with and without companion

There was no effect of the presence of a companion on individual scores on PC1 or PC2 (Fig. 2b). On PC3 P-pigs had higher positive scores (more time nosing the floor, rearing and vocalising, less time idle) than did AL-pigs (Table 6).

3.4.4. Experiment 3: OF twice

Pig scores increased from the first to the second test (increased time idle, reduced exploration i.e. long latencies to the ball and feeder) on PC1 (Table 6; Fig. 2c). There was no effect of test repetition on PC2 or PC3. Females were more active (0.58 ± 0.72) (PC3; more time nosing the floor, increased rearing and vocalising, and less time idle) than were males (-0.49 ± 1.36).

4. Discussion

4.1. Experiment One: Effect of Azaperone on open-field behaviour

Azaperone had clear effects on pig behaviour in the open field. Effects of Azaperone at 1 mg/kg were appropriate to induce psychological and not sedative effects in the pigs. Azaperone-treated pigs spent more time lying down yet were more active, explored the arena and novel objects more, and vocalised less than when they had received a saline injection. Azaperone-treated pigs also spent almost twice as long engaging in object and arena exploration than did untreated pigs. These results agree with the finding that pigs treated with the anxiolytic Midazolam were less fearful of novel stimuli [16] and with increased exploratory behaviour in Azaperone-treated sheep tested in an open field [17].

The increase in activity seen here, however, contrasts with a study where Diazepam did not affect pig activity (lines crossed) or the number of entries into the centre of an open field [8]. In the current study, A-pigs spent more time in the periphery than did S-pigs. Time spent in the periphery or in the centre of an open field is a measure used to assess fear and anxiety in rodents [11]. The usual interpretation is that because rodents tend to remain close to the walls (thigmotaxis, an anti-predatory strategy), increased time in the periphery is indicative of a higher level of anxiety [11]. However, given that A-pigs spent more time with the objects (which were located in the arena corners) it appears that the longer duration in the periphery by A-pigs is more likely to be due to reduced fear levels.

Azaperone reduced the frequency of vocalising more than threefold. Vocalisations in pigs during isolation may reflect negative emotional valence [18–20]. Low tone vocalisations may be used to maintain social contact, while high-pitched vocalisations may relate more to excitement [19]. High pitched vocalisations (squeals) were distinguished from low tone vocalisations in the present study, but were rare. Vocalisations in the present study might therefore relate more to the social isolation component of the open-field than response to novelty as such [8].

In line with the data on individual variables, Azaperone-treated pig scores differed from untreated pigs on the first two components in the PCA. We interpret PC1 as reflecting the degree of neophobia shown by the animal as measured through general activity in the arena and exploration of its components. Low scores on this dimension imply a pig that is moving around the arena and spending a lot of its time interacting with the wall and with the two novel objects. High scores on this dimension both reflects a pig taking longer to make contact with the novel objects and one that spends a lot of time behaviourally idle. The second dimension could be interpreted as relating primarily to arousal level as reflected in time spent standing and frequency of vocalising. Vocalisations loaded highly on PC2 and the view that the vocalisations of pigs reflect the animal's “level of excitement” [8] supports the suggestion that PC2 may reflect level of emotional reactivity. Although Fraser [8] found increased locomotor activity was associated with sustained vocalisation in pigs, we did not.

Azaperone-treated pigs, compared to saline-treated pigs, had lower scores on dimensions associated with neophobia/exploration, (PC1) and arousal (PC2) (i.e. high levels of object exploration and activity and reduced vocalisations). The shift across both dimensions can be understood to reflect a move from a fearful/anxious (combination of inactivity/neophobia and high arousal level) behavioural profile in saline-treated pigs to a bold (active exploration and low arousal) behaviour profile in Azaperone-treated pigs.

