Binge Exposure to Ethanol Modulation of Stress Genes in the Hypothalamus of F344 Rats

Presenter: Nikhil Kota
Seton Hall University
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Background

- In the United States, 25% of people ages 12 years or older have reported binge drinking, drinking to BAC) higher than 0.08[8].
- Alcohol causes spleen atrophy, the shrinking of the spleen[3] , an immune organ that filters red/white blood cells
- Microgravity suppresses one's immunity,
- The hypothalamus contains stress related genes including Corticotrophin Releasing Hormone(CRH), Mineralocorticoid Receptor(MR), and Glucocorticoid Receptor(GR).

Hypothesis

Binge exposure to ethanol induces age-dependent spleen atrophy and increases the expression of stress-related genes.

Materials

Animals

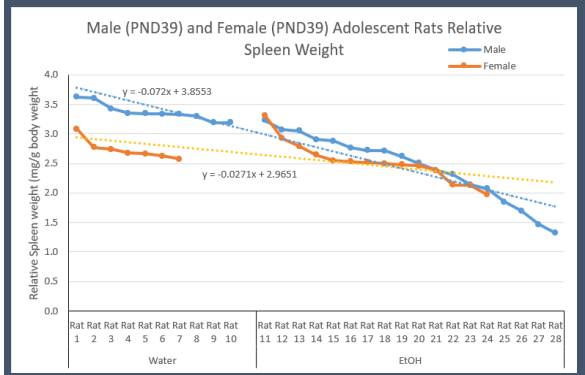
The F344 rat model is a strain of rats that was developed for immunology research[1]. All rats were purchased from Envigo RMS, Inc. (Indianapolis, IN) and housed in vivarium with a maintained 12:12-h light-dark cycle (7am-7pm). All procedures were conducted in accordance with the Seton Hall University Institutional Animal Care and Use Committee.

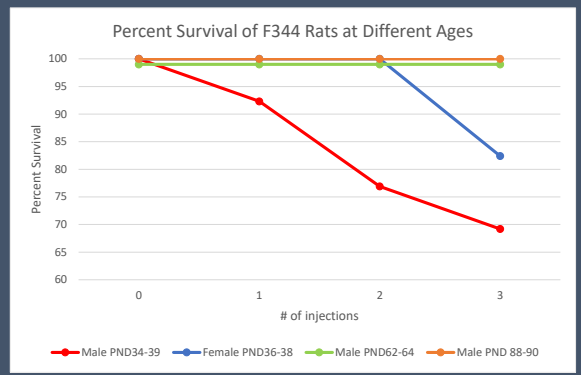
F344 Age Groups Tested

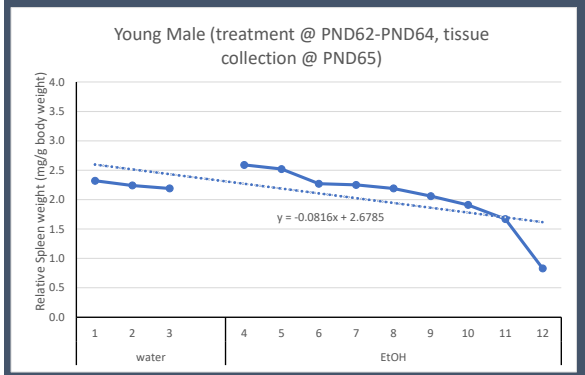
Group	Post-Natal Day (PND)
Male/Female Adolescent Rat*	34-39
Male Young Rat	60-65
Male Adult Rat	86-91

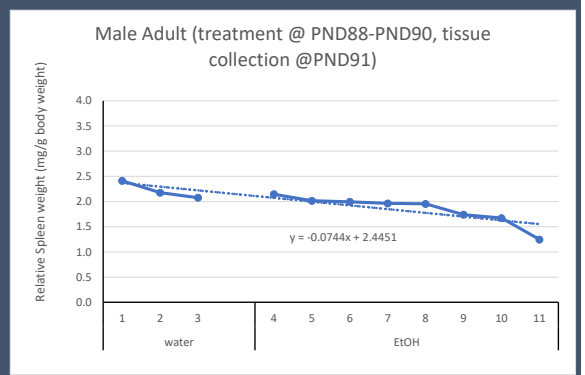
*12 rats tested in each group, 3 control

