Brain-derived neurotrophic factor as an indicator of environmental enrichment effectiveness

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Executive Summary

Environment enrichment for pigs is a rising topic. However, measures to define what constitute environment enrichment are lacking. Recent neuroscience evidence suggests that environmental enrichment increases a molecule called brain-derived neurotrophic factor (BDNF), the most abundant molecule that simulates the growth of neurons (brain cells). Research in humans and rodents also suggest that BDNF is linked with higher stress resilience. Compared to usual neuroscience measures, BDNF presents the advantage that it can be measured in blood, and studies have shown that concentration of BDNF in the blood is well correlated with BDNF concentration in the brain. If blood levels of BDNF specifically increases after environmental enrichment is provided, BDNF could offer an objective physiological indicator of successful enrichment programs in pigs.

This study investigated whether BDNF concentration differed between piglets housed in different systems during lactation (enriched vs. barren) and after weaning (enriched vs. barren), using a 2 × 2 factorial design and the provision of a Ridley foraging block as enrichment. The best matrix to sample BDNF was also tested, comparing BDNF concentration in serum, plasma and saliva samples. We also examined BDNF concentration as an indicator of longer-term effects (from pre-weaning to 11 weeks of age in the same individuals), or transient states (comparing 24 h before and after pigs were subjected to a cognitive maze test). Finally, we analysed the correlation between BDNF concentration and other behavioural (maze test) measures.

Overall, serum BDNF concentration was higher in pigs provided with environmental enrichment, in the form of a foraging block, compared to pigs housed in a barren environment. The provision of enrichment early in life, before weaning, resulted in a more pronounced BDNF concentration increase than enrichment provided later in life, after weaning. BDNF concentration reduced as the pigs aged from 3 to 11 weeks of age. There was no correlation between BDNF and the performance of the pig in a maze test. BDNF could be reliably measure in serum, but we could not reliably measure in plasma and it was not detectable in saliva.

In conclusion, measuring BDNF in pig serum is promising to assess the effect of providing pigs with environmental enrichment. However, further research is needed to determine whether various forms of enrichment are more or less effective, and links this increase in BDNF to other indicators of improved pig welfare.

Table of Contents

1. Background

Environment enrichment for pigs is a rising topic. However, the topic of environment enrichment lack rigorous and validated scientific approaches to evaluate the best enrichment interventions and to assess their effects on pigs (van de Weerd et al. 2009).

Furthermore, there is no good measure of effective enrichment. Until now, most studies have focused on behavioural changes, such as quantifying the time animals spend interacting with the enrichment. However, changes in behaviour are a normal response to change, a way for animals to adapt to their environment. Use of the enrichment per se does not necessarily equate to beneficial outcomes for the pig. In contrast, few studies have tackled the important question of the effects of environmental enrichment programs on pigs (the functional benefits enrichment brings), such as improving biological function or improving stress resilience.

Environmental enrichment has been hypothesised to increase learning, memory and stress resilience, which could theoretically carry beneficial effects for pigs such as reduced aggression (if memory is enhanced), better adaptation (if learning is enhanced), and lower stress which is linked to reduced impact on growth, reproduction and disease susceptibility (if stress resilience is enhanced).

Recent neuroscience evidence suggests that the effect of environmental enrichment on the brain may be mediated by brain-derived neurotrophic factor (BDNF), the most abundant molecule in the brain that stimulates neuronal growth, especially in the hippocampus, a brain region involved in learning and memory (Bekinschtein et al., 2011). Studies also suggest that BDNF may be linked with higher stress resilience in humans and other animal species, with for instance rats housed in enriched cages post-weaning having higher serum BDNF and being more resilient to a repeated stressor (Mosaferi et al., 2015).

BDNF can be measured in the blood, and studies report good correlation between the blood and brain BDNF, as BDNF easily crosses the blood-brain barrier, supporting that blood BDNF can be used as a proxy for brain BDNF. Klein et al (2011) reported a significant correlation between BDNF in pig's brain and plasma.

