Altered Hippocampal Epigenetic Regulation Underlying Reduced Cognitive Development in Response to Early Life Environmental Insults

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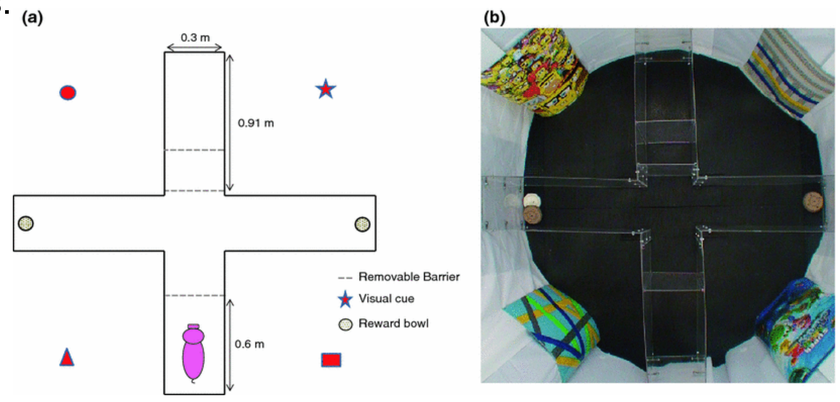
Introduction

- The hippocampus is involved in learning and memory and undergoes significant growth and maturation during the neonatal period.
- Environmental insults during neonatal developmental can affect epigenetic patterns and have lasting effects on brain function.
- Both iron deficiency and viral infection are common issues during childhood, and have been linked to cognitive impairments.
- Previous studies have demonstrated reduced hippocampal-based spatial learning and memory in piglets suffering from neonatal iron deficiency[1] and porcine reproductive and respiratory syndrome virus (PRRSv) infection[2].
- The aim of this study was to identify the epigenetic mechanisms responsible for reduced cognitive performance in response to early life environmental insults (iron deficiency and PRRSv infection).

Materials and Methods

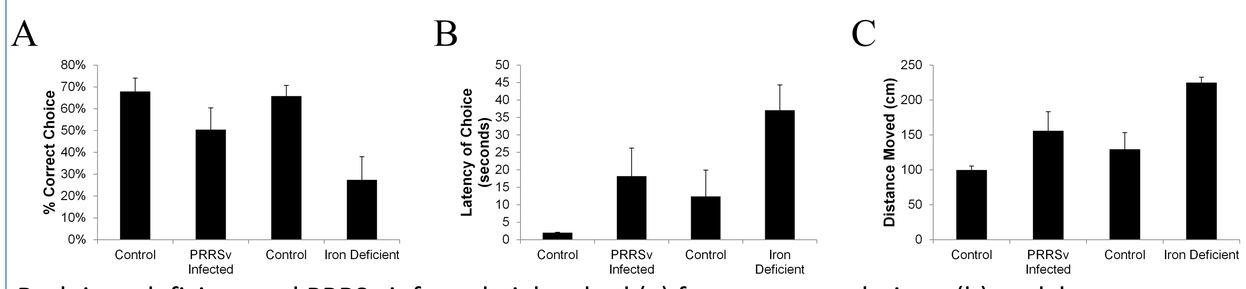
- Piglets were either fed an iron deficient diet (n=3), infected with PRRSv at 7 days of age (n=4), or remained untreated (control; n=9).
- Spatial learning and memory was assessed using a clear plastic T-maze with visual cues.



- Hippocampal DNA and RNA was extracted from 4 week old pigs.
- DNA methylation and gene transcription was assessed via reduced representation bisulfite sequencing (RRBS) and RNA-seq, respectively.
- Reads were aligned to Sscrofa10.2 using BS-Seeker2 (RRBS) or Tophat (RNA-seq)
- Quantifications were performed using BS-Seeker2 (RRBS) and Cufflinks (RNA-seq).
- The Boruta machine learning approach was utilized to identify DNA methylation and gene expression features associated with the reduced cognition phenotype.

