Judgement bias testing in group-housed gestating sows

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ABSTRACT

Societal concerns about animal welfare have triggered the movement of gestating sows from individual stalls to group housing in many countries. Common methods of assessing sow welfare focus on overt physical ailments, and potentially neglect psychological stressors. A judgement bias task may allow researchers to evaluate an animal's subjective mental or affective state to provide a more comprehensive welfare assessment. Thus, group housed sows were trained to a spatial differentiation task to evaluate their ability to be assessed for individual judgement bias. A total of 45 sows were trained to the task across two replicates, with 24 successfully meeting the learning criteria required to be tested for a judgement bias. In the first replicate, 60% of sows displayed positive bias while 40% displayed negative biases. In the second replicate, 52% of sows displayed positive biases while 33% of sows displayed negative biases. A linear mixed effects model revealed that feed rank affected the latency to approach the ambiguous stimulus (χ^2 (1) = 9.47, p = 0.002) with more dominant animals being more likely to exhibit a positive bias. Given that all sows in the present study were group housed, as well as fed and managed similarly, these findings highlight the complexities underlying judgement bias outcomes.

1. Introduction

Farm animal welfare has become a matter of increased scrutiny over the past 50 years, and as a result, a wealth of biological and psychological research has been performed in an effort to define "good" welfare. Animal welfare can be defined as the biological and psychological state of an animal as it attempts to cope with its environment (Broom et al., 1995). In this definition, welfare includes both pleasurable and unpleasant affective states (e.g., psychological welfare) such as contentment, anxiety, and fear. The housing of pregnant gilts and sows in stalls is currently one of the most controversial animal welfare concerns in the swine industry as the impact of prolonged confinement on the animal's psychological health is poorly understood (Rhodes et al., 2005). Government legislation has banned the practice of housing of gestating gilts and sows in stalls in many countries, including the EU (EU Council Directive, 2001/88/EC), Australia, New Zealand, Canada, South Africa and nine states in the US (Weaver and Morris, 2004; Schulz and Tonsor, 2015). Market initiatives also are impacting gestation stall use as over 60 nationally or internationally branded companies have promised to remove gestation stalls from their US supply chains (Shields et al., 2017)

In group housing, sows can experience a greater freedom of movement and a more complex social environment. Welfare assessment of group-housed sows has predominately been limited to traditional metrics of poor biological welfare including skin lesions, lameness, body condition and productivity (Broom et al., 1995; Arey and Edwards, 1998; Barnett et al., 2000; Bench et al., 2013a,b). While preference, motivation and aversion testing can be used to infer an animal's subjective experience (Kirkden and Pajor, 2006), there is currently no universal method to gauge the affective state, or psychological welfare, in non-human animals objectively. The evaluation of affective state in animals can be deduced from physiological indicators (e.g. heart rate, hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) activity), however, interpretation of these measurements is confounded by the fact that these metrics evaluate the relative arousal level of the animal's affective state, rather than the valence (i.e., positive or negative). For example, an increase in heart rate or cortisol activity all indicate high arousal, but can be associated with escape from predation (negative valence) and with anticipatory behaviors (positive valence).

An alternative metric for assessing psychological welfare in animals may be the judgement bias test. This psychological theory underlying this test proposes that an animal will appraise a certain ambiguous stimulus as predicting either positive or negative outcome depending on the individual's affective state (Harding et al., 2004; Mendl et al., 2009). For example, an animal experiencing a negative state such as

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anxiety or fear will appraise an ambiguous stimulus as threatening and demonstrate a negative or "pessimistic" bias. Judgement bias assessments are frequently carried out via go/no-go tests as they have the advantage of being relatively easy to train and allow for observable responses by the animals. Previous studies have utilized the go/no-go task to assess judgement bias in young piglets housed in barren and enriched pens (Douglas et al., 2012), following social isolation (Düpjan et al., 2013), and following a change to the stocking density (Scollo et al., 2014). Despite this interest, little is known about the effectiveness of using judgement bias testing to assess the affective states of gestating sows.

The current study used a spatial differentiation task to evaluate whether group housed, gestating sows could be trained a go/no-go task and thus be assessed for individual judgement bias as well as to identify factors that impact judgement bias in gestating sows. A subset of the sows in the initial replicate of the study participated in a second replicate and provided the opportunity to evaluate whether sows maintained their biases after one year, as well as investigated whether repetition of the operant conditioning increased an animal's ability to learn the task.

2. Materials and methods

2.1. Animals and housing

At the Swine Teaching and Research Center of the University of Pennsylvania Veterinary School, approximately 120–140 gestating sows are housed in a dynamic group pen and fed via electronic sow feeding stations (ESF, Schauer Agrotronic Compident 7). The sow pen allows for 1.9 m^2 per sow, and includes nine 3.0 × 2.1 m concrete lying areas, and a total of 183.9 m^2 of slatted flooring. Forty PIC 1050 Landrace-Yorkshire gestating sows (mostly parities 4–5) were trained to a go/no-go task and assessed for judgement bias. One year after the first replicate, 21 of the original 40 PIC 1050 Landrace-Yorkshire gestating sows (mostly parities 6–7) were available for re-testing. In order to maximize the number of animals in the study, five additional and naïve sows were recruited into the second replicate. The care and use of all animals in this study was approved by the University of Pennsylvania's Institutional Animal Care and Use Committee.

