ALARM PHEROMONES IN URINE MODIFY THE BEHAVIOUR OF WEANER PIGS

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Abstract

Pigs undergoing an alarming experience may produce olfactory stimuli (pheromones) in their urine, which may have consequences for the welfare of other pigs exposed to these stimuli. Twelve young adult female pigs (gilts) were restrained for 30min in feeding stalls (a potentially alarming experience) and any urine produced was collected. Six gilts had previously experienced the procedure (experienced gilts), and six gilts had not (inexperienced gilts). The gilts' behaviour was recorded over the 30min period. The inexperienced gilts exhibited significantly more escape behaviour ($P < 0.001$) and less exploratory behaviour ($P < 0.05$) than those which had prior experience of restraint.

Twelve weaner pigs (30 days old) were subsequently individually exposed to the urine from inexperienced gilts, the urine from experienced gilts and a water control in an open field test. The ambulation score in the open field test was significantly higher ($P < 0.01$) when weaners were exposed to the urine from inexperienced gilts compared to the other two treatments. The weaners were then presented with a feeder containing feed sprayed with the treatment samples. The weaners took significantly longer to approach the feeder sprayed with the urine from the inexperienced gilts ($P < 0.05$) and performed significantly more eating bouts ($P < 0.05$) that were significantly shorter compared with the water control ($P < 0.05$). Total number of vocalizations ($P < 0.01$) and grunts ($P < 0.05$) were significantly greater in those pigs exposed to the inexperienced gilts' urine. These results indicate that urinary alarm pheromones were produced by the inexperienced gilts during restraint in the feeding stall and that these pheromones significantly modified the behaviour of weaner pigs subsequently exposed to them. Exposure to such pheromones is likely to adversely affect the welfare of pigs.

Keywords: *alarm pheromones, animal welfare, behaviour, feeding motivation, pigs*

Introduction

Olfactory stimuli play an important role in social communication in pigs. Pheromones released in urine and saliva have been shown to be important in controlling various aspects of reproduction (Pearce & Hughes 1987a, b), aggression (McGlone *et al* 1987) and suckling behaviour (Morrow-Tesch & McGlone 1990) in this species.

It has recently been suggested that pheromones may be important in communicating aspects of fear in pigs. Vieuille-Thomas and Signoret (1992) demonstrated the presence of an alarm pheromone in the urine of adult gilts (8–11 months old) following restraint in a feeder station. Other gilts subsequently avoided the feeder when it contained urine from gilts restrained for the first time, but did not avoid it when it was contaminated with urine from gilts accustomed to restraint. It is possible that the urinary pheromones released by the inexperienced gilts during this stressful situation may be the same as those reported by McGlone (1985) as being released by young pigs in response to injections of adrenocorticotrophic hormone (ACTH) and being important in mediating submissive behaviour in fighting pigs.

Alarm pheromones are known to have important roles in other species, for example immunosuppressive effects in mice (*Mus domesticus*; Cocke & Thiessen 1990) and inhibition of feeding behaviour in black-tailed deer (*Odocoileus hemionus*; Müller-Schwarze 1971). Unavoidable exposure to 'alarm pheromones' in the environment may reduce the welfare of pigs by inducing chronic stress responses and immunosuppression. This may be of importance in the increased morbidity seen in recently weaned pigs reared under commercial conditions (van Veen *et al* 1985) when these animals are kept at high stocking densities at a time considered particularly stressful (eg Blecha & Kelley [1981]; Pluske & Williams [1996]). The present study was designed to examine changes in behaviour associated with the production of alarm pheromones in the urine of restrained gilts and to determine the effects of exposure to these pheromones on the behaviour of weaner pigs.

Materials and methods

This study was carried out as two replicates using a total of 12 gilts and 12 weaner pigs. Part 1 of each replicate utilized six gilts and part 2 six weaner pigs.

