Neuronal Development in the Perinatal FGR Brain

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Introduction

Fetal growth restriction (FGR) is commonly caused by reduced availability of oxygen and nutrients from the placenta to the fetus. FGR may result in poor brain development and has been associated with a spectrum of adverse childhood outcomes. There are no current treatment options available. Inflammation is a key mechanism contributing to neuropathology in FGR newborns. Understanding the possible impact on neuronal development during the perinatal period may allow for better therapeutic treatments in these vulnerable cell populations.

Our aim was to investigate the temporal profile of neuronal maturation in the fetal and neonatal FGR pig brain.

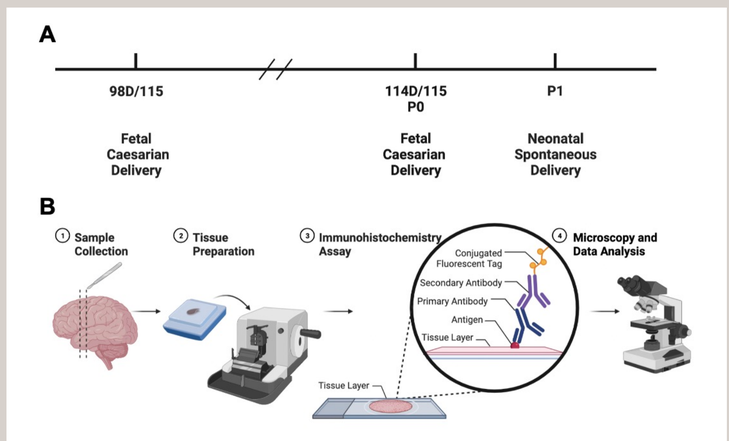

Figure 1: Representation of methods followed. A Experimental timeline for tissue sample collection. **B** Whole brains were flushed, collected, coronally sliced and fixed. Regions were embedded and sectioned using a microtome. Primary and Secondary antibodies were used to label cell markers of interest. Pictographs of the frontal cortex were subject to cell counts and density analyses.

Methods

The FGR piglet is a well-established preclinical model used to examine neuropathology and mechanisms underlying brain injury associated with FGR. Fetal FGR (<10th percentile) and normally grown (NG) piglets were delivered via caesarean on gestational days 98/115 (98D; n=14, equivalent to 26 weeks' gestation in humans) and 114/115 (P0; n=16). Neonatal piglets were spontaneously born and examined on the first day of life (P1; n=15, <24hrs).

Immunohistochemical Assay

Fixed brain tissue was labelled with immunohistochemical markers and counter stained with 4',6-diamidino-2-phenylindole (DAPI). Negative controls (omitting primary antibody) were processed in parallel. All staining was conducted in triplicates.

