Relationship between Environmental Enrichment and the Response to Novelty in Laboratory-housed Pigs

Brittany L Backus,^{1,*} Mhairi A Sutherland,² and Tiffanie A Brooks¹

Environmental enrichment is the enhancement of the physical or social environment in which an animal lives with the goal to improve its quality of life. Our objective was to investigate the effect of providing environmental enrichment in the home pen on responsiveness to novelty in laboratory-housed pigs. Pigs were housed (4 pigs per pen) in enriched (n = 32) or barren (control; n = 32) pens for 3 wk total and tested in 2 anxiety behavioral tests, the novel object (NOT) and human interaction (HIT) tests. Pigs were placed in a novel arena for a 5-min familiarization period, after which either a novel object (NOT) or an unfamiliar human (HIT) was introduced for a 5-min interaction period. Behavior in the home pen and during NOT and HIT was monitored through direct observations and videorecording. In the home pen, enriched pigs spent more time active and interacting with the environment, whereas control pigs spent more time inactive and in social interactions. In addition, enriched pigs crossed more squares during the familiarization period, tended to freeze more, and interacted less with the novel object or person than control pigs. In conclusion, enrichment may improve welfare by stimulating activity and decreasing aggressive behaviors in the home pen. However, enriched pigs may experience increased anxiety when exposed to novelty, whereas pigs housed without environmental enrichment—due to lack of stimulation in the home pen—may be more motivated to interact with sources of novelty or enrichment during testing than their enriched counterparts.

Abbreviations: HIT, human interaction test; NOT, novel object test.

The environment in which an animal is reared is well known to influence its physical and psychologic development. Environmental enrichment can reduce pain perception, anxiety, and agonistic behaviors and is protective against stressors, thus potentially improving an animal's overall welfare.^{3,11,18,33,44} In rodents, environmental enrichment can reduce chronic stress and decrease the response to acute stressors. In the rat model, environmental enrichment improves the ability of rats to cope with acute stressors and reduces anxiety-related behaviors in multiple behavioral tests.²⁶

When animals are faced with novelty, they are often conflicted between their instinctive desire to explore novel environments and neophobia (that is, their fearfulness of the environment).^{15,18} Environmental enrichment allows animals an opportunity to explore and interact with their environment, thereby enhancing cognition, sensorimotor skills, physical activity, and a positive affective state.^{1,18,19} The ability to explore may be particularly important in pigs, which are highly intelligent and curious and can become bored easily.42 Environmental enrichment can increase explorative and play behaviors.^{31,41} Play behavior is seen in pigs starting at birth^{6,31} and helps them to train for unexpected events.⁴¹ Consequently, increased play behavior at an early age may enable pigs to cope with and adapt to future novelty and social challenges.

Laboratory animals undergo several procedures that can be perceived as stressful, including handling, blood sampling, exposure to novelty, and surgical procedures. Excess stress during

laboratory research trials may affect an animal's physiology and behavior, potentially altering research conclusions.¹⁰ Therefore, scientists should strive to maintain a neutral or positive affective state in their research animals. Domestic pigs are more commonly used in biomedical research in light of their anatomic, physiologic, psychologic, and social similarities to humans. This high similarity has led to an increase in the number of pigs housed in laboratory research settings. Currently, there is research describing the effects of enrichment on pigs that are being commercially reared for food, and therefore most of these enrichment studies use environmental enrichment to decrease abnormal behaviors^{3,35} (for example, tail biting) or aggression when pigs are forming new social hierarchies,^{6,11} such as during mixing of herds and weaning. Limited research has compared the relationship between rearing environment and the ability of pigs to cope with novelty in a laboratory setting.9 Using environmental enrichment as a means to create a positive affective state in laboratory pigs is a novel approach to decrease the reactivity and stress associated with routine laboratory research procedures.

Providing pigs with environmental enrichment in their home pens might enhance their positive affective state and mitigate how they perceive potentially negative or novel stimuli. In addition, animals that are more readily able to cope with novel stimuli are likely to be calmer and, therefore, easier to handle or train during routine research procedures, thus improving the overall welfare of pigs used in laboratory animal research. Therefore, our objective was to determine whether rearing pigs in an environmentally enriched laboratory research setting improved the animals' welfare and reduced the stress responses to novelty by creating a positive affective state. We hypothesized that environmental enrichment will lead to calm pigs that are less anxious than their unenriched counterparts.

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^{*}Corresponding author. Email: brittany.backus@ttu.edu

Materials and Methods

All animal procedures were approved (protocol no. 15002-01) by the IACUC prior to beginning the study, which was conducted in 2015 at a Texas Tech University animal facility (Lubbock, TX). Texas Tech University is AAALAC-accredited. All pigs were obtained from a herd certified by the Texas Animal Health Commission to be free of brucellosis and pseudorabies; in addition, the herd is tested quarterly for porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus.