Part 1

Twelve gilts (Newsham N-21, 7 months of age, 120kg liveweight) from the commercial herd at Harper Adams University College were used to assess the production of the putative alarm pheromones. Six of the gilts were restrained in feeding stalls, having had no prior experience of similar treatment (inexperienced gilts) while six were restrained after they had previously experienced the procedure six times over the previous 2 weeks (experienced gilts). Temporary restraint in a feeder was a normal husbandry procedure used on the unit to allow the gilts to become accustomed to being confined in the individual feeders that they would encounter in the sow house. Prior observations had shown that once the inexperienced gilt tried to exit from the stall but was unable to do so, the procedure appeared to become stressful in some individuals, being accompanied by vocalizations, urination and escape attempts. Such behaviour has been suggested to indicate stress in pigs (von Borell & Ladewig 1992) and be accompanied by an enhanced adrenal response to ACTH (von Borell & Ladewig 1992).

The feeding stalls used for urine collection were fitted with slatted floors covering plastic trays into which the urine drained immediately after urination. The behaviour of each gilt was recorded using scan sampling every minute for 30min. The behaviours recorded are shown in Table 1.

Urine samples were only used from those gilts that urinated within the 30min behaviour recording period (all the gilts under study urinated in the requisite time period). The gilts' urine samples were then ranked according to the percentage of time spent in exhibiting

Table 1 Behaviours recorded in part 1.

Postures	
standing	supported by legs only
lying	recumbent or sternal lying
Activities	
feeding	nose in contact with food/chewing immediately following contact with food in trough at front of feeder
defecating	self-explanatory
urinating	self-explanatory
exploratory	active nose or mouth contact with pen, floor or fittings
comfort	stretching, shaking, rubbing or scratching
alert (still)	motionless, with eyes open and individual apparently aware of the environment
inactive	eyes shut and individual apparently asleep/unaware of the environment
escape	attempts at turning around, pushing on back gate or attempts to leave the stall over the front wall

escape behaviour. The urine samples used in the inexperienced and experienced treatments were those collected from gilts exhibiting the most and the fewest escape attempts respectively. These samples were subsequently used in part 2 of the experiment.

Part 2

Twelve weaner pigs aged 30–32 days (1 week post-weaning) of similar weight (9.31 ± 0.25 kg) were deprived of food for 6h from 0800h to 1400h. The pigs were then individually transferred to one of three testing areas at 1400h. The pigs were exposed to three consecutive tests, one at each testing area. The tests were presented according to a balanced order design so that each possible permutation of the three tests was presented to two pigs (tests 1, 2, 3; tests 1, 3, 2; tests 2, 1, 3; etc). The tests were:

test 1 – exposure to a trough sprayed with 50ml of the inexperienced (high escape behaviour) urine sample;

test 2 – exposure to a trough sprayed with 50ml of the experienced (low escape behaviour) urine sample;

test 3 – exposure to a trough sprayed with 50ml water.

Each weaner pig was observed during a 2min open field test immediately following entry to the testing pen, with the trough suspended 1.2m above the ground (see Figure 1 for layout of testing pen). This was based on the open field test technique described by von Borell and Ladewig (1992) for use with pigs. An ambulation score was determined by counting the number of sections which the front right foot of the pig entered. In addition, the frequency of all vocalizations (classified as grunts or squeals) and the frequency of urination and defecation were also recorded. After 2min, the trough was lowered onto the ground and the following data recorded over the subsequent 3min period: i) latency to approach to 1m from trough; ii) latency to approach to 0.5m from trough; iii) latency to first contact with trough; iv) latency to start of eating; v) time spent at trough (nose protruding over edge of trough); vi) number of eating bouts (separated by at least 5s); vii) number of grunts; viii) number of squeals; and ix) number of urinations and defecations.