We have validated a lab assay using the enzyme-linked immunosorbent assay (ELISA) technique, which is a common lab technique, and can successfully and reliably

measure BDNF in piglets' serum (Rault et al. unpublished). It is unknown how saliva, plasma and serum BDNF concentrations relate to each other in pigs. Assessing the potential of BDNF as a biomarker of environmental enrichment efficacy requires a more thorough investigation to assess its validity (sensitivity, specificity), and in various sample matrix (blood vs. saliva).

BDNF has also been hypothesised to reflect mental states, with meta-studies suggesting that BDNF may reflect an individual's mood (i.e. a long-lasting emotional state). There is good parallel between the extent of peripheral BDNF decrease and the severity of manic and depressive episodes in humans (Fernandes et al., 2015), and no difference between maniac or depressive patients in euthymic state (normal non-depressed, reasonably positive mood) and control humans (Fernandes et al. 2015, Munkhold et al. 2016). Similarly, to the provision of enrichment, successful antidepressant treatment raises serum BDNF (Vinogradov et al., 2009). However, BDNF is also important in both fear learning and extinction (Andero and Ressler 2012), demonstrating its complex role in the regulation of emotional learning and memory.

2. Introduction

The provision of environment enrichment is a rising concern to safeguard animal welfare, especially for species kept in indoor or barren environments, but the mechanisms through which enrichment benefits animals remain poorly understood. Despite the growing implementation of environmental enrichment programs in captive environments, there are still few rigorous and validated scientific approaches to evaluate enrichment interventions and assess their effects. Environmental enrichment is defined as an improvement in the biological functioning of captive animals resulting from modifications to their environment (Newberry 1995). Most studies have focused on behavioural changes, such as quantifying the time animals spend interacting with the enrichment provided (e.g. Guy et al 2013). Conversely, few studies have tackled the functional benefits of enrichment, such as improved biological functioning or stress resilience. In particular, the effect of enrichment on neurobiological development and neural processes remains to be elucidated (van de Weerd and Day, 2009).

Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain and it plays an important role in neuronal survival, growth and function. In particular, BDNF is important for hippocampal growth and development, stimulating learning and memory (Bekinschtein et al., 2011). Higher BDNF has been linked to improved cognitive functions (Bekinschtein et al., 2011; Novkovic et al., 2015) and higher stress resilience (Mosaferi et al., 2015; Berton et al., 2006), sharing features with the intended effects of successful environment enrichment programs. In fact, environment enrichment has been shown to increase BDNF concentration, including physical exercise (Rasmussen et al., 2009), early social enrichment (Branchi et al., 2006) and other housing conditions (Zhu et al., 2006).

Knock out and transgenic mice models demonstrated that BDNF is required for the enhancement of hippocampal neurogenesis following environmental enrichment (Rossi et al., 2006; Novkovic et al., 2015). Hence, BDNF has been hypothesised to mediate the effect of environmental enrichment on the brain (Bekinschtein et al., 2011; Choubarji et al., 2011). As such, BDNF may reflect the functional benefits of environmental enrichment. For instance, rats housed in enriched cages post-weaning have higher serum BDNF and are more resilient to a repeated stressor (Mosaferi et al., 2015), and the enrichment-related prevention of stereotyped behaviour is associated with increased BDNF in the striatum of deer mice (Turner and Lewis 2003).

An appealing feature for the use of BDNF as an indicator of effective enrichment is that have BDNF concentrations in blood and brain are correlated in various species (Klein et al., 2011; Karege et al., 2002), as BDNF can cross the blood brain barrier (Pan et al. 1998). Hence, blood BDNF can be used as a proxy for brain BDNF, allowing for a less invasive assessment of neuronal changes and repeatable sampling overtime. BDNF has been extensively studied for its involvement in the mechanisms of neurogenesis and resulting mental health implications for humans (Chourbaji et al., 2011; Autry and Monteggia 2002; Tapia-Arancibia et al. 2008). However, changes in blood BDNF as a potential indicator of animal welfare has not been investigated so far.

This study investigated changes in serum BDNF concentration according to the provision or not of enrichment before or after weaning, age, and the link between BDNF and other animal welfare measures: performance in a cognitive maze test and physiological and immune measures.

3. Methodology

All methods and animal use were approved by the South Australian Research and Development Institute Animal Ethics Committee.

