

## Early human contact and housing for pigs – part 1: responses to humans, novelty and isolation



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### ABSTRACT

The development of fear and stress responses in animals can be influenced by early life experiences, including interactions with humans, maternal care, and the physical surroundings. This paper is the first of three reporting on a large experiment examining the effects of the early housing environment and early positive human contact on stress resilience in pigs. This first paper reports on the responses of pigs to humans, novelty, and social isolation. Using a  $2 \times 2$  factorial design, 48 litters of pigs were reared in either a conventional farrowing crate (FC) where the sow was confined or a loose farrowing pen (LP; PigSAFE pen) which was larger, more physically complex and allowed the sow to move freely throughout the farrowing and lactation period. Piglets were provided with either routine contact from stockpeople (C), or routine contact plus regular opportunities for positive human contact (+HC) involving 5 min of scratching, patting and stroking imposed to the litter 5 days/week from 0–4 weeks of age. The positive handling treatment was highly effective in reducing piglets' fear of humans, based on +HC piglets showing greater approach and less avoidance of an unfamiliar person at 3 weeks of age. There was evidence that this reduction in fear of humans lasted well beyond when the treatment was applied (lactation), with +HC pigs showing greater approach and less avoidance of humans in tests at 6, 9 and 14 weeks of age. The +HC treatment also reduced piglets' fear of a novel object at 3 weeks of age, and for pigs in FC, the cortisol response after social isolation at 7 weeks of age. Rearing in FC compared to LP reduced piglets' fear of novelty at 3 weeks of age, as well as their vocalisations and cortisol response to isolation at 7 weeks of age. The FC pigs showed greater approach and less avoidance of humans compared to LP pigs at 3, 4 and 6 weeks of age, but not at 9 and 14 weeks of age. These results show that positive handling early in life can reduce pigs' fear of humans, fear of novelty and physiological stress response to social isolation. The LP pigs were reared in a more isolated environment with less overall contact with stockpeople and other pigs, which may have increased their fear responses to humans and novel situations, suggesting that different housing systems can modulate these pigs' responses.

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### Implications

High levels of fear in farm animals, including fear of stockpeople, can impair animal welfare and productivity. This research showed that the development of fear responses in pigs can be

impacted by the housing environment and contact with people early in life. Providing opportunities for positive interactions with humans early in life produced the most sustained reductions in pigs' fear of humans. Rearing pigs in farrowing crates as opposed to the loose farrowing and lactation treatment used in the present experiment reduced fear of humans, highlighting the importance of the housing environment on the development of the human-animal relationship.

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## Introduction

Farm animals are regularly exposed to situations that induce fear. Fear responses can be adaptive in that they involve physiological and behavioural responses that allow animals to avoid potentially harmful situations (Jones, 1997). However, in animal production systems, these adaptive responses are highly restricted, for example through the prevention of escape behaviour, which results in fear being a major source of stress for farm animals (Jones, 1996). Stress induced by high levels of general fearfulness, defined as an individual's propensity to be more or less easily frightened (Jones, 1996), as well as stimulus-specific fearfulness, such as fear of humans, can impair farm animal welfare and productivity (reviewed by: Jones, 1996; Rushen et al., 1999; Hemsworth, 2003; Acharya et al., 2022).

Fear responses can be measured by assessing behavioural responses to specific stressors, such as avoidance of human presence and novel situations, and as a consequence, reduced exploration and attraction to humans and novelty (Hemsworth and Coleman, 2011). While it is not clear exactly how fear and exploration are related (Feenders et al., 2011), it is generally agreed that high levels of fear inhibit other motivational systems including exploration, and consequently the presence of exploratory behaviour can be interpreted as evidence of relatively low levels of fear (Forkman et al., 2007; Hemsworth et al., 2018). Fear can also be measured using physiological responses, for example through assessing the magnitude of glucocorticoid and immune responses to stressors (Moberg, 2000).

These behavioural and physiological indicators of fear can provide valuable insight into how resilient animals are to stressors. Resilience is characterised by low behavioural and physiological responses to stressors (Iacoviella and Charney, 2019), suggesting that animals more adept at coping with stress are likely to show lower levels of fear in response to stressors such as close human presence. In the context of animal production systems where routine stressors often involve intense or close contact with stockpeople, animals with lower fear of humans may be better positioned to cope with these challenges. Furthermore, increased approach and reduced avoidance of humans may be indicative of positive emotional responses to humans (Nowak and Boivin, 2015; Hemsworth et al., 2018).

There is increasing evidence in pigs that positive human interactions such as patting, stroking and talking softly to pigs decreases fear of humans, can foster a positive-human animal relationship (Hemsworth and Coleman, 2011; Hemsworth et al., 2018; Rault et al., 2020), and may also improve the flexibility of pigs to a range of challenges. Talking to and patting sows daily alleviated the cortisol response of sows to tether housing (Pederson et al., 1998), and the presence of a human that had positively interacted with weaner pigs reduced their vocalisations on reunion with the human after social isolation (Villain et al., 2020). Additionally, piglets that were held and stroked regularly were more accepting of physical interaction from familiar and unfamiliar people, but also demonstrated more play behaviour and less vocalisation in a novel arena, indicating reduced fear responses to both humans and novel situations (de Oliveira et al., 2015; Zupan et al., 2016). Pigs may be more sensitive to the effects of handling early in life, given that patting and stroking pigs from 0–3 weeks of age has been shown to be more effective in reducing subsequent fear responses to humans at 18 weeks of age, compared to positive handling imposed from 3 to 6 or 6–9 weeks of age (Hemsworth and Barnett, 1992). This suggests that the period from 0 to 3 weeks of age may represent an important stage of development in pigs, during which positive handling can produce the most benefit in terms of improving resilience to stressors involving humans.

Fear is known to be transmitted to piglets from their dams (Rooney et al., 2021), but surprisingly, there has been little research examining the effects of housing systems and sow confinement on fear responses in pigs. It has been suggested that through greater maternal care, opportunity for sow-interaction, space and/or environmental complexity, pigs reared in loose pens where the sow is free to move during lactation may be better prepared to cope with stress compared to pigs reared in standard farrowing crates (Oostindjer et al., 2011). In contrast to this, recently, we found that piglets reared in loose farrowing and lactation pens (PigSAFE pens) showed greater reactivity during capture by a stockperson, and based on more avoidance, greater fear of novelty and humans (Hayes et al., 2021). One possible explanation for these effects is that piglets from the loose housing system were reared in a more isolated environment than piglets in farrowing crates, and that reduced contact with people and other pigs and less visual stimulation in general in the loose system contributed to greater fear responses. With increasing interest in reducing the use of farrowing crates (Baxter et al., 2018; Hemsworth, 2018), there is a clear need to better understand the effects of rearing pigs in alternative farrowing and lactation housing systems on their resilience to stressors. In addition, a better understanding of both short- and long-term effects of early housing and human contact on the fear and stress responses of pigs is required.