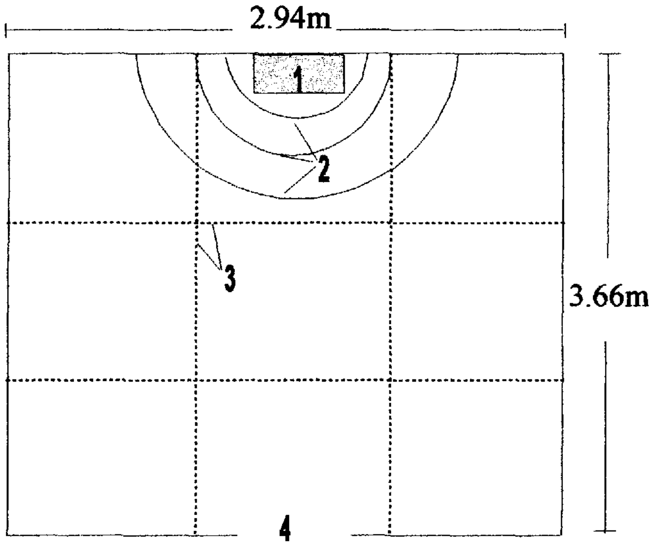

Figure 1 Layout of testing pen: 1 – feeder; 2 – 0.5m, 1m and 2m graduated distances from feeder; 3 – lines dividing test area; 4 – entrance to test area.

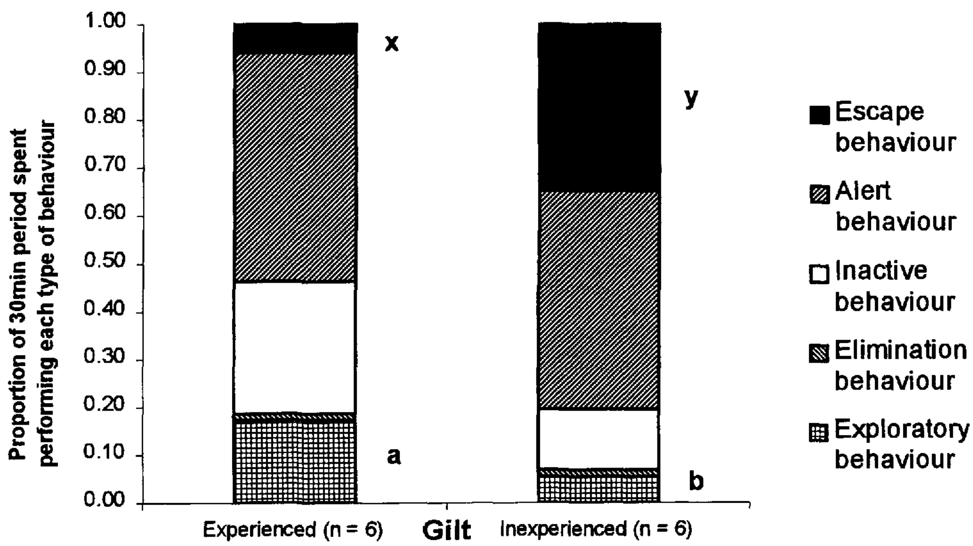

Figure 2 The means of the different behaviours performed by gilts during the urine collection period whilst restrained in feeding stalls. Means with different letters are significantly different: a vs b $P < 0.05$; x vs y $P < 0.001$.

Statistical analysis

There was no significant effect ($P > 0.05$) of order of presentation of the tests, so analysis of the weaner pigs' behaviour in response to the three different treatments was carried out using a randomized block analysis of variance with individual pigs as a blocking factor. Treatment differences between means were identified using protected least significant differences (Snedecor & Cochran 1980). In order to normalize the data, they were transformed according to the equation:

$y = \log_e(x + 1)$.

Non-parametric data (number of urinations, number of defecations and number of squeals) were analysed using the Kruskal-Wallis technique (Siegel 1956). All analyses were carried out using the Genstat for Windows (version 3.2) statistics package (Lawes Agricultural Trust 1995).