2.2. Data collection

2.2.1. Operant conditioning

For both the positive and negative stimuli, a single 25.4 cm in diameter metal pet food bowl was filled with 200 g of corn-soy meal and placed in the far left or right corner of a 2.4 × 8.3 m experimental field (Fig. 1). The first 3.7 m of this field was walled with steel gating, while the remainder of the field was walled with cement. The designation of positive versus negative stimulus location (left or right) was randomized for each sow. Using operant conditioning of paired associations, sows were trained to distinguish between the two spatial stimuli based

Fig. 1. Schematic representation of cognitive bias experiment. Sow was held behind gate at starting line. During training, the conditioned feed bowls were place in a location that was either to the sow's far left or right in the arena. Only one feed bowl was present at a time. Testing trials placed feed bowl in the ambiguous stimulus position (?), equidistant from rewarded (+) and punished (-) locations.

on the location of the food bowl. Only one bowl was present in the field during each trial, with a primary researcher standing behind the bowl, holding the red flag behind their back, in order to keep a consistent protocol. This primary researcher kept their gaze forward and did not interact with the sow. A second researcher acted as the animal wrangler; releasing the animal from the starting gate and retrieving the animal from the experimental area after each trial. Animals were trained at approximately the same time each day (1000–1400) for a maximum 12 trials or a maximum time of 30 min. The order in which the animals were trained and tested was dependent upon the time of their last meal, in order to ensure that each animal was trained at least 4 h after eating their daily feed at the ESF stations.

On the first day of training, the sows were conditioned to approach the bowl in the positive location over a series of ten trials. Approach was defined as the latency for the sow to leave the starting gate and cross the goal line of a 1 × 1 m area containing the feed bowl with at least one front limb (snout was within 12.7 cm of the bowl). The distance from the starting gate to the goal line was 7.3 m. If the sow approached the positive stimulus, she was allowed to consume the corn-soy feed for 10 s. The second day of training began with a positive trial for reference, followed by a minimum of four negative trials, and ending with one positive trial. If the sow approached the feed bowl in the negative stimulus location, the standing researcher would wave a red flag over the bowl for 20 s, preventing the sow from consuming the feed. A 20 s duration for the flag wave was chosen as this was sufficient time for a secondary researcher to guide the sow out of the experimental arena. The third and fourth training day began and ended with a positive trial, with eight randomized positive or negative trials in between. Sows were considered trained if they approached the positive stimulus within 20 s if they did not approach the negative stimulus for at least 50 s over at least three trials on the last day of training (Fig. 2). We chose 20 s as maximum latency for "go" response based on average walking speed to approach bowl from starting gate for a sow. The minimal successful no-go response of 50 s of avoidance of the negative cue was determined empirically based on observation of the sows and distribution of the data (see Fig. 2A).

2.2.2. Judgement bias testing

The experiment day included three trials: a positive trial, a negative trial, and an ambiguous trial. In this ambiguous trial, the feed bowl was placed in the middle of the experimental field, equidistant to the positive and negative stimuli (Fig. 1). The latency of approach to this ambiguous stimulus was recorded for a maximum time of 60 s (i.e., no-go).

2.2.3. Production data

On the day of judgement bias testing the sow's current duration of gestation was recorded as well as her parity (number of previous litters she had farrowed). Feed order data was also collected during the week of training and testing. The time of day that each sow entered the electronic sow feeding stations (ESF) was recorded automatically by ESF computer software. Each sow was assigned a daily feed order based on the time she entered the feeder, and consumed the majority of her daily feed allotment. A feeding rank value was calculated for each individual sow by averaging her daily feed order over the 7 days bracketing the sow's training and testing.

