10/03/23

# Choline: a potential neuroprotective agent for microglia recovery from prenatal ethanol exposure

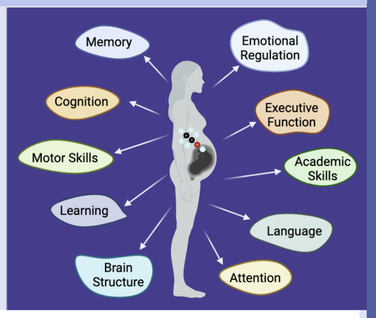
Charuta Saha, Fiona Ramnaraign, and Dr. Brian Christie

*This research was supported by the Jamie Cassels Undergraduate Research Awards, University of Victoria," and "Supervised by Department of Medical Sciences, University of Victoria, Victoria, BC.*

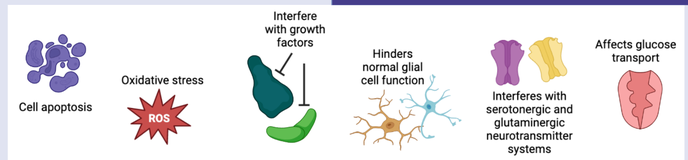
## How does Fetal Alcohol Spectrum Disorder affect learning and memory?

Fetal alcohol spectrum disorder (FASD) is a collection of neurobehavioral and cognitive effects arising from prenatal exposure to alcohol.[1,2] A particular brain area highly susceptible to FASD-related neurodegeneration is the hippocampus; an area essential for learning and memory.[3]

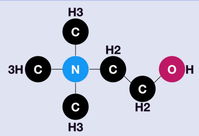
**The current prevalence of FASD in the general Canadian population is 4%.**[3]



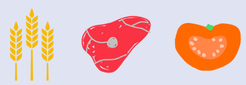

**Prenatal ethanol exposure affects synaptic plasticity through the following mechanisms:**[5,6,7]




## How might choline supplementation help recover effects of prenatal ethanol exposure?




Choline is an essential nutrient present naturally in foods such as red meat, legumes, wheat germ and even pumpkin seeds.[8]



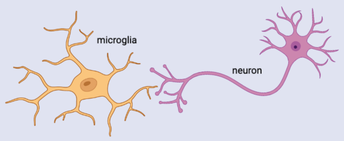
Precursor to neurotransmitter, acetylcholine.

Promotes lipid transport and membrane integrity

Methyl donor

**Choline and alcohol overlap in their pathways of action but the exact molecular mechanism by which choline can reverse the neurodegenerative effects of alcohol is not yet well understood.**

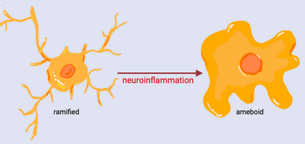
## The role of microglia in mediating synaptic plasticity during development.




**Microglia mediate synaptic pruning during development to control formation of precise neuronal circuitry.**[9]

**Chronic microglia up-regulation leads to:**[10]

- ↓ Synaptic plasticity
- ↑ TNF-α, and MCP-1 mediated cell apoptosis



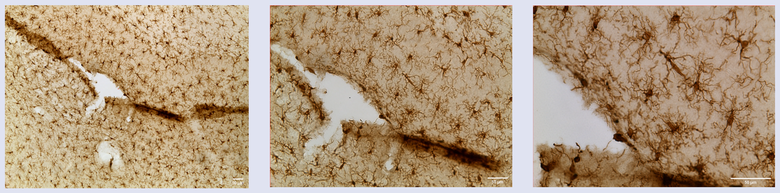



**Figure 1. IBA-1 staining of microglia in the hippocampus at 10X, 20X, and 40X magnification.** Cell counting will be performed at 10X magnification. Magnifications ranging from 60X to 100X will be used for the morphology analysis in order to visualize the morphology of microglia from a ramified to amoeboid shape.

**References.** (1) Noor 2018; (2) Mattson 2019; (3) Gil-Mohapel 2010; (4) Flannigan 2018; (5) Welch-Carre 2005; (6) Morris 2019; (7) Fonatine 2016 (8) Ernst 2022; (9) Kane 2021; (10) Lana 2021; (11) Ohsawa 2000

## Treatment Paradigm

**PD0: Birth**

79 Sprague Dawley pups randomly assigned to one of four treatment groups

**PD4-9: EtOH Exposure**

**Dose:** 5.25g/kg EtOH

**Control:** equivalent milk diet

Administered via gastric intubation

**PD10-30: Choline Supplementation**

**Dose:** 100mg/kg choline solution

**Control:** saline solution

Administered via subcutaneous injection

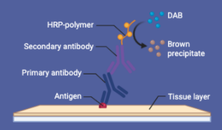
**PD36: Euthanasia and Tissue Collection**

Intracardial Perfusion performed and brain tissue fixed via paraformaldehyde before collection..

**Tissue Sectioning**

Vibratome used to section tissue into **50 µm** coronal slices.