This paper examined the effects of early experiences during lactation, by imposing early positive interactions with humans and by changing the housing environment, on pigs' fear and stress responses during lactation but also beyond. This research is part of a larger experiment investigating the impacts of early experiences on stress resilience. The effects of early experiences on the stress resilience of pigs to routine farming practices and on the ability of pigs to cope with their general environment are discussed in the rest of the series of papers (Lucas et al., 2024a and 2024b).

## Material and methods

### Experimental design

The experimental design (allocation of treatments to experimental material) of this multiphase experiment had more elements, and thus complexity, as the animals in the experiment grew from piglets to finisher pigs. The methodology of the first of the three phases was very similar, but not identical, to a separate previous study (Hayes et al., 2021). The present research was conducted at the same research and innovation unit of a large commercial piggery in Corowa, NSW, Australia, as Hayes et al. (2021).

### Preweaning phase: experimental set-up

Preweaning, the experiment consisted of 24 litters from a farrowing crate housing system (FC), where the sow was confined throughout the farrowing and lactation period, and 24 litters from a larger and more structurally complex loose pen (LP), where the sow was free to move throughout the farrowing and lactation period. All litters were born from second parity Landrace × Large White sows that previously farrowed in a similar housing system, either farrowing crates or loose housing, at their first parity. Twelve FC litters and 12 LP litters were assigned to a routine human contact (C) treatment, and the remaining 12 FC litters and 12 LP litters were assigned to a positive human contact (+HC) treatment. This resulted in a two housing system × two human contact treatment factorial design during the lactation period. Further details on the housing systems and human contact treatments are described in subsequent sections.

The two housing systems were in separate but adjacent rooms managed by the same stockpeople. As fear of people is reduced

in pigs that observe positive handling of neighbouring pigs (Luna et al., 2021), an important consideration when allocating positions of C and +HC litters in the rooms was minimising the amount of visual contact with people that C litters received when the +HC treatment was being imposed in surrounding pens. Thus, C litters were allocated to areas of both rooms where the experimenter could avoid walking past during the delivery of the positive handling treatment. Additionally, in the farrowing crate room where piglets had greater visual contact outside of the home pen, non-experimental litters were allocated in between +HC and C litters. Within these constraints, the allocation of human contact treatments to litters, within each housing system, was carried out so that the spatial variation of +HC and C litters were each as representative of the housing system room as possible (Fig. 1).

There was a 7-day farrowing spread across all treatments. Some cross-fostering occurred in the first 24 h of life, within the same housing system and before handling treatments began, when it was necessary to match litter size with the sow's ability to nurse. All piglets were processed at 4 days of age which involved vaccination, administration of iron, tail docking, and ear tagging, and at 3 weeks of age, all piglets were vaccinated and males were immunocastrated. All pigs received another vaccination at 12 weeks of age.

#### Preweaning phase: housing treatment

Any details relating to the housing treatments not reported here are described in Hayes et al. (2021). The farrowing crate and loose pen housing treatments had similar overhead lighting and ambient temperatures and no bedding or enrichment was provided. Each farrowing crate contained a  $2.3 \times 1.7$  m area for the piglets with slatted steel flooring, and a  $1.1 \times 0.41$  m solid creep mat heated by an overhead lamp (Fig. 2a). The surrounding walls of each farrowing crate allowed sows and piglets to have visual contact with people in the aisles. Each loose pen (PigSAFE design; Baxter et al., 2015) contained a  $3.6 \times 2.4$  m area for piglets and sows (Fig. 2b). The pens had a combination of solid and slatted plastic flooring and contained a covered piglet-only triangular creep area heated by a lamp. Although rarely used, there was a stalled area within each pen where the sow could be confined briefly to allow safe entry for stockpeople. The walls in the central and back areas of the pen contained sloped sides to reduce the risk of overlay by the sow (see light grey partitions depicted in Fig. 2b). There were windows between pens which allowed limited interaction between adjacent pigs, and piglets' visual contact with people in the room was also minimal. Due to recent design modifications, at the back of each pen, there was a  $2.4 \times 0.4$  m area of space which was not accessible to sows or piglets (pictured in Fig. 2b, not included in the length dimension of the pen).

#### Preweaning phase: human contact treatment

As with the housing treatments, any details relating to the routine and positive human contact treatments not reported here are described in Hayes et al. (2021). The routine contact treatment

involved human contact with stockpeople through the imposition of routine husbandry and management that was typical of a commercial environment. This included visual contact with stockpeople during health and welfare checks and sow feeding twice per day, and when piglet creep food was provided once per day after 14 days of age.

In the positive human contact treatment, piglets received regular opportunities to interact with an experimenter in addition to routine contact with stockpeople. 5 days per week from 1 day of age until weaning, the +HC treatment involved an experimenter gently patting, stroking and scratching piglets. Two experimenters, one male and one female, were responsible for delivering the treatment, but only one of these experimenters imposed it each day. The treatment was delivered to any piglets that approached the experimenter or were sleeping in the creep area, and the experimenter attempted to interact with as many different piglets in the litter as possible. The experimenter remained silent during imposition of the treatment. The +HC treatment was delivered to the litter for a duration of 5 min in the morning, between 0700 and 1000 h. In farrowing crates, the experimenter crouched behind the sow's crate and next to the creep area to deliver the +HC treatment from inside the pen, and in loose pens, the experimenter crouched outside the pen and interacted with piglets over the pen wall by removing the creep roof.

#### Weaner transition phase

Weaning occurred at 4 weeks of age (mean age = 27 days; SD = 1.5; no difference between treatments). The process for weaning was somewhat complex, involving a two-stage mixing and moving procedure. The rationale behind this was to minimise disruption to other pigs during the collection of blood samples after weaning (reported in Lucas et al., 2024a), and to observe the behavioural response of pigs in smaller groups during the human test at 4 weeks of age. The process is described in further detail in Part 2 of this series of papers where responses to weaning are discussed (Lucas et al., 2024a). In summary, half of the pigs were mixed at the beginning of the 'weaner transition phase' which lasted from 0–48 h postweaning, and the remaining half were mixed 48 h postweaning at the beginning of the 'weaner-finisher phase'.