2.3. Data analysis

2.3.1. Judgement bias index

A judgement bias index (JBI) was calculated for each animal that was trained by day five. The index can range from 0 to 1, with 0 reflecting a response to the ambiguous stimulus analogous to a negative conditioned response, and 1 being analogous to a positive conditioned response, and is defined by the following equation:
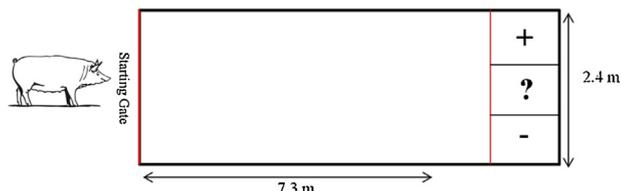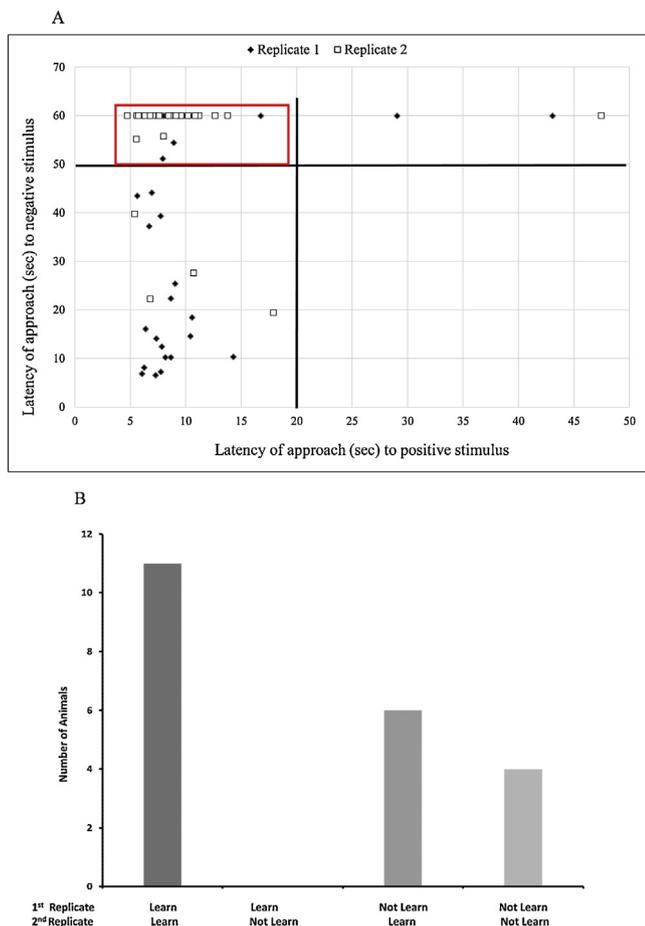
Fig. 2. Performance of group-housed gestating sows in operant conditioning task. (a) Learning criteria for go/no-go task was based on speed of approach to positive and negative stimuli on final day of training. Sows which approached the positive stimuli in < 20 s and avoided the negative stimulus for > 50 s met the learning criteria are outlined in red box. Almost all of the sows learned to rapidly approach the positive cue, but many still failed to avoid the negative cue. (b) Impact of previous training. Eleven sows which met learning criteria in the first replicate also met the criteria in the second replicate. No sows met the learning criteria in the first replicate, but not in the second. Six sows which did not learn in the first replicate did learn in the second replicate. Four sows did not meet the learning criteria for go/no-go task in both replicates. Key: (♦) – First replicate; (☐) – Second replicate.

$$JBI = 1 - ([L_{amb}-L_{pos}]/[L_{neg}-L_{pos}])$$

where:

L_{amb} = approach latency for ambiguous stimulus (sec)
L_{pos} = approach latency for positive stimulus (sec)
L_{neg} = approach latency for positive stimulus (sec)

The JBI index was designed to normalize a sow's response to the ambiguous stimulus based on her responses to the positive and negative stimuli and interpreted that a score of less than 0.2 was a negative bias and greater than 0.8 was a positive bias. This elucidation of the index was empirically determined as the sow behaviors largely diverged into two populations that clustered within two deciles of the theoretical minimum value of the index (if an animal's ambiguous cue latency was identical to their negative conditioned latency) maximum value (if an animal's ambiguous cue latency was identical to their positive conditioned latency).

2.3.2. Comparison of latency means

An independent-samples *t*-test was conducted to compare latencies to approach for either the positive or the negative cue between animals

that exhibited either a positive or negative bias approaching the ambiguous cue.

2.3.3. Modeling of study outcomes

A linear mixed effects analysis was implemented in R (R Core Team, 2012) using the lme4 package (Bates et al., 2012) to better understand the relationship between key variables and to identify factors that influence sow behavior related to operate conditioning and judgement bias. For operant conditioning, a model was developed to predict the outcome of training. Specifically the latency to approach the negative conditioned stimulus was modelled as it was the best differentiator between sows that learned or failed to learn the go-no go task. Given that repeated testing of ambiguous trials can lead to a habituation of cognitive bias test in animals (Doyle et al., 2010), we chose to consider response latency as a differentiator rather than proportion of response to each conditioned and ambiguous trials. This is similar to the Stroop effect recently examined in rhesus macaques, where emotional valence can impact the speed of cognitive processing (Bethell et al., 2016). With regard to the judgement bias task, both the latency to approach the ambiguous stimulus and our judgement bias index (JBI described above) were selected as outcome variables for the models. A similar model structure was used for each outcome variable which included as fixed effects, sow feed rank, days of gestation at testing, and prior learning experience as well as interactions between these fixed effects and as random effects, sow parity and replicate number. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question.