3.1. *Animals and experimental design*

This project was an add-on to existing APL project 2015/038 ("Does enriching the lactation and weaner environment of piglets alter their longer-term utilisation of enrichment substrates and devices, and are there life-time benefits for welfare and production? P.I. Cameron Ralph). The overarching project investigated the effects of enrichment in the lactation phase and in the grower phase with measurement of behavioural, health and immune responses relevant to welfare and production, as well as investigate the pigs' utilisation of enrichment during the pre- and post-weaning period.

Large White × Landrace cross piglets from 24 litters were randomly allocated to a 2 × 2 factorial design experiment at the Roseworthy Piggery. Piglets were born and reared in conventional farrowing crates with no added enrichment, or in the same farrowing crates with the addition of enrichment foraging blocks from 7 days of age. Enrichment blocks originally produced for use by sows were reformulated specifically for use in sucker and weaner pigs (Ridley AgriProducts, Pakenham, Victoria, Australia). Unfortunately, the nutritional composition of this block was not shared by the supplier. Blocks were replaced every 7 days with new blocks and the size of block increased relative to the age of the pigs. The pigs never finished a block within 7 days, and therefore there were always some of the block present. Two female and two male focal piglets were randomly selected and tagged at 24 hours after birth from each litter. At weaning at 21 days of age, piglets were split across treatments such that half of the piglets from each treatment before weaning (enriched or barren) were weaned into a barren pen environment and the other half into an enriched environment containing new enrichment blocks of the same type as during lactation. This created four treatments combinations before and after weaning: Enriched-Enriched (E-E), Barren-Barren (B-B), Enriched-Barren (E-B) and Barren-Enriched (B-E), with 24 pigs per treatment combination. Food and water were provided *ad libitum* during the weaner phase. Pigs remained in these pens until 11 weeks of age.

Pigs were split in two groups. The first group of pigs was composed of 48 of the 96 focal pigs, with 12 pigs per treatment combination. Blood samples were collected from these pigs 24 hours before weaning (with weaning at 3 weeks of age), 24 h after weaning, at 5 weeks and at 11 weeks of age, and the pigs were weighed after blood samples were collected. This experimental group is referred to as the 'longitudinal' group from here on. Saliva was also collected from these pigs at 11 weeks of age.

The second group of pigs was composed of another 24 pigs from the 96 focal pigs, different pigs but from the same litters as the longitudinal group, with six pigs per treatment combination. These pigs were subjected to a cognitive maze test 40 days post-weaning, around 8.5 week of age. The test arena consisted of two traps (L-shaped walls) with a start box at one end of the maze and two familiar pigs and the reward (canned cream in a bowl) at the other end of the maze. The starting box in the maze test had a transparent door such that the pigs could see the maze while in the start box, and held then for 1 min and then released into the maze. The pigs were first submitted to three training runs at 52 and 53 days of age, and exposed to four test runs 3 days later. Variables recorded including time to exit the starting box, time taken to navigate through the maze and reach the reward, the number of times that pig entered a trap and the total time spent in the trap, and the difference in time taken to solve the maze between test run 1 and test run 4. Blood samples were collected 24 h prior to the test (between 1047h and 1227h) and immediately after completion of the maze test (between 1018h and 1424h). Time to complete the test varied depending on the pig's performance, from 148 ± 6.13 seconds to 22.67 ± 6.09 seconds for each testing run, with the test being repeated four times successively. Blood samples were collected within 10 min after completion of the test. This experimental group is referred to as the 'cognition maze test' group from here on.