Prior to weaning, 2 litters of the same lactation housing system and human contact treatment were selected to be paired and mixed. Four same-sex pigs from each pair ('cohort 1') were mixed on the day of weaning and housed in  $1.8 \times 0.8$  m pens during the 48 h duration of the weaner transition phase. Thus, during this phase, there were 24 pairs of pens. Each pair consisted of a pen of four male pigs and a pen of four female pigs, with all eight pigs in a pair originating from 2 litters of the same preweaning housing system and human contact treatment. The remaining pigs from each pair ('cohort 2', comprised of another four same-sex pigs from the pair) stayed in their lactation housing without their sows during this period. At 48 h after weaning, the cohort one and two pigs from each pair were mixed in the weaner facility which marked the start of the weaner-finisher phase.

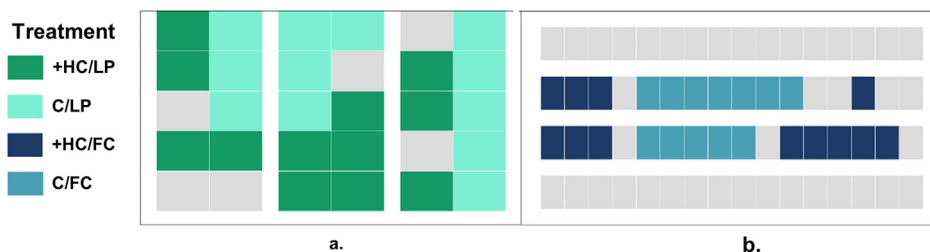
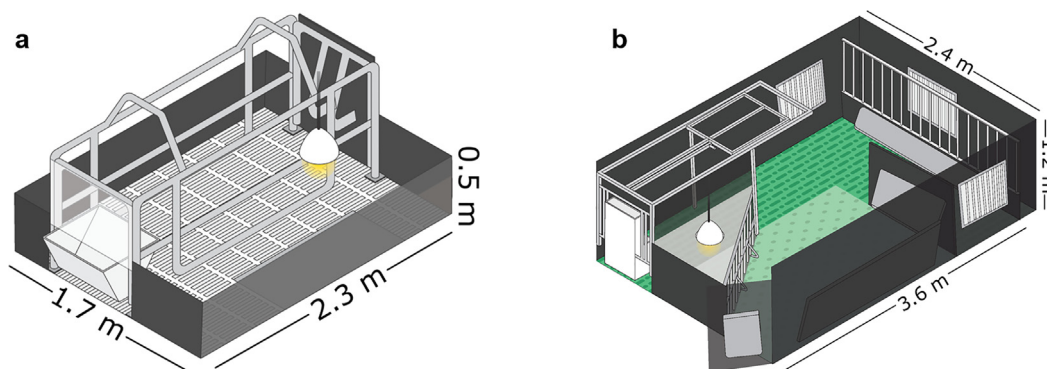


Fig. 1. Allocation of positive human contact (+HC) and routine human contact (C) litters of pigs in (a) the loose pen (LP) room; (b) the farrowing crate (FC) room for the preweaning phase of the experiment. Grey boxes in both housing systems represent non-experimental litters.



**Fig 2.** Diagrams of the two housing systems pigs were reared in: (a) farrowing crate (FC); (b) loose pen (LP).

### Weaner-finisher phase

After cohorts 1 and 2 had been mixed in the weaner facility, there were 24 pairs of pens each made up of a pen of eight male pigs and a pen of eight female pigs, with all 16 pigs in a pair of pens being from 2 litters of the same preweaning housing system and human contact treatment. At 10 weeks of age, pigs remained in the same groups but were moved to the grower/finisher facility where they stayed until the conclusion of the experiment. Males and females from the same pair were housed in adjacent pens. The weaner and grower/finisher pens were spatially arranged in a six-block split-plot design with housing system associated with main plots, human contact treatment associated with subplots and each subplot being two adjacent pens (one pen containing eight males, one pen containing eight females). The two pens in a subplot contained only pigs from 2 litters that were being paired.

The pens in the weaner facility were  $3.0 \times 1.5$  m, and the pens in the grower/finisher facility were  $3.7 \times 2.6$  m. All pens contained  $\frac{3}{4}$  slatted steel flooring and  $\frac{1}{4}$  solid concrete flooring. The pens had four open barred sides, with the exception of pens located on the two outer rows of the room which contained solid-sided back walls. Pigs were provided with *ad libitum* access to water and food and received daily health and welfare checks by stockpeople. No environmental enrichment was provided in accordance with typical commercial practice in Australia.

### Measurements

The behavioural responses of pigs to humans were measured in human tests at various time points from 3–14 weeks of age, and in all three design phases of the experiment (Fig. 3). Furthermore, the behavioural responses of pigs to novelty were measured in the novel arena and novel object tests at 3 weeks of age, and the behavioural and physiological responses to social isolation were measured at 7 weeks of age. Methodologies for each of the human tests differed in terms of test location, movement and posture of the human, and whether pigs were tested in groups or as individuals. The rationale for this was to reduce the chance of pigs habituating to the testing context over time, and to examine whether pigs' responses to humans were generalised to different contexts. Different experimenters were used as the human stimulus in each of the human tests. The experimenter was always unfamiliar to pigs; they were not involved in imposition of the +HC treatment or routine husbandry of pigs. The test experimenters wore the same clothing as everyone else on the farm, including that of the people responsible for delivering the +HC treatment and daily management of the pigs.