Results

Part 1

As shown in Figure 2, the inexperienced gilts exhibited significantly more escape behaviour ($P < 0.001$) and less exploratory behaviour ($P < 0.05$) when restrained in the feeders compared to the experienced gilts. The actual behaviours performed by the individual gilts are shown in Figure 3.

Part 2

As shown in Table 2, the mean ambulation score in the open field test was significantly higher when the pigs were exposed to the inexperienced gilts' urine compared to both the

Table 2 The effect of presence of urine from an inexperienced or experienced gilt and a water control on the behaviour of weaner pigs undergoing an open field test and subsequent presentation of a feeder (values are means for 12 pigs). Inexp – inexperienced; Exp – experienced.

	Inexp	Exp	Water	SEM
Open field test				
ambulation score	18.5^x	9.7^y	12.3^y	1.40
total vocalizations	51.5	25.3	29.6	5.55
grunts	41.6	25.3	27.6	4.52
squeals	9.9	0.0	2.0	2.45
no. urinations	0.0	0.1	0.0	0.03
no. defecations	0.2	0.5	0.1	0.09
Food presentation				
latency to 1m from feeder (s)	88.4^a	72.1	43.0^b	8.00
latency to 0.5m from feeder (s)	98.4^a	78.6^a	48.7^b	7.90
latency to 1st contact with feeder (s)	112.3^a	103.8^a	57.4^b	7.89
latency to start of eating (s)	126.6	113.5	72.5	9.66
no. eating bouts	2.8^a	1.5^b	1.8^b	0.27
average duration of eating bout (s)	16.6^a	36.7	49.9^b	6.00
total time spent eating (s)	47.8	62.1	97.9	10.42
total vocalizations	63.0^x	41.9^y	41.4^y	9.44
grunts	59.3^a	41.9^b	40.8^b	9.10
squeals	3.7	0.0	0.6	0.78
no. urinations	0.0	0.0	0.2	0.04
no. defecations	0.2	0.3	0.2	0.07

Means with different superscripts in the same row are significantly different: a vs b, $P < 0.05$; x vs y, $P < 0.01$.

