Biomarkers of Pigs' Emotional Valence

Subjects: Agriculture, Dairy & Animal Science

Contributor: Michelle Hebart

It is widely recognized that the assessment of animal welfare should include measures of positive emotional (affective) state. Existing behavioral and physiological indicators of a positive affective state frequently lack sensitivity, objectivity or are unsuitable in a production environment. Therefore, there is a need to develop new approaches to accurately and objectively measure a positive emotional state in animals, including novel molecular markers such a miRNA. These biomarkers must be measurable in the peripheral circulation and provide an accurate account of the physiological and molecular activity in regions of the brain associated with emotional processing. Further, such markers require validation against established behavioral and physiological indices. Here we investigated the efficacy of circulating miRNA as biomarkers of emotional state in the pig.

Keywords: welfare ; biomarkers ; positive affective state ; miRNA ; pigs

1. Introduction

The assessment of emotional or affective state in animals can be challenging, particularly the assessment of positive emotion since there are fewer identified behaviors or biomarkers specific to these states. Emotions have been operationally defined as “specific, intense and short-lived responses to stimuli” whilst mood refers to “longer, more ambiguous, and nonattributable affective feelings of lower intensity” [1][2], both of which can vary along two main axes, including arousal, or strength of response, and valence (direction of response, being positive or negative) [3]. Emotions are recognized as complex, multifaceted phenomena, that give rise to rapid physiological and behavioral changes which likely evolved to achieve goals related to survival, such as attainment of valuable resources/rewards and avoidance of harm/punishment [4]. Animal welfare encompasses a long-lasting state comprising the summed-up experiences of the individual [5] and can be defined in terms of affective states and their relative weighting over time [6]. Therefore, the assessment of animal welfare should include measures of animal emotion [7]. However, in order to study animal emotional state, it is first imperative to identify methods that accurately and objectively measures the emotional state of animals.

A number of physiological and behavioral indices are currently used to infer the emotional state of animals. For example, physiological indices including hypothalomo–pituitary–adrenal axis (HPA) activity, sympathetic and autonomic functioning, endocrine function, as well as behavioral parameters have been used as makers of emotional state in animals. However, although these measures can indicate emotional arousal, they are often unable to distinguish between the valence or direction of the emotion being elicited. Furthermore, these measures tend to relate to negative affect, with less focus on, and development of, indicators of positive emotional state [8]. One assessment tool recently shown to have value in this respect is the judgment bias test (JBT), which use an animal’s behavioral response as an indicator of its underlying affective state in response to an unknown stimulus. [9]. Animals first learn to discriminate between a positive stimulus, such as a high value reward, and an aversive or nonrewarding stimulus, such as no reward or punishment [10]. Once animals have learnt to discriminate between positive and aversive stimuli, they are then tested on an ambiguous stimulus, intermediate between the two learned stimuli. These tests are based on the assumption that if, under ambiguity, the animal behaves in a manner normally associated with a positive reward, that animal has an enhanced expectation of a positive outcome that, thus, implies a positive emotional state [11]. Conversely, if the animal displays behaviors consistent with an aversive outcome, that animal has reduced anticipation of a positive outcome, which implies the animal is in a negative affective state [11]. The JBP has been used successfully in a variety of species including rats [12], sheep [13], dogs [14], chickens [15], and pigs [16][17][18], but while JBPs are considered to have good validity [19], they are less suited to production environments due to the time it takes to train animals to perform the test [20]. There is therefore an urgent need to identify and validate objective physiological or molecular markers of positive affect [21][22], in order to complement or even replace existing behavioral and physiological measures [23][24]. Following validation, new technologies may be able to be developed to analyze these biomarkers rapidly on farm using relatively noninvasive sampling, thus making them applicable for production environments (i.e., sensor-based technologies in blood or saliva).

MiRNA are small, noncoding RNA molecules involved in the regulation of genes post-transcriptionally. These molecules are ubiquitous throughout the body, including the brain, and are involved in the regulation of genes, including those associated with emotional processing [22]. For example, dysregulations of specific miRNAs have been used as diagnostic tools for a number of psychological conditions including anxiety [25][26], major depressive disorder (MDD) [27], post-traumatic stress disorder (PTSD) [28], bipolar disorder [29], and schizophrenia [30]. These molecules are involved in the regulation of emotional processes, and are released into the circulation, enabling measurement in the blood, urine or saliva [31][32]. As a result, they have the potential to be biomarkers of the activity associated with emotional processing, including those neuronal systems involved in the regulation of positive emotions such as the serotonergic and dopaminergic reward pathways [22][33][34]. For example, miRNA-16 has recently been implicated in the modulation of serotonergic transmission in the mouse brain [35]. In another mouse study, specific miRNAs, including miRNA-212, were shown to regulate the motivational properties of drug addiction within the prefrontal cortex (PFC) and striatum following the self-administration of addictive drugs [36]. Nevertheless, most miRNA research conducted in humans and rodents has focused on negative physiological or disease related conditions [37], including neuropathic pain or psychological conditions that can impact emotional state. Few studies have investigated miRNA with the specific intention to identify miRNA as correlates of positive emotional state, and to our knowledge no such studies have been conducted in pigs.

To identify and validate novel measures of positive emotion in the pig, including molecular markers such as miRNA, requires an accurate assessment of different affective states in the animal as well as the implementation of a robust means to manipulate affective state in a controlled experimental setting. Husbandry practices are known to influence production outcomes and impact welfare parameters. For example, increased floor space was shown to produce healthier pigs with high immunity and increased comfort and play behavior [38]. Pigs that are socially isolated from pen mates have shown increased behaviors indicative of stress and a decrease in behaviors indicative of positive welfare such as play [39]. The provision of enrichment to animals in farmed systems is suggested to improve biological functioning, as well as increase overall wellbeing, as it allows the animal to perform rewarding and motivated species-specific behaviors [40][41]. Furthermore, the provision of enrichment to pigs has been shown to induce a positive judgment bias compared to animals housed in barren systems, suggesting pigs provided enrichment were in a more positive emotional state [16].