### Novelty and human tests at 3 weeks of age

At 3 weeks of age, the behavioural responses to novel and human stimuli were assessed in two male and two female piglets selected from each litter using a random number generator. The four piglets from each litter were tested as a group. Following the protocol described by Hayes and colleagues (2021), testing involved consecutive 60 s exposures to 1. An empty novel arena (length  $\times$  width  $\times$  height,  $1.8 \text{ m} \times 0.6 \text{ m} \times 0.6 \text{ m}$ ) located adjacent to the home pen, 2. A novel object (orange traffic cone) introduced to the arena, 3. An unfamiliar female experimenter extending their hand inside the arena, and 4. The same experimenter standing stationary inside the arena. Piglets were initially placed into the arena along one of the 0.6 m walls, and the novel and human stimuli were presented at the opposite end of the arena. From video footage (GoPro Hero8 camera), the number of entries each piglet made into  $0.6 \times 0.6$  m sections of the arena was recorded in addition to the approach and interaction behaviour of piglets towards the novel object, the human hand, and the stationary human. Approach behaviour was defined by a piglet entering within 0.6 m of the stimulus, and interaction was defined by any physical contact with the stimulus. A maximum response time of 60 s was imputed if a piglet did not approach or interact.

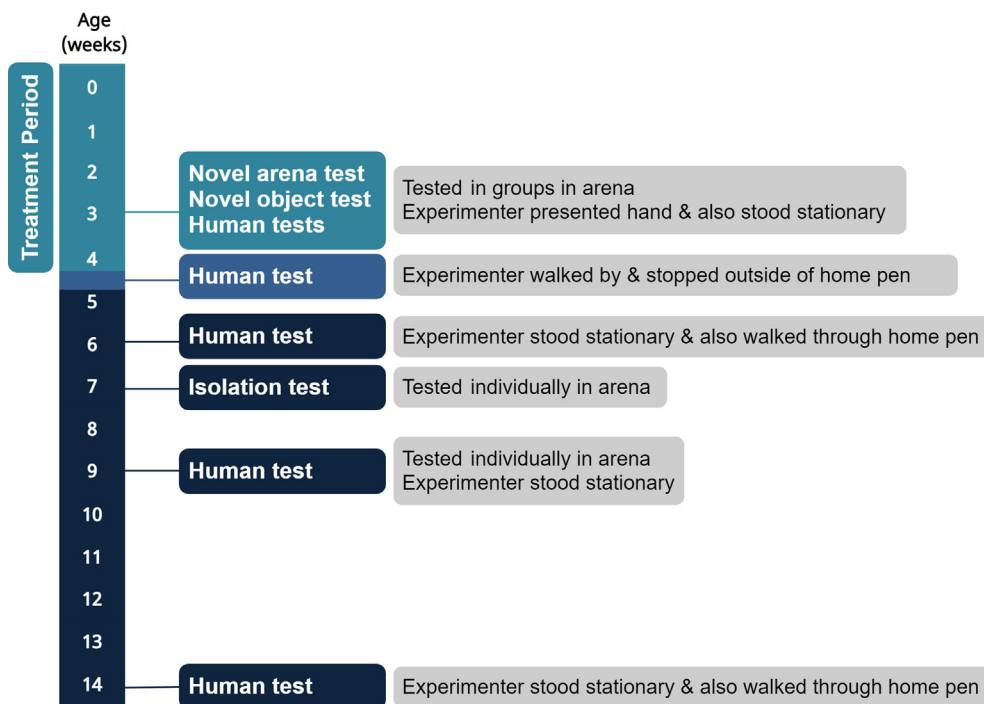
### Human test at 4 weeks of age

One day after weaning and mixing at 4 weeks of age, the behavioural responses to an unfamiliar human walking past the pen were assessed in all pigs from cohort 1. A female experimenter entered the shed and completed six laps of the room in succession, walking at a pace of 1 step per s and stopping in front of each pen for 3 s. Using video footage (GoPro Hero8 camera), the position of each pig in the pen (front, middle or back third of the pen) was measured 3 times in each lap as follows. The first recording was taken when the experimenter started moving forward from the previous pen, the second when the experimenter reached the pen, and the third when the experimenter started moving away from the pen. The position of each pig was determined based on the position of the pig's two front legs.

### Human test at 6 weeks of age

At 6 weeks of age, the behavioural responses to an unfamiliar human walking inside the home pen were assessed in all groups of pigs. A female experimenter slowly entered the pen and stood stationary in front of the gate for 5 s, before completing one lap of the pen walking in a clockwise direction at a pace of 1 step per s. After reaching the starting point in front of the gate, the experimenter stood stationary for an additional 30 s before slowly





**Fig. 3.** Timeline of different behaviour tests conducted in the experiment to measure the responses of pigs to humans, novelty, and social isolation. Each of the three shades represents a different phase of the experiment; from top to bottom, preweaning phase (treatment period), weaner transition phase and weaner-finisher phase.

exiting and moving on to test the adjacent pen. Using video footage (GoPro Hero8 camera), instantaneous point sampling was used to record the proximity of each pig to the experimenter. During the beginning and end phases of the test when the experimenter was stationary, the proximity of each pig was recorded at 5 s intervals. During the middle of the test, the proximity of each pig was recorded at every step the experimenter took around the pen. The pens contained ten rows of flooring that were each 0.3 m wide. The rows of flooring were used to record proximity, such that if a pig was recorded as being 1 row away from the experimenter, there was at least 0.3 m between the pig and the experimenter. Pigs in the same row of the experimenter were recorded as being 0 rows away. The position of each pig was determined by the row the pig's two front legs were in.

#### Social isolation test at 7 weeks of age

At 7 weeks of age, the behavioural and physiological responses to social isolation were assessed in the four cohort one pigs from each pen. Pigs were tested individually in a portable 0.7 × 0.7 × 0.7 m arena positioned in the aisle adjacent to the home pen. The arena was constructed of black wooden board and was elevated 0.9 m off the floor, secured to a trolley. Pigs in the arena had no visual contact with the experimenters or with other pigs. To conduct the isolation test, an experimenter lifted a test pig from the home pen and gently placed the pig in the centre of the arena. The 2-min test commenced once the experimenter moved away from the arena and at the end of the test, the pig was lifted up and gently placed in the home pen and the next pig from the pen was tested. Using video footage that also captured audio (GoPro Hero8 camera), the latency of the pig to vocalise and attempt to escape and the total number of vocalisations and escape attempts were recorded. An escape attempt was defined as the pig lifting at least two legs from the floor simultaneously, either in an attempt to jump or climb out of the arena. A maximum response time of 120 s was imputed if a pig did not vocalise or make an escape attempt. There was poor reliability in manually counting

vocalisations for pigs that vocalised almost continuously throughout the test (due to difficulties in being able to distinguish between one vocalisation stopping and another starting), but there was excellent reliability in counting 10 vocalisations or less. Thus, vocalisations in the isolation test were analysed by recording the number of pigs that vocalised more than 10 times throughout the test. Blood samples for subsequent analysis of serum cortisol and immunoglobulin A (**IgA**) were collected from all test pigs 45 min after isolation testing (see section on 'Validation and Quality Assurance' for information pertaining to the blood sampling procedure and assay characteristics).