3.2. *Data Collection and Analysis*

3.2.1. **Brain-derived neurotrophic factor assay**

Blood samples were withdrawn within 2 min via jugular venipuncture with 21 Ga × 2.5 cm needles through manual arm restraint with a bleeder and a second person holding the pig. We followed the BDNF kit manufacturer recommendations for handling of the samples. Blood samples were collected in a 4mL EDTA tubes (BD vacutainer, North Ryde, NSW, Australia) and a 4ml serum tubes (BD vacutainer,

North Ryde, NSW, Australia) for each pig. The serum tubes were left to clot at room temperature for 1 hour, and then centrifuged at 1,000 × g for 15 min. The serum fraction was then pipetted into Eppendorf tubes and stored at -20°C until assayed. For plasma collection, collection tubes were stored on ice at all times and blood samples were centrifuged for the first time within 30 minutes of collection at 1,500 × g for 15 min at 4°C. The plasma fraction was pipetted into Eppendorf tubes, kept on ice, and centrifuged a second time at 10,000 × g for 10 min at 4°C within 2 to 3.5 h. The last plasma fraction was pipetted into another Eppendorf tube and stored at -20°C until assayed.

A saliva sample was collected from each pig from the longitudinal group at 11 weeks of age. Samples were collected in the home pen by allowing the pig to chew on a salivette (Sarstedt, Nümbrecht, Germany) until the salivette was saturated. Salivettes were stored on ice and then centrifuged at 10,000 × g for 10 min at 4°C to extract the saliva. Saliva was then stored at -20°C until assayed.

For assaying, samples were thawed on ice and assayed using a commercial Enzyme-linked Immunosorbent Assay kit (Mature BDNF *Rapid*™ ELISA kit, BEK-2211-1P, Biosensis Pty Ltd, Thebarton, SA, Australia). We conducted serial dilution trials to assess the reliability of BDNF measurements in pig serum, plasma and saliva. Our serial dilutions trials confirmed that for parallelism remained good (means ± SE: 108.2 ± 7.1 %) for dilution concentrations that remained above 2 pg/mL. However, in discussion with the kit manufacturer technical staff, we also created two additional standards to obtain a range of detection from 1.95 to 125 pg/mL to optimise detection in the lower range. Samples were incubated for 8 min prior to reading the sample with the ELISA plate reader to optimise the sensitivity of the readings. Samples for which the duplicates returned a coefficient of variation superior to 10% were run a second time, and discarded from the dataset if the coefficient of variation still exceeded 10%. The average intra-assay CV was 4.6% and the average inter-assay CV was 10.0%.

3.3. *Statistical analyses*

Data were checked for the criteria for normality and homogeneity of variance. Serum BDNF data were log transformed, which normalised the data and reduced the right-skewed distribution common in the BDNF literature. Serum BDNF concentration were analysed using a Mixed Models in SAS (version 9.3, SAS Institute

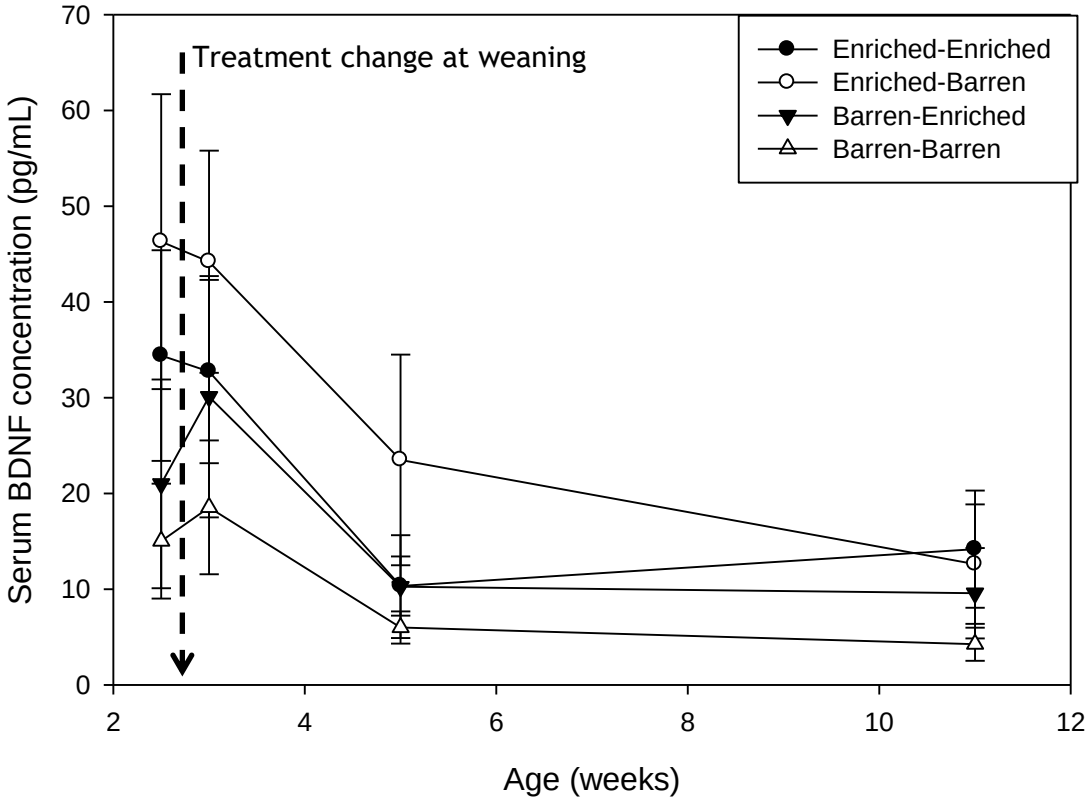
Inc., Cary NC, USA), with the fixed effects of enrichment before weaning, enrichment after weaning, their interaction, litter nested within pre-weaning treatment, and age at sampling to account for the repeated sampling over time with the pig as random factor. Sex and weight were never significant and were subsequently not removed from the model. The Tukey-Kramer adjustment method was applied to correct for the number of post-hoc comparisons. Correlations between the log-transformed BDNF concentration and behavioural measures were tested using Pearson correlation. Results are expressed as least-square (LS) means ± standard error of the mean (S.E.M.), and P-values less than or equal to 0.05 were considered significant.

