Sex differences in pubertal LPS-induced changes in adult neurogenesis

LeBel, Nicholas[1]; Kolmogorova, Daria[1]; Ismail Nafissa[1]
[1]NISE Laboratory, School of Psychology, University of Ottawa, Ottawa, Ontario, Canada

Introduction

- **Puberty** is a pivotal stage of neurological & sexual maturation. The developing pubertal brain exhibits an increased vulnerability to external stressors, and activation of the **immune response** during puberty has immediate and lasting effects on **neurocognitive processes** involved in **learning** and **memory**[1]. Emergence of immune-regulating male and female sex hormones during puberty create sex differences in the neural sequelae of immune responses.

Objectives:

- Analysis of the long-term effects and sex differences of pubertal immune stress on adult **neurogenesis**.

Hypotheses:

- Decreased expression of **DCX$^+$ cells** is expected among (a) **LPS-treated** mice compared to their saline-treated counterparts and (b) **gonadectomized** mice compared to their sham-operated counterparts.

Key Terms

Lipopolysaccharide (LPS): Major outer surface membrane components present in almost all gram-negative bacteria; extremely strong stimulators of innate or natural immunity in diverse eukaryotic species[2].

Gonadectomy (Gx): The surgical removal of the gonads.

Neurogenesis: Generation of new neurons from neuronal stem cells.

Doublecortin (DCX): Protein expressed by neuronal precursor cells; a biomarker of neurogenesis.

Methods

CD-1 Mice (n= 90)							
Male (n= 45)				Female (n= 45)			
Saline (n= 21)		LPS (n= 24)		Saline (n= 21)		LPS (n= 24)	
Sham (n= 9)	Gx (n= 12)	Sham (n= 12)	Gx (n= 12)	Sham (n= 9)	Gx (n= 12)	Sham (n= 12)	Gx (n= 12)

- **3 wks old:** Arrival of mice to the lab.
- **6 wks old:** Single *i.p.* injection of LPS (1.5 mg/kg) or 0.9% saline (1.5 mg/kg)
- **9 wks old:** Gonadectomy (Gx) & sham-operations
- **17 wks old:**
  - Brains were extracted & perfused with 4% PFA. Free-floating brain tissue (40 um) underwent fluorescent immunocytochemistry staining for DCX.
  - DCX$^+$ expression was inferred from ImageJ analysis of DCX fluorescence intensity in the neurogenic subgranular zone of the hippocampus.

Results

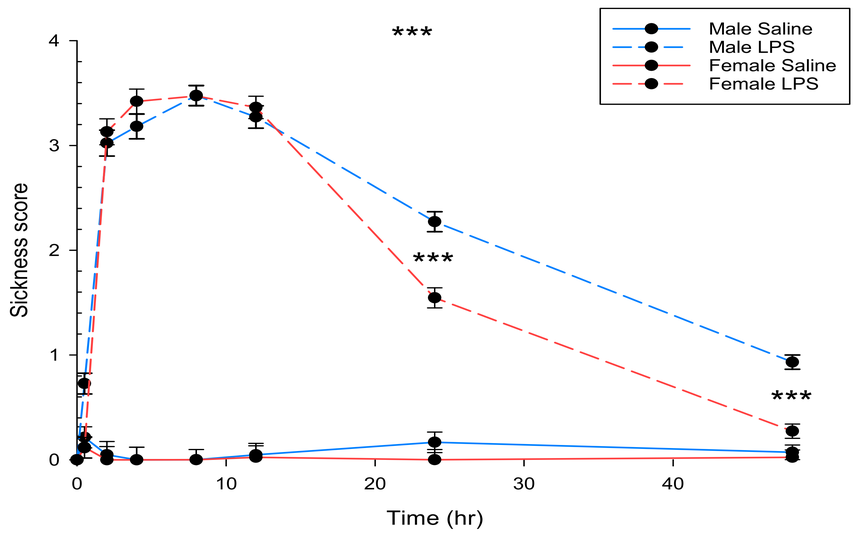

Figure 1. Mean sickness ratings of pubertal males and females exposed to either 0.9% saline (1.5 mg/kg, *ip*) or lipopolysaccharide (1.5 mg/kg, *ip*). Note: *= p<.05, **= p<.01, ***= p<.001

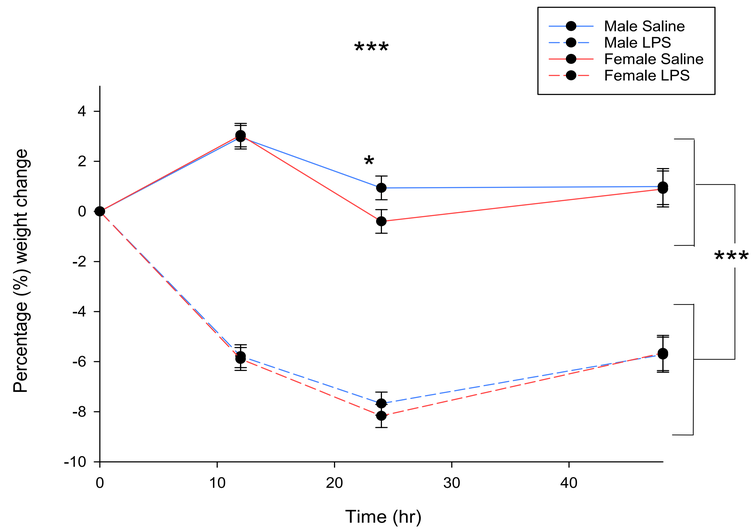

Figure 2. Percentage weight change from time of exposure to either 0.9% saline (1.5 mg/kg, *ip*) or lipopolysaccharide (1.5 mg/kg, *ip*). Note: *= p<.05, **= p<.01, ***= p<.001