Housing. The study population comprised Large White \times Landrace female pigs (PIC North America, Hendersonville, TN) that were weaned at 28 d and then transported to the Animal Care Services animal facility and allowed to acclimate for 1 wk prior to starting studies. Pigs were housed in 1.83 m \times 1.22 m research pens with epoxy resin slatted floors at a minimum stocking density of 0.9 m². Food and water were provided without restriction, and husbandry staff performed health checks and cleaned the pens every morning. Nonlittermate, weightmatched gilts $(7.63 \pm 0.95 \text{ kg})$ were randomly assigned to either barren, standard (control; n = 32) home pens or environmentally enriched pens (n = 32) at 4 pigs per pen. All treatments groups were present in the same room, but different treatment groups did not have nose-to-nose contact with each another. Control pigs were reared in standard research pens with no enrichment or change in environment; the pen contained only a galvanized feeder and nipple waterer.

In contrast, enrichment (Figure 1) included an apple-scented Porcichew (Ketchum, Brockville, Ontario, Canada) that hung from the middle of the pen and a large (10-in.) Best Ball (Bio-Serv, Flemington, NJ). These items remained in the pen throughout the study and were cleaned of any fecal matter daily. Other enrichment was changed every 24 h to maintain novelty, interest, and cleanliness or because it was a destructible item. Toys to chew and play with included rubber boots, twisted rope, and DNA Flexer (Bio-Serv). Substrates (soil, peat moss, and shredded paper) were placed in a rubber pan to allow pigs to perform rooting behavior. Feed bags were given to manipulate and destroy. Towels were dragged, chewed, and used as bedding. Treats were placed in Kongs (Bio-Serv) and rubber footballs to allow pigs to forage for treats. The treats included in the toy were Prima Treats (Bio-Serv), cookies, and marshmallows. In addition, Bunny Blocks (Bio-Serv) were hung in the cage to gnaw. Daily positive human contact included scratching and stroking of the pigs for at least 10 min per pen; an effort was made to spend equal time with each individual pig. During this period, pigs also received treats by hand, including dried and fresh fruits and vegetables, peanut butter, strawberry Boost, marshmallows, and cookies. The same person changed the enrichment and interacted with the pigs daily. Items were chosen to allow pigs to display species-specific behaviors of chewing, rooting, manipulating, and foraging. Enrichment toys, substrates, and treats were selected daily by using a random-number generator for a 2-wk treatment period prior to and throughout behavioral testing (1 wk), for a total of 3 wk.

The daily behavioral repertoire of pigs in the control and enriched environments was recorded in the home pen for 48 h at the end of the 2-wk treatment period. Behaviors coded included (Figure 2) : maintenance (lying, sitting, standing, walking, eating, drinking), locomotor play (running, scampering, pivoting, tossing head, flopping), social interactions (pushing and nudging when involving 2 or more pigs), and substrate-oriented behaviors. Substrate-orientated behaviors were categorized as interactions with the pen (pen fittings, walls, or floor) and interaction with enrichment (enrichment items were provided to the enriched pigs only). Behavior was recorded by using scan sampling every 5 min over 48 h (Figure 2)

Behavioral tests. After 2 wk in the rearing environment, pigs underwent 2 behavioral tests (10 min each) over a 1-wk period to determine the effect of environment on anxiety-like behavior. Specifically, pigs completed one (randomly selected) of the 2 behavioral tests, followed by a rest day; the second test was completed during another 2-d testing period. The behavior testing room was adjacent to the housing rooms, and pigs were carried over and placed in the test arena.

Novel object test (NOT). Each pig was placed in one corner of a small arena (2.4 m × 2.4 m) that was divided into 9 equal squares (Figure 3). The pigs were given a 5 min-familiarization period to explore the novel environment. After the familiarization period, a novel object (bucket) was slowly lowered into the arena, and pigs were then given 5 min to interact with the object. Neither control nor enriched pigs had previously experienced a bucket as part of their daily routine, so this object was completely novel to both treatment groups. Direct observation and videocameras were used to record activity—the frequency of crossing each of the squares and number and duration of 'freezes'—and interactions: latency to approach the novel object and the frequency and duration of interactions (Figure 2).

Human interaction test (HIT). A small arena $(2.4 \text{ m} \times 2.4 \text{ m})$ was divided into 9 equal squares (Figure 3). Pigs received a 5-min familiarization period same as for NOT, but during the 5-min interaction period an unfamiliar person entered the pen and sat in a nonthreatening pose (eyes cast down). This person did not have daily contact with the pigs in the form of husbandry care or enrichment. The person wore the same clothes each time to avoid any association with clothes or their color and took the same path to enter and exit the arena, walking along the edge of the arena opposite to the entrance corner. The interaction period ended as soon as the person stood up to exit the arena. When approached by the pig, the person did not engage the pig but allowed it to direct the interaction (aggressive, playful). Direct observations and videorecordings of activity and interactions were obtained (Figure 2).