2. Behaviour Data

2.1. Identification of Positive and Aversive Cue

During the learning phase (weeks 1–10) pigs were able to successfully identify the positive cue as shown by the decreased mean latency to approach the positive cue over time (χ^2 (9) = 117.7, p = 0.000, **Figure 1**A). During the learning phase from weeks 6–10 there was a significant difference in the latency towards the aversive cue over time (χ^2 (4) = 12.99, p = 0.012, **Figure 1**B). During week ten of training the latency to approach the positive cue was significantly lower compared to the aversive cue (Z = −5.8, p = 0.000, **Figure 2**).

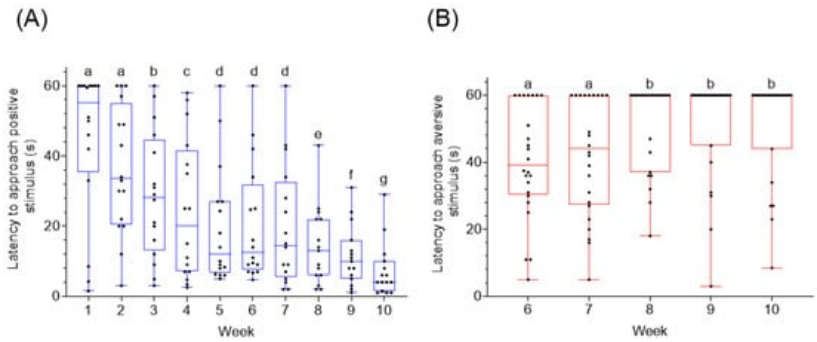

Figure 1. (**A**) Latency to approach (s) the positive stimulus during training weeks (1–10) in pigs (n = 24), (**B**) indicates latency to approach aversive stimulus during training weeks (6–10) in pigs (n24). Data are medians with range. Significant difference is indicated with differences in subscripts (p < 0.05).

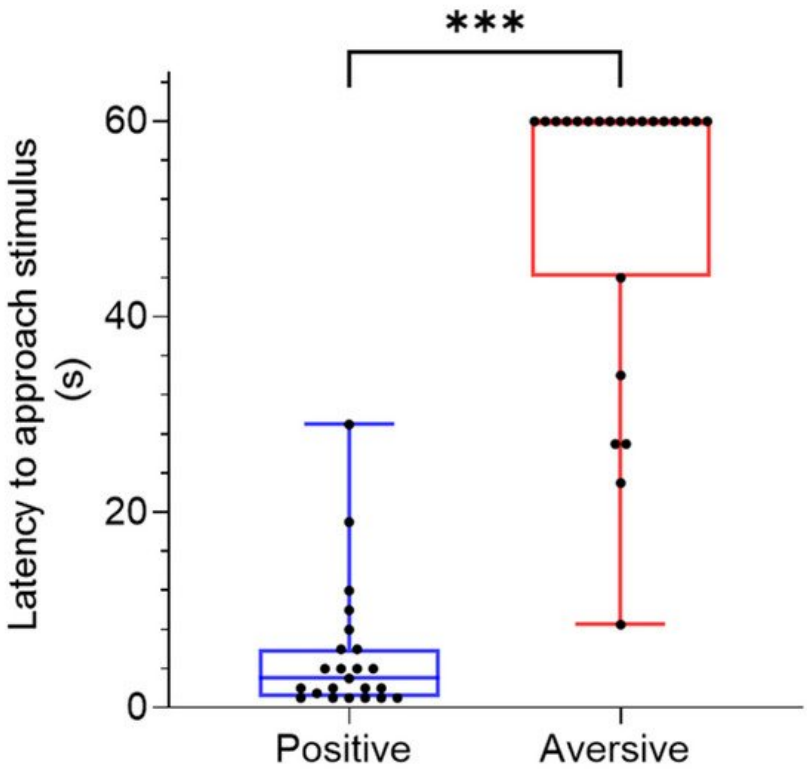

Figure 2. Latency to approach (s) positive and aversive stimulus at week ten of training in pigs (*n* = 23). Data are medians with range. Significant difference is indicated with presence of asterisk ($p < 0.05$).

2.2. Cue Location and Latency to Approach

An overall effect of cue location on latency to approach was observed in all pigs in both JBT1 and JBT2 ($\chi^2(2) = 21.7$, $p = 0.000$; **Figure 3**). During JBT1, an increased latency to approach was observed towards the aversive location compared to both the ambiguous ($Z = -404.0$, $p = 0.000$) and positive ($Z = -3.88$, $p = 0.000$) locations. Pigs further had increased latency towards the ambiguous location compared to the positive location ($Z = -3.6$, $p = 0.020$; **Figure 3**A). During JBT2, an increased latency to approach was observed towards the aversive location compared to both the ambiguous ($Z = -3.99$, $p = 0.000$) and positive ($Z = -3.7$, $p = 0.001$) locations, but no increased latency towards the ambiguous location compared to the positive location was observed ($Z = -1.4$, $p = 0.16$; **Figure 3**B). Between JBT1 and JBT2, there was no difference in latency to approach the ambiguous location in pigs exposed to either enriched or barren housing treatments ($Z = -1.2$, $p = 0.250$ and $Z = -1.22$, $p = 0.360$ $p = 0.36$; **Figure 3**C). There was no significant effect of treatment on latency towards the ambiguous cue during JBT2 ($Z = 2.11$, $p = 0.48$; **Figure 3**D).