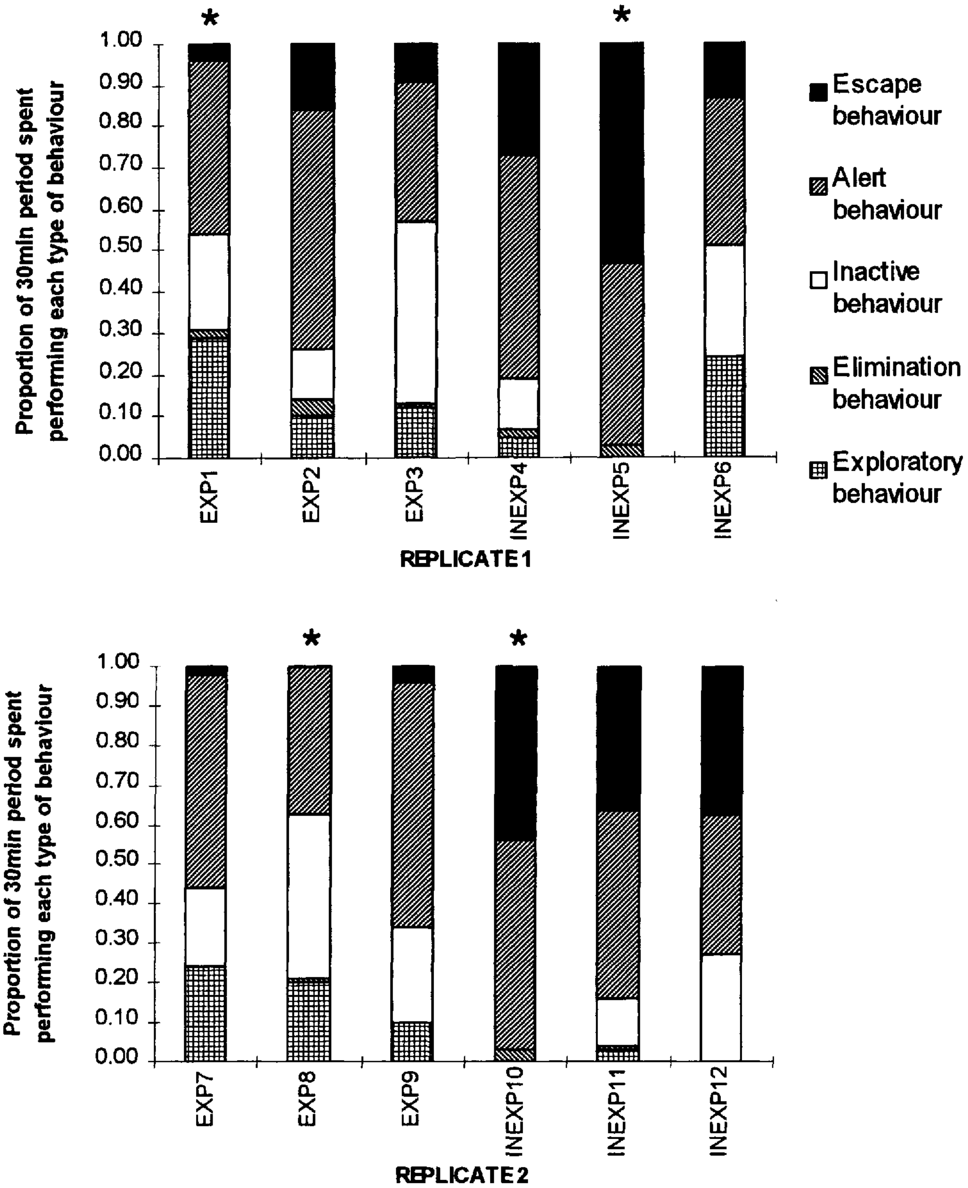

Figure 3 **The behaviour performed by individual gilts during the urine collection period whilst restrained in feeding stalls (replicates 1 and 2). * indicates gilts providing samples for use in part 2 of the investigation.**

experienced gilts' urine and water treatments ($P < 0.01$). On presentation with a feeder sprayed with one of the three types of sample, there were a number of significant treatment effects. The latency to approach to 1m and 0.5m was significantly increased in the inexperienced treatment when compared to the water treatment ($P < 0.05$). There were no significant differences between the approach latencies of both urine treatments but the experienced treatment also had a significantly increased latency to approach to 0.5m when compared with the water treatment ($P < 0.05$). Latency to first contact with the feeder was significantly increased in both the treatments involving exposure to urine compared to water ($P < 0.05$). The number of eating bouts was higher in the inexperienced treatment compared with the other treatments ($P < 0.05$). However, the average duration of these bouts was significantly less in the inexperienced treatment ($P < 0.05$) compared with the water treatment, with the experienced treatment not being significantly different from either of the other two treatments. Both total vocalizations ($P < 0.01$) and number of grunts ($P < 0.05$) were significantly higher in those pigs exposed to the inexperienced gilts' urine compared to the experienced and water treatments.

Discussion

The results of the present study support the suggestion of Vieuille-Thomas and Signoret (1992) that gilts restrained in a feeder stall release olfactory stimuli in their urine which are perceived as aversive by other pigs. The present study extends these findings by demonstrating that young pigs (weaners) are also able to perceive these pheromones and that they also perceive them as aversive.

Examination of the behaviour exhibited by the gilts during their restraint in the feeding stalls indicated that the inexperienced animals with high escape behaviour were clearly alarmed by this restraint whereas the experienced gilts with low escape behaviour generally remained quiet during the observation period (see Figure 2). It is therefore considered appropriate in the following discussion to refer to exposure to urine collected from inexperienced gilts as the 'alarm' treatment and urine from the experienced gilts as the 'quiet' treatment.