Statistical analysis. All data were tested for constant variance and departures from the normal distribution by using a univariate procedure (SAS Institute, Cary, NC). All variables except frequency of squares crossed were square-root transformed. Data underwent analysis of variance by using the mixed model procedure of SAS. For behaviors measured in the home pen, the model included treatment (control and enriched) as the main fixed effect and pen (1 to 16) as a random effect. The behaviors analyzed included inactive (lying and sitting combined), active (eating, drinking, standing, walking, playing, and interacting with pen or enrichment combined), locomotor play (running, scampering, pivoting, head tossing, and flopping combined), social interactions (pushing and nudging combined), interacting with the pen, interacting with the enrichment and total interactions (interacting with the pen and enrichment combined). For behaviors measured in the NOT and HIT tests, the model included treatment (control and enriched), period (familiarization and interaction), and test (NOT and HIT) and their interactions as fixed effects and order of test (first and second) and pen (1 to 16) as random effects. Multiple comparisons were calculated by using the PDIFF option in SAS. Statistical significance was determined at a *P* value less than 0.05, and trends defined as a P value less than 0.10. Graphs, tables, and text present actual data (not transformed) summarized by least-square means (\pm SEM).

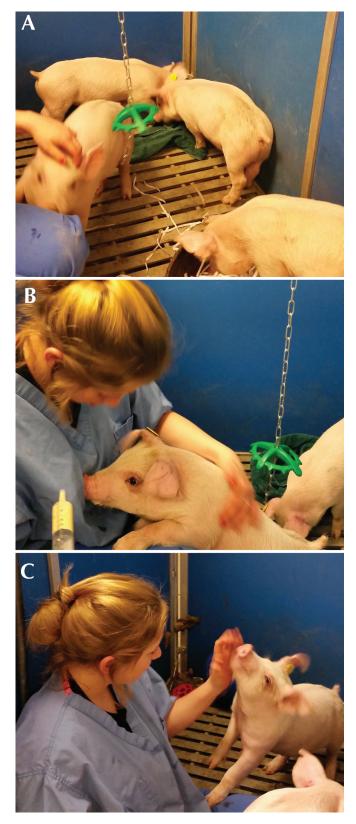


Figure 1. Environmentally enriched pigs (A) interacting with toys and substrates, (B) getting treats, and (C) interacting with a person in the home pen.

Results

Home pen behavior. All data are presented as the percentage of total observations (Figure 4). Pigs provided environmental enrichment spent more time interacting with enrichment (control, 0.0% ± 0.5%; enriched, 6.6% ± 0.5%; *P* < 0.001), whereas control pigs spent more time interacting with the pen (control, 3.4% ± 0.4%; enriched, 1.0% ± 0.4%; *P* < 0.001). Overall, enriched pigs spent more time interacting with the environment (enrichment and pen combined; control, 3.4% ± 0.6%; enriched, 7.5% ± 0.6%; *P* < 0.001). Although control pigs spent more time in social interactions (control, 3.8% ± 0.3%; enriched, 1.8% ± 0.3%; *P* < 0.001), treatment had no effect (*P* > 0.05) on the performance of locomotor play behaviors (control, 0.9% ± 0.5%; enriched, 1.9% ± 0.5%). Control pigs spent more time inactive (control, 77.9% ± 1.3%; enriched, 73.9% ± 1.3%; *P* = 0.048) and tended to spend less time active (control, 22.1% ± 1.3%; enriched, 26.1% ± 1.3%; *P* = 0.065).

Behavior tests. Both control and enriched pigs crossed more (P < 0.001) squares during the familiarization period than during the interaction period, and enriched pigs tended to cross more squares (P = 0.051) during the familiarization period than control pigs (Figure 5).

Enriched pigs showed a tendency (P = 0.068) to freeze more often than control pigs (control, 30.8 ± 9.5 freezes; enriched, 38.7 ± 9.5 freezes). Moreover, pigs froze more (P = 0.028) during the familiarization than the interaction period, regardless of the test (familiarization, 37.9 ± 9.46 freezes; interaction, 31.6 ± 9.46 freezes).

Enriched pigs tended (P = 0.063) to interact less with the novel entity, whether it was an object or human, compared with control pigs (control, 10.4 ± 1.2 interactions; enriched, 8.6 \pm 1.2 interactions). However, the duration of interaction with the novel object or human did not differ between control and enriched pigs (P > 0.05). Overall, latency to approach an unfamiliar person was longer (P < 0.001) than to approach a novel object (HIT, 131.3 ± 34.5 s; NOT, 36.3 ± 34.5 s).