4. Outcomes

4.1. *Longitudinal group*

Pigs provided with foraging enrichment before weaning tended to have higher serum BDNF concentration than pigs housed in barren pens before weaning ($F_{(1,112)}$=3.32, P=0.07, Figure 1). Pigs provided with foraging enrichment after weaning did not differ in serum BDNF concentration from pigs housed in barren pens after weaning ($F_{(1,112)}$=0.05, P=0.82). The interaction between foraging enrichment before and after weaning was not significant ($F_{(1,112)}$=0.31, P=0.58). Litter had a significant effect ($F_{(20,112)}$=2.84, P<0.001). Age at sampling had a significant effect ($F_{(3,112)}$=24.66, P<0.001), as serum BDNF concentration did not differ before and after weaning, significantly reduced by 5 weeks of age (P < 0.001), and then did not differ between 5 and 11 weeks of age (P=0.19).

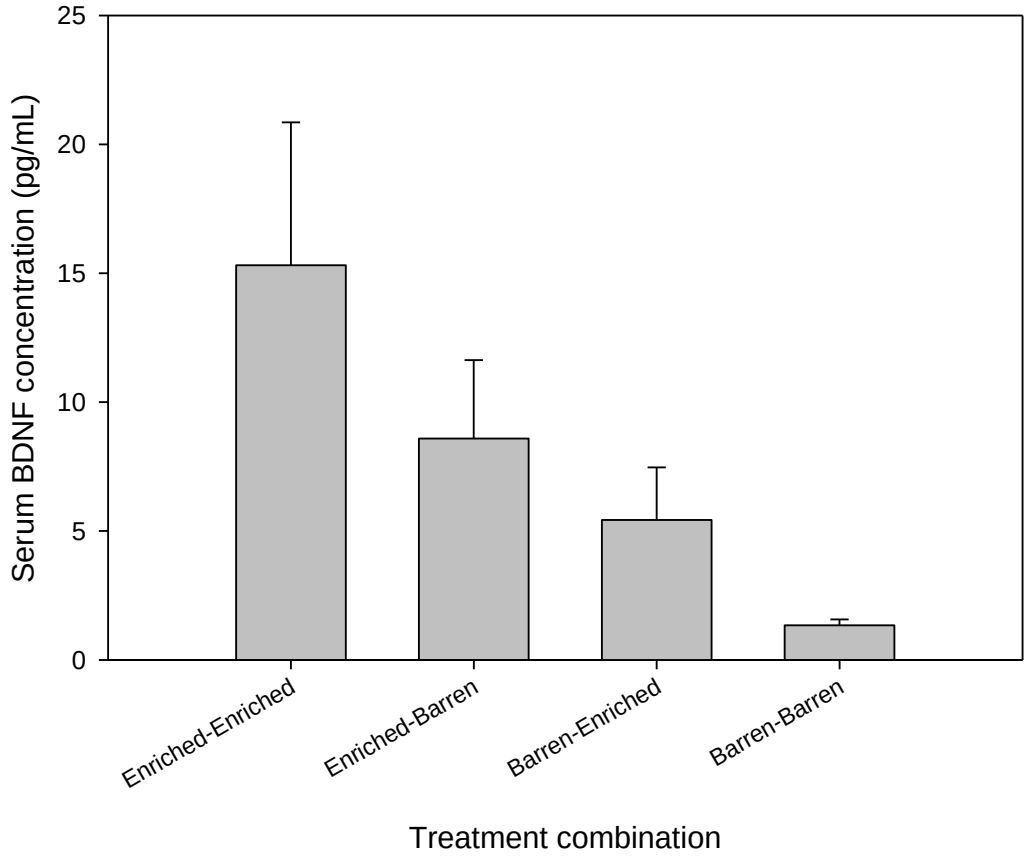
Figure 1 – Serum BDNF concentration from before weaning until 11 weeks of age. Data were analysed using the logarithmic transformation log(x+1) are presented as non-transformed (N = 8-12 pigs per time point per treatment). Treatments changed at weaning at three weeks of age, creating the four-treatment combination: Enriched-Enriched, Enriched-Barren, Barren-Enriched and Barren-Barren.