3. Results

3.1. Operant conditioning of group housed gestating sows

A large variation was observed in the ability of group housed sows to learn the go/no-go task over the brief 4 day training period (Fig. 2). In the first replicate, 20 of the 40 sows trained demonstrated sufficient learning ability on experiment day 4 to be tested for judgement bias on experiment day 5. During the second replicate, conducted one year later, 21 of the 26 sows subjected to the training regime achieved the learning criteria. Trained sows approached the positive conditioned stimulus in < 20 s and refrained from approaching the negative conditioned stimulus for at least 50 s (Fig. 2A – upper left hand quadrant; mean L_{pos} = 8.7 ± 0.4 s and mean L_{neg} = 59.3 ± 0.3 s; n = 41). Of the remaining 'non-trained' sows, majority of the sows learned to respond appropriately to the positive conditioned stimulus but failed to be deterred by the negative conditioned stimulus. Eighteen of the 20 animals in the first replicate and five of six animals in the second replicate were unable to learn to stay away from the negative stimulus for at least 50 s (Fig. 2 A – lower left hand quadrant; L_{pos} = 8.5 ± 0.6 s and mean L_{neg} = 20.8 ± 2.7 s; n = 22). This is in contrast to only two sows in the first replicate and one in the second replicate that learned to avoid the feed bowl given the negative stimulus, but failed to learn to approach the positive stimulus in the required time (Fig. 2 A – upper right hand quadrant; L_{pos} = 46.5 ± 11.3 s and mean L_{neg} = 60 s: n = 3). No animals in either replicate failed both criteria.

In the second replicate, 21 of the original 40 animals were available for retesting (Fig. 2B). All 11 animals that had met learning criteria in the first replicate also met learning criteria in the second replicate. Six animals that failed to train in the first replicate were able to meet the learning criteria in the second replicate whereas four animals failed to train in both years. Note that no animals succeeded the training regime in the first replicate but then failed to train in the second replicate. A linear mixed effects model revealed that prior learning experience affected the latency to approach the negative stimulus (χ^2 (3) = 7.90, p = 0.048), as previous exposure to training delayed the approach to
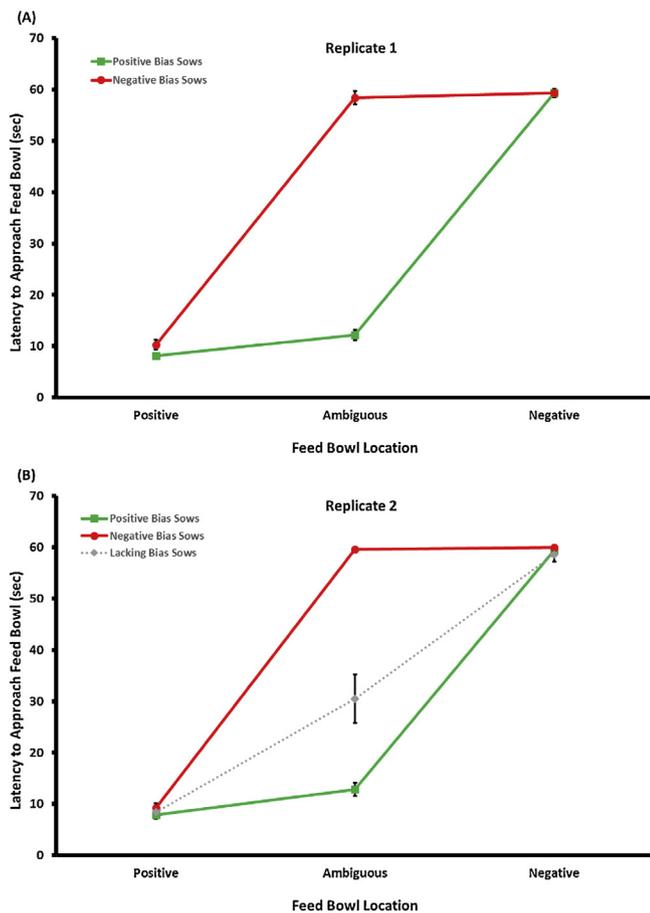
Fig. 3. Performance of group housed gestating sows in a judgement bias task. (a) Mean latency for sows to approach the positive, ambiguous, and negative stimuli is displayed for animal exhibiting either a positive bias (green dots; $L_{amb} < 20$ s) or a negative bias (red dots; $L_{amb} > 50$ s) during the (a) first and (b) second replicate of the study. Both positive and negative bias animals approach the positive and negative conditioned stimulus with similar latencies independent of their judgement bias. Standard error of the mean (s.e.m) is also depicted for each data point when larger than the respective symbol. Findings from the three sows that failed to express a clear bias (L_{amb} greater than 20 but less than 50 s) are shown in grey for the second replicate.

the negative stimulus by 17.9 ± 4.3 s. Neither feed rank nor day of gestation yielded a statistically significant impact on latency to approach the negative stimulus.