Discussion

Many animals are unable to cope with the novelty of research procedures and thus display fear and anxiety-like behaviors. This increased stress response can affect research results. Environmental enrichment is used to provide lab animals with a complex environment of objects, and conspecifics for motor, cognitive, and social stimulation.43 Enrichment attenuates anxiety-like behavior responses and has both physical and psychologic benefits. In the present study, enriched pigs spent more time interacting with enrichment objects and the environment overall in the home pen but tended to interact fewer times with a novel object or person in an arena test. Consequently enriched pigs may have been more anxious toward novelty, compared with control pigs. Alternately, the novelty (novel object or person) we used in the arena test may not have been sufficiently stimulating or novel for enriched pigs, given that they were already used to a complex environment. Conversely, control pigs tended to interact more times with the novelty in the test arena, thus perhaps reflecting the lack of stimulation in the home pen.

Enrichment reduces inactivity and aggression in pigs as it increases exploratory and play behaviors.^{3,6,9,11} In the current study, control pigs were more inactive and tended to spend less time displaying active behaviors in the home pen, compared with enriched pigs. These findings are in line with other studies in which pigs housed in barren environments were less active than those provided enrichment.^{3,9} Piglets housed with rope toys were more active, and piglets provided with paper were less inactive, than pigs housed in barren environments.²⁷ Pigs' curiosity can be stimulated by external stimuli, such as enrichment.⁴² Increased inactivity might be due to a lack of Vol 56, No 6 Journal of the American Association for Laboratory Animal Science November 2017

| Behavior | Description |
|--|---|
| Home pen | |
| Maintenance | |
| Walk | Low-speed locomotion in which propulsion derives from action of legs |
| Eat | Time spent with head in feeder (assumed to be feeding) |
| Drink | Mouth on water spout |
| Sit | Resting on the caudal part of the body |
| Stand | Assuming or maintaining upright position on extended legs |
| Play – individual | |
| Run | High-speed locomotion in which propulsion derives from action of legs |
| Scamper | Sequence of at least 2 forward hops in rapid succession |
| Pivot | Jump or whirl with vertical and horizontal bouncy movements |
| Toss head | Vigorous lateral-rotationary movements of the head |
| Flop | Rapid drop from upright position to sternal or lateral recumbency |
| Social interaction (at least 2 pigs in | volved) |
| Push | Pushing of the opponent with the head or shoulder |
| Nudge | Gentle snout contact |
| Substrate interactions | |
| Pen | Rooting or touching of any of the pen fittings: walls, floor, door, feeder, or waterer |
| Enrichment | rooting, touching, or pulling any of the available enrichment items |
| Behavior tests | |
| Cross square-freeze | Head and shoulders have entered a new outlined square, with massive body flexion and temporary immobility |
| Interaction | Physical contact with the object or unfamiliar human with the snout, mouth, or front hooves (standing on) |



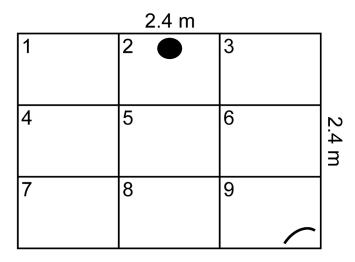


Figure 3. Arena layout for the novel object and human interaction tests. The arc indicates the entrance–exit, and the filled circle indicates the location of the novel object or unfamiliar person.

opportunity to increase the behavioral repertoire prompted by enrichment.

Increased play behavior may be an indicator of positive welfare and emotion.^{21,30,38} Play behaviors occur more under enhanced housing conditions and nonthreatening environments.³⁰ However, we found no difference in locomotor play behaviors between control and enriched pigs in the home pen. This finding is similar to another study²⁷ that found no effect on play behavior but contrasts with a report⁹ in which a straw-bedded enriched environment increased locomotor play behaviors, such as gamboling, pivoting, rolling, and shaking enrichment items. One limitation to our study may be the use of 5-min scan sampling to measure play behavior due to the normal low incidence of this behavior. Continuous sampling might have been a better method to detect significances in short-duration behaviors such as locomotor play. We measured play behavior in the home pen, but another colleague³⁷ found increased play only when pigs were released into a corridor near their home pen,

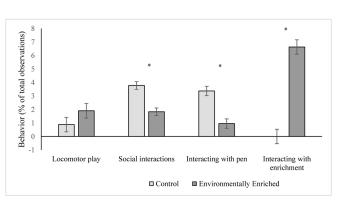


Figure 4. The percentage of total observations (least-squares mean \pm SEM) that pigs spent performing different behaviors over a 48-h observation period in standard (control, CON) or environmentally enriched (EE) pen. *, Values differ significantly (P < 0.05) between groups.

due to the novelty of the environment and increased space that allowed for the associated bursts of energetic activity. It would be interesting to look for play behaviors in the novel arena, where the pigs had more room for locomotor play behaviors, because our enriched pigs tended to be more active during the familiarization period.