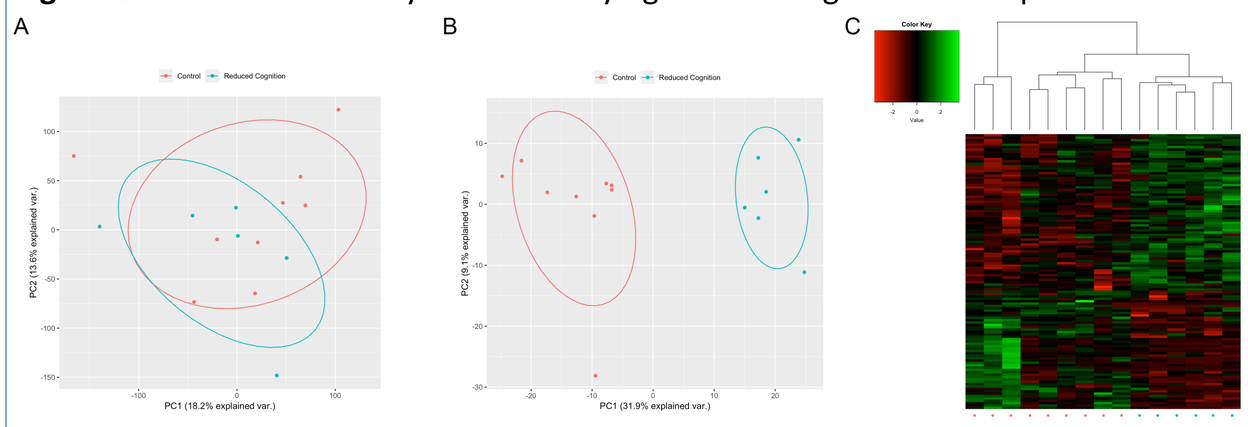
Results

Figure 1. Effects of early life environmental insults on cognitive development.



Both iron deficient and PRRSv infected piglets had (a) fewer correct choices, (b) took longer to locate the reward, and (c) covered more distance in the maize, all indicative of reduced cognitive performance.

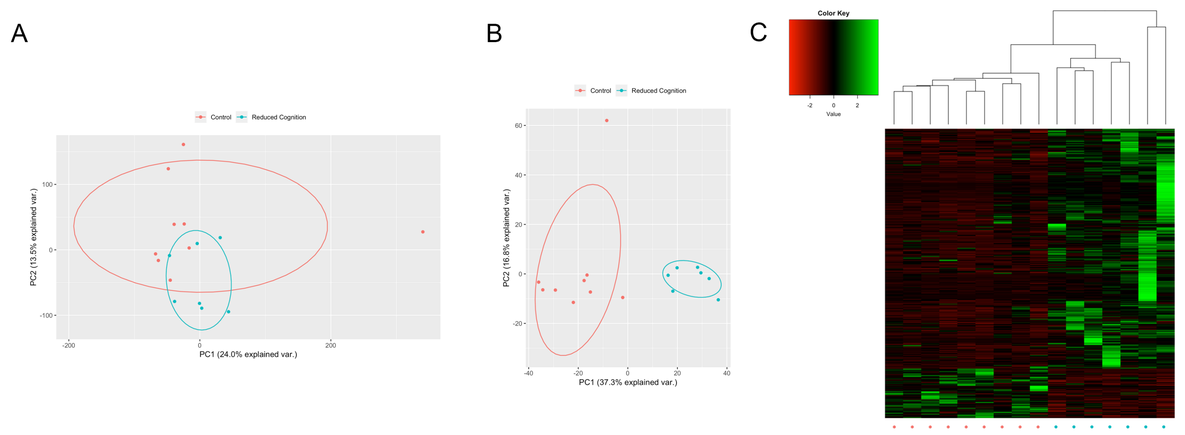
Figure 2. Altered DNA methylation underlying reduced cognitive development.



(a) Samples did not cluster by group when comparing methylation levels of the 30,696 tested regions (ANOSIM R = -0.06899, p-value = 0.731). (b) Samples clustered by group when comparing methylation levels of the 116 differentially methylated regions (DMRs; ANOSIM R = 0.7741, p-value = 0.001). (c) Heatmap displaying methylation levels at the 116 DMRs, represented as z-scores.

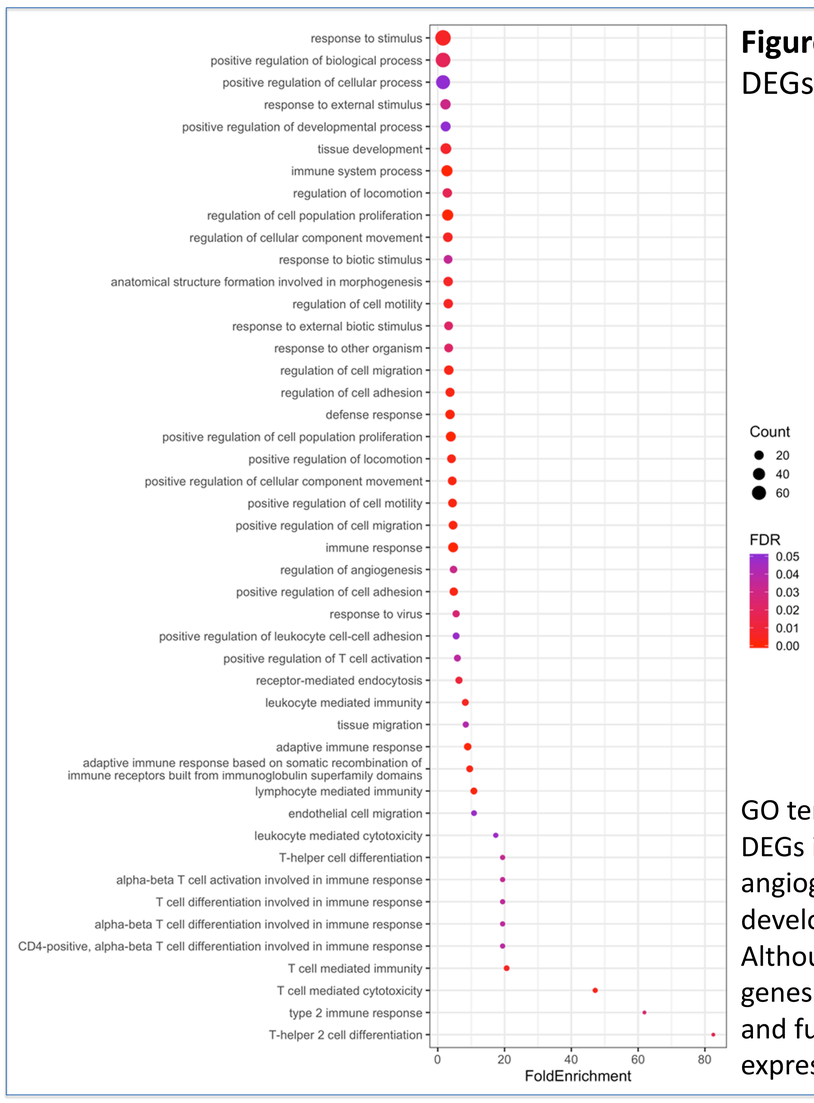
Results (cont.)