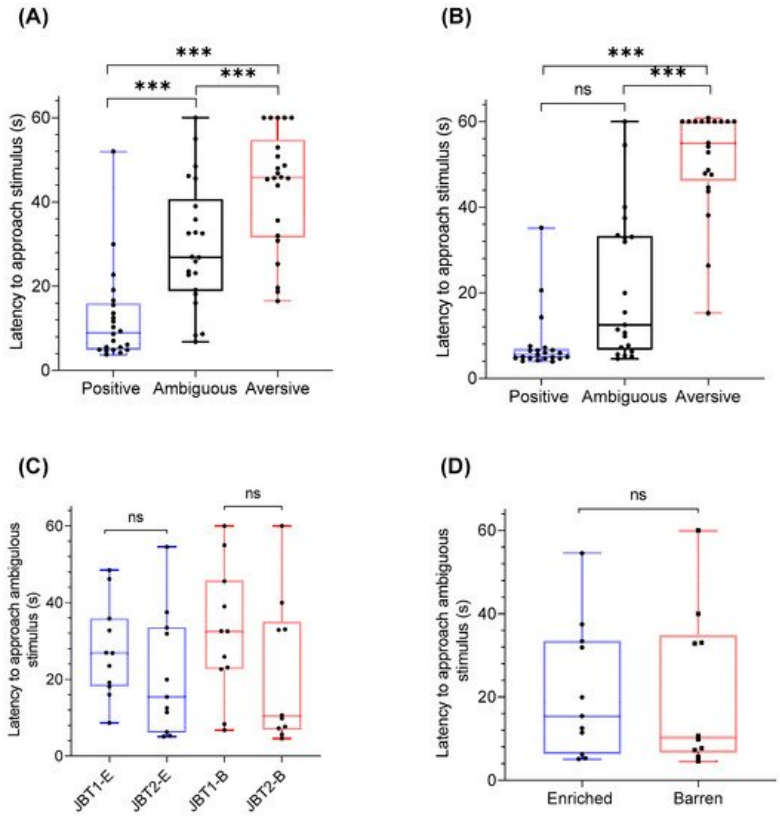

Figure 3. (**A**) Latency for pigs (*n* = 23) to approach positive, aversive and ambiguous stimulus at judgment bias test 1 (JBT1), (**B**) latency to approach positive, aversive and ambiguous stimulus in judgment bias test 2 (JBT2) in pigs exposed to enriched housing (*n* = 12), or barren housing (*n* = 11), (**C**) indicates latency to approach ambiguous stimulus between JBT1 and JBT2 in pigs exposed to enriched (*n* = 12), or barren housing (*n* = 11), and (**D**) indicates latency to approach ambiguous stimulus during JBT2 in pigs exposed to enriched (*n* = 12) or barren housing (*n* = 11). Data are medians with range. Significant difference is indicated with presence of asterisk ($p < 0.05$).

2.3. Treatment Effects on Judgment Bias

No effect of treatment on JBI between JBT1 and JBT2 was observed (χ^2 (20) = 2.0, $p = 0.5$).

2.4 Blood and Brain MiRNA

At bleed 1 there were 51 differentially expressed miRNA between pigs exposed to enriched and barren housing (14 up regulated and 37 down regulated) but none were significant (FDR $p > 0.05$). Similarly, following bleed 2 there were 71 differentially expressed miRNA between pigs exposed to enriched and barren housing (43 up regulated and 28 down regulated) but none were significant at the FDR threshold (FDR $p > 0.05$). Within the amygdala, a total of 185 miRNA were differentially expressed (122 up regulated and 63 down regulated), but no significant effect of treatment was observed (FDR $p > 0.05$). The top 10 genes that were closest to achieving statistical significance, for each comparison, are listed in Tables S1–S3 (Supplementary Materials).

2.5. Dopamine, Serotonin and Metabolites

Pigs exposed to enriched housing had an increased concentration of dopamine (DA) (2838.8 ng/g vs. 1002.3 ng/g, $Z = -2.26$, $p = 0.02$) and its metabolite DOPAC (620.1 ng/g vs. 266.6 ng/g, $Z = -2.26$, $p = 0.02$) within the striatum, compared to pigs housed in barren conditions (**Figure 4**). No significant effect on DA or DOPAC was observed in the amygdala ($Z = -0.94$, $p = 0.37$ and $Z = -0.53$, $p = 0.68$) or prefrontal cortex ($Z = -1.60$, $p = 0.37$ and $Z = -1.60$, $p = 0.37$). Furthermore, treatment had no significant effect on serotonin (5HT) or its metabolite 5-HIAA in the striatum ($Z = -0.8$, $p = 0.12$), amygdala ($Z = -1.60$, $p = 0.13$), or prefrontal cortex ($Z = -1.2$, $p = 0.68$).