We detected intergroup differences in social interaction, including pushing and nudging,¹¹ which can be considered unpleasant, aggressive behaviors.²¹ We found that control pigs participated in more social interactions and aggressive encounters than enriched pigs, consistent with others who have found that enrichment decreased aggression in weaned pigs.^{28,32} Enriched pigs might have spent less time performing social behaviors because they spent more time interacting with the enrichment and were overall more interactive with their environment: enriched pigs spent 6.6% of the time interacting with enrichment than did control pigs, who spent 3.4% of the time interacting with enrichment the pen fittings. Our findings are similar to those of a previous study²⁷ in which piglets reared with paper spent more time interacting with enrichment and less time exploring the pen fittings than pigs reared with rope toys or in barren

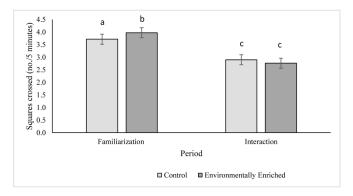


Figure 5. Number of squares crossed (least-squares mean ± SEM) by pigs during the familiarization and interaction period of the novel object and human interaction tests. Pigs were either housed in enriched (n = 32; EE) or barren, control (n = 32; CON) pens prior to testing. Different superscripted lowercase letters indicate values that differ ($P \le 0.051$).

environments. In addition, interacting with enrichment items might be considered object play because it involves physically manipulating the inanimate items provided,²² and therefore is an indicator of positive welfare.^{21,30,38} Control pigs interacted with the pen fittings and penmates more than enriched pigs did, possibly due to the lack of other items to manipulate. Others have found that pigs reared in enriched environments displayed more exploratory behaviors after weaning and less oral manipulative behaviors than barren-reared pigs.^{3,9,28} The enrichment items we provided in the present study might have been a distraction that decreased interaction with pen fittings and encounters with conspecifics or that reduced retaliation from penmates.^{3,35,40} Conspecifics and pen components can become unintentional enrichment when no other objects are provided and can cause self-inflicted wounds or harm to other pigs.²² By providing enrichment, aggressive behaviors can be redirected to exploratory behaviors.²⁸ Increased interaction with enrichment items and the environment, more active behaviors, and decreased negative social interactions in enriched pigs may have created a positive affective state and improved the pigs' welfare by allowing them opportunity to expand their behavioral repertoire.24,27

Enrichment affects the stress response of animals by reducing hypothalamic–pituitary–adrenal axis reactivity^{4,18,29} and enhancing hippocampal neurogenesis.^{25,48} These molecular, anatomic, and behavioral changes stimulate a positive effect on the brain and therefore can create a positive affective state.⁴³ Although beyond the scope of our current study, other measures of positive affective states would be worth incorporating into future studies.

The anxiety of an unfamiliar environment can often override exploration of it,⁵⁰ but environmental enrichment might protect against and reverse the effects of stress responses. In the behavioral tests we used, the increased activity observed as crossing more squares during the familiarization period may indicate that—compared with controls—enriched pigs were less fearful of or stressed by the novel environment, allowing them to be more explorative of the novel environment. Different results regarding locomotor activity in a novel arena have been reported, and the interpretation of this behavior—whether it represents fear or exploration—varies in the literature. Some found no differences in the exploration of novel environment in pigs;¹⁴ others concluded that less activity indicated less fear in enrichment-reared pigs.¹² In addition, enriched pigs spend more time active in novel environments.^{23,49} Enrichment decreased fear and increased exploration in multiple anxiety behavioral studies in mice^{5,44} and rats.^{26,48,50} Activity across multiple behavioral tests may be directly related and suggests that increased activity indicates low anxiety.³⁰ The familiarization period in our study is considered an open-field test, which typically is used as a measure of activity and not fear in swine behavioral research.¹⁷ Furthermore, interpreting activity in a novel environment as fear or exploration may be dependent on species, with ground-foraging species such as pigs eliciting explorative behaviors.²² We also found that pigs housed with environmental enrichment spent less time inactive and tended to be more active than control pigs in their home pens, indicating that provision with environmental enrichment may stimulate pigs to be more active in general. Other colleagues³⁰ have indicated that locomotor activity and exploration are not easily differentiated and are often grouped together.

In our study, enriched pigs tended to freeze more than control pigs, and pigs froze more during familiarization than in the interaction period. Freeze behaviors are often considered a negative emotion.³⁸ Our finding is opposite to that reported elsewhere,²⁷ in which piglets raised in barren environments froze more in a novel arena. Freeze behavior in a novel arena is often interpreted as a fear response. The increased activity and freezing in our current study may suggest that enriched pigs were more anxious toward novelty than control pigs, opposite to our hypothesis. Enriched pigs may be more alert or vigilant in novel environments than controls.²³ However, freezing is just one component of a pig's response to a stimulus. In that regard, some colleagues⁷ have proposed that freeze behavior reflects that the animal is orientating itself toward the stimulus to investigate, thus suggesting that freeze behavior might also be seen as a state of arousal and not necessarily fear. However, others⁸ argued that because pigs are not prey animals, freeze behavior in open field tests cannot be interpreted as a fear response, as in rodents. If activity is interpreted as exploration, then freezing more during the same familiarization period may suggest pigs were not fearful. Another study³⁸ revealed that pigs froze more during a rewarding event than they did during a training period because they were alerted by the presence of the naïve pig used in the study.