4.2. *Cognitive maze group*

At 8 weeks of age, pigs provided with foraging enrichment before weaning had higher serum BDNF concentration than pigs housed in barren pens before weaning ($F_{(1,21)}$=6.92, P=0.02, Figure 2). Pigs provided with foraging enrichment after weaning tended to have higher serum BDNF concentration than pigs housed in barren pens after weaning ($F_{(1,21)}$=3.26, P=0.09). The interaction between foraging enrichment before and after weaning was not significant ($F_{(1,21)}$=2.33, P=0.14). Litter was not significant ($F_{(16,21)}$=1.08, P=0.43), but the 24 pigs originated from 18 different litters, which reduced power to account for this effect. Age at sampling, before or after performing the cognitive maze test, was not significant ($F_{(1,21)}$=2.33, P=0.14). Indeed, the correlation between serum BDNF before and after the cognitive maze test was strong and significant ($r_{(22)}$=0.728, P<0.001).

Figure 2 – Serum BDNF concentration during the maze test at 8.5 weeks of age (40 days post-weaning) per treatment combination: Enriched-Enriched, Enriched-Barren, Barren-Enriched and Barren-Barren (n=6 pigs per treatment combination). Data were analysed using the logarithmic transformation log(x+1) are presented as non-transformed (N = 6 pigs per treatment). Serum BDNF concentration results before and after the test were pooled as there was no effect of time.



There were no significant correlation between serum BDNF concentration before or after the cognitive maze test and the behavioural performance of the pigs during the test, based on the time pigs took to exit the starting box, to reach the reward, the number of times that pig entered a trap and the total time spent in each of the trap.

4.3. *Matrix of sampling*

Across our assay runs in the laboratory, BDNF could be reliably assess in serum, with concentrations within the detection range of the kit standards and good parallelism between serial dilutions.

BDNF concentration was low in plasma samples (mean = 6.76 pg/mL), with concentrations often below the detection limit of 2 pg/mL (40% of samples assayed), and poor parallelism in serial dilutions (expected concentrations between 109 and 300%) which was likely related to the low concentration being too close to the lower detection limit, and therefore unreliable or undetectable concentration below this limit.

BDNF was also low and often undetectable in saliva, and with significance matrix interference as assessed by poor recovery rates after samples were spiked (i.e. added) with known concentration of BDNF standards. The kit manufacturer's technical services also communicated to us that their kit was not validated for assaying BDNF in saliva, and that they were still themselves having difficulty to find an appropriate protocol for preparation of the samples to obtain reliable results in measuring BDNF in human saliva. This echoes a recent paper that could not reliably measure BDNF in human saliva, testing three different kits (Vrijen et al, 2017).