3.2. Judgement bias of group housed gestating sows

3.2.1. Latency to approach to valenced cues

Distinct differences were observed in the response of group housed sows to a judgement bias task where animals were first trained to differentiate a positive versus negative conditioned stimulus and then presented with an ambiguous stimulus (Fig. 3). In the first replicate, all animals exhibited a latency to approach the ambiguous stimulus that was either < 20 s ($L_{amb} = 12.2 \pm 1.0$ s, n = 12) or > 50 s ($L_{amb} = 58.4 \pm 1.3$ s, n = 8; Fig. 3A). This suggests that individual sows expressed a bias by judging the ambiguous stimulus as either a positive or negative conditioned stimulus based on the performance criteria established in the operant conditioning phase of the study. Animals that expressed a positive bias exhibited no difference in either their latency to approach the negative or positive cue when compared to those animals that expressed a negative bias. There was not significant difference for the approach to the positive conditioned stimulus between positively biased sows ($L_{pos} = 8.1 \pm 0.4$ s, n = 12) versus negatively

biased sows ($L_{pos} = 10.3 \pm 1.0$ s, n = 8) (t(9) = -2.10, p = 0.07) nor for the approach to the negative conditioned stimulus (positively biased sows: $L_{neg} = 59.3 \pm 0.7$ s, n = 12 versus negatively biased sows: $L_{neg} = 59.3 \pm 0.8$, n = 8; (t(18) = -0.04, p = 0.97)).

Similar results were observed in the second replicate when comparing latency to approach to the ambiguous cue between sows displaying a positive bias ($L_{amb} = 12.8 \pm 1.3$, n = 11) versus sows displaying a negative bias ($L_{amb} = 59.6 \pm 0.4$, n = 7; Fig. 3B). Again in the second replicate, animals judging the ambiguous stimulus as positive exhibited no difference in either their latency to approach the negative or positive cue when compared to those that judged the ambiguous cue as negative. Sows approaching the positive cue with a positive bias ($L_{pos} = 7.9 \pm 0.8$ s, n = 11) were not different from those with a negative bias ($L_{pos} = 9.2 \pm 1.0$ s, n = 7) (t(10) = -1.33, p = 0.21))or sows approaching the negative cue with a positive bias ($L_{neg} = 59.4 \pm 0.5$ s, n = 11) versus sows with a negative bias ($L_{neg} = 60.0 \pm 0.0$, n = 7) (t(13) = -1.08, p = 0.30). Interestingly, however, in the second replicate three animals which met the learning criteria exhibited a mean latency to approach the ambiguous stimulus of 30.5 ± 4.7 s (range = 23.9 to 39.5 s); which is not a clear bias based on our criterion for judging a stimulus as either positive or negative. A linear mixed effects model revealed that feed rank affected the latency to approach the ambiguous stimulus (χ^2 (3) = 16.73, p = 0.0008) by changing this latency to approach by about 8.7 ± 2.4 s for every ten places in the feed rank with animal eating earlier in the day taking less time to approach the ambiguous stimulus and more likely to express a positive bias. Neither day of gestation nor prior learning experience yielded a statistically significant impact on latency to approach the ambiguous stimulus.

3.2.2. Judgement bias indices

Across both replicates, sows largely separated into one of two responses that we interpreted as either a negative bias (JBI < 0.2) or positive bias (JBI > 0.8) (See Fig. 4). In the first replicate, eight sows exhibited a negative bias ($JBI_{neg} = 0.02$ +/- 0.01) whereas 12 sows responded with a positive bias ($JBI_{pos} = 0.92$ +/- 0.02). Similarly in the second replicate seven sows had a negative bias ($JBI_{neg} = 0.01$ +/- 0.01) while 11 sows displayed with a positive bias ($JBI_{pos} = 0.90$ +/- 0.08). In addition, three animals in the second replication had JBI's that ranged between 0.4 and 0.7 with a mean of 0.57 ± 0.09. A linear mixed effects modeling revealed that feed rank affected the JBI (χ^2 (3) = 16.43, p = 0.0009). The JBI increased 0.17 ± 0.06 for every ten place change in feed rank, with animals eating earlier in the day having a higher JBI and more likely to express a positive bias. Neither day of gestation nor prior learning experience yielded a statistically significant impact on the JBI.