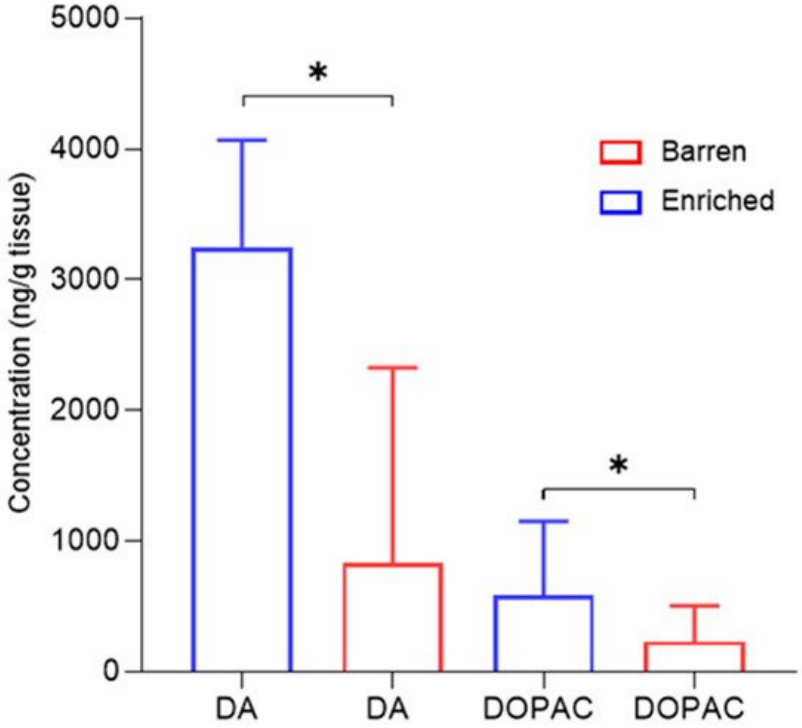

Figure 4. Concentration (ng/g tissue) of dopamine (DA), and its metabolite (DOPAC), in the striatum of pigs exposed to either enriched (n = 6) or barren (n = 6) housing treatments. Data are median ± range. Significant differences are indicated by presence of asterisks ($p < 0.05$).

3. Discussion

In this study we investigated the suitability of circulating miRNA as biomarkers to distinguish valence of emotional state in the pig. We proposed that miRNA would be differentially expressed in the brain and blood during positive emotional states, and that a change in miRNA could be corroborated with already existing behavioural and physiological indices of emotional valence. We hypothesized that (i) exposing pigs to enriched housing conditions would result in a more positive judgment bias, increased neurotransmitter concentration, and differential miRNA patterns in the brain and blood compared to pigs exposed to barren environments, (ii) that changes in the expression of miRNA in the brain could be corroborated with changes of miRNA expression in blood, allowing peripheral miRNA response to be used as a proxy marker for emotional state in the pig. We found that treatment had no effect on behaviour during the JBT, nor did we observe differences in miRNA profiles in the brain or blood of pigs. There was an increase in concentrations of DA and its metabolite DOPAC in the striatum, but this increase was not observed in amygdala or prefrontal cortex. No difference in the neurotransmitter serotonin (5-hydroxytryptophan or 5-HT), nor its metabolite 5-HIAA, was found in any brain region between treatment groups. The results of this study imply that either (a) miRNAs are not likely to be valid biomarkers of positive affective state, at least under the type of conditions employed in this study, or (b) that the study design employed with enriched housing versus barren housing as a modifier of affective state was not sufficient to create differential affective states, and therefore establish the validity of miRNA as biomarkers.

With regard to the first possible interpretation—that miRNAs are not likely to be valid biomarkers of affective state—there is some limited evidence from the porcine literature on the validity of miRNAs, at least as biomarkers of negative states. Weaning stress [42], and heat stress [43], altered miRNA expression in intestinal and muscle tissue respectively. Lecchi et al. 2020 [44], also demonstrated that certain miRNA expression changes in saliva were present following castration and tail docking without analgesia. Our null finding, in contrast to these studies, might be explained by the assumed relatively low impact on physiological processes created in our study. The effects of heat and pain variously create cell damage, tissue degradation and inflammatory pathway activation which may not occur as a result of environmental change. MiRNA may therefore only be useful biomarkers where a relatively invasive change occurs that has a notable effect on physiology.

A significant increase in the tonal concentration of DA, and its metabolite DOPAC, in the striatum of animals exposed to enriched housing conditions was observed. This finding is consistent with our hypothesis and suggests that the provision of enrichment resulted in a chronic shift in affective state, leading to a more positive emotional state in the animals. It is difficult to know if the relationship between the treatment and increased DA was a causative effect, or perhaps a response elicited by another biological process. Given that DA is implicated in behavioural control and is essential for reward related processes including reward learning [45][46], we anticipated this same difference to be reflected in the judgment bias data.

For example, here we observed a treatment effect on tonal DA (i.e., a sustained level of DA neuron firing) where enriched housing increased tonal DA compared to animals housed in barren conditions. Subsequently, we would anticipate that the tonal increase in DA would influence behaviour, where pigs would, under ambiguity, have an enhanced expectation of a positive outcome and behave in a manner normally associated with a positive reward. Here, we did not detect a treatment effect on behavioural parameters; however, potential issues with the design of the behaviour paradigm may account for this and are discussed below. Furthermore, it is interesting that we did not see an increase in DA in the amygdala or the prefrontal cortex. Following rewarding experiences, dopaminergic neurons project widely throughout the brain. The ventral striatum is the region of the brain most closely associated with reward processing such as reward-based learning [47], and is directly innovated by the orbital prefrontal cortex and amygdala [48]. The amygdala plays a critical role in the coordination of the conscious experience of emotion and, along with the prefrontal cortex, forms reciprocal connections that allow learning and experience of the cognitive aspects of emotion [49]. It is unusual, then, given the interconnections between these regions, that no increase in DA was apparent in the amygdala or prefrontal cortex. However, there is some evidence from human studies that an increased reactivity in the ventral striatum occurs during adolescence, leading to stronger striatal activation in response to primary, secondary and social rewards [47]. We speculate that the age of the pigs used in the present study may have resulted in similar effects, where enhanced activity within the striatum may have occurred but was obscured in other brain regions (i.e., amygdala and prefrontal cortex) due to potential developmental differences in the brain. Further research is necessary to clarify and confirm this.