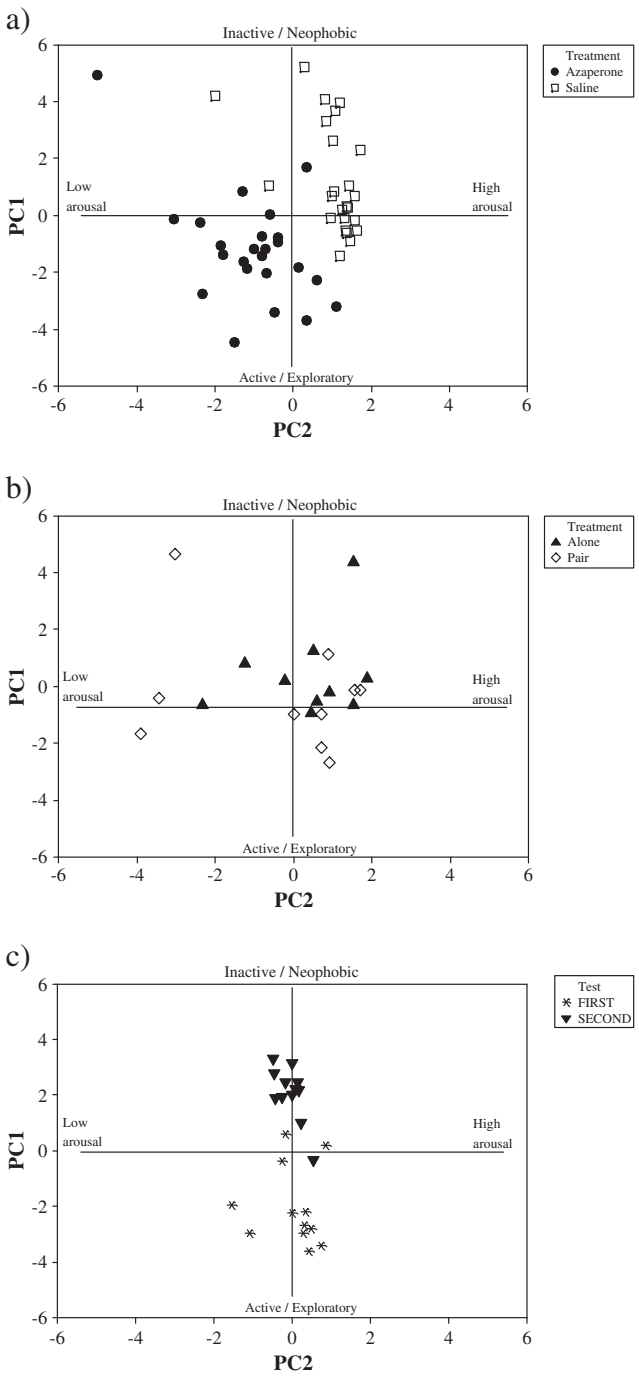

Fig. 2. PCA scores of pig behaviour in an open field test when: a) treated with saline or Azaperone (n = 24), b) tested in isolation or with a companion (n = 20), or c) on two occasions (n = 12).

4.2. Experiment Two: Effect of paired exposure to the open field

We hypothesised that if the behaviours that changed in Experiment One when pigs were given Azaperone reflected underlying emotional state, then similar changes would be seen if the threat posed by the open field was altered through the presence of a companion. Although the different test manipulations are unlikely to produce exactly analogous emotional states, individual open-field behaviours were indeed altered in the direction predicted to reflect reduced fear in pigs tested with a companion. Pigs tested in the open field in pairs spent less time idle, more time exploring and lying down, and less time vocalising than did isolated controls. However, as these effects were rather small it is not clear that Azaperone has similar effects on behaviour as does the presence of a companion. From the PCA data it appears that the behavioural profiles of paired or solitary pigs were more similar than were saline versus Azaperone-treated pigs. Scores on PC1 (exploration/activity) and PC2 (arousal) were unaffected by whether pigs were tested alone or with a companion while P-pigs were more exploratory than were AL-pigs (PC3).

There may be several explanations as to why pairs engaged in more general exploration (PC3) but did not show reduced arousal (PC2) or reduced fear by exploring the novel objects (PC1). The outcome of Experiment One suggests that behavioural measures are related to underlying states of emotionality. However, it is possible that the aspects of emotionality being assessed are not related to the social isolation component of the open field. Although the presence of a companion may alter the extent to which pigs view the open field as threatening, the arena is still unfamiliar to them. So the fact that the magnitude of behavioural change was less than that seen in Experiments One and Three does not necessarily indicate a failing of the underlying construct.

4.3. Experiment Three: Effect of repeat exposure to the open field

Pigs were less active, less exploratory and less vocal on their second exposure to the open field than they had been on their first exposure. In terms of PCA scores this resulted in a clear shift in dimension one (Fig. 2c) but no change on the arousal axis, despite the change in vocalisation frequency. This could reflect a breed effect as a different sire line was used in this experiment. Alternatively, there are two other possible interpretations of this result. Firstly, it may be that pigs reduce their exploratory behaviour during their second exposure to the open field because the arena and its contents are no longer novel and the lack of exploration represents disinterest. Alternatively, pigs may actually be more anxious/fearful during a second exposure to the OF.

4.4. General discussion

The three experiments have shed light on how open field behaviour in pigs can be interpreted with a view to assessing the emotional state of individuals. However, when looking at the three studies combined there are some differences in outcome that require further exploration. The increase in activity in Azaperone treated pigs is interpreted here as lowered emotionality: Azaperone caused pigs to sacrifice time standing idle for increased locomotor activity, lying, and exploring the arena and the novel objects. By contrast, activity was unaffected by social manipulation (Experiment 2), and was reduced in pigs exposed to the arena a second time (Experiment 3). Exploration of the pen and its contents was also reduced in pigs exposed to the open field a second time, which might be interpreted as an increased fear/anxiety profile upon second exposure. However, the level of vocalising was also reduced, which based on other trials suggests a lower level of distress [8], so an alternative interpretation of the activity/exploration reduction is that pigs were less interested in exploring the arena second time around. Such alternatives highlight the difficulty in interpreting individual variables of pig behaviour in relation to putative underlying emotionality. It is also well known in rodent studies that behaviours seen during a second test exposure often reveal information (about an individual's motivational and emotional state) separate to first test exposure. For instance, a prior undrugged exposure to an elevated-plus-maze can attenuate or even abolish the effects of anxiolytic drugs in later exposures [21,22].

The clear effects of Azaperone on pig behaviour appear to be due to a reduction in fear/anxiety levels, which occurs through a combination of lowered neophobia (and consequently increased exploration) and a lower arousal level. As it has been done for rodents, we interpret

the combination of high arousal (emotional reactivity) and a neophobic behavioural style in our pigs as indicating fear/anxiety [23,24]. Conceptually, fear is distinguished from anxiety as a physiological and behavioural response to actual rather than potential danger [25]. However, we do not distinguish here between fear and anxiety because although no specific immediate threat exists in the open field, isolation in the wild would represent a time of increased risk for social animals.

Many studies that use tests such as the open field to assess emotionality in pigs make use of single behavioural parameters (often based on rodent studies) and there is no consensus within the literature as to how particular measures should be interpreted. Here we have reported the benefits, when assessing pig emotionality, of considering a combination of different behavioural dimensions rather than assessing individual measures, which could be interpreted in different ways when considered separately. The results of this work further emphasise the fact that rodent behavioural parameters indicative of emotionality may not be directly relevant to pigs and that behavioural measures taken during open-field exposure can be useful for making assessments of pig emotionality.

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