Ethanol-Induced Neuroinflammation in the Developing Hippocampus: Mast Cells and Microglia

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Fetal Alcohol Spectrum Disorders

FASD afflicts up to 5% of American school age children (May et al., 2009), with an estimated cost of $6 billion annually in the US (Popova et al., 2011).

Structural and functional neurological deficits are a common outcome in FASD, including impairments in executive function, learning, and memory (Fuglestad et al., 2015; Mattson et al., 2011).

FASD model rats demonstrate impaired hippocampal-dependent learning and memory as juveniles and adults, including deficits in one-trial context fear conditioning and trace fear conditioning (DuPont et al., 2014; Goodfellow and Lindquist, 2014; Hunt et al., 2009; Murawski and Stanton, 2010).

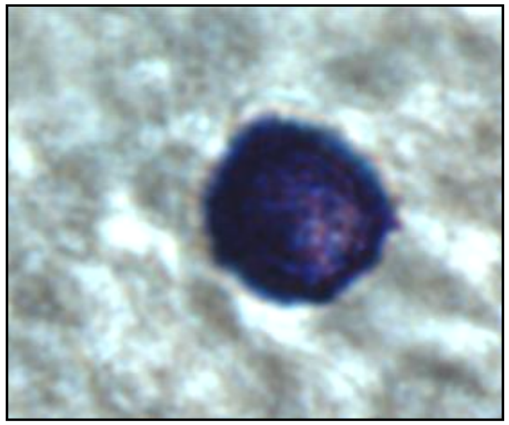
FASD & Inflammation

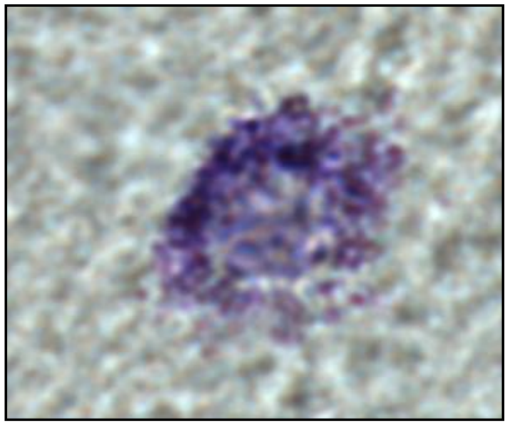
Perinatal ethanol exposure in rodents induces wide-ranging deleterious effects, including activation of neuroimmune cells and a coordinated inflammatory response (Boschen et al., 2016; Bodnar et al., 2016) that can persist long after the exposure period (Tiwari and Chopra, 2011).

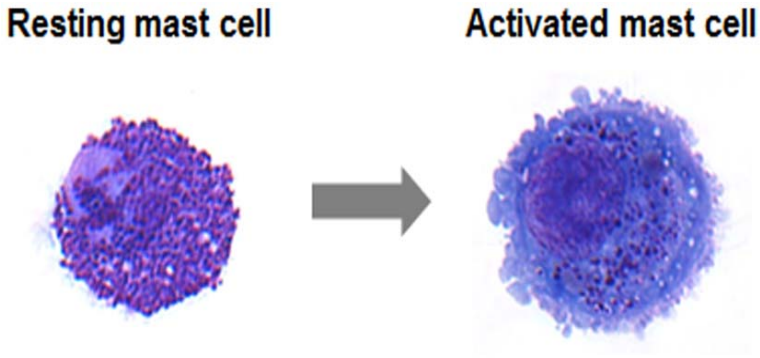
Microglia play a pivotal role in the development and progression of neonatal neuroinflammation, and their activation in response to early-life ethanol is a topic of increasing study (e.g., Drew and Kane, 2014; Guizzetti et al., 2014).

Mast Cells

Mast cells (MCs), an understudied central immune cell, may also be activated by ethanol, contributing to neuroinflammation in the neonate hippocampus.







Lorentz et al. 2012

Mast Cell in resting (granulated) state and activated (degranulated) state.
Image courtesy of Dr. Kathryn Lenz.

Mast Cells

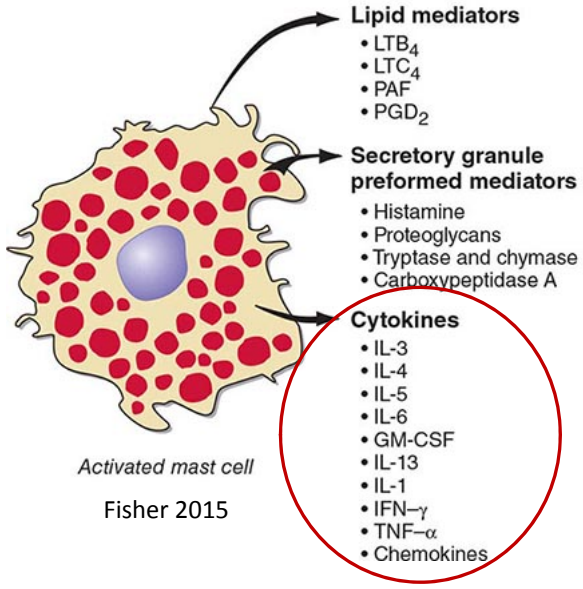
Part of the innate immune system—with a long recognized role in allergy and asthma—MCs form from hematopoietic stem cells in bone marrow (Gilfillan et al., 2011; Lambracht-Hall et al., 1990).

MCs gain rapid entry to the developing brain via blood-brain barrier (BBB) passage, with ~97% of mast cells found on the brain side (Silverman et al., 2000) in close association with cerebral blood vessels (Michaloudi et al., 2003).

MCs are most abundant during early brain development in humans (Dropp, 1979) and, in rodents, a large proportion of total brain MCs are located in and around the hippocampus, peaking around PD5 (Nautiyal et al., 2012).

Mast Cells

MCs act as '**first-responders**' at sites of pathogenic injury or infection, releasing pre-formed and newly synthesized pro-inflammatory mediators, including cytokines (Marshall, 2004; Silver and Curley, 2013).