Serotonin is a key neurotransmitter abundant throughout the body and involved in a number of biological systems. Central 5-HT, however, is implicated in behavioural and neuropsychological processes including, but not limited to, mood regulation, appetite, sexuality and attention. In humans, chronic dysregulation of serotonergic activity, including alterations in serotonergic tone, is considered a key component underlying a number of affective disorders including anxiety and depression [50][51]. Serotonergic neurons originating from the raphe nucleus project to multiple brain structures involved in emotional regulation and behaviour response; this includes the amygdala [52], striatum [53], and prefrontal cortex [54]. Previously, administration of the 5-HT antagonist pCPA resulted in pessimistic judgment bias in sheep [55] and pigs [56], and depleted 5-HT concentration in brain regions including the rostral anterior cingulate cortex, prefrontal cortex, striatum, amygdala, hippocampus, hypothalamus and brain stem [57]. Furthermore, pharmacologically induced increases in 5-HT led to a positive judgment bias in rats with a dose dependent response [57]. Unexpectedly, we observed no difference in tonal 5-HT concentrations in the brain of pigs housed in enriched conditions. An explanation for this may be that the duration animals were exposed to the enriched treatment (four weeks), or the enrichment itself, was not sufficient to alter tonal 5-HT concentrations. Another factor may be that alterations in 5-HT levels are more closely associated with the body's stress systems, including HPA activity in response to negative stimuli [58]. For example, following acute handling stress, 5-HT has been shown to be reduced from baseline levels in hippocampus and amygdala in fearful pigs, with the same reduction not occurring following non-stressful handling [58]. Another study has shown hippocampal 5-HT is positively correlated with standing alert time (freezing) during a novel object test, indicating a higher level of anxiety or fear in pigs [59]. It is plausible that the effect of enrichment was not sufficient to stimulate the bodies HPA axis, and thus no chronic changes in 5-HT levels were observed.

That animals housed in enriched conditions would experience a more positive emotional state leading to the judgment of ambiguous stimuli with an enhanced expectation of a positive outcome, and, therefore, result in reduced time to approach the ambiguous cue provided. However, in this study no change in judgment bias was observed in response to enriched housing. There are two likely reasons for this: (i) there was no change in affective state in response to the treatments and/or (ii) the possibility that factors related to the training and test design may have compromised the JBT results.

Whilst increased space allowance, as provided in the enriched housing, has been shown to have beneficial effects on welfare in several studies [60], enrichment may be a determining factor in effects observed. Although the provision of enrichment has been previously shown to improve welfare outcomes and induce a positive bias in pigs [16][41][61], the type of enrichment given in this trial may not have been considered a rewarding stimulus by the pigs, and thus not been integrated at a cellular level. For example, for enrichment to be effective it should stimulate an animal's visual, somatosensory, and olfactory systems whilst maintaining its novelty [62], where natural substrates, such as straw, green fodder, root vegetables and pressed or chopped miscanthus, are considered optimal for animal welfare. Unfortunately, the use of natural substrates for enrichment was not feasible in this trial due to the negative impact this may have had on the effluent system on this particular farm. Consequently, the substrate used may not have been sufficient to provide a rewarding stimulus. Furthermore, the provision of enrichment may have, in fact, affected the pigs in a negative manner, perhaps leading to aggression due to competition for the limited resource. Furthermore, the social structure of pigs is based on a dominance hierarchy, which is vigorously established through fighting when unacquainted pigs are brought together [63]. Although pen mates in the enriched housing group remained the same throughout this experiment, there may have been some incidences of aggression following training or testing, as individual animals were frequently

removed from and then reintroduced to the group. Competition for resources could also have been a factor of disturbance for the pigs housed in groups. If the objects provided were insufficient then the social competition from pen mates may not allow all animals to use the enrichment at the same time, leading to adverse events such as aggression and tail biting [64]. It would have been beneficial to make additional behavioural observations of individuals in the enriched housing treatment to gain a better understanding of the level of activity and types of behaviour shown toward enrichment objects, as well as an account of behaviours considered to reflect positive emotions such as play behaviours [65][66].

Similar issues may have arisen in pigs housed in barren conditions. We would expect that that the effect of isolation in a barren environment would have a negative impact on the pigs and result in a more negative judgment bias. It may be that the animals exposed to barren environments did not find the environment extreme enough to alter behavioural outcomes in the judgment bias test. This has been observed in piglets where repeated social isolation had no effect on behaviour parameters toward ambiguous stimuli [67]. It may also be the case that the pigs housed in the negative environment were displaying rebound behaviour during the test. Rebound behaviour can be described as an increased tendency to perform a specific behaviour, i.e., an activity rebound, after a period of prevention [68]. If pigs were unable to perform locomotive behaviour due to the isolated and restricted housing, they may have developed or built up the urge to display increased locomotive behaviours once released into the test arena. If the pigs that were confined showed increased locomotive behaviour due to rebound effects, some may have touched the ambiguous probe (through choice or accidentally) quicker than if they were not confined, and thus confounded the latency to approach results. The test design itself may also have not been sensitive enough to successfully identify differences in affective state in the pigs in response to the housing treatment. During testing, a number of factors may have arisen which could have affected latency outcomes. It is common for judgment bias trials, including the present study, to leave the ambiguous cue unrewarded [9]. However, such an approach has, in some cases, led to loss of ambiguity towards the ambiguous cue and pigs learn to associate the ambiguous stimulus with an unrewarded outcome [9]. If pigs in this trial learned that the ambiguous stimulus was unrewarded during JBT1, and then remembered this during JBT2, their responses may have led to false measures of judgment bias, as seen previously in sheep [69] and pigs [70]. It has been suggested that rewarding ambiguous cues may maintain optimistic choices throughout testing [70], although similar issues may still arise through associative learning in relation to ambiguous trials that are rewarded. Furthermore, it has been suggested that the measurement of latency alone may lead to the false detection of pessimism in cases where animals are exposed to repeated ambiguous trials [71]. This was observed in rats, where exposure to repeated ambiguous trials was associated with increased latency. However, this increase in latency was also associated with optimistic responses in an active choice test [71].

