Necrotizing enterocolitis is associated with acute brain responses in preterm pigs - DTU Orbit (24/07/2018)

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**BACKGROUND:** Necrotizing enterocolitis (NEC) is an acute gut inflammatory disorder that occurs in preterm infants in the first weeks after birth. Infants surviving NEC often show impaired neurodevelopment. The mechanisms linking NEC lesions with later neurodevelopment are poorly understood but may include proinflammatory signaling in the immature brain. Using preterm pigs as a model for preterm infants, we hypothesized that severe intestinal NEC lesions are associated with acute effects on the developing hippocampus.

**METHODS:** Cesarean-delivered preterm pigs (n = 117) were reared for 8 days and spontaneously developed variable severity of NEC lesions. Neonatal arousal, physical activity, and in vitro neuritogenic effects of cerebrospinal fluid (CSF) were investigated in pigs showing NEC lesions in the colon (Co-NEC) or in the small intestine (Si-NEC). Hippocampal transcriptome analysis and qPCR were used to assess gene expressions and their relation to biological processes, including neuroinflammation, and neural plasticity. Microglia activation was quantified by stereology. The neuritogenic response to selected proteins was investigated in primary cultures of hippocampal neurons.

**RESULTS:** NEC development rapidly reduced the physical activity of pigs, especially when lesions occurred in the small intestine. Si-NEC and Co-NEC were associated with 27 and 12 hippocampal differentially expressed genes (DEGs), respectively. These included genes related to neuroinflammation (i.e., S100A8, S100A9, IL8, IL6, MMP8, SAA, TAGLN2) and hypoxia (i.e., PDK4, IER3, TXNIP, AGER), and they were all upregulated in Si-NEC pigs. Genes related to protection against oxidative stress (HBB, ALAS2) and oligodendrocytes (OPALIN) were downregulated in Si-NEC pigs. CSF collected from NEC pigs promoted neurite outgrowth in vitro, and the S100A9 and S100A8/S100A9 proteins may mediate the neuritogenic effects of NEC-related CSF on hippocampal neurons. NEC lesions did not affect total microglial cell number but markedly increased the proportion of Iba1-positive amoeboid microglial cells.

**CONCLUSIONS:** NEC lesions, especially when present in the small intestine, are associated with changes to hippocampal gene expression that potentially mediate neuroinflammation and disturbed neural circuit formation via enhanced neuronal differentiation. Early brain-protective interventions may be critical for preterm infants affected by intestinal NEC lesions to reduce their later neurological dysfunctions.