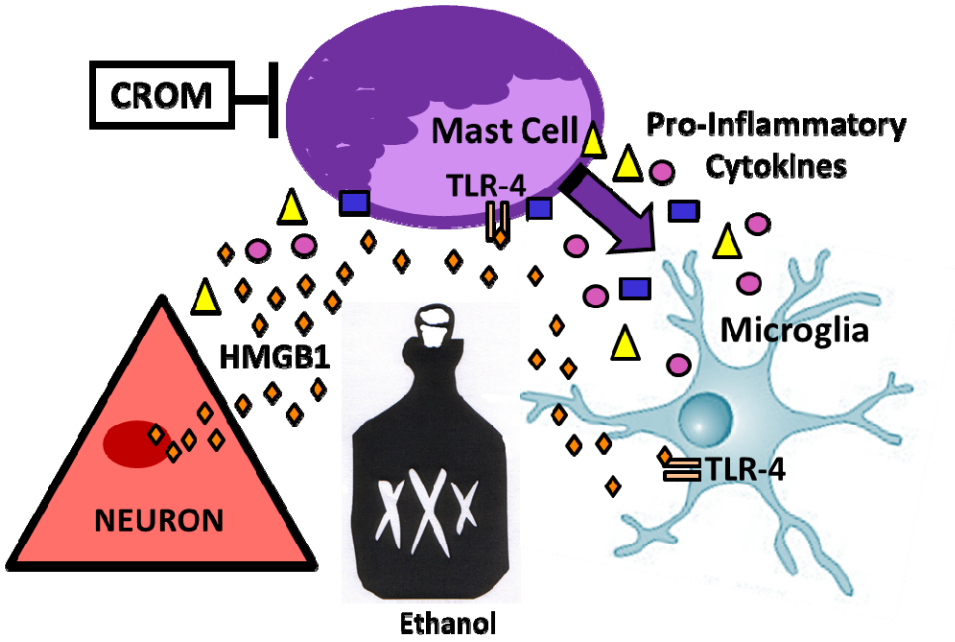
Fisher 2015

MCs (like microglia and astrocytes) express Toll-like receptor (TLR)-4 (Fernandez-Lizarbe et al., 2013; Pietrzak et al., 2011).

TLR-4 can be directly activated by ethanol or indirectly activated via the dose-dependent release of high mobility group box 1 (HMGB1) from neurons and other cells (Montesinos et al., 2016).

Mast Cells & Microglia

Ethanol is hypothesized to induce rapid MC degranulation in the neonate hippocampus, catalyzing the recruitment, amplification, and propagation of other neuronal and neuroimmune responses, including microglia.



Three Questions

1. Does postnatal ethanol induce MC degranulation in and around hippocampus?
2. Does MC degranulation increase the morphological activation of hippocampal microglia?
3. Does inhibition of MC degranulation (via Sodium Cromolyn) attenuate microglia activation?

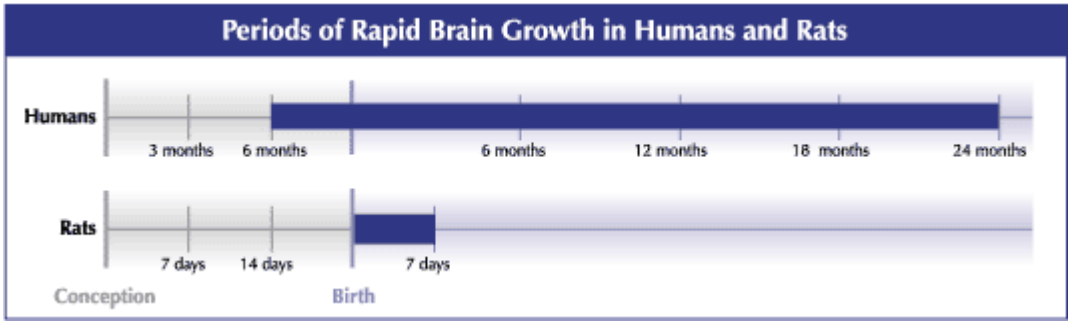
Experimental Methods

FASD: Binge-like third trimester-equivalent ethanol exposure (PD4-6) in rats

Ethanol/milk solution administered via intragastric intubation:

- **5E:** 5 g/kg/day (11.33% v/v)
- **SI:** sham intubated
- **UC:** unhandled control

Third trimester 'brain growth spurt'



Male rats only
Group sizes = 4 to 6
Blood Alcohol Concentration: ~360 mg/dl

Tyler Dause

Experimental Methods

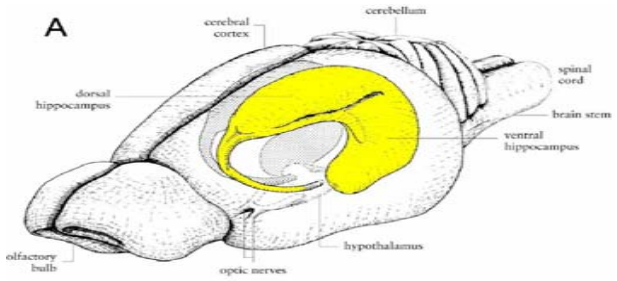
FASD: Binge-like third trimester-equivalent ethanol exposure (PD4-6) in rats

Approximately 30 min prior to first intubation across PD4-6, **SI** and **5E** rats cryoanesthetized; sterile saline (VEH) or sodium cromolyn (**CROM**; 100 µg/µl) administered via bilateral ICV injections (1.5µl per side); **UC** rats do not undergo surgery

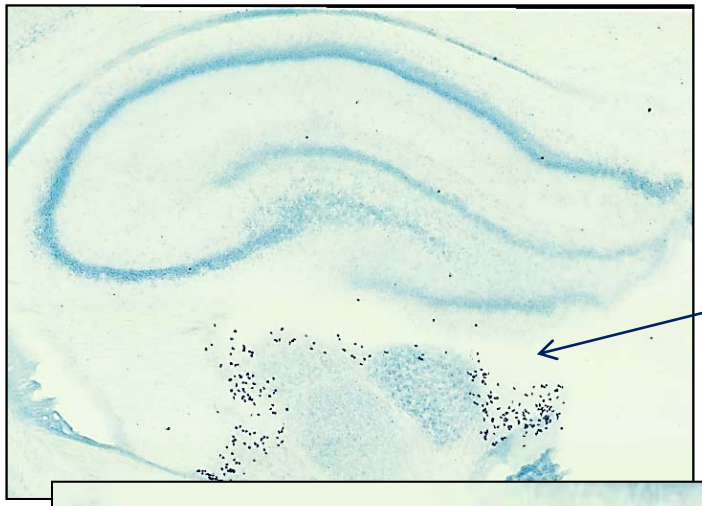
On PD6, 2 h after last intubation, all rats sacrificed and whole (dorsal and ventral) hippocampus sectioned (50 µm)