One year after the first replicate, 21 of the original 40 sows were available for re-testing. In the first replicate ten of these animals failed to meet training criteria, whereas 11 met the training criteria and were tested with an ambiguous cue. Six of these animals judged the ambiguous cue in the first replicate as positive (JBI = 0.90 ± 0.04) whereas 5 viewed it as negative (JBI = 0.02 ± 0.02). All 11 of these animal meet the training criteria in the second trial and were retested with an ambiguous cue in the second replicate (Fig. 5). Three of original positive animal maintained a positive bias (JBI = 0.95 ± 0.03) in the second trial while one of the original positive animals tested negative (JBI = 0) and two others failed to express a clear bias (JBI = 0.51 ± 0.12) in second replicate. Of the five original negative animals, four remained negative (JBI = 0.01 ± 0.01) in the second replicate and one expressed a positive bias (JBI = 0.82) in second replicate. Of the 10 sows that failed to learn the task in the first replicate, six were successful in training a second time and yielded four positive biases (JBI = 0.90 ± 0.05), one negative bias (JBI = 0), and one failed to establish a bias (JBI = 0.67) in the second replicate. Thus, the judgement biases re-assessed in the second replicate were eight positive biases (JBI = 0.91 ± 0.03), six negative biases (JBI = 0.01 ± 0.01),
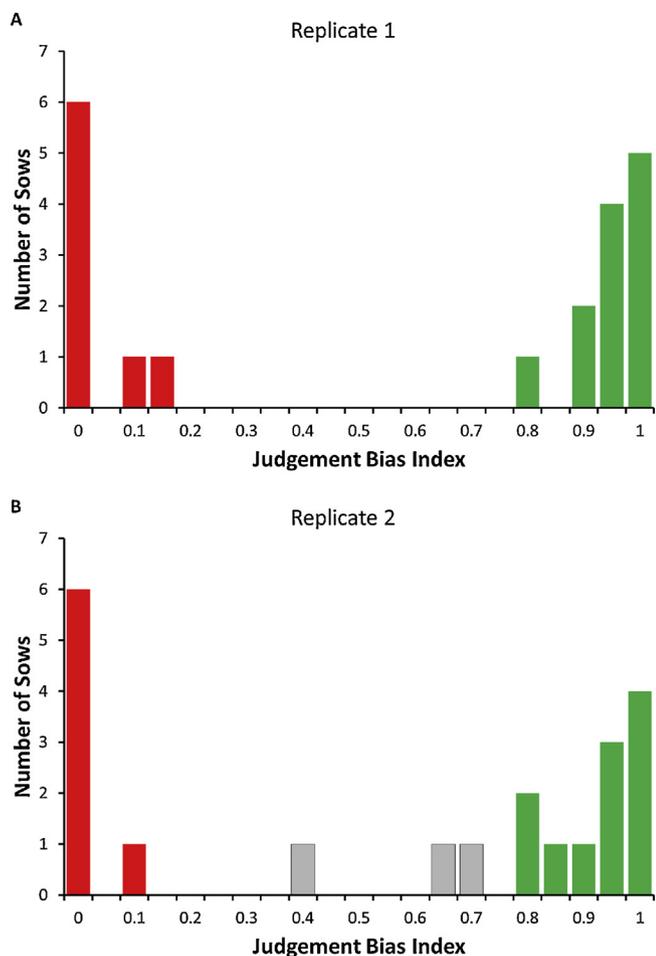
Fig. 4. Predominantly bimodal distribution of judgement bias index (JBI) across (a) first and (b) second replicates. Sows displaying positive biases (JBI > 0.8) are depicted with green bars and sows with negative biases (JBI < 0.2) are in red. Sows failing to exhibit a clear positive or negative bias (JBI ranging between 0.4 and 0.7) are shown in grey.
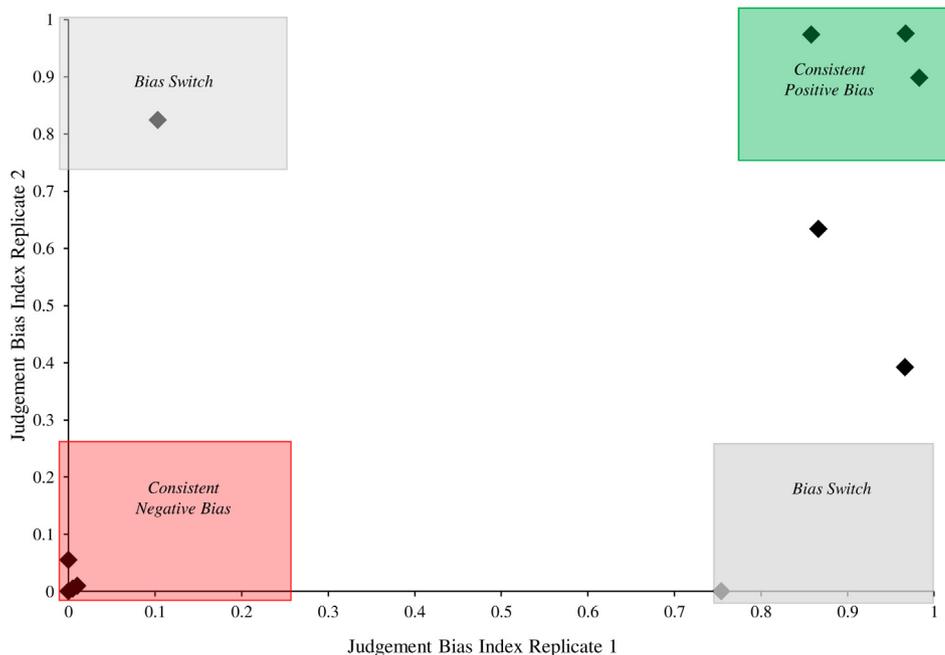
three that failed to establish a bias (JBI = 0.57 ± 0.09) and four not trainable both years.

4. Discussion

To our knowledge this is the first application of a judgement bias task to probe the affective state of mature swine, and in particular, gestating sows. A significant challenge with judgement bias testing is interpreting the results. Despite the growing body of work describing the application of judgement bias tasks to the assessment of animal welfare, many questions remain about the methodology and implications of an animal's behavior in this task (Roelofs et al., 2016). In order to address these issues, we will first discuss the caveats or limitations of our approach and then our interpretation of the results.