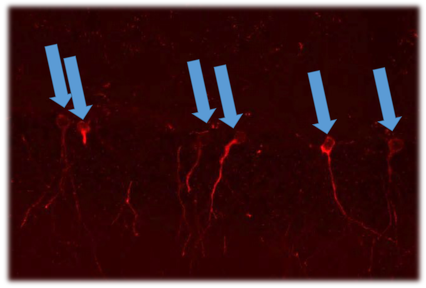

Figure 3. Enlarged image of DCX$^+$ cells in a female saline sham mouse obtained at 40x with a confocal microscope. Image manipulated to enhance contrast and brightness of DCX$^+$ cells (red).

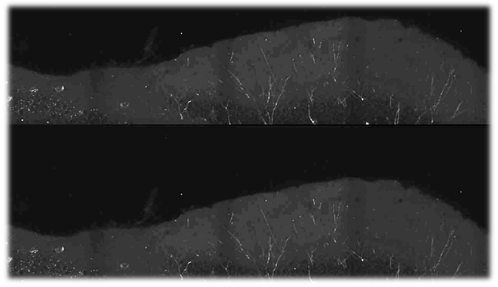

Figure 4. Subgranular zone of the dorsal hippocampus in a female saline sham mouse. Image obtained at 40x with a confocal microscope. Image manipulated into a black-and-white image.

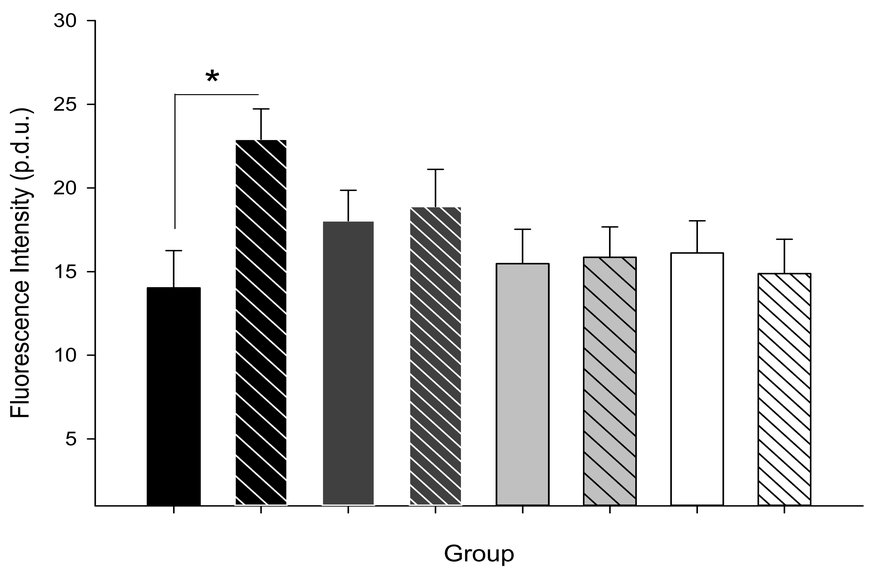

Figure 5. Mean DCX fluorescence intensity (program-derived units [p.d.u.]) in the subgranular zone of the dorsal hippocampus. Note: *= p< .05

Discussion

Sickness

As expected, LPS-treated mice exhibited sickness symptoms and significant weight loss over the 48 hr observation period post-injection. Males showed greater sickness and slower recovery than females. These findings demonstrate the significant sexual dimorphism in the regulation of immune responses by male and female sex hormones during puberty.

Hippocampal Neurogenesis

Interestingly, there was no significant difference in DCX fluorescence intensity within the SGZ between mice injected with LPS during puberty versus saline control. Main effects of treatment and sex were also not observed. These results may be a result of the low dosage and/or the acute (i.e., single injection) vs. chronic stress exposure paradigm. Nevertheless, the data revealed tendencies towards significant interactions between sex, treatment, and surgery.

Recommendations for Future Research

- Improvement upon methodological issues (e.g., sample size).
- Analysis of the morphological differences in DCX$^+$ cells between the groups (saline vs. LPS, male vs. female, sham-operation vs. Gx, etc.).
- Comparison of acute versus chronic LPS exposure during puberty.

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References

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[2] Ryan, S. M., & Nolan, Y. M. (2016). Neuroinflammation Negatively Affects Adult Hippocampal Neurogenesis and Cognition: Can Exercise Compensate? Neuroscience and Biobehavioral Reviews, 61, 121-131.