#### Human test at 9 weeks of age

At 9 weeks of age, the behavioural responses to a stationary unfamiliar human were assessed in four pigs from each pen. Pigs were selected for testing on the basis of the first pig sighted at the front of the pen when the experimenter opened the pen gate, therefore, a mix of cohort one and two pigs were tested. Pigs were tested individually in a 3.0 × 1.5 × 1.5 m arena constructed of black wooden boarding inside an empty pen in the same room where the pigs were housed. The arena contained a solid 1.5 m high gate on one of the 1.5 m long walls. Three painted lines were marked on the arena floor to separate it into four equal sections. Two teams of experimenters conducted testing simultaneously in identical arenas located on opposite sides of the room. In each team, one experimenter (a male experimenter in one team, a female in the other) moved the pig from the home pen to the test arena with the assistance of a solid stockboard. Once the pig was inside the arena, an observer recorded the number of entries the pig made into sections of the empty arena. After 2 min, the experimenter slowly entered the arena and stood stationary in front of the gate for 3 min and quietly relayed the pig's approach and interaction behaviour to the observer. Approach behaviour was defined by the pig entering within 0.5 m of the experimenter, and interaction was defined by any physical contact the pig initiated with the experimenter (sniffing, nosing, chewing or stepping on experi-

menter). A maximum response time of 180 s was imputed if a pig did not approach or interact with the experimenter. At the end of the test, the pig was walked back to the home pen and the next pig from the pen was tested.

#### Human test at 14 weeks of age

At 14 weeks of age, the behavioural responses to an unfamiliar human walking inside the home pen were assessed in all pigs. A female experimenter slowly entered the pen and stood stationary in front of the gate for 5 s, before completing one lap of the pen walking in a clockwise direction at a pace of 1 step per s. After reaching the starting point in front of the gate, the experimenter stood stationary for an additional 30 s before slowly exiting and moving on to test the adjacent pen. Using video footage (GoPro Hero8 camera), instantaneous scan sampling at 5-s intervals was used to record the number of pigs within 1 m of the experimenter at each phase of the test.

#### Validation and quality assurance

##### Observer reliability

Observers conducting video observations were blind to treatment. For each video-recorded behaviour test, the observer repeated observations for at least six different pigs from different litters/pens. Intra-observer reliability was assessed for all video observations using intraclass correlation coefficient estimates based on single measure, absolute agreement, two-way mixed effects models analysed in SPSS (IBM Corp, 2020). Intraclass correlation coefficient estimates were all above 0.92, with 95% confidence intervals for the estimates between 0.85 and 0.99, indicating excellent reliability.

##### Blood sampling procedure and assay characteristics

Approximately 45 min after the isolation test, a team of experienced technicians collected blood samples from the test pigs. Pigs were held inverted for sample collection which took place within 2 min of securing the pig. Samples were collected in the aisle in front of the home pen of the sample pig. Samples were collected into serum tubes (BD Vacutainer, New South Wales, Australia), inverted 5–6 times and left to clot for at least 1 h before being centrifuged for 10 min at  $1\,300 \times g$ . After centrifugation serum was transferred to polypropylene tubes and stored in a  $-20\text{ }^{\circ}\text{C}$  freezer before being moved to a  $-80\text{ }^{\circ}\text{C}$  freezer. All samples were assayed in duplicate. Serum concentrations of cortisol were determined using a commercial radioimmunoassay kit (Cortisol Coated Tube RIA Kit, MP Biomedicals Australia Pty Ltd, Seven Hills, New South Wales, Australia). The intra-assay coefficients of variation for samples containing 20.2 and 53.2 ng/L were 6.9 and 8.0% and the inter-assay coefficients of variation were 8.7 and 9.2%, respectively. Serum concentrations of IgA were determined using a pig immunoglobulin A ELISA kit (#CSB-E13234p, Cusabio, Houston, Texas, USA). The samples were diluted 1:1500 or 1:1000 in serum diluent as recommended by the manufacturer. The intra-assay CV was 6.4%, and the inter-assay CV was 8.5%.

#### Statistical analysis

For each test, a set of summary measurements to assess aspects of fear was calculated for each litter during lactation (preweaning phase), or for a pair of adjacent pens (one all males; one all females) from weaning onwards (weaner transition and weaner-finisher phases). When measurements were assessed on individual pigs, an average value of the pigs for the litter (preweaning phase) or pair of pens (weaner-transition and weaner-finisher phases)

was calculated. When measurements were assessed on a pen basis postweaning, an average value of the two pens in a pair was calculated. Each measurement was analysed using ANOVA with one of the structures presented in Table 1. The split-plot ANOVA in the weaner and grower/finisher facilities was used to account for the spatial distribution of the pens in the sheds. The only exception was the square root of the number of interactions with the standing human in the human test at 3 weeks of age, in which both the means and residual variation differed between treatments. With this measurement a restricted maximum likelihood (REML) analysis, that included a separate residual variance for each treatment, was used.

Prior to analyses of variance, many measurements were transformed to ensure the distribution of the residuals was not markedly skewed and/or that the amount of residual variation did not increase as the mean increased. Details of transformations used are presented in the tables in the Results section of this paper (Tables 2–5).

Non-parametric permutation tests, based on the usual ANOVA  $F$  values, were used to calculate  $P$ -values for the number of escape attempts, latency to make an escape attempt and the proportion of pigs that made an escape attempt and vocalised more than 10 times in the social isolation test. In all these cases, there were many pens with no pigs partaking in the behaviour, or there was discreteness in the data, and thus, the usual parametric  $P$ -values could not be considered as reliable.

Analyses were carried out using the ANOVA directive, the REML directive and the APERMTEST procedure of Genstat for Windows 19th edition (VSN International, 2018).

## Results

Of the 32 behaviour measurements examined, only one had a significant ( $P < 0.05$ ) interaction between human contact treatment and housing system, and the  $P$ -value for this interaction was only 0.04 (Tables 2–4). Thus, the main effects of human contact and housing system on behaviour measurements are reported separately.

**Table 1**  
ANOVA structures for statistical analysis of measurements collected during different phases of the experiment examining early human contact and housing for pigs.