4.4. *Discussion*

Overall, serum BDNF concentration was higher in pigs provided with environmental enrichment, in the form of a foraging block, compared to pigs housed in a barren environment. The provision of enrichment early in life, before weaning, resulted in a more pronounced BDNF concentration increase than enrichment provided later in life, after weaning.

Nevertheless, there was substantial inter-individual variation between pigs, even within treatments. This may reflect functional variation in the effects of enrichment on each pig, possibly depending on their individual interaction or use of the enrichment device, as the variation in BDNF concentration was proportionally higher in the pigs provided with enrichment compared to the pigs housed in the barren environment. We did not record use of the enrichment in the present experiment to test this hypothesis. Hence, variation in BDNF could reflect either variation on

enrichment use or the biological inter-individual variation in BDNF reported commonly in the BDNF literature in other species, and usually necessitate a relatively large sample size.

BDNF concentration were stable when measured before and immediately after the completion of a cognitive maze test, despite its half-life of 10 min in blood (Sakane and Pardridge, 1997). Hence, serum BDNF appear to reflect relatively stable traits, rather than transient states, which could be influenced by acute events, in agreement with similar stability across days in humans (Trajkovska et al. 2007).

We also did not find any relationship between BDNF concentrations, before or after the maze test, and the pig's performance in the maze test. Therefore, we could not confirm that higher serum BNDF is linked to increased cognitive performance, as other studies have shown before (Bekinschtein et al., 2011; Novkovic et al., 2015). It could be that the effects of the enrichment were not pronounced enough. Alternatively, the function of the enrichment provided (foraging device) might have been irrelevant to help the pig in this particular task.

Through repeated sampling on the same individuals, BDNF concentration was shown to reduce with age. This is likely related to BDNF's neurotrophic role and the more intense brain development in early life. Brain plasticity reduces later in life, as does plasma BDNF (Lommatzsh et al., 2005), and indeed BDNF has received a lot of attention for its potential involvement in age-related decline neural plasticity and neurodegenerative problems such as Alzheimer and Parkinson diseases (Tapia-Arancibia et al. 2008). However, in our study, the pigs were followed from early development to pre-pubertal stage, and as such, it is rather focused on early life development rather than the entire lifespan of a pig.

The dynamics of change in BDNF concentration according to the timing and type of environment enrichment provided merits further research, given the mediating role of BDNF in neuronal plasticity. For instance, pigs exposed for five daily sessions of 15 min to an enriched pen already showed a trend for higher BDNF1 gene expression in the frontal cortex (Brown et al., 2017). The temporal response in BDNF concentration following the animal's interaction with the enrichment, the sustainability of BDNF concentration following removal of this enrichment, and whether BDNF concentration reduces after the interest by the animal in the environmental enrichment wane merit further research. Of note, BDNF is known to increase following increased sensorimotor activity (Rasmussen et al., 2009).

Exercise-induced BDNF increase is also a relevant aspect for enrichment *per se*, given that most animal species spend the majority of their active time foraging in the wild. However, it may be important to dissociate the effects of different forms of enrichment, such as those stimulating physical activity, exploration, or other sensory stimulation on BDNF. Comparative studies on the effect of different types of environmental enrichment on BDNF would be particularly informative to determine the mechanism by which various environmental enrichment interventions stimulates BDNF synthesis.

The fact that BDNF concentration in blood correlates well with brain concentrations in a number of species, including pigs (Klein et al., 2011), its generic function as the most abundant neurotrophin, and with a particular role in the hippocampal development, makes BDNF an ideal candidate as an indicator of environmental enrichment. The measurement of peripheral BDNF offers unique opportunities to assess the influence of husbandry practices on brain development, without requiring killing the animal for brain measurement.