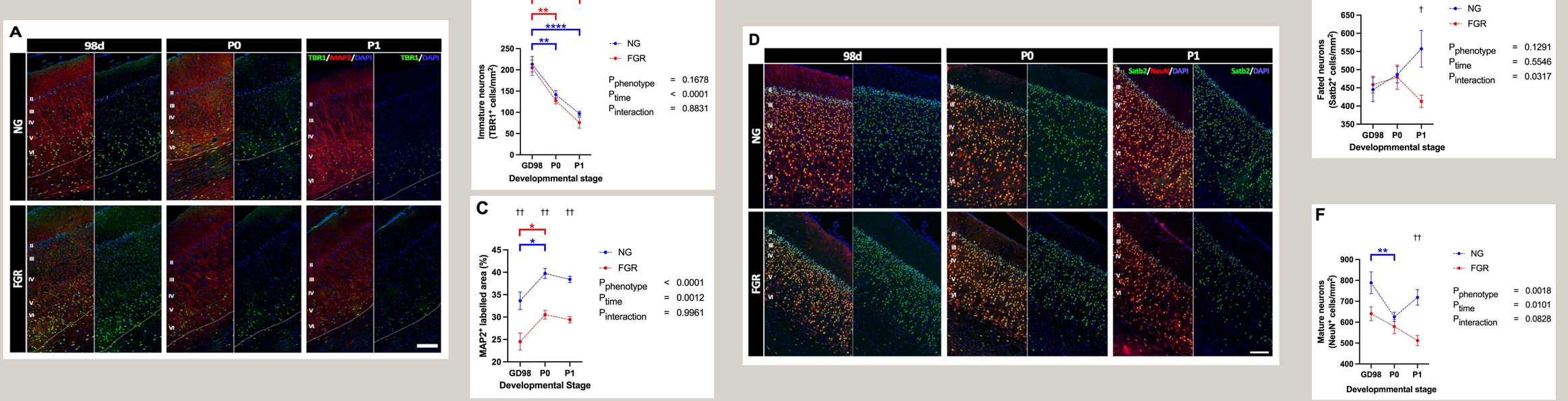

Figure 2: Representative labelling of neuronal markers across all cortical layers during the perinatal period with graphs of percentage area labelled (MAP2) and density cell counts (TBR1, NeuN, STAB2). Significance within phenotype is represented by colour (NG: blue, FGR: red), symbol represents significance between phenotypes. All data presented as mean ± SEM (minimum n=7 for all groups). Mixed effects two-way analysis of variance (ANOVA) with the post-hoc Sidak. **A.** TBR1 positive cells (Green) and MAP2 positive area coverage (Red) in NG and FGR cortex across all stages of development. Both phenotypes expressed TBR1-positive cells with decreasing density at later stages of development. MAP2 density was reduced in the FGR cortex across all time points. Scale bar: 100um. **B.** TBR1 density with a mains effect for developmental stage. **C.** MAP2 coverage was significantly reduced in the FGR cortex at each stage. **D.** SATB2 (Green) and NeuN (Red) positive cells in NG and FGR cortex across all stages of development. SATB2 and NeuN labelling in the FGR cortex had altered distribution across cortical layers during the perinatal period, and decreased labelling density at P1. **E.** NeuN density with a mains effect for developmental stage. **F.** SATB2 density reduced in FGR at P1.

Primary Antibody Marker	Labelling
Microtubule-associated protein 2 (MAP2)	Neuron Soma and Dendrites
T-Box brain transcription factor 1 (TBR1)	Intermediate Neurons and Laminar Identity
Neuronal nuclei (NeuN)	Mature Cortical Neurons
Special AT-rich sequence-binding protein 2 (SATB2)	Calloso-Cortical Fated Neurons

Discussion

These findings indicate early reductions in neuronal structure in the FGR fetal cortex that are also evident in the neonatal brain. Most alterations to the density and distribution of mature neuronal cell populations appear to occur in the neonatal brain.

Studies have previously identified upregulation of the inflammatory response in the neonatal brain. It is currently unknown if this is influencing neuronal development prior to birth. Characterising the inflammatory profile during the perinatal period may support the working hypothesis that inflammation is upregulated during birth.

Therefore, endorsing early administration of anti-inflammatory treatments after spontaneous delivery to support vulnerable neuronal populations in the FGR cortex. This may be an effective intervention strategy to improve neurodevelopmental outcomes in FGR newborns.

Results

Intermediate neurons regulate layer specification at a consistent density across both phenotypes.

TBR1 was consistently expressed across both phenotypes and reduced in density at subsequent time points.

Impaired neuronal structure was observed in the FGR cortex across all time points.

MAP2 labelling indicated reduced somato-dendritic coverage in the FGR cortex across all time points. Both phenotypes demonstrate similar trends that increased in the fetal cortex and then refined coverage following spontaneous delivery.

Differentiated neuronal cell populations were reduced in density in the neonatal FGR brain following spontaneous delivery.

The fetal brain demonstrated no difference in SATB2 density between phenotypes. A significant decrease in the FGR cortex was observed at P1. The FGR cortex presented high-density labelling across a wider area of outer cortical layers and had a greater reduction of cells in the deeper layers than NG.

The density of mature neuronal cell populations was reduced in the neonatal FGR cortex.

NeuN labelling demonstrated no difference between NG and FGR phenotypes in the fetal brain at 98D and P0. The density of mature cortical neurons was reduced in the FGR cortex at P1.

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