In response to the novel object or person, our control pigs tended to interact more often than the enrichment pigs. However, neither the latency to interact nor the duration of interactions with the novel object or person differed between groups. This outcome is opposite to our hypothesis and the findings of others who report that enriched housed pigs^{2,39} and rats^{15,16,50} were quicker to make contact or interacted more with a novel object or person. Our enriched pigs might have been more anxious to interact with a novel object or person. In line with our results, others have found that pigs in enriched conditions displayed more avoidance behavior toward a novel object, with barren-housed pigs showing a shorter latency to contact a novel object³⁴ or person.¹⁴ Conversely, barren-reared pigs have demonstrated increased exploration of novel objects,²³ suggesting that pigs reared without enrichment show greater motivation to explore a novel object or person because, compared with pigs reared with enrichment, they lack the opportunity to increase their behavioral repertoire^{24,36,39} or satisfy their intrinsic motivation to explore.³⁰ We found no difference between groups in the total time spent interacting with either the object in NOT or the person in HIT, but our observations suggest that enriched pigs interacted for prolonged periods of or the entire time, whereas control pigs typically had multiple, brief interactions. This difference might be one reason why enriched pigs tended to display fewer interactions. Numerically, control and enriched pigs reacted oppositely to the HIT and NOT tests, thus cancelling out each other overall. Using a larger sample size might have yielded significant differences.

The novel object used in our experiment might have been of more interest to pigs raised in barren environments than those housed in complex environments. The bucket might not have been sufficiently complex to keep the attention of enriched pigs, given that they were used to more intricate objects that allowed for greater manipulation. Different objects and substrates used for enrichment have different values to pigs;²⁰ for example, rope was more satisfying than wood,⁴⁷ but paper more so than rope.²⁷ Not providing enrichment in the home pen might have had a rebound effect, in that control pigs were more inactive in the home pen owing to lack of stimulation and therefore might have been more encouraged to play and interact with the novelty than were enriched pigs.³⁰ Furthermore, minimal handling of animals might increase novelty-seeking behaviors in pigs.¹³ One group¹³ found that the type of enrichment used (a chain that was not replenished compared with straw and a toy) affected the number of touches with a handler, when pigs were handled minimally. Enrichment paradigms differ between laboratories in many aspects (for example, types of objects, targeted behavior, frequency of changing enrichment, duration with the items), thus complicating comparison of the effects of enrichment across the literature.43

Another reason we might have observed increased interactions in the control pigs was that, according to our general observations, pigs became 'frustrated' when the person did not interact, because the pigs nudged the person's arms and legs in attempts to get the person to touch them. Enriched pigs were used to climbing on top of and being scratched by a person as they received treats in their home pen. Pigs might have become frustrated when they were denied the contact they sought.^{45,46} Even though the test person had no previous interaction with the pigs, all pigs were used to human contact through daily husbandry and health checks, so they might have habituated to the presence of a person. One study¹³ found that pigs who received straw or a toy overcame their fear of humans because those provisions had to be changed, whereas pigs exposed to enrichment by using a chain (which did not require replenishment) and nonenriched pigs would not associate with humans. Alternately, an unfamiliar person might still have been perceived as a slight threat, given that both treatment groups demonstrated longer delays until interaction with the person compared with the object.

In conclusion, environmental enrichment of pigs appears to stimulate activity and decrease aggressive behaviors in the home pen, potentially improving overall animal welfare. However, conflicting data from NOT and HIT may indicate that enriched pigs are more anxious when exposed to novelty than control pigs. Alternatively, control pigs may be more motivated to interact with sources of novelty or enrichment during testing, due to the lack of stimulation in their home pen. Differences in the behavioral response of pigs to NOT and HIT in the present study compared with those in other studies might reflect the experimental setting (commercial farm compared with laboratory) or variability in enrichment programs across laboratories.43 Industry-driven studies typically do not use as robust of an enrichment program as we did here, with daily alternating of items, due to constraints associated with facility design. It is important to note that the present study was designed to assess an enrichment program appropriate for laboratory-housed research pigs, with an emphasis on creating a positive affective state, rather than just reduction of fighting or stereotypical behaviors, as is the predominant focus of enrichment programs for commercially reared pigs.^{3,6,11,35} Our inconclusive results in regard to the effect of enrichment on the responsiveness of pigs to novelty may reflect the behavioral tests used and the brevity of the study, which might have been suboptimal for evaluate affective state and response to novelty.³⁰ Future studies should test pigs' responsiveness to actual laboratory procedures (for example, blood collection, training, injections) to better test the hypothesis that environmental enrichment will enable pigs to better cope with these practices, making the animals easier to work with and handle.