4.1. Limitations

4.1.1. Sow training

Due to logistical constraints on the study, we used a relatively brief training procedure (4 days) for the sows. Nearly 50% of the sows in the first cohort learned the go/no task in the allotted time while closer to two-thirds of the sows were trained successfully in the second cohort. Repeat training did increase the number of sows learning the task as most, but not all, of the sows that were trained for a second time achieved our learning criteria. However there did remain four animals that never learned the task even after a second year of training. Our training protocol likely selected for sows that were "fast" learners. One of the challenges with judgement bias testing is that researchers can only test the animals which can be classically conditioned to learn the task. Therefore, such studies are inherently biased toward including a subset of the initial study animals.

Go/no-go behavioral responses have the advantage of being relatively easy to train and allow for observable responses by the animal. The type of reinforcers used in the training of conditioned stimuli may influence the sensitivity of the judgement bias test, questions it addresses as well as its interpretation. Food reinforcers have been used in all animal studies to date but are vulnerable to the effects of individual differences of feeding motivation. Tests using reinforcers that are similar in affective terms (e.g. small vs. large quantity of food) may fail to reveal judgement biases. Tests using positive and neutral reinforcers

Fig. 5. Consistency of judgement bias between replicates. Twenty-three sows were available for training and testing in both replicates. Eleven sows met the learning criteria in both replicates. The majority of these sows exhibited a consistent bias from year to year. Three sows maintained a positive bias in the second trial, while four sows maintained a negative bias in the second replicate. One sow switched from positive to negative bias and one sow switched from a negative to positive bias in the second replicate. Two sows failed exhibit a clear bias in the second replicate.

(e.g. food vs no food) focus on changes in anticipation of positive events (i.e., depression), while the use of negative and neutral reinforcers targets changes in anticipation of negative events (i.e., anxiety). For that reason, we intentionally used clearly positive and negative re-inforcers (e.g., reward and punishment) as they engage a number of affect-related influences on decision making. Future research may evaluate the effects of different neutral, positive and negative reinforces on affective state assessment, and well as investigate other types of positive reinforcers (such as access to social mates, enrichment items).

Almost all of the sows that failed to learn the task did not demon-strate the appropriate restraint for the negative conditioned stimulus. This indicates that the aversive consequence (flag wave, deny access to feed) failed to be a sufficient deterrent to these twenty-two sows. Only three sows failed to learn to approach the positive stimulus quickly suggesting that, perhaps for these animals, the negative reinforcer may have been interpreted as extremely aversive that they refused to ap-proach any cue. These failures emphasizes the possible importance of individual differences when it comes to choosing species-specific re-wards and punishments for assessing judgement biases and highlights how more attention needs to be paid to the individual experience of different sows during judgement bias testing, such as age, size, duration in the pen and behavioral tendencies.

4.1.2. Feed motivation

As standard for commercial swine, all gilts and sows in the present study were fed approximately 30% of ad-libitum consumption every day, which equals to about 2.3 kg of corn-soy feed (Patterson-Kane et al., 2011). This means that the sows were most likely never sated following their feeding time at the ESF station. However, to ensure that each animal was motivated to engage in the operant task for access to the feed reward, all animals were trained and tested at least 4 h after their feeding time. While there is the potential for individual differences in food motivation to contribute to the variation in behavioral re-sponses exhibited by the gilts and sows, the experimental design of the present study aimed to keep the level of hunger as consistent as possible across the study animals.

4.1.3. Repeat of ambiguous stimulus

Some of the previous studies on judgement bias in swine utilized multiple ambiguous cues as the judgment test in order to compare the percent of approach or response among the differently-valence stimuli (Douglas et al., 2012; Düpjan et al., 2013; Murphy et al., 2013; Scollo et al., 2014). As previously stated, the animals in the present study were fed the standard commercial diet of approximately 30% of ad-libitum consumption every day. By the time of operant conditioning, four hours after their daily feed time, each animal demonstrated strong interest in gaining access to the feed reward. This strong food motivation influ-enced the animals' response to repeated presentations of the un-rewarded ambiguous stimulus. Murphy et al. (2013) suggest that the decreased frequency and speed of response by both commercial and Göttingen minipigs in their repeated tests was most likely due to the animals' learning about the outcomes of ambiguous trials, rendering them no longer ambiguous. Doyle et al. (2010) also found that sheep reduced their responsiveness to ambiguous stimuli with repeated testing over weeks and suggest that there may be a limit on the re-peatability of the judgement bias test. Because animals can learn that the ambiguous stimuli are unreinforced, some studies have used partial reinforcement of reference locations (Bateson and Matheson, 2007; Matheson et al., 2008). In the present study, we chose to use the first response to the first ambiguous stimuli as the unit of measurement in order to assess the animals' appraisal of a truly ambiguous stimulus.