Male adolescent Fischer 344 rats experienced the greatest spleen atrophy compared to female adolescent, young male, and adult male rats. Natural Killer (NK) cells could be the underlying cellular mechanism for spleen atrophy as NK cells are strongly correlated with relative spleen weight. Spleen atrophy is puberty-dependent as female adolescent rats are developmentally more mature and therefore experienced less severe spleen atrophy than male adolescent rats.

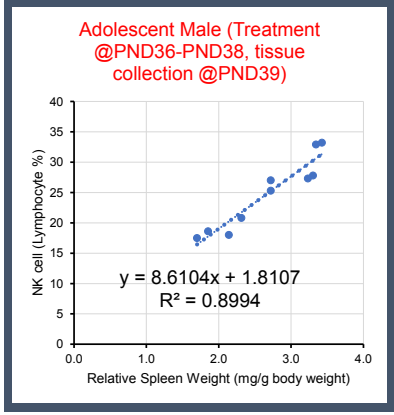


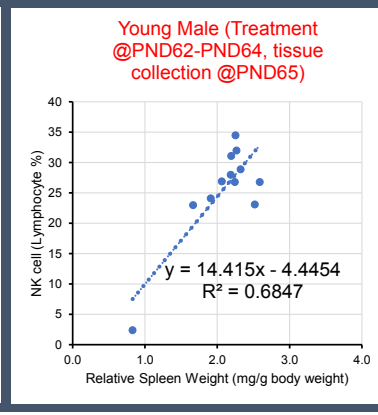


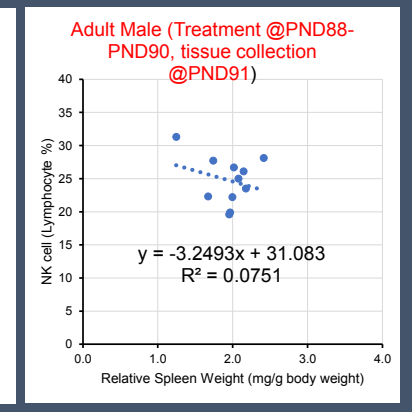




Correlation Between Percentage of Natural Killer (NK) Cells and Relative Spleen Weight







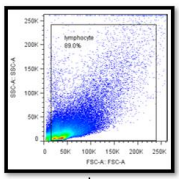
Methods

Treatment Schedule

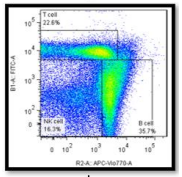
Day	1	2	3	4	5	6
Treatment	Conditioning (Water)		EtOH Treatment (4.8 g/kg/d 52% w/v EtOH)*chosen based off previous research[4, 5,6]			Sacrifice & Tissue Collection

Flow Cytometry

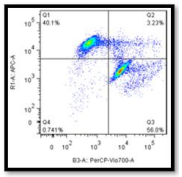
- Following a single spleen cell isolation, flow cytometry was used to characterize the immune cell population
- Removed dead/nonviable cells via FSC and SSC



89% Lymphocytes

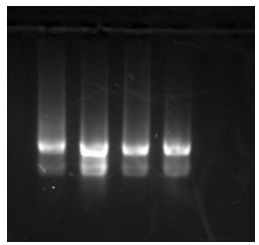


22.6 % T Cells
16.3 % NK Cells
35.7% B Cells



40% Tc Cells
56% Th Cells

Gel Electrophoresis



After collecting the hypothalamus, its RNA was extracted, visualized via gel electrophoresis and was finally converted to cDNA ready for qRT-PCR.

References

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Project contributors: C. Hodgkinson, PhD and D. Goldman. MD (NIAAA/NIH); W. Huang, MS, X. Liu, MD, PhD and S.L. Chang, PhD (Institute of Neuroimmune Pharmacology and Department of Biological Sciences at Seton Hall University)