References

1. Mendl, M.; Burman, O.; Paul, E. An integrative and functional framework for the study of animal emotion and mood. Pro c. R. Soc. B Biol. Sci. 2010, 277, 2895–2904.
2. Schnall, S. Affect, mood and emotions. In Social and Emotional Aspect of Learning; Elsevier: Oxford, UK, 2010; pp. 59 –64. ISBN 9780123814777.
3. Paul, E.; Harding, E.; Mendl, M. Measuring emotional processes in animals: The utility of a cognitive approach. Neuros ci. Biobehav. Rev. 2005, 29, 469–491.
4. Mendl, M.; Burman, O.; Parker, R.; Paul, E. Cognitive bias as an indicator of animal emotion and welfare: Emerging evi dence and underlying mechanisms. Appl. Anim. Behav. Sci. 2009, 118, 161–181.
5. Mellor, D.; Beausoleil, N. Extending the ‘Five Domains’ model for animal welfare assessment to incorporate positive we lfare states. Anim. Welf. 2015, 24, 241–253.
6. Mason, G.; Mendl, M. Why is there no simple way of measuring animal welfare? Anim. Welf. 2013, 2, 301–319.
7. Duncan, I. Science-based assessment of animal welfare: Farm animals. Rev. Sci. Tech. 2005, 24, 483–492.
8. Kremer, L.; Klein Holkenborg, S.; Reimert, I.; Bolhuis, J.; Webb, L. The nuts and bolts of animal emotion. Neurosci. Bio behav. Rev. 2020, 113, 273–286.
9. Roelofs, S.; Boleij, H.; Nordquist, R.; van der Staay, F. Making decisions under ambiguity: Judgment bias tasks for asse ssing emotional state in animals. Front. Behav. Neurosci. 2016, 10, 1–16.
10. Bateson, M.; Emmerson, M.; Ergün, G.; Monaghan, P.; Nettle, D. Opposite effects of early-life competition and develop mental telomere attrition on cognitive biases in juvenile European starlings. PLoS ONE 2015, 10, e132602.
11. Whittaker, A.; Barker, T. A consideration of the role of biology and test design as confounding factors in judgment bias t ests. Appl. Anim. Behav. Sci. 2020, 232, 105–126.

12. George, R.P.; Barker, T.H.; Lymn, K.A.; Bigatton, D.A.; Howarth, G.S.; Whittaker, A.L. A Judgement Bias Test to Assess Affective State and Potential Therapeutics in a Rat Model of Chemotherapy-Induced Mucositis. Sci. Rep. 2018, 8, 819 3.

13. Doyle, R.; Fisher, A.; Hinch, G.; Boissy, A.; Lee, C. Release from restraint generates a positive judgment bias in sheep. Appl. Anim. Behav. Sci. 2010, 122, 28–34.

14. Mendl, M.; Brooks, J.; Basse, C.; Burman, O.; Paul, E.; Blackwell, E.; Casey, R. Dogs showing separation-related beha viour exhibit a 'pessimistic' cognitive bias. Curr. Biol. 2010, 20, 839–840.

15. Iyasere, O.; Beard, A.; Guy, J.; Bateson, M. Elevated levels of the stress hormone, corticosterone, cause 'pessimistic' ju dgment bias in broiler chickens. Sci. Rep. 2017, 7, 1–12.

16. Douglas, C.; Bateson, M.; Walsh, C.; Bédué, A.; Edwards, S. Environmental enrichment induces optimistic cognitive bia ses in pigs. Appl. Anim. Behav. Sci. 2012, 139, 65–73.

17. Scollo, A.; Gottardo, F.; Contiero, B.; Edwards, S. Does stocking density modify affective state in pigs as assessed by c ognitive bias, behavioural and physiological parameters. Appl. Anim. Behav. Sci. 2014, 153, 26–35.

18. Brajon, S.; Laforest, P.; Schmitt, O.; Devillers, N. The way humans behave modulates the emotional state of piglets. PL oS ONE 2015, 10, e133408.

19. Lagisz, M.; Zidar, J.; Nakagawa, S.; Neville, V.; Sorato, E.; Paul, E.; Bateson, M.; Mendl, M.; Løvlie, H. Optimism, pessi mism and judgment bias in animals: A systematic review and meta-analysis. Neurosci. Biobehav. Rev. 2020, 118, 3–1 7.

20. Bethell, E. A "how-to" guide for designing judgment bias studies to assess captive animal welfare. J. Appl. Anim. Welf. Sci. 2015, 18, 18–42.

21. Si, Y.; Song, Z.; Sun, X.; Wang, J. microRNA and mRNA profiles in nucleus accumbens underlying depression versus r esilience in response to chronic stress. Am. J. Med. Genet. B Neuropsychiatr. Genet. 2018, 177, 563–579.

22. Wingo, A.; Almli, L.; Stevens, J.; Jovanovic, T.; Wingo, T.; Tharp, G.; Li, Y.; Lori, A.; Briscione, M.; Jin, P.; et al. Genome-wide association study of positive emotion identifies a genetic variant and a role for microRNAs. Mol. Psychiatry 2017, 22, 774–783.

