Effect of pubertal exposure to probiotic and immune challenge on c-Fos expression following restraint stress in adult mice

Émilie Frenette[1]; Emma Murray[1]; Lauren Arber[1]; Michael Swenson[1]; Nafissa Ismail[1]
[1]NISE Laboratory, School of Psychology, University of Ottawa, Ottawa, Ontario, Canada

Introduction

Puberty is particularly vulnerable to the impact of stress. Exposure to immune challenge (lipopolysaccharide; LPS) during this critical period causes long-term effects on cognitive functioning and anxiety[1]. Recently, studies have shown a link between the gut microbiome and the central nervous system with respect to altered behaviours such as anxiety or stress[2,3]. Altering the gut microbiota through probiotics decreases anxiety and, stress response[2,3]. However, little research has been done during the pubertal period.

Objective:
Examine the effects of pubertal exposure to probiotics and an immune challenge on brain activation following restraint stress in adulthood in male and female mice.

Hypothesis:
- Mice exposed to LPS will show greater c-Fos expression following restraint stress in the paraventricular nucleus of the hypothalamus (i.e. a brain regions involved in the stress response) compared to saline-injected controls.
- Mice treated with the probiotic *Lactobacillus reuteri* will display less c-Fos expression in this area compared to mice that were not exposed to probiotics during puberty.
- Probiotic treatment will serve as a protective factor against LPS-induced c-Fos reactivity.

Methodology

Probiotic treatment (from 5-7 weeks of age):
- Mice were given Broth containing *Lactobacillus reuteri* or a control broth for 2 weeks during development

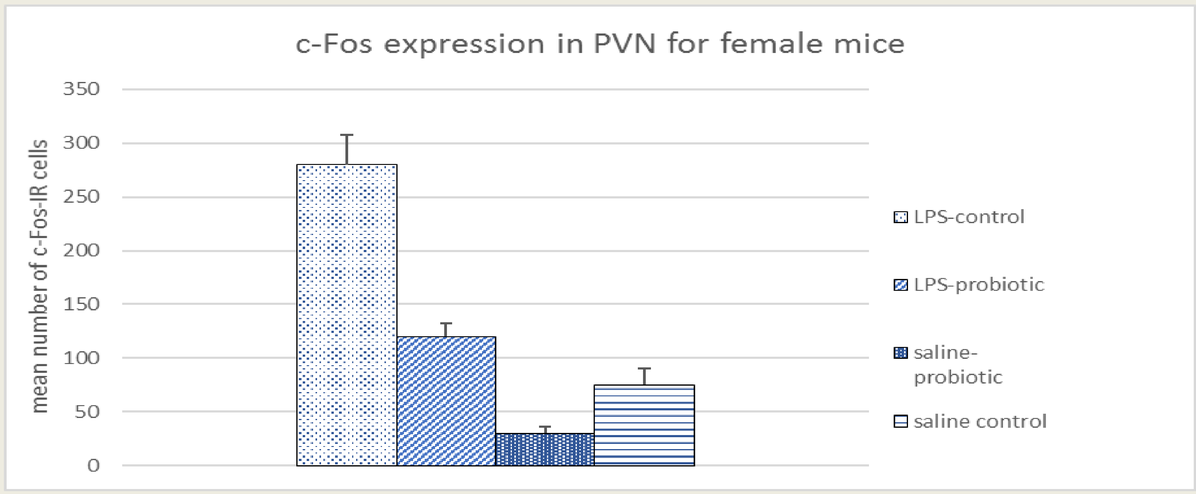
Intraperitoneal injection (6 weeks of age):
- Mice received an intraperitoneal injection (IP) of LPS or saline.