Source of variation	df
Measurements collected during the preweaning phase <sup>1</sup>	
Housing system	1
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	44
Measurements collected during the weaner transition phase (Week 4 human test) <sup>2</sup>	
Housing system	1
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	20
Measurements collected during the weaner-finisher phase <sup>3</sup>	
Block stratum	5
Row within block stratum	
Housing system	1
Residual	5
Pair within row stratum	
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	10

<sup>1</sup> Unit of analysis is litter/lactation pen.

<sup>2</sup> Unit of analysis is a pair of temporary pens containing only cohort 1 pigs.

<sup>3</sup> Unit of analysis is a pair of pens; one pen containing females; one pen containing males.

**Table 2**

Effects of early housing and human contact on the behavioural responses of piglets to novelty and humans at 3 weeks of age during the preweaning phase. The latency to approach and interact and the time spent within 0.6 m of each stimulus were converted to a proportion of the 60 s observation time and then angularly transformed, and the number of interactions with each stimulus was square root transformed prior to analysis; back-transformed means are presented in parentheses.

	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Empty arena									
Number of entries	3.7	2.7	0.36	3.0	3.4	0.36	0.0066	0.26	0.24
Novel object									
Latency to approach (s)	34 (19)	59 (44)	4.1	53 (38)	40 (25)	4.1	$3.4 \times 10^{-7}$	0.0041	0.58
Latency to interact (s)	42 (26)	62 (47)	4.4	59 (44)	45 (30)	4.4	0.000024	0.0018	0.91
Number of interactions	2.1 (4.5)	1.4 (2.0)	0.18	1.4 (1.9)	2.1 (4.6)	0.18	0.00040	0.00019	0.18
Time within 0.6 m (s)	44 (29)	27 (12)	3.6	28 (13)	42 (27)	3.6	0.000024	0.000025	0.43
Human hand									
Latency to approach (s)	35 (20)	56 (41)	6.2	54 (40)	36 (21)	6.2	0.0018	0.0052	0.13
Latency to interact (s)	58 (44)	69 (53)	5.7	73 (55)	55 (40)	5.7	0.061	0.0037	0.67
Number of interactions	1.1 (1.2)	0.7 (0.47)	0.17	0.5 (0.29)	1.3 (1.6)	0.17	0.016	0.000084	0.72
Time within 0.6 m (s)	31 (16)	21 (8.0)	4.6	20 (7.1)	33 (17)	4.6	0.038	0.0091	0.31
Standing human									
Latency to approach (s)	36 (21)	56 (41)	5.5	55 (41)	37 (22)	5.5	0.00064	0.0017	0.58
Latency to interact (s)	38 (23)	63 (47)	5.4	60 (45)	40 (26)	5.4	0.000042	0.00059	0.52
Number of interactions	2.3 (4.1)	1.4 (2.0)	0.25	1.5 (2.2)	2.3 (5.1)	0.21	0.0015	0.00089	0.70
Time within 0.6 m (s)	43 (28)	27 (12)	5.3	27 (12)	43 (28)	5.3	0.0032	0.0033	0.80

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

**Table 3**

Effects of early housing and human contact on the behavioural responses of pigs to an unfamiliar human experimenter walking by and stopping in front of the pen, 1 day after weaning and mixing at 4 weeks of age during the weaner transition phase of the experiment. Pigs in the front of the pen were closer in proximity to the experimenter than pigs in the middle and back of the pen. All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses.

	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
% Pigs in front of pen	28 (22)	16 (7.3)	4.4	24 (17)	19 (11)	4.4	0.014	0.24	0.28
% Pigs in middle of pen	22 (15)	19 (10)	2.3	22 (13)	20 (11)	2.3	0.13	0.44	0.50
% Pigs in back of pen	52 (63)	64 (81)	4.4	55 (68)	61 (77)	4.4	0.016	0.21	0.31

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

### Effect of human contact treatment on behavioural responses to humans, novelty and social isolation

There was strong evidence ( $P < 0.01$ ) of a human contact treatment effect on all variables measured in the novel object and human tests at 3 weeks of age (Table 2). This effect was in the direction of lower fear responses in +HC piglets, based on +HC piglets showing greater approach and interaction with the novel and human stimuli compared to C piglets. There was no effect of human contact treatment on the responses of pigs to a human at 4 weeks of age during the weaner transition phase (Table 3). However, there was evidence of +HC pigs showing less fear of humans than C pigs after this time, with +HC pigs in closer proximity to the experimenter in the human test at 6 weeks of age, +HC pigs approaching the experimenter faster in the human test at 9 weeks of age, and a greater proportion of +HC pigs within 1 m of the stationary experimenter at the end of the human test at 14 weeks of age ( $P < 0.05$ , Table 4). There was no evidence ( $P > 0.05$ ) of a human contact treatment effect on the behavioural responses of pigs in the social isolation test at 7 weeks of age (Table 4).

### Effect of housing system on behavioural responses to humans, novelty and social isolation

There was strong evidence of a housing system effect on most variables measured in the novelty tests at 3 weeks of age and in the human tests at 3, 4 and 6 weeks of age, in the direction of greater fear responses in LP pigs than in FC pigs, based on LP pigs

showing less approach and interaction and more avoidance of the novel and human stimuli ( $P < 0.05$ , Tables 2–4). In the social isolation test at 7 weeks of age, there was an indication ( $P < 0.1$ ) that rearing in LP rather than FC led to a greater proportion of pigs attempting escape from the test arena, a shorter latency of pigs to vocalise and a greater proportion of pigs vocalising more than 10 times (Table 4). There was no evidence ( $P > 0.05$ ) of a housing system effect on the behavioural responses of pigs in the human tests at 9 and 14 weeks of age (Table 4).

### Effect of human contact treatment and housing system on physiological responses to social isolation

Serum cortisol concentrations 45 min after the social isolation test at 7 weeks of age were lower in pigs that had been reared in FC compared to LP (Table 5). In FC pigs, but not LP pigs, these cortisol concentrations were further lowered for pigs reared with +HC (Human contact treatment × housing system interaction,  $P = 0.006$ ). There was no evidence ( $P > 0.05$ ) of a housing system or human contact treatment effect on serum IgA concentrations after the social isolation test.