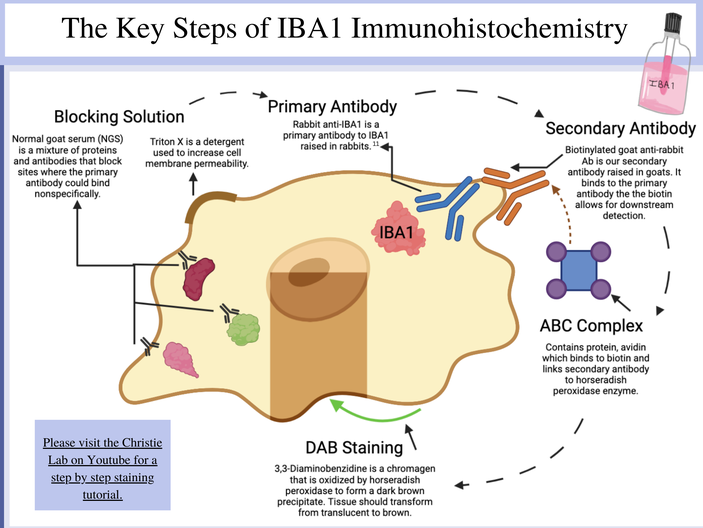
**IBA1 Immunohistochemistry**




**Imaging and Analysis**

Imaged at 10X using brightfield microscopy. Cell density and morphology analysis performed.

## The Key Steps of IBA1 Immunohistochemistry




Please visit the Christie Lab on Youtube for a step by step staining tutorial.

## Regions of Interest

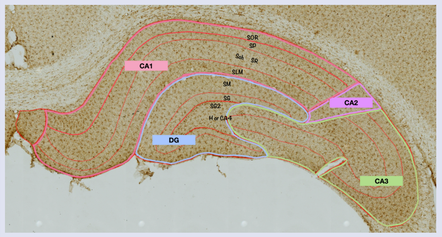



**Figure 7. Regions of interest.** The regions of interest (CA1, CA3 and DG) and molecular layers within the hippocampus.

**Regions of Interest**

- CA1
- DG
- CA3

## Parameters for Analysis

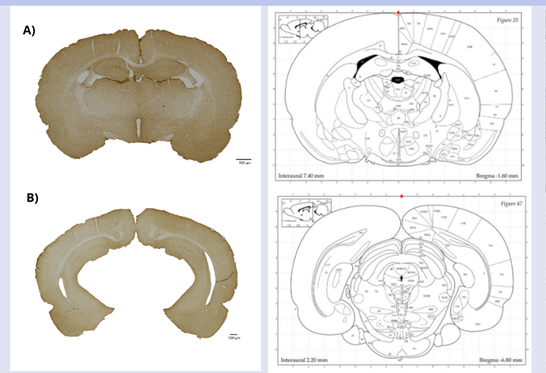



**Figure 4. Range of Hippocampal Tissue.** Brightfield microscopy images of dorsal (A) and ventral (B) IBA-1 stained PD36 rat brains. BREGMA coordinates (-1.60mm to -6.80 mm) were used to define the range of hippocampal regions.

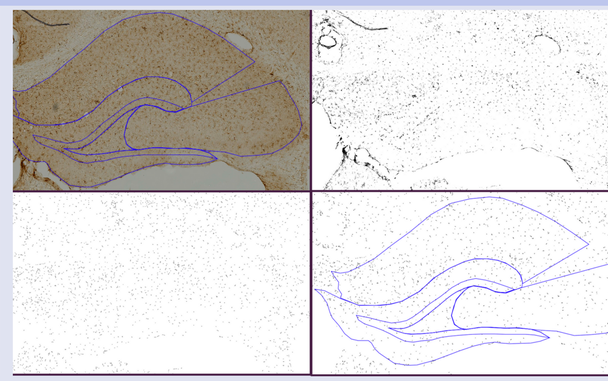


**Figure 5. Automated processing and analysis of microglial cell densities.** (A) Regions of interest outlined (B) Background subtraction and processing of binary image (C) Outlines of analyzed particles (D) Cell density output: IBA1 particle count/area of region of interest.

## Preliminary Results

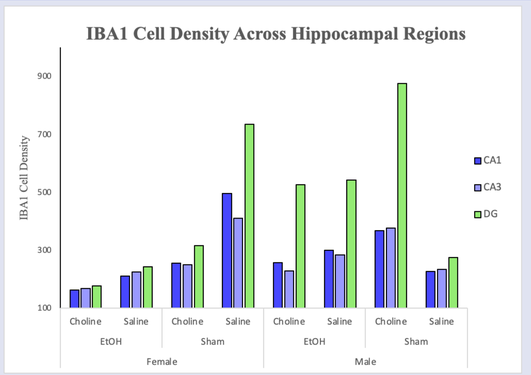



**Figure 2. Average IBA1 cell densities across CA1, CA3 and DG regions.** In both male and female subjects, the cell densities were lower in the EtOH-choline condition than the EtOH-Saline condition. In females, cell densities were highest in the saline-sham condition and in males in the choline-sham condition. Across all conditions, cell densities were highest in the DG region.

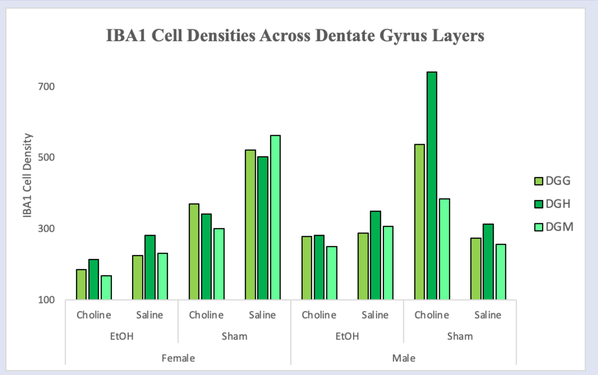



**Figure 3. Average IBA1 cell densities across dentate gyrus layers.** In male subjects, the DG hilus generally had the highest cell densities across all conditions. In female subjects, cell densities were more varied across the DG layers. In all DG layers, cell densities in the EtOH-choline condition were lower than in the Saline-EtOH and Choline-Sham conditions.