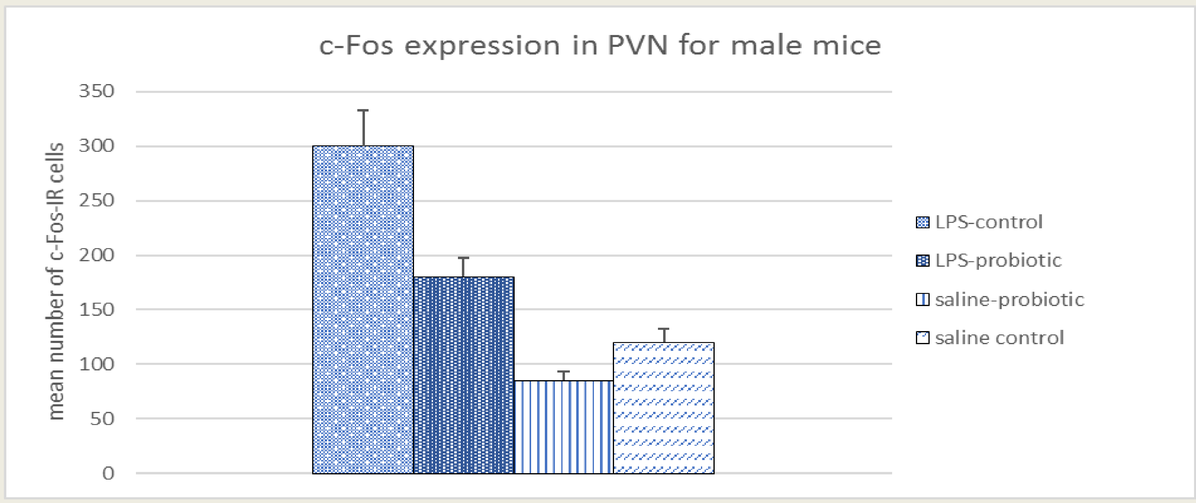
30-minute restraint stress (10 weeks of age, adulthood):
- Mice were exposed to a restraint stress followed by brain collection for analysis of the c-Fos marker

Males *(n=20)*	LPS	Probiotic *(n=5)*
		Control *(n=5)*
	Saline	Probiotic *(n=5)*
		Control *(n=5)*
Females *(n=20)*	LPS	Probiotic *(n=5)*
		Control *(n=5)*
	Saline	Probiotic *(n=5)*
		Control *(n=5)*

Expected results

We expect that:
- Probiotic treatment will serve as a protective factor against LPS-induced c-Fos reactivity
- Mice exposed to LPS will display greater c-Fos expression following restraint stress in the paraventricular nucleus of the hypothalamus
- Mice treated with the probiotic will show less c-Fos expression in this area then mice that were not exposed to probiotics during puberty
- No significant diffrences in c-Fos expression between males and females





Conclusion

c-Fos expression:
- The results should support the hypothesis previously mentioned.
- This research will provide a better understanding of the effects of pubertal immune exposure and of the protective effects of probiotics by providing an understanding of the role of gut bacteria diversity on brain development during puberty and understand the mechanism of gut-brain interaction. Results could propose a potential preventative measure for anxiety and other stress-related disorders

Future directions:
- Examine other brain regions for c-Fos expression and biomarker that respond to stress
- Examine commercially available probiotics containing greater strains of bacteria
- Will brain differences exist if probiotic was taken for a short period of time (one week) vs throughout lifetime

References and acknowledgements

I would like to thank everyone who helped me complete this project. A big thanks to professor Nafissa Ismail who gave me the opportunity to do UROP in her laboratory (NeuroImmunology, Stress, and Endocrinology laboratory). Thank you for the support and the great experience! Also, a special thanks to Emma Murray who guided me, helped me, and answered all my questions throughout this project. And thank you to Jacky Liang for all of the technical support.

References:
[1]Ismail, N. and Blaustein JD. (2013). Pubertal immune challenge blocks the ability of estradiol to enhance performance on cognitive tasks in adult female mice. Psychoneuroendocrinology., 38(7), 1170-1177. doi:10.1016/j.psyneuen.2012.11.003
[2]Bravo, J.A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryana, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl Acad. Sci. USA*, *108*(38), 106050-16055. doi: 10.1073/pnas.1102999108
[3]Messaoudi, M., Lalonde R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … Cazaubiel, J. M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br. J. Nutr., 105(5), 755-764. doi: 10.1017/S0007114510004319

Contact
Émilie Frenette
NISE Laboratory, University Of Ottawa
Email: efren083@uottawa.ca