Ambulation score and vocalization frequency in open field tests have been suggested to reflect a pig's current 'level of excitement' (Fraser 1974; von Borell & Ladewig 1992) and to indicate its 'behavioural arousal' (von Borell & Hurnik 1991). In the present experiment, the significantly higher ambulation score in the alarm treatment compared with the quiet and water treatments ($P < 0.01$) may indicate a higher state of arousal in these pigs, possibly resulting from an increased motivation to retreat from the aversive stimulus of the alarm pheromones. In addition, on presentation with the feeder there was a significant increase in vocalizations ($P < 0.01$) associated with exposure to the alarm treatment. Increased vocalizations in isolated piglets have recently been reported to be associated with changes in plasma levels of adrenaline and cortisol (Schrader & Todt 1998), indicating that the level of vocalizations may be a viable indicator of stress in young pigs. The calls made by piglets separated from the sow have also been suggested to be an indicator of welfare by Weary *et al* (1997b), with longer and higher pitched calls having been shown to be induced by various stressful situations such as hunger (Weary & Fraser 1995) and cold (Weary *et al* 1997b) and it has been suggested that these act as an aid to the sow in locating her offspring. In the present experiment, the increased vocalizations observed in response to the urine of the inexperienced gilt (alarm treatment) may represent the newly weaned piglet's attempts to reunite itself with the sow. Indeed, an increased rate of calling by piglets, with higher frequency calls of longer duration, has been shown to result in stronger responses in sows, including orientation towards the source of the vocalizations (Weary *et al* 1997a), indicating a survival advantage for this response to aversive stimuli such as alarm pheromones.

In the food presentation test, exposure to urine per se (both alarm and quiet treatments) resulted in significantly reduced approach behaviour towards the feeder ($P < 0.05$). However, exposure to the alarm treatment resulted in a significant increase in the number of eating bouts ($P < 0.05$) and these were significantly shorter ($P < 0.05$) compared to the other treatments. The increased number of feeding bouts observed in the alarm treatment indicate a strong motivation to feed, but the reduced bout duration may indicate inhibition of this motivation by exposure to the urinary pheromone. In addition, this observation indicates that the perception of the alarm pheromone may require close proximity to its source as has been shown to be the case for other pig pheromones (Pearce & Paterson 1992). However, the differences in behaviour between treatments observed during the open field test, where the weaner was not in close proximity to the treatment sample, indicate that either a different or additional perception mechanism is operating and/or there may be more than one pheromone present in the alarmed gilts' urine. McGlone (1985) proposed that urine collected from pigs after injection of ACTH contained a pheromone that induced submissive behaviour when sprayed in the air above regrouped, fighting pigs. After a follow-up study (McGlone *et al* 1987), it was proposed that there were at least two urinary factors that modulated aggressive behaviour: one produced in the urine of fighting pigs which decreased aggression; and another produced in the urine of handled pigs which increased aggression. These observations demonstrate that at least two urinary pheromones are produced by pigs in response to alarming stimuli and support the possibility that at least two urinary factors are responsible for modifying the behaviour of the weaners in the alarm treatment in the present study. Further research in this area will be required in order to clarify the involvement of pheromones in controlling the response of pigs to stressful stimuli.

Animal welfare implications

The production of alarm pheromones in pigs may have important consequences for the welfare of these animals in husbandry systems where pigs are subjected to a number of alarming events such as mixing, regrouping and rehousing, particularly when these occur under the confined conditions associated with intensive husbandry. Previous work on this species has suggested that these pheromones may also be involved in mediating the heightened levels of aggression seen under such conditions (McGlone *et al* 1987). Furthermore, evidence from other species (Cocke & Thiessen 1990) may suggest that exposure to such pheromones can significantly impair immune function and therefore may be involved in the increased morbidity commonly seen in recently weaned pigs (van Veen *et al* 1985). Therefore, further elucidation of the role of these pheromones in pigs would allow a greater understanding of the factors influencing welfare in this species.

Acknowledgements

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