Figure 3. Altered gene expression underlying reduced cognitive development.



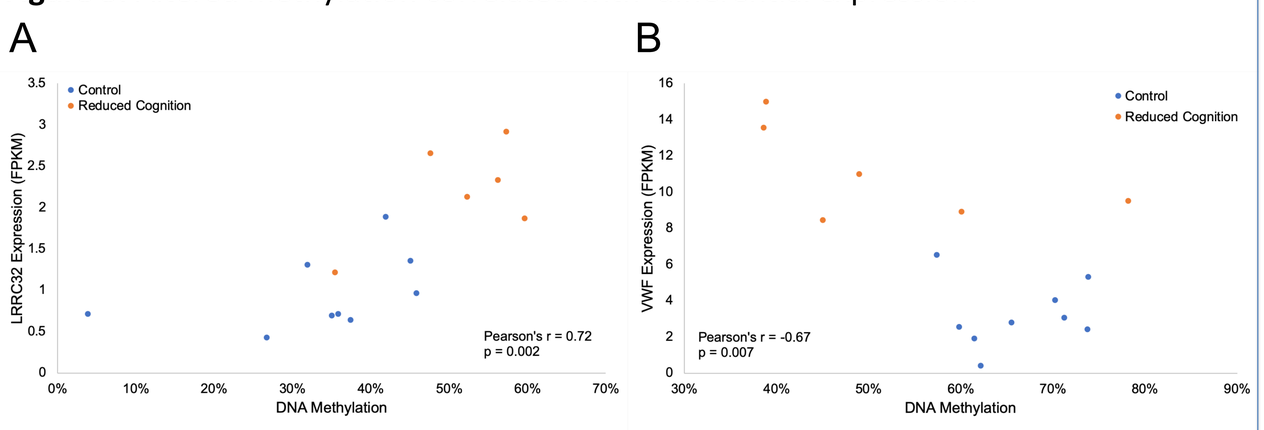
(a) Samples did not cluster by group when comparing methylation levels of the 38,522 tested genes (ANOSIM R = 0.1189, p-value = 0.075). (b) Samples clustered by group when comparing expression levels of the 420 differentially expressed genes (DEGs; ANOSIM R = 0.6981, p-value = 0.001). (c) Heatmap displaying expression levels at the 420 DEGs, represented as z-scores.

Figure 4. GO terms enriched for DEGs.



GO terms enriched for 217 known DEGs included immune responses, angiogenesis, and cellular development and proliferation. Although not enriched, a number of genes related to neurodevelopment and function were either differentially expressed or methylated.

Figure 5. Altered methylation correlated with differential expression.



Two DMRs overlapping two DEGs were identified. (a) A 60 bp region in the 2nd intron of *LRRC32* was hypermethylated and overexpressed in the reduced cognition group. (b) A 28 bp region in the 11th intron of *VWF* was hypomethylated and overexpressed in the reduced cognition group.

Conclusions and Future Work

- Altered hippocampal gene expression and DNA methylation associated with reduced cognition was observed across the two independent studies.
- GO term analysis identified enrichment of genes involved in immune response, angiogenesis, and cellular development and proliferation.
- Two DEGs were associated with 2 DMRs:
  - LRRC32 is a key regulator of TGF-B, controls activation of TGF-B1 on activated regulatory T cells.
  - VWF expression is associated with increased BBB permeability.
- Altered expression or methylation of genes involved in neurodevelopment and function were also identified.
- Further investigation of genes involved in neurodevelopment and function is required to identify disruptions resulting in cognitive deficiency.

References

[1]Rytych JJL et al. J Nutr (2012) 142:2050–2056.
[2]Elmore MRP et al. J Neurosci (2014) 34:2120–2129.

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