Microglia: 1/8 sections stained for Iba-1 antibody

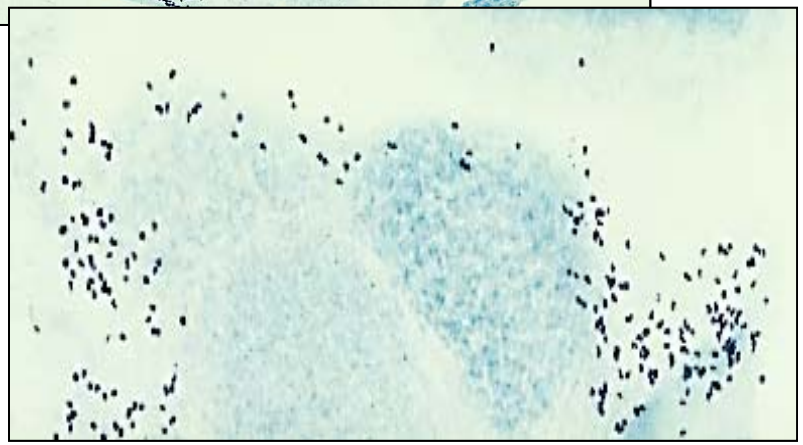
MCs: remaining sections stained with Toluidine Blue