## Discussion

The treatment providing regular opportunities for positive interactions with humans early in life was highly effective in reducing piglets' fear of humans, based on the approach and proximity of piglets to an unfamiliar human at 3 weeks of age. The pos-

**Table 4**

Effects of early housing and human contact on the behavioural responses of pigs in various tests examining fear responses in the weaner-finisher phase of the experiment. Details of each test in Material and Methods. Data transformation details in footnotes; back-transformed means in parentheses.

	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Human test in the home pen at 6 weeks of age (proximity of pigs to experimenter in # rows of flooring; 1 row = 0.3 m)									
Human stationary at start of test	2.9	5.9	0.44	5.2	3.5	0.58	0.00098	0.013	0.76
Human moving during middle of test	3.2	4.0	0.11	3.8	3.4	0.16	0.00076	0.039	0.51
Human stationary at end of test	3.1	5.8	0.36	5.0	3.9	0.50	0.00064	0.040	0.70
Social isolation test at 7 weeks of age									
% Pigs attempting escape <sup>2</sup>	5.2 (0.82)	12.9 (5.0)	3.69	10.1 (3.1)	8.0 (1.9)	2.43	0.099 <sup>1</sup>	0.39 <sup>1</sup>	0.073 <sup>1</sup>
Latency to escape attempt (s)	120	120	2.2	110	120	1.8	0.62 <sup>1</sup>	0.072 <sup>1</sup>	0.64 <sup>1</sup>
Number of escape attempts <sup>3</sup>	0.4 (0.13)	0.5 (0.22)	0.19	0.5 (0.28)	0.3 (0.086)	0.15	0.59 <sup>1</sup>	0.14 <sup>1</sup>	0.86 <sup>1</sup>
Latency to vocalise (s)	41	23	7.3	35	28	6.1	0.055	0.32	0.43
% Pigs vocalised > 10 times <sup>2</sup>	49 (57)	69 (88)	4.5	58 (71)	61 (76)	3.00	0.057 <sup>1</sup>	0.34 <sup>1</sup>	0.14 <sup>1</sup>
Human test in an arena at 9 weeks of age									
Number of section entries in empty arena	17	17	1.6	17	17	0.9	0.77	0.68	0.041
Latency to approach human (s) <sup>2</sup>	24 (29)	28 (40)	4.0	30 (45)	22 (25)	2.7	0.33	0.013	0.55
Latency to interact with human (s) <sup>2</sup>	40 (74)	42 (79)	3.3	44 (85)	38 (68)	5.2	0.61	0.30	0.99
Time within 0.5 m of human (s) <sup>2</sup>	41 (78)	39 (70)	3.9	37 (64)	43 (84)	3.7	0.54	0.12	0.98
Number of interactions with human <sup>3</sup>	3.0 (9.2)	2.9 (8.4)	0.34	2.8 (7.6)	3.2 (10)	0.34	0.67	0.25	0.81
Human test in the home pen at 14 weeks of age (% pigs within 1 m of experimenter)									
Human stationary at start of test	67	67	4.4	63	71	6.7	0.90	0.27	0.34
Human moving during middle of test	62	62	4.0	58	67	6.5	0.78	0.20	0.71
Human stationary at end of test	75	76	5.4	69	82	5.7	0.88	0.037	0.43

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

<sup>1</sup> P-values calculated using permutation tests.

<sup>2</sup> Data were angularly transformed. Data for the human test at 9 weeks of age were converted to a proportion of the 180 s observation time and then transformed.

<sup>3</sup> Data were square root transformed.

**Table 5**

Effects of early housing and human contact on the physiological responses of pigs to 2 min of social isolation at 7 weeks of age. Serum cortisol and IgA concentrations 45 min after testing were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. Note that this table contains means of each of the four treatment combinations, rather than the main effect means.

	Treatment				SED		P-value		
	C/FC	+HC/FC	C/LP	+HC/LP	Same Housing	Other	Housing System	Human Contact	Housing × Contact
Cortisol concentrations 45 min after testing (ng/ml)	1.4 (23)	1.2 (17)	1.5 (30)	1.5 (31)	0.03	0.06	0.014	0.046	0.0063
IgA concentrations 45 min after testing (µg/ml)	3.4 (2500)	3.3 (1900)	3.3 (1900)	3.4 (2400)	0.10	0.09	0.88	0.99	0.23

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact; IgA = immunoglobulin A.

itive handling treatment was also effective at reducing pigs' fear of a novel object at 3 weeks of age. There was evidence that some reduction in fear of humans lasted well beyond the period of application of the treatment (lactation; 0–4 weeks of age), with all measures of fear of humans indicating less fearful responses in +HC pigs, with considerably more of these measures being statistically significant than would be expected by chance (5 out of 10 measures with  $P < 0.05$ ).

There were several methodological differences between each of the tests conducted from 3 to 14 weeks of age, including the location where testing was conducted (home pen or novel arena), the posture of the human (walking, standing stationary or crouching and extending hand) and whether pigs were tested individually or in a group. Although it is unclear why there were no human contact treatment effects on the behavioural response of pigs to an unfamiliar human walking in front of the pen after weaning at 4 weeks of age, the results from other behavioural tests in the experiment indicate that the +HC pigs generalised their response to different people and a range of contexts.

Hemsworth and Barnett (1992) found that individual patting and stroking in an arena from 0 to 3 weeks of age increased pigs'

approach and interaction with an unfamiliar human at 18 weeks of age. However, the effect of the early handling treatment appeared to weaken over time with subsequent human contact, as the authors found no differences in fear of humans at 20, 22 or 24 weeks of age, between pigs handled from 0 to 3 weeks of age and pigs that received only routine human contact. Subsequent human contact may have also weakened the effects of the early handling treatment on fear of humans in the present experiment given that the effects on fear responses at 9 and 14 weeks of age were not as strong as at 3 and 6 weeks of age. Differences in the methodology of the tests and their sensitivity to measure fear behaviour may have also played a role. However, the positive handling treatment in this experiment still reduced fear of humans for most of the study period.

In a previous experiment, we found that the effect of 3 min of regular patting, stroking and scratching on the latency of pigs to approach and interact with a standing human, human hand and traffic cone at 2 weeks of age, led to smaller reductions in fear responses than observed in the present experiment at 3 weeks of age (Hayes et al., 2021). In fact, in the previous experiment, no effect was observed to the introduction of a human hand or traffic



cone. In the present experiment, +HC pigs that received 5 min of regular patting, stroking, and scratching were faster than C pigs to approach and interact with a standing human, a human hand and a traffic cone. Differences in the duration of the positive handling treatment as well as in the age at which piglets were tested may be responsible for the stronger responses observed in the present experiment.