In this study, BDNF was measured in serum, which is assumed to reflect the total pool of BDNF (Polacchini et al., 2015), as platelets contain large amount of BDNF that are released when platelets are activated during the clotting process through degranulation (Fujimura et al., 2002). We confirmed through our analysis with pig blood that BDNF is more reliably measured in serum than in plasma (Lommatzh et al. 2005), which could be due to the blood sample collection method and handling (Polacchini et al., 2015; Fujimura et al., 2002). It is also known that BDNF is present in higher concentration in serum compared to plasma (Radka et al., 1996), presumably due to that platelet activation during the clotting process (Fujimura et al., 2002). We were unsuccessful at measuring BDNF in pig saliva, and other recent attempts have also failed to date to reliably measure BDNF in human saliva (Vrijen et al., 2017), seemingly due to matrix interference. We analysed samples within 5 months of collection, and a previous study suggests that serum BDNF stored at -20°C progressively degrade, especially after 6 months of storage (Trajkovska et al. 2007). This could explain the much lower concentration observed in the present study compared to a previous preliminary trial (Rault et al., unpublished; 3250 pg/mL in 4 weeks old pig serum) and a previous study (Klein et al. 2011; 1000 pg/mL in 8-12 weeks old pig plasma), possibly due to differences in sample handling, temperature or centrifugation between studies.

From a methodological point of view, based on the measures collected in the present study and preliminary research (Rault et al., unpublished), the largest factors to consider when designing experiments on BDNF are:

Controlling for litter effect (due to genetic and/or early life experience)

Deciding on the matrix to sample (serum or plasma)

Sample processing (centrifugation due to the influence of platelet-stored BDNF), Sample storage (temperature and time; Trajkovska et al., 2007; Tsuchimine et al., 2014)

Appropriate sample size, and whether the assay measures pro-BDNF, mature BDNF or both forms (Polacchini et al. 2015) considering the two forms may activate different intracellular signalling pathways (Autry and Monteggia, 2012). Notably, BDNF is a protein hormone, but BDNF in whole blood BDNF does not appear to be particularly sensitive to freeze-thaw cycles (Trajkovska et al., 2007).

In conclusion, serum BDNF offers novel opportunities to clarify the functional effects of environmental enrichment and its links to animal welfare. The ability to measure BDNF in blood brings an ethical approach to the study of the effects of environmental enrichment on neurobiological development. A better understanding of the factors modulating BDNF and its link to welfare states may bring insight into the benefits of stimulating an animal's life.

5. Application of Research

At present, there is no reliable, validated and objective measure of successful environmental enrichment programs apart from labour intensive behavioural observations combined with the need for extensive physiological tests to assess the potential effects of enrichment use. This project showed that brain-derived neurotrophic factor (BDNF) measured in serum increases following the provision of foraging enrichment, and supports BDNF as a physiological indicator of effective enrichment programs in pigs. Furthermore, previous literature in humans and rodents supports the link between BDNF and positive welfare effects such as increased stress resilience, learning, memory and possibly positive affective states,

although we could not find relationship between pig serum BDNF and their performance in a specific maze test in the present study.

BDNF can be used by industry to assess and demonstrate the effectiveness of their enrichment programs, and by researchers and industry to assess new means of providing environmental enrichment. Commercial ELISA physiological kits are currently available and reliable. Hence, blood samples can be easily assayed for serum BDNF by University or diagnostic labs or with in-house self-diagnostic if the equipment is available. However, standardisation of the collection method, sample centrifugation is important to obtain rigorous results, and in that respect sample collection and centrifugation, probably more care than steroid hormones like cortisol that is more robust to handling and storage.

6. Conclusion

Brain-derived neurotrophic factor represents a promising physiological indicator of effective environmental enrichment interventions, which can be collected in serum and likely reflects its brain concentration and resulting effects.

7. Limitations/Risks

The present project only assessed BDNF in one herd of pigs, provided with one type of enrichment aimed to encourage foraging. Further research should assess the variation of BDNF concentration according to various contexts: farms, breeds, enrichment types, season, type of blood collection tubes etc.

The literature also suggests that large sample size be preferred, given the variability in BDNF concentration between individuals. The marked effects of litter, possibly reflecting genetic effects, is important to take into account in the experimental design to minimise variability.

8. Recommendations

The measure of BDNF could be included in future research on the benefits of enrichment for pigs.

9. Acknowledgements

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