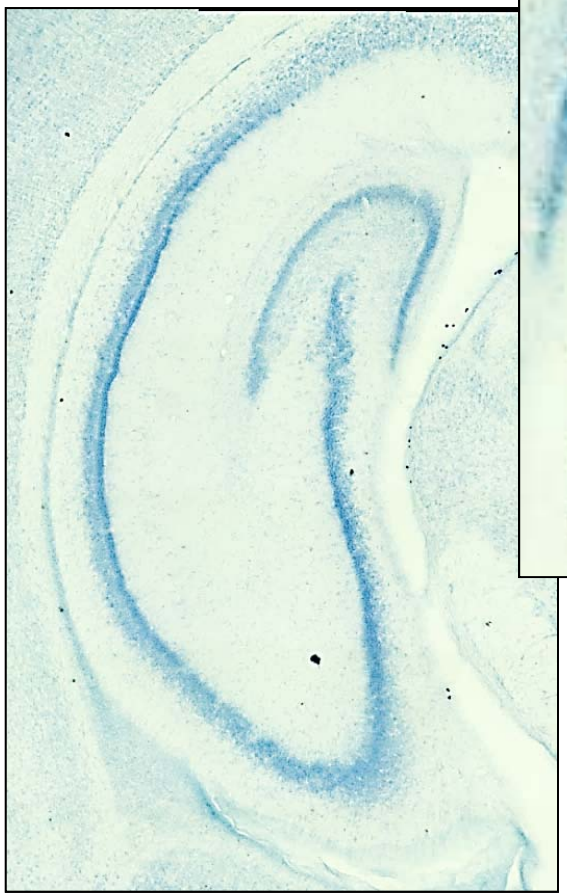


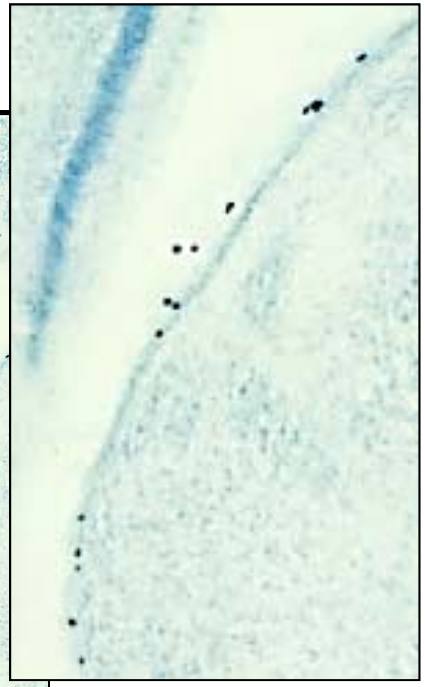
Mast Cells



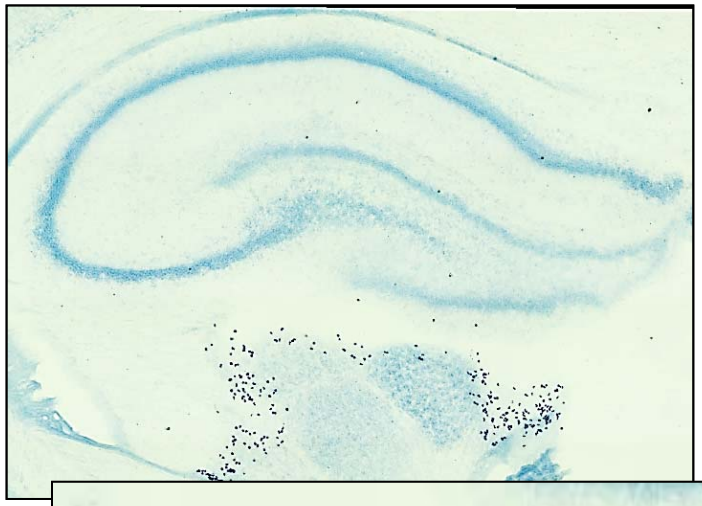
Velum Interpositum

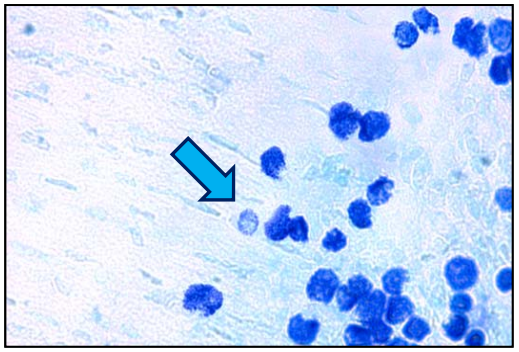


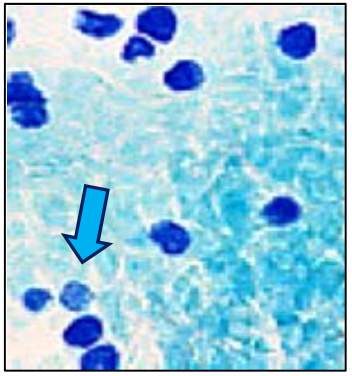


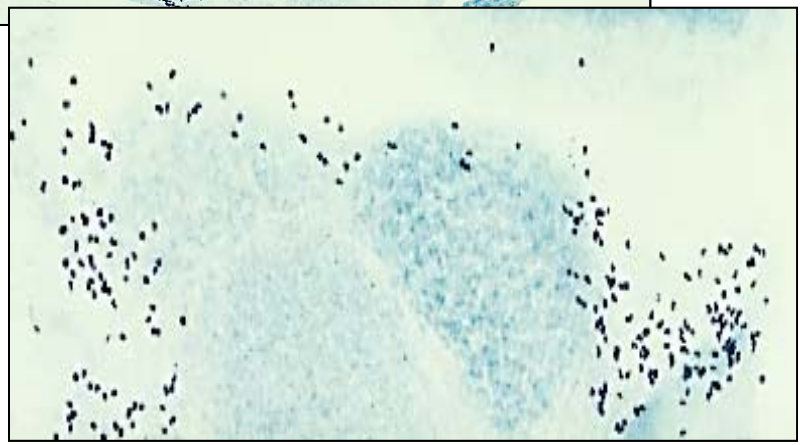


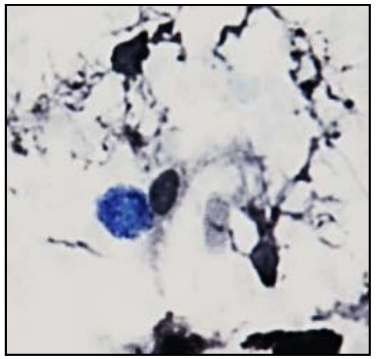
Mast Cells







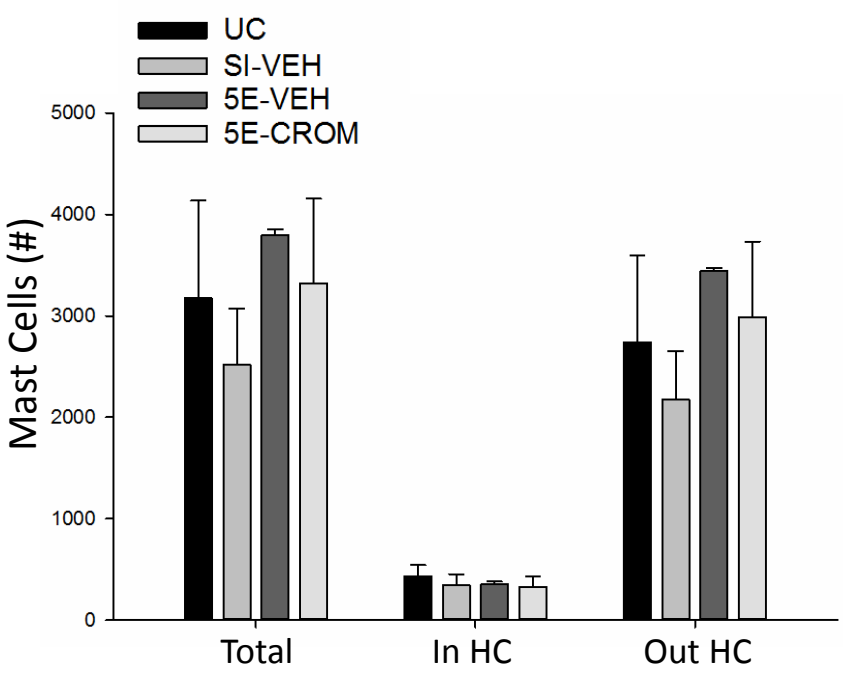


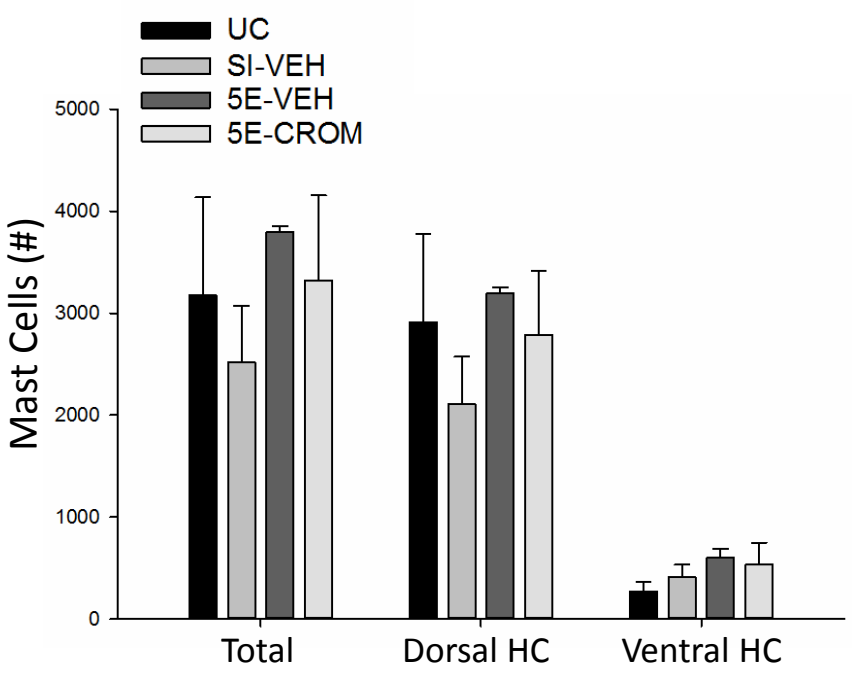


Criteria for MC degranulation includes loss of blue stain, fuzzy appearance, or visible granules in the vicinity of the cell (Dong et al., 2016).

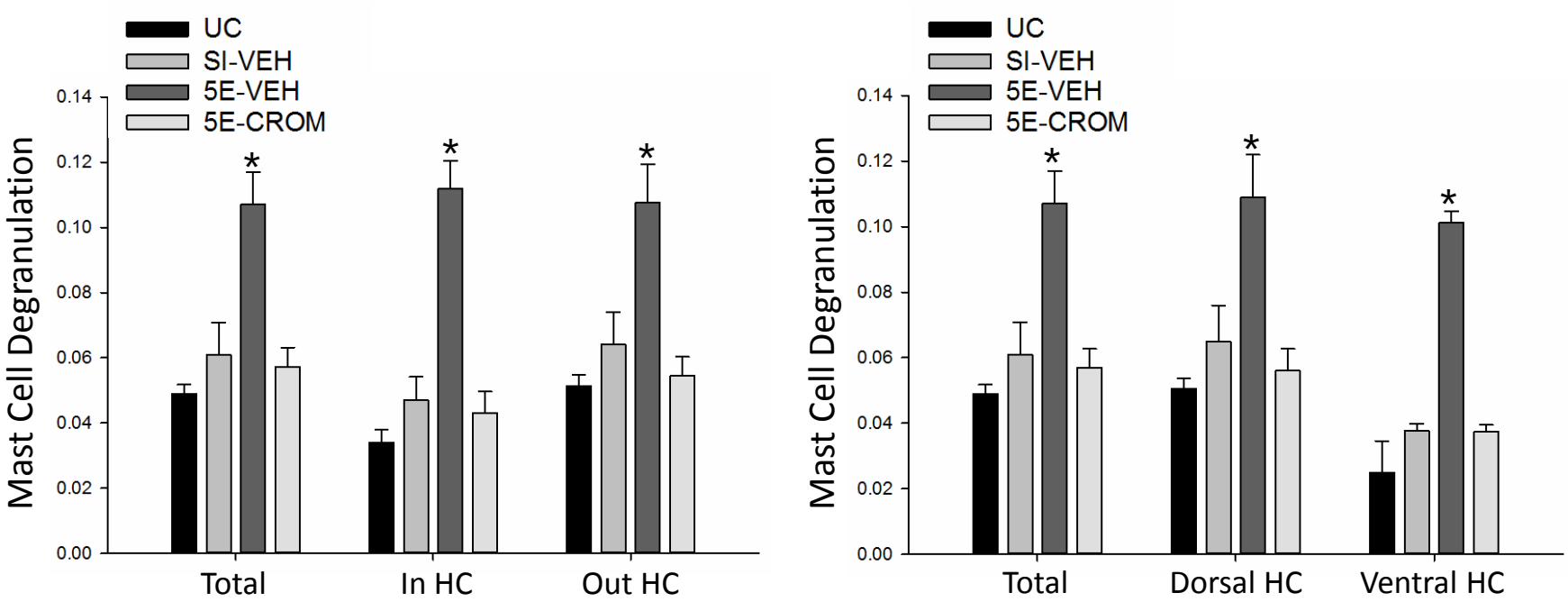
Inter-rater coefficient of determination of r^2=0.99