4.1.4. Changes in feed rank over time

The order through which individual sows enter and eat at the ESF station has been reported to be correlated with her social rank within the group such that high ranking sows eat earliest and low ranking sows eat latest in the feeding cycle (Chapinal et al., 2008; O'Connell et al., 2003). It is also believed that social hierarchy in the pen can be related to both weight and age of the animal (parity) (Arey, 1999). Thus it is possible to hypothesize that part of the effect that we see on the animals that were tested two years in a row might be related to their increase social status and presumably change feed rank during the second year of the study. Interestingly of the eleven animals tested for judgement bias in both years of the study only six of the sows improved their feed rank while the remaining five ate later in the day than they did during the first year. Clearly there are many other factors such as who the other sows are in the pen that contribute to feed order at an ESF station in pen of gestating sows.

4.2. Interpretation of results

4.2.1. Judgement bias differences

Sows in our study exhibited both positive and negative judgement biases. Judgement bias has been used previously assess the affective state of animals living in either enriched or barren environments (Douglas et al., 2012; references from other species). However all sows in the present study were housed similarly and with some degree of enrichment (mobility, social interaction, and access to straw). Thus, we would argue that judgement bias outcomes have a more complex de-terminant than the influence of housing type alone. Each sow experi-ences her environment differently; which can result in different affec-tive states. Such individual differences add complexity to the challenges of improving sow welfare when these animals are housed in groups. This may serve as a cautionary tale for future judgement bias research, where significant weight is given to the environment as a sole factor in influencing individual affective state. The results of the present study raise the possibility that, like with growing pigs (Asher et al., 2016), individual differences in coping styles of sows may play a role when assessing affective state; with certain individuals predisposed to cope better in complex environments (Horback and Parsons, 2016). These apparent differences in affective state also serve to highlight the chal-lenges in designing and implementing group housing systems to meet the potentially underappreciated diverse needs of gestating gilts and sows.

It is also important to realize that judgement bias in these sows, defined by differences in rate of approach to an ambiguous stimulus, was not related to their overall speed or activity levels. The experi-mental design allows us to quantify the approach latencies to positive, negative, and ambiguous stimuli. All sows which met the training cri-teria exhibited similar latencies to approach the both conditioned ne-gative and positive stimuli independent of whether they had a positive or negative bias.

4.2.2. Role of social hierarchy

Sows eating early in the day the electronic sow feeder were more likely to have a positive bias in our study. Feed rank or order is believed to be related to social hierarchy in group housed sows (Chapinal et al., 2008; O'Connell et al., 2003). Exactly how feed rank relates to social hierarchy, or if individual preferences for various limited resources such as feed, resting areas, and enrichment might result in multiple hierarchies is unknown. Even with this limitation, it is interesting to consider if the animals with higher feed rank have shorter latencies and higher JBI as a consequence of their more positive affective state or because of other underlying causes. One possibility is that a high ranking sow may have had the previous experience of having privileged access to feed when being successful at eating earlier in the day. If the high feed ranking sows were more positively inclined about feeding in general then we might expect to see a relationship between feed rank and the latency to approach the positive cue, but we did not. The im-pact of feed rank on JBI further highlights the complexity in inter-preting the outcomes of judgement bias tasks.

4.2.3. Temporal consistency of judgement bias

More sows were successful at learning the judgement task with repeated training. Sows that were able to learn the task both years needed fewer trials in order to learn the paradigm than sows unable to learn in the first replicate. Consistent with the notion of swine being intelligent mammals (Kornum and Knudsen, 2011), the results from the second replicate suggest that the sows retained knowledge about the conditioned stimuli during the training hiatus. It is also interesting that the majority of sows retained their JBI for one year from the first replicate to the second replicate. Three of the six sows that were initially positive remained positive while four of five sows that were originally negative remained negative. Two animals failed to express a bias in the second trial, but only two animals actually reversed their bias from replicate one to two. One switched from positive to negative and one from negative to positive. Why two animals exhibited a positive bias in the first replicate, but failed to express a bias in the second replicate is unclear. However, it is possible, as discussed above, that failure to reward the ambiguous cue which they interpreted as positive in the first replicate may have influenced their assessment of the ambiguous cue in the second replicate. The fact that two animals reversed their judgement biases highlights how the assessment of affective state in swine may not be a permanent element of the animal's welfare, and may be transient as other environmental and social variables fluctuate.

5. Conclusions

Many of the commonly used metrics for farm animal welfare such as skin lesions, lameness, and body condition scoring focus on the physical welfare of the animal and are useful in quantifying negative affective states (e.g. degrees of bad welfare). However, measurements that address the psychological welfare of farm animals are much less common and as such it is much harder to measure positive affective states (e.g. degrees of good welfare). In the present study, twenty-four gestating sows were trained successfully to a go/no-go task in order to evaluate their judgement bias toward an ambiguous stimulus. A range of responses was observed, indicating that sows living in the same large group pen can experience both positive and negative affective states. Given that all sows in the present study were housed similarly, we would argue that the influence of housing conditions on psychological welfare is not the same for all sows. Each sow copes with environment and social stress differently and thus experiences different affective states. Such individual differences add complexity to the challenges of improving sow welfare when these animals are housed in groups and are likely important to understanding the affective state of gestating sows. Continued research in this area promises to help determine optimal group composition for loose housing systems based on individual traits in order to improve animal welfare.

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