Neurobiological effects of probiotic-supplemented diets in acutely stressed male Long-Evans rats: Evidence of enhanced resilience

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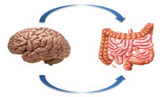
Introduction:

~Considering that the human intestine is home to almost 100 trillion microorganisms including bacteria (Cryan, 2011), recent research has focused on the role of the microbiome in neurobiological functions such as stress, anxiety and coping responses.



~Focusing on animal models, previous findings indicate that modifications of the gut microbiota via antibiotics and certain probiotics alter the anxiety response via the vagus nerve & immune system mediation (MacQueen et al., 2017).

~Recently, the concept of ***Psychobiotics*** has been introduced to refer to the use of microbiota to positively influence mental health outcomes (Foster et al., 2017).

Purpose and Hypothesis

The purpose of the current study was to investigate the effects of probiotics and milk fat on various stress/anxiety responses in male rats exposed to acute (i.e. brief) stress. Given previous findings, it was hypothesized that the probiotic supplement would alter the animals' behavior, hormones, and neurobiological markers in a direction consistent with emotional resilience.

Method:

Experimental Design

Probiotic (n=8) | Maltodextrin (n=8) | Milk Fat (n=8)

Habituation to lab

Probiotic Treatment begins (10^9 colony forming units per milliliter of water)*

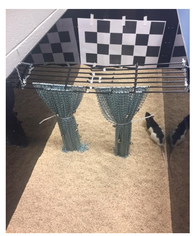
Behavioral Tasks – Open Field (5 minutes in arena), Uncertainty Challenge Task (5 minutes or until reward is eaten).

Behavioral Tasks with Mild Stress / Immunological Stress with Puncture Wound

N=24 Long-Evans Rats; 5 weeks old

Open Field Test

Uncertainty Challenge Test

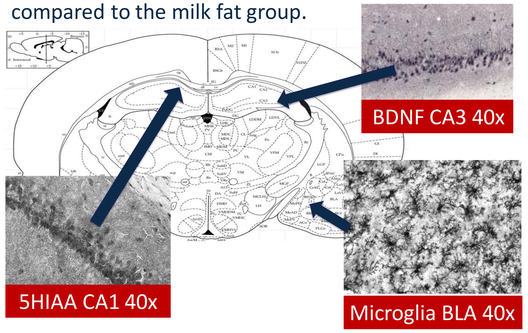


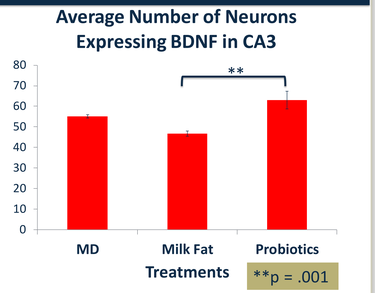
*Probiotic Combination: *L rhamnous* (R0011) + *L helveticus* (R0052) (Lacidofil, generously provided by Lallemand Health Solutions, Montreal, QC) ** Maltodextrin (MD) solution & Milk Fat solution were used for control groups.

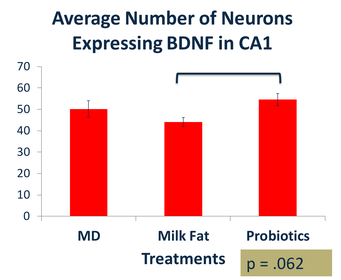
Neural Results:

BDNF-immunoreactivity

The probiotic group had significantly more BDNF-immunoreactive cells in the CA3 of the hippocampus compared to the milk fat group.



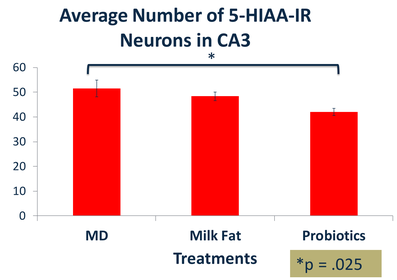




5-HIAA Immunoreactivity

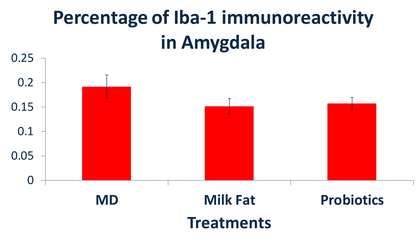
The probiotic group had significantly less serotonin metabolites in the CA3 of the hippocampus compared to the MD group

Results consistent with previous research trends (Desbonnet et al., 2008; Desbonnet et al., 2010)



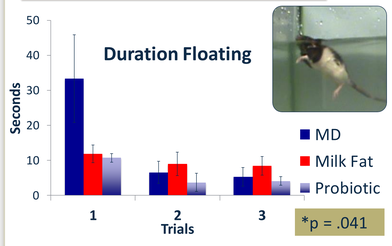
Microglia Immunoreactivity

No significant differences in immunoreactive microglia in the BLA of the amygdala



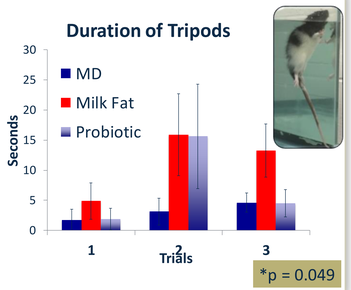
Behavior Results

Forced Swim Test



The MD animals floated significantly more than the Milk fat and Probiotic animals, which is considered a depressive behavior.

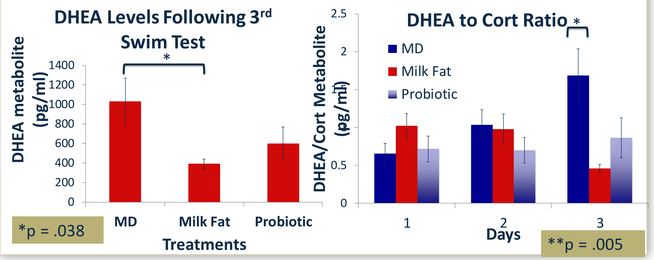
MD rat's tripod behavior was significantly reduced between trials 2 and 3. Similar to floating, tripod is recognized as an energy-conserving behavior (Hawley et al., 2014; Molenkijk & de Kloet, 2015).



Endocrine Results:

ELISA: Fecal samples

For the DHEA/CORT levels, the MD animals had significantly higher DHEA