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References

- 1. Bateson M, Matheson SM. 2007. Performance on a categorization task suggests that removal of environmental enrichment induces 'pessimism' in captive European starlings (*Sturnus vulgaris*). Anim Welf 16:33–36.
- Beattie VE, O'Connell NE, Kilpatrick DJ, Moss BW. 2000. Influence of environmental enrichment on welfare-related behavioral and physiological parameters in growing pigs. Anim Sci J 70:443– 450.
- Beattie VE, O'Connell NE, Moss BW. 2000. Influence of environmental enrichment on the behavior, performance, and meat quality of domestic pigs. Livest Prod Sci 65:71–79.
- Belz EE, Kennell JS, Czambel K, Rubin RT, Rhodes ME. 2003. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. Pharmacol Biochem Behav 76:481–486.
- Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I, Pick CG. 2004. Environmental enrichment in mice decreases anxiety, attenuates stress responses, and enhances natural killer cell activity. Eur J Neurosci 20:1341–1347.
- 6. **Blackshaw JK, Thomas FJ, Lee J-A.** 1997. The effect of a fixed or free toy on the growth rate and aggressive behavior of weaned pigs and the influence of hierarchy on initial investigation of toys. Appl Anim Behav Sci **53**:203–212.
- 7. Blackshaw JK, Blackshaw AW, McGlone JJ. 1998. Startle–freeze behavior in weaned pigs. Int J Comp Psychol **11**:30–39.
- Boissy A, Bouissou M-F. 1995. Assessment of individual differences in behavioral reaction of heifers exposed to various fear-eliciting situations. Appl Anim Behav Sci 46:17–31.
- 9. Bolhuis JE, Schouten WGP, Schrama JW, Wiegant VM. 2005. Behavioral development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. Appl Anim Behav Sci 93:213–228.
- 10. **Broom DM.** 1988. The scientific assessment of animal welfare. Appl Anim Behav Sci **20:**5–19.
- 11. Chaloupková H, Illmann G, Bartos L, Spinka M. 2007. The effect of preweaning housing on the play and agonistic behavior of domestic pigs. Appl Anim Behav Sci **103**:25–34.
- Chaloupková H, Illmann G, Neuhauserová K, Tománek M, Valis L. 2007. Preweaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. J Anim Sci 85:1741–1749.
- 13. Day JEL, Spoolder HAM, Burfoot A, Chamberlain HL, Edwards SA. 2002. The separate and interactive effects of handling and environmental enrichment on the behavior and welfare of growing pigs. Appl Anim Behav Sci **75:**177–192.
- 14. de Jong IC, Prelle IT, van de Burgwal JA, Lambooij E, Korte SM, Blokhuis HJ, Koolhaas JM. 2000. Effects of environmental enrichment on behavioral responses to novelty, learning, and memory and the circadian rhythm in cortisol in growing pigs. Physiol Behav 68:571–578.

- 15. Fernández-Teruel A, Driscoll P, Gil L, Aguilar R, Tobena A, Escorihuela RM. 2002. Enduring effects of environmental enrichment on novelty seeking, saccharin, and ethanol intake in 2 rat lines (RHA/Verh and RLA/Verh) differing in incentive-seeking behavior. Pharmacol Biochem Behav 73:225–231.
- Fernández-Teruel A, Escorihuela RM, Castellano B, Gonzalez B, Tobena A. 1997. Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: focus on the Roman rat lines. Behav Genet 27:513–526.
- 17. Forkman B, Furuhaug IL, Jensen P. 1995. Personality, coping patterns, and aggression in piglets. Appl Anim Behav Sci 45:31–42.
- Fox C, Merali Z, Harrison C. 2006. Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. Behav Brain Res 175:1–8.
- Gabriel AF, Paoletti G, Seta DD, Panelli R, Marcus MAE, Farabollini F, Carli G, Joosten EAJ. 2010. Enriched environment and the recovery from inflammatory pain: social versus physical aspects and their interaction. Behav Brain Res 208:90–95.
- Guy JH, Meads ZA, Shiel RS, Edwards SA. 2013. The effect of combining different environmental enrichment materials on enrichment use by growing pigs. Appl Anim Behav Sci 144:102–107.
- 21. Held SDE, Spinka M. 2011. Animal play and animal welfare. Anim Behav 81:891–899.
- 22. Horback KM. 2014. Nosing around: play in pigs. Anim Behav Cog 1:186–196.
- Jansen J, Bolhuis JE, Schouten WGP, Spruijt BM, Wiegant VM. 2008. Spatial learning in pigs: effects of environmental enrichment and individual characteristics on behavior and performance. Anim Cogn 12:303–315.
- Kelly HRC, Bruce JM, English PR, Fowler VR, Edwards SA. 2000. Behavior of 3-wk weaned pigs in Straw-Flow, deep straw, and flat-deck housing systems. Appl Anim Behav Sci 68:269–280.
- 25. Kempermann G, Kuhn HG, Gage FH. 1997. More hippocampal neurons in adult mice living in an enriched environment. Nature **386**:493–495.
- Larsson F, Winblad B, Mohammed AH. 2002. Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. Pharmacol Biochem Behav 73:193–207.
- 27. Lewis E, Boyle LA, O'Doherty JV, Lynch PB, Brophy P. 2006. The effect of providing shredded paper or ropes to piglets in farrowing crates on their behavior and health and the behavior and health of their dams. Appl Anim Behav Sci 96:1–17.
- Melotti L, Oostindjer M, Bolhuis JE, Held S, Mendl M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl Anim Behav Sci 133:144–153.
- Morley-Fletcher S, Rea M, Maccari S, Laviola G. 2003. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behavior and HPA axis reactivity in rats. Eur J Neurosci 18:3367–3374.
- Murphy E, Nordquist RE, van der Staay FJ. 2014. A review of behavioral methods to study emotion and mood in pigs, *Sus scrofa*. Appl Anim Behav Sci 159:9–28.
- Newberry RC, Wood-Gush DGM, Hall JW. 1988. Playful behavior of piglets. Behav Processes 17:205–216.
- 32. Nowicki J, Klocek C. 2012. The effect of aromatized environmental enrichment in pen on social relations and behavioral profile of newly mixed weaners. Annals of Animal Science **12:**403–412.
- 33. O'Connell NE, Beattie VE, Sneddon IA, Breuer K, Mercer JT, Rance KA, Sutcliffe MEM, Edwards SA. 2005. Influence of individual predisposition, maternal experience, and lactation