23. Whittaker, A.; Marsh, L. The role of behavioural assessment in determining positive affective states in animals. CAB Re v. 2019, 14, 1–13.

24. Mellor, D. Animal emotions, behaviour and the promotion of positive welfare states. N. Z. Vet. J. 2012, 60, 1–8.

25. Fonken, L.; Gaudet, A.; Gaier, K.; Nelson, R.; Popovich, P. MicroRNA-155 deletion reduces anxiety-and depressive-like behaviours in mice. Psychoneuroendocrinology 2016, 63, 362–369.

26. Haramati, S.; Navon, I.; Issler, O.; Ezra-Nevo, G.; Gil, S.; Zwang, R.; Hornstein, E.; Chen, A. MicroRNA as repressors o f stress-induced anxiety: The case of amygdalar miR-34. J. Neurosci. 2011, 31, 14191–14203.

27. Lopez, J.; Lim, R.; Cruceanu, C.; Crapper, L.; Fasano, C.; Labonte, B.; Maussion, G.; Yang, J.; Yerko, V.; Vigneault, E.; et al. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant tre atment. Nat. Med. 2014, 20, 764–768.

28. Balakathiresan, N.; Chandran, R.; Bhomia, M.; Jia, M.; Li, H.; Maheshwari, R. Serum and amygdala microRNA signatur es of posttraumatic stress: Fear correlation and biomarker potential. J. Psychiatr. Res. 2014, 57, 65–73.

29. Rong, H.; Liu, T.; Yang, K.; Yang, H.; Wu, D.; Liao, C.; Hong, F.; Yang, H.; Wan, F.; Ye, X.; et al. MicroRNA-134 plasma l evels before and after treatment for bipolar mania. J. Psychiatr. Res. 2011, 45, 92–95.

30. Lai, C.; Yu, S.; Hsieh, M.; Chen, C.; Chen, H.; Wen, C.; Huang, Y.; Hsiao, P.; Hsiao, C.; Liu, C.; et al. MicroRNA expres sion aberration as potential peripheral blood biomarkers for schizophrenia. PLoS ONE 2011, 6, e21635.

31. Wiegand, C.; Savelsbergh, A.; Heusser, P. MicroRNAs in psychological stress reactions and their use as stress-associa ted biomarkers, especially in human saliva. Biomed. Hub. 2017, 2, 1–15.

32. Weber, J.; Baxter, D.; Zhang, S.; Huang, D.; Huang, K.; Lee, M.; Galas, D.; Wang, K. The microRNA spectrum in 12 bo dy fluids. Clin. Chem. 2010, 56, 1733–1741.

33. Tavares, G.; Torres, A.; de Souza, J. Early life stress and the onset of obesity: Proof of microRNAs' involvement throug h modulation of serotonin and dopamine systems' homeostasis. Front. Physiol. 2020, 11, 925.

34. Dash, S.; Balasubramaniam, M.; Martínez-Rivera, F.; Godino, A.; Peck, E.; Patnaik, S.; Suar, M.; Calipari, E.; Nestler, E.; Villalta, F.; et al. Cocaine-regulated microRNA miR-124 controls poly (ADP-ribose) polymerase-1 expression in neur onal cells. Sci. Rep. 2020, 10, 11197.

35. Baudry, A.; Mouillet-Richard, S.; Schneider, B.; Launay, J.; Kellermann, O. miR-16 targets the serotonin transporter: A n ew facet for adaptive responses to antidepressants. Science 2010, 329, 1537–1541.

36. Kenny, P. Epigenetics, microRNA, and addiction. Dialogues Clin. Neurosci. 2014, 16, 335–344.

37. Podolska, A.; Kaczkowski, B.; Kamp Busk, P.; Søkilde, R.; Litman, T.; Fredholm, M.; Cirera, S. MicroRNA expression pr ofiling of the porcine developing brain. PLoS ONE 2011, 6, e14494.

38. Lee, J.; Choi, H.; Heo, Y.; Chung, Y. Effect of floor space allowance on pig productivity across stages of growth: A field-scale analysis. Asian-Australas. J. Anim. Sci. 2016, 29, 739–746.

39. Herskin, M.; Jensen, K. Effects of different degrees of social isolation on the behaviour of weaned piglets kept for exper imental purposes. Anim. Welf. 2000, 9, 237–249.

40. Puppe, B.; Ernst, K.; Schön, P.; Manteuffel, G. Cognitive enrichment affects behavioural reactivity in domestic pigs. App l. Anim. Behav. Sci. 2007, 105, 75–86.

41. Van de Weerd, H.; Day, J. A review of environmental enrichment for pigs housed in intensive housing systems. Appl. An im. Behav. Sci. 2009, 116, 1–20.

42. Tao, X.; Xu, Z.; Men, X. Analysis of serum microRNA expression profiles and comparison with small intestinal microRN A expression profiles in weaned piglets. PLoS ONE 2016, 11, e162776.

43. Hao, Y.; Liu, J.; Zhang, Y.; Yang, P.; Feng, Y.; Cui, Y.; Yang, C.; Gu, X. The microrna expression profile in porcine skelet al muscle is changed by constant heat stress. Anim. Gen. 2016, 47, 365–369.

44. Lecchi, C.; Zamarian, V.; Gini, C.; Avanzini, C.; Polloni, A.; Nodari, S.; Ceciliani, F. Salivary mirnas are potential biomark ers for the accurate and precise identification of inflammatory response after tail docking and castration in piglets. J. Ani m. Sci. 2020, 98.

45. Cools, R. Dopaminergic control of the striatum for high-level cognition. Curr. Opin. Neurobiol. 2011, 21, 402–407.

46. Flagel, S.; Clark, J.; Robinson, T.; Mayo, L.; Czuj, A.; Willuhn, I.; Akers, C.; Clinton, S.; Phillips, P.; Akil, H. A selective ro le for dopamine in stimulus-reward learning. Nature 2011, 6, 53–57.

47. Tottenham, N.; Galván, A. Stress and the adolescent brain: Amygdala-prefrontal cortex circuitry and ventral striatum as developmental targets. Neurosci. Biobehav. Rev. 2016, 70, 217–227.

48. Gottfried, J. Neurobiology of Sensation and Reward; CRC Press: New York, NY, USA, 2011; pp. 348–349.

49. Hensler, J. Serotonin in Mood and Emotion. Handb. Behav. Neurosci. 2010, 21, 367–378.

50. Berger, M.; Gray, J.; Roth, B. The expanded biology of serotonin. Annu. Rev. Med. 2009, 60, 355–366.

51. Brummelte, S.; Mc Glanaghy, E.; Bonnin, A.; Oberlander, T. Developmental changes in serotonin signaling: Implications for early brain function, behaviour and adaptation. Neuroscience 2017, 342, 212–231.

52. Murray, E. The amygdala, reward and emotion. Trends Cogn. Sci. 2007, 11, 489–497.

53. Dalley, J.; Everitt, B.; Robbins, T. Impulsivity, compulsivity, and top-down cognitive control. Neuron 2011, 24, 680–694.

54. Puig, M.; Gulledge, A. Serotonin and prefrontal cortex function: Neurons, networks, and circuits. Mol. Neurobiol. 2011, 44, 449–464.

55. Doyle, R.; Hinch, G.; Fisher, A.; Boissy, A.; Henshall, J.; Lee, C. Administration of serotonin inhibitor p-Chlorophenylala nine induces pessimistic-like judgment bias in sheep. Psychoneuroendocrinology 2011, 36, 279–288.

56. Stracke, J.; Otten, W.; Tuchscherer, A.; Puppe, B.; Düpjan, S. Serotonin depletion induces pessimistic-like behaviour in a cognitive bias paradigm in pigs. Physiol. Behav. 2017, 174, 18–26.

57. Rygula, R.; Papciak, J.; Popik, P. The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on t he cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm. Eur. Neuropsychopharmacol. 2014, 2 4, 1103–1111.

58. Arroyo, L.; Carreras, R.; Valent, D.; Peña, R.; Mainau, E.; Velarde, A.; Sabrià, J.; Bassols, A. Effect of handling on neur otransmitter profile in pig brain according to fear related behaviour. Physiol. Behav. 2016, 1, 374–381.

59. Ursinus, W.; Bolhuis, J.; Zonderland, J.; Rodenburg, T.; de Souza, A.; Koopmanschap, R.; Kemp, B.; Korte-Bouws, G.; Korte, S.; van Reenen, C. Relations between peripheral and brain serotonin measures and behavioural responses in a novelty test in pigs. Physiol. Behav. 2013, 118, 88–96.

60. Whittaker, A.L.; Van Wettere, W.H.; Hughes, P.E. Space requirements to optimize welfare and performance in group ho used pigs: A review. Am. J. Anim. Vet. Sci. 2012, 7, 48–54.

61. Beattie, V.; O'Connell, N.; Kilpatrick, D.; Moss, B. Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. Anim. Sci. 2000, 70, 443–450.

62. EU Council. Council Directive 2016/336/EC of 8 March 2016. Laying down Minimum Standards for the Protection of Pigs. Off. J. Eur. Union. 2016, pp. 5–13. Available online: https://eur-lex.europa.eu/eli/reco/2016/336/oj (accessed on 14 June 2021).
63. Puppe, B.; Langbeina, J.; Bauer, J.; Hoyb, S. A comparative view on social hierarchy formation at different stages of pig production using sociometric measures. Livest. Sci. 2008, 113, 155–162.
64. Docking, C.; Van de Weerd, H.; Day, J.; Edwards, S. The influence of age on the use of potential enrichment objects and synchronisation of behaviour of pigs. Appl. Anim. Behav. Sci. 2008, 110, 244–257.
65. Bolhuis, J.; Schouten, W.; Schrama, J.; Wiegant, V. Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. Appl. Anim. Behav. Sci. 2005, 93, 213–228.
66. Zupan, M.; Rehn, T.; de Oliveira, D.; Keeling, L. Promoting positive states: The effect of early human handling on play and exploratory behaviour in pigs. Animal 2016, 10, 135–141.
67. Düpjan, S.; Ramp, C.; Kanitz, E.; Tuchscherer, A.; Puppe, B. A design for studies on cognitive bias in the domestic pig. J. Vet. Behav. 2013, 8, 485–489.
68. Jensen, M.B. Effects of confinement on rebounds of locomotor behaviour of calves and heifers, and the spatial preferences of calves. Appl. Anim. Behav. Sci. 1999, 62, 43–56.
69. Doyle, R.; Vidal, S.; Hinch, G.; Fisher, A.; Boissy, A.; Lee, C. The effect of repeated testing on judgment biases in sheep. Behav. Proc. 2010, 83, 349–352.
70. Murphy, E.; Nordquist, R.; van der Staay, F. Responses of conventional pigs and Göttingen miniature pigs in an active choice judgment bias task. Appl. Anim. Behav. Sci. 2013, 148, 64–76.
71. Barker, T.H.; Howarth, G.S.; Whittaker, A.L. Increased latencies to respond in a judgment bias test are not associated with pessimistic biases in rats. Behav. Proc. 2018, 146, 64–66.