In addition to reduced fear of novelty and humans, for piglets that had been reared in farrowing crates, the positive handling treatment reduced the physiological stress response to isolation at 7 weeks of age, based on lower serum cortisol concentrations 45 min after isolation. Although earlier research suggested that positive handling of pigs has a stimulus-specific effect on fear (Hemsworth et al., 1986), other studies have reported that human interaction can reduce pigs' fear of humans as well as reduce fear more generally. For instance, holding piglets and stroking them led to less fear of people and less fear of novelty, based on levels of play behaviour and vocalisations in a novel arena (de Oliveira et al., 2015; Zupan et al., 2016). One interpretation of reduced fear of novelty and reduced cortisol after isolation in the present experiment is that the positive handling treatment improved general stress resilience. As stress resilience is typically fostered by overcoming experiences that are "challenging but not overwhelming" (Lyons et al., 2009; Lyons et al., 2010; Parker and Maestriperi, 2011; Lyons and Schatzberg, 2019), it is possible that close human contact during imposition of the handling treatment provided pigs with a minor challenge to overcome, that led to improved coping with other types of challenges. Furthermore, as humans were involved in conducting the novel object test and the isolation test, reduced fear of humans may have reduced the stressfulness of the test for the +HC pigs. This has implications for pigs in a commercial setting, as the presence of a human that pigs have had previous positive interaction with may reduce the stressfulness of routine farming challenges. We discuss this in more detail in Part 2 of this series which reports on the responses of pigs to routine husbandry practices, that appear to align with the fear levels of pigs reported here (Lucas et al., 2024a).

No effect of positive handling on serum IgA concentrations postisolation was observed. However, salivary concentrations of IgA have previously been shown to increase in pigs after a longer period of isolation than in the present experiment (Escribano et al., 2015), and thus, it is possible that the length of isolation in our experiment was insufficient for any effect to become apparent. There was also no evidence of effects of the handling treatment on the behaviour of pigs during the isolation test. A longer test duration could have resulted in stronger differences between the behaviour of +HC and C pigs.

Fear of humans was higher in LP pigs than FC pigs in the present experiment as shown by more avoidance and less approach and interaction with an unfamiliar human at 3, 4 and 6 weeks of age. Counter to these results, Kinane and colleagues (2021) found no effect of housing pigs in farrowing crates compared to loose lactation pens on fear of humans; however, the loose system differed markedly from that in the present experiment in terms of space and opportunities for sow-piglet interaction and human-pig interaction. Also, in the present experiment, LP pigs showed less approach and interaction with a novel traffic cone at 3 weeks of age, indicating greater fear of novelty. And at 7 weeks of age during the social isolation test, there was some evidence that LP pigs vocalised more and faster than FC pigs and had an increased likelihood of attempting escape from the test arena. At least in +HC pigs, the cortisol response 45 min after isolation was also lower in FC pigs. Similarly, Brajon and colleagues (2017) found that piglets reared in straw-enriched pens showed greater stress in response to isolation at 3–4 weeks of age, based on more escape behaviour and vocalisation compared to crate-reared piglets.

One explanation, which we have previously speculated (Hayes et al., 2021), for the increased fear and stress response of LP pigs to humans, novelty and isolation is that pigs in the PigSAFE loose system were reared in a more isolated environment with fewer opportunities to learn to cope with stress. In this loose system, the high pen walls limited piglets' contact with people outside of the pen, whereas in the farrowing crate system, piglets could more easily observe people in the room. There was also more opportunity for interactions at an earlier age between adjacent FC litters over the short dividing walls, compared to between adjacent LP litters through the windows in dividing walls, as LP piglets could only reach the pen windows at 2–3 weeks of age. Furthermore, there was less routine contact with stockpeople in the loose system than in the farrowing crate system due to the layout of both rooms. For example, many of the loose pens were positioned along aisles which were dead ends while the farrowing crates were positioned along aisles that stockpeople used to access neighbouring sheds. Stockpeople also spent more time in the farrowing crate room conducting routine inspections and feeding as there were twice as many litters housed there than in the loose pen room.

It is important to highlight that these features, including high solid walls and restricted human contact, may have been unique to the loose housing system studied in the present experiment. These specific features may have led to LP pigs being more vulnerable to stress due to fewer opportunities during rearing to 1. Habituate to humans, and/or 2. Learn to cope with stressors such as close exposure to stockpeople, their equipment and other pigs. Overall, this emphasises that the design of the farrowing and lactation environment, in terms of both pen and room design, can have significant implications for the human-animal relationship and animal welfare. This should be considered carefully given the expected increase in adoption of loose housing systems.

It is also possible that the maternal behaviour of the sows played a role in the differing fear responses of LP and FC pigs given that sows in pens show better maternal behaviour and more interactions with their piglets compared to sows from farrowing crates (Cronin et al., 1996; Thodberg et al., 2002; Chidgey et al., 2016; Singh et al., 2017). Therefore, it may be that sows in the loose system showed heightened maternal responses when stockpeople were close, which increased their piglets' fear of stockpeople. The design of the present experiment does not allow the contributions of effects of maternal behaviour of the sow, contact with humans, contact with neighbouring pigs and visual stimulation in general, as well as other differences between the housing systems to be determined. Detailed experimental research is required to disentangle the specific characteristics of the housing systems that may affect fear and stress responses of piglets. There were no effects of the farrowing and lactation housing system on the fear responses of pigs to an unfamiliar person in the human tests at 9 or 14 weeks of age. It may be that the effect of the early housing system on fear of humans was ameliorated by pigs' subsequent interactions with humans, as previously discussed.

While the present experiment showed that the loose housing treatment increased pigs' fear of humans during lactation and after weaning, positive handling during lactation resulted in more profound and sustained reductions in fear of humans. These results emphasise the significance of the design of the early housing environment on the development of the human-animal relationship, and more broadly, highlight the importance of early experiences in shaping the later life fear responses of pigs.

### Ethics approval

All animal procedures were conducted with prior institutional ethical approval under the requirements of the New South Wales

Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes (Rivalea Animal Ethics Committee #19B018C).

### Data and model availability statement

The data were not deposited in an official repository but are available from the corresponding author upon request.

### Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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### Declaration of interest

None.

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