environment on the responses of pigs to weaning at 2 different ages. Appl Anim Behav Sci **90:**219–232.

- 34. Olsson IAS, de Jonge FH, Schuurman T, Helmond FA. 1999. Poor rearing conditions and social stress in pigs: repeated social challenge and the effect of behavioral and physiological responses to stressors. Behav Processes **46**:201–215.
- 35. **Oostindjer M, van den Brand H, Kemp B, Bolhuis JE**. 2011. Effects of environmental enrichment and loose housing of lactating sows on piglet behavior before and after weaning. Appl Anim Behav Sci **134**:31–41.
- 36. **Pearce GP, Paterson AM.** 1993. The effect of space restriction and provision of toys during rearing on the behavior, productivity and physiology of male pigs. Appl Anim Behav Sci **36**:11–28.
- 37. Rauw WM. 2013. A note on the consistency of a behavioral play marker in piglets. J Anim Sci Biotechnol 4:1–6.
- Reimert I, Bolhuis JE, Kemp B, Rodenburg TB. 2013. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol Behav 109:42–50.
- Reimert I, Rodenburg TB, Ursinus WW, Kemp B, Bolhuis JE. 2014. Selection based on indirect genetic effects for growth, environmental enrichment, and coping style affect the immune status of pigs. PLoS One 9:1–11.
- 40. Scott K, Taylor L, Gill BP, Edwards SA. 2007. Influence of different types of environmental enrichment on the behavior of finishing pigs in 2 different housing systems. 2. Ratio of pigs to enrichment. Appl Anim Behav Sci 105:51–58.
- Spinka M, Newberry RC, Bekoff M. 2001. Mammalian play: training for the unexpected. Q Rev Biol 76:141–168.
- 42. Studnitz M, Jensen MB, Pedersen LJ. 2007. Why do pigs root and in what will they root? A review on the exploratory behavior of pigs in relation to environmental enrichment. Appl Anim Behav Sci 107:183–197.
- 43. Sztainberg Y, Chen A. 2010. An environmental enrichment model for mice. Nat Protoc 5:1535–1539.
- 44. Sztainberg Y, Kuperman, Y, Tsoory M, Lebow M, Chen A. 2010. The anxiolytic effect of environmental enrichment is mediated via amygdalar CRF receptor type 1. Mol Psychiatry 15: 905–917.
- 45. **Terlouw EMC, Porcher J.** 2005. Repeated handling of pigs during rearing. I. Refusal of contact by the handler and reactivity to familiar and unfamiliar humans. J Anim Sci **83:**1653–1663.
- 46. **Terlouw EMC, Porcher J, Fernandez X.** 2005. Repeated handling of pigs during rearing. II. Effect of reactivity to humans on aggression during mixing and on meat quality. J Anim Sci **83:**1664–1672.
- 47. Trickett SL, Guy JH, Edwards SA. 2009. The role of novelty in environmental enrichment for the weaned pig. Appl Anim Behav Sci 116:45–51.
- 48. Veena J, Srikumar BN, Raju TR, Rao BSS. 2009. Exposure to enriched environment restores the survival and differentiation of newborn cells in the hippocampus and ameliorates depressive symptoms in chronically stressed rats. Neurosci Lett 455:178–182.
- Wemelsfelder F, Haskell M, Mendl MT, Calvert S, Lawrence AB. 2000. Diversity of behavior during novel object tests is reduced in pigs housed in substrate-impoverished conditions. Anim Behav 60:385–394.
- 50. Zimmermann A, Stauffacher M, Langhans W, Wurbel H. 2001. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav Brain Res **121**:11–20.