Total Mast Cells



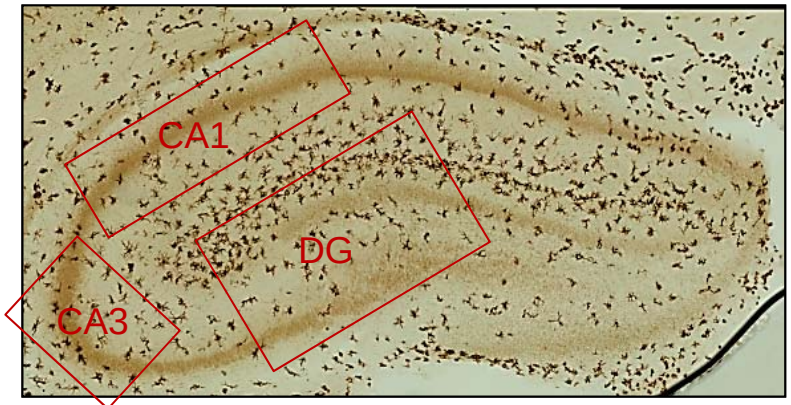


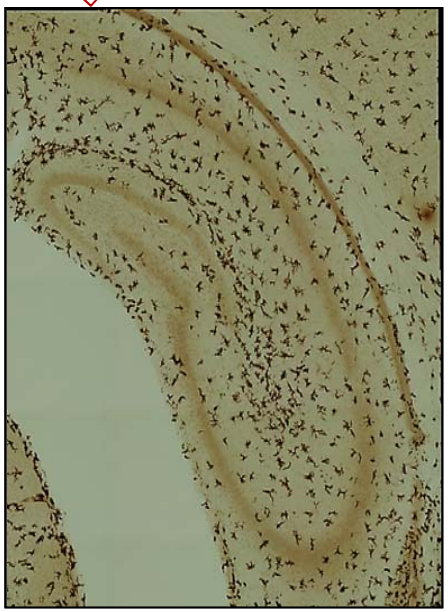
Proportion of MC Degranulation

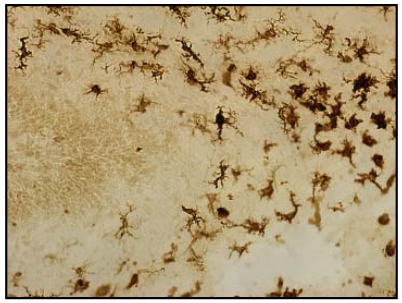


Postnatal ethanol roughly doubles the proportion degranulated Mast Cells
Pre-treatment with Sodium Cromolyn inhibits ethanol-induced degranulation

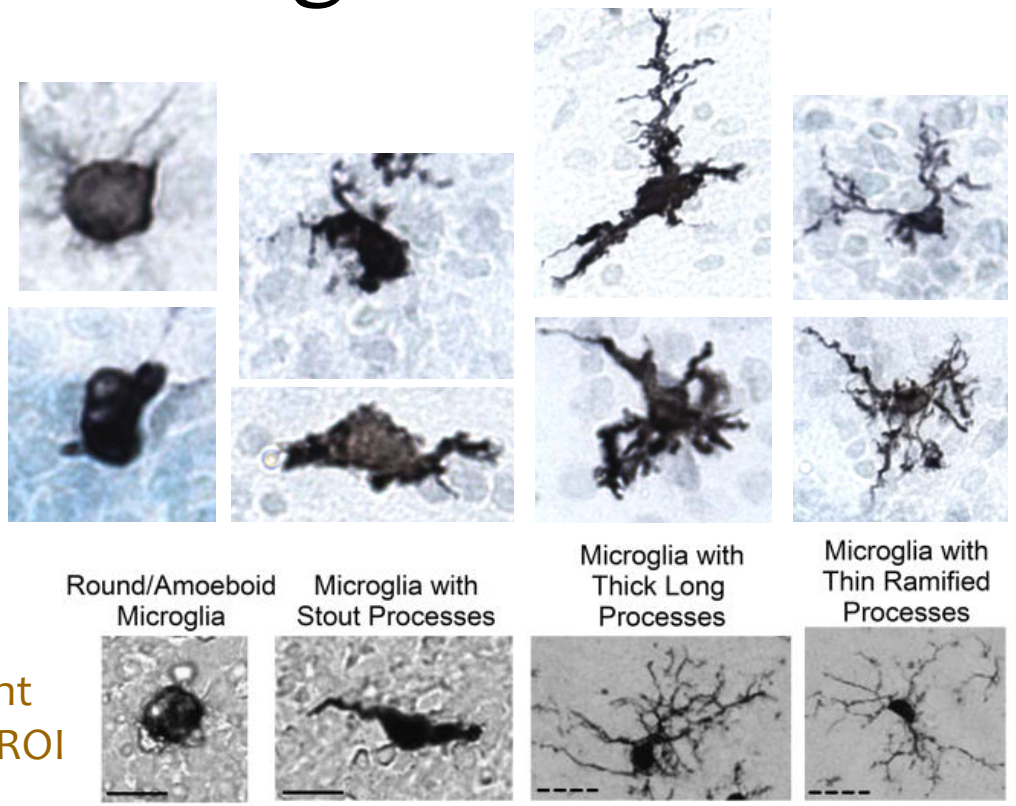
Microglia





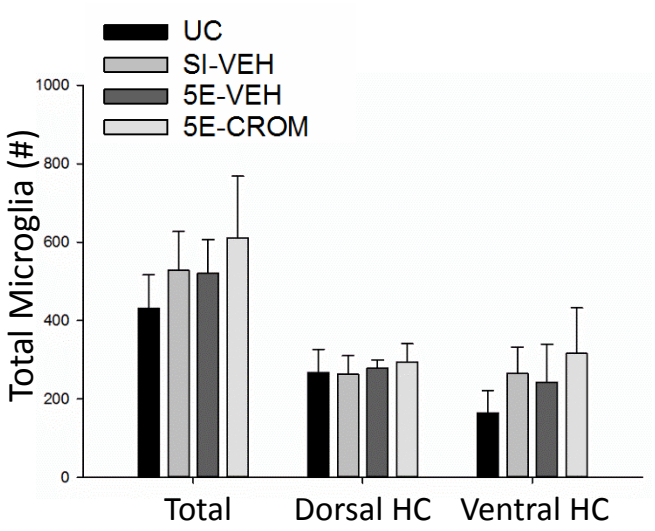


Unbiased Stereology
ROI area held constant
~10 counting frames/ROI

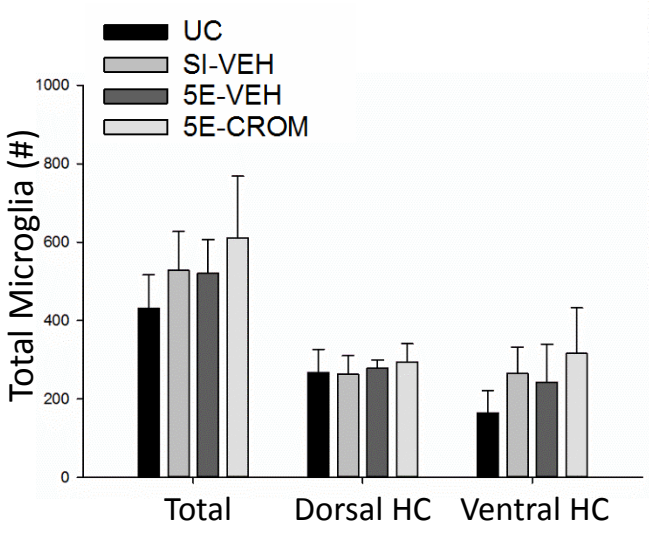


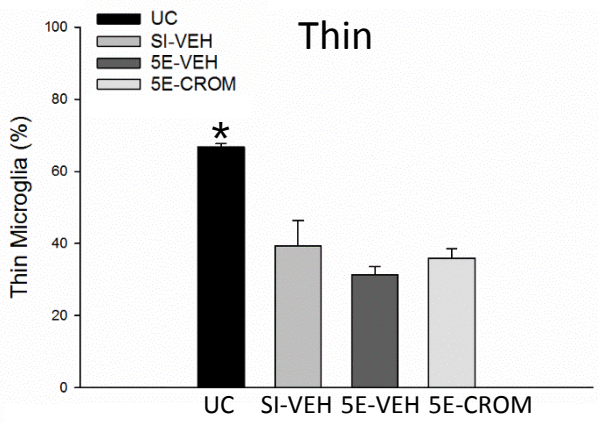
Schwarz, Sholar, Bilbo (2012)

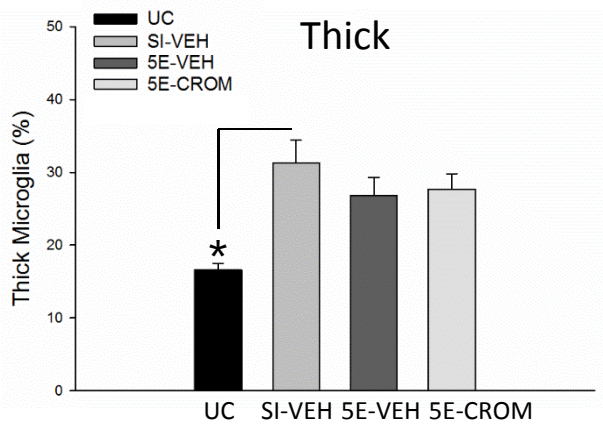
Microglia: Whole HC

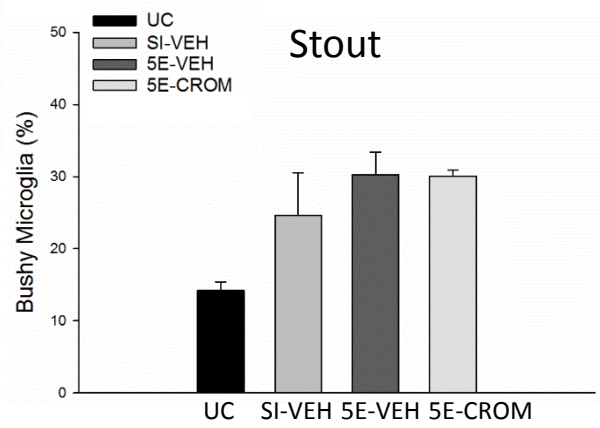


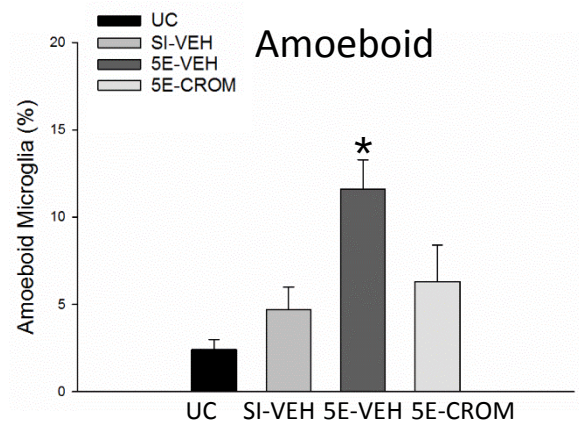
Microglia: Whole HC





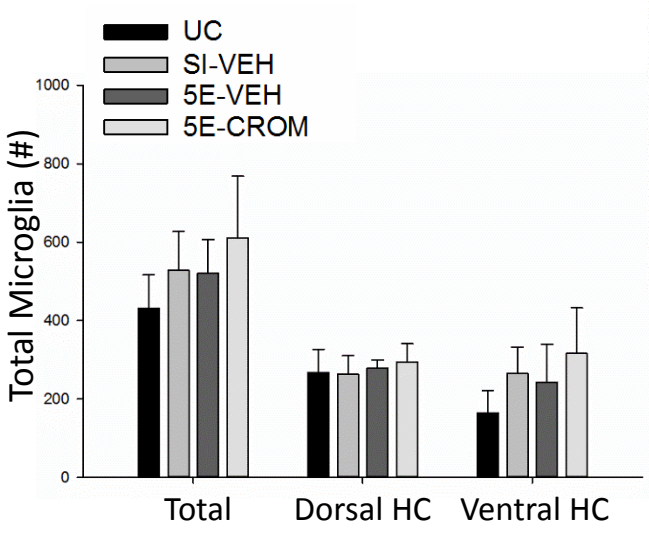




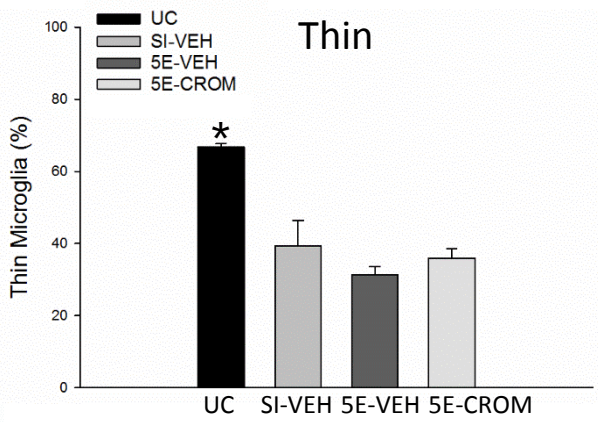


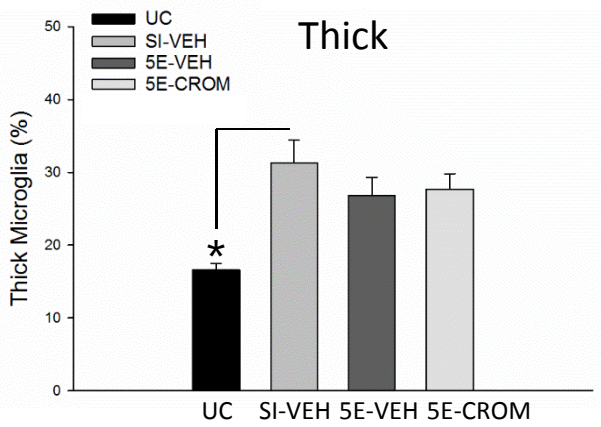
Postnatal ethanol promotes the morphological activation of microglia—i.e., more amoeboid phenotype

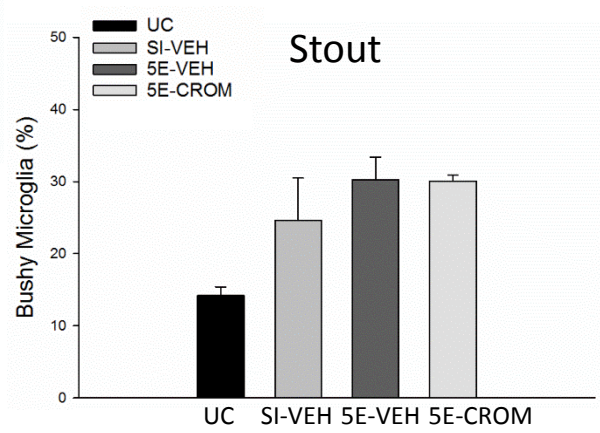
Microglia: Whole HC

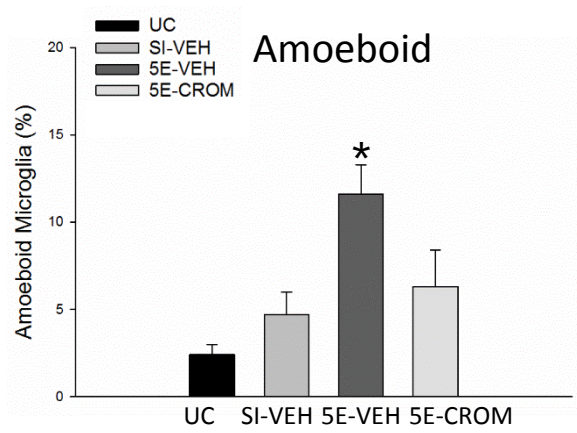


UC results suggest microglia transition driven in part by intubation procedure or surgery….

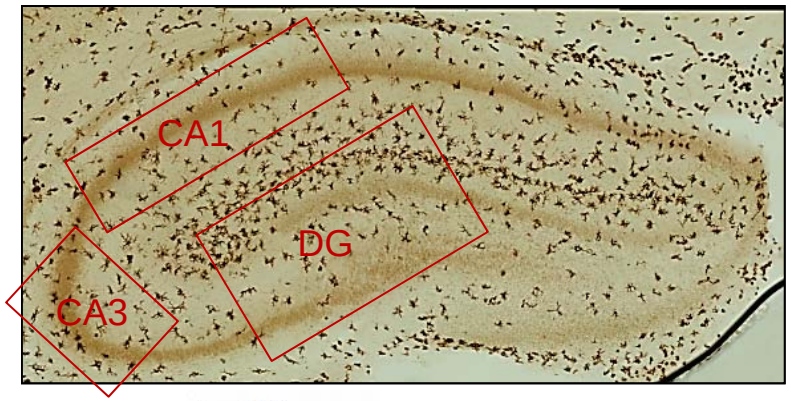


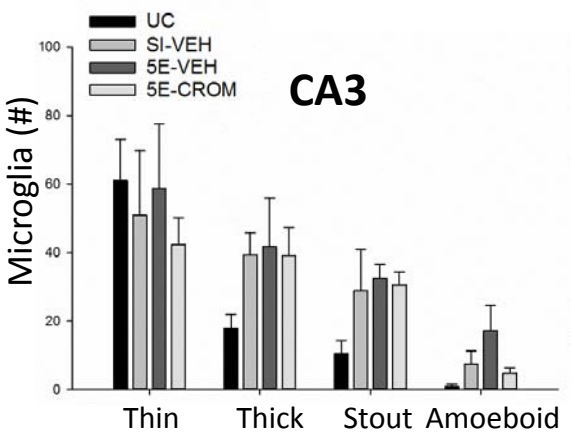


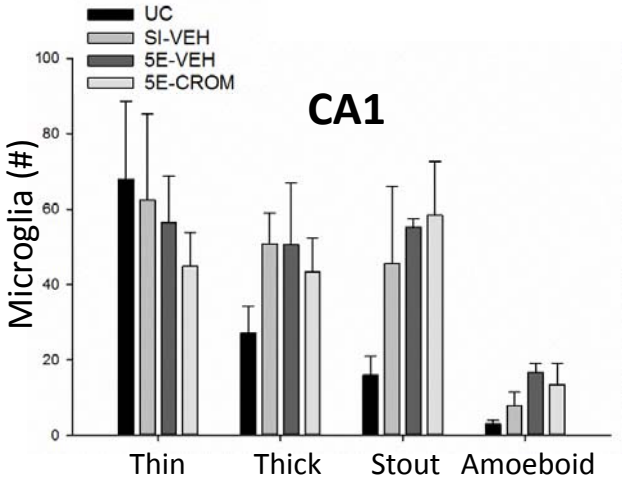


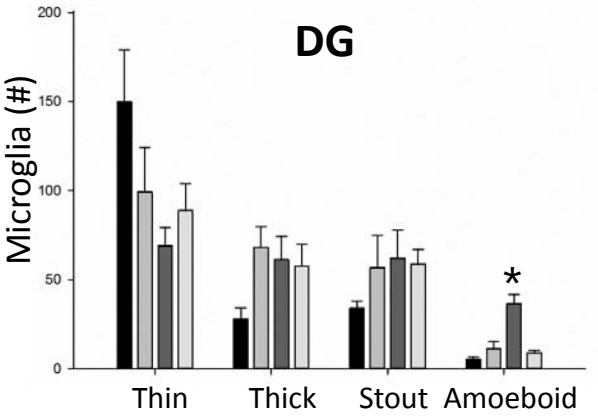


Microglia: By Region









FASD / MCs / Microglia

PD4-6 binge-like ethanol exposure in male pups significantly increased MC degranulation in and around the neonate hippocampus.

- Proportion degranulated: 3-6% in UC and SI rats; 10-12% in 5E-VEH rats
- Rates roughly comparable for MCs inside and outside hippocampus

Postnatal ethanol significantly increased the proportion of amoeboid microglia in whole hippocampus, and by region, in the dentate gyrus.

Cromolyn significantly diminished MC degranulation in 5E rats and the proportion of hippocampal amoeboid-shaped microglia.

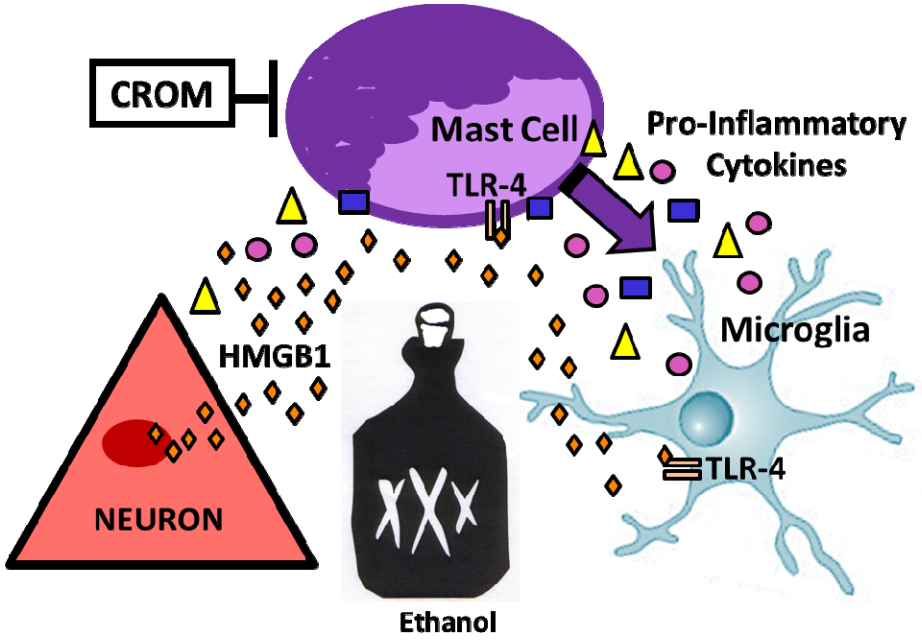
Next up: Female rats

FASD / MCs / Microglia

Early in development, cytokine release by MCs and microglia can produce positive feedback loops (i.e., chronic neuroinflammation) that persist long after the initiating inflammatory event (Dong et al., 2016).

In turn, prolonged cytokine release in early development can disrupt later-life behavior and cognitive function (e.g., Skaper et al., 2014; Williamson et al., 2011).

Studying the effects of perinatal ethanol exposure on microglia in isolation from MCs may miss important therapeutic targets that arise from MC-microglia interactions.



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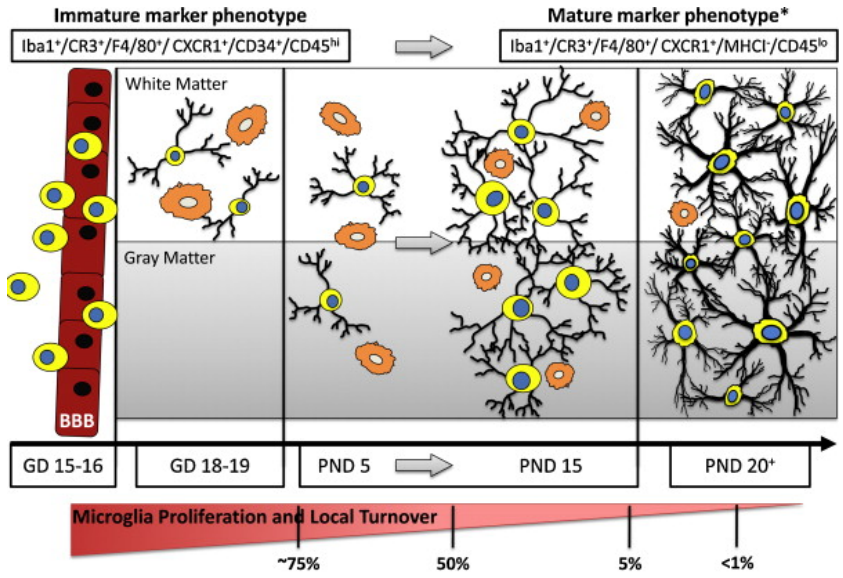

During a critical period of postnatal microglia development (PD5-15), the number of microglia increases dramatically... an increased ratio of ramified versus amoeboid microglia becomes apparent, with the cells having noticeably more complex process arbors and cytoplasmic material (Harry and Kraft, 2012)

Postnatal ethanol may delay the natural developmental transition between microglia phenotype, leading to an increased proportion of amoeboid-shaped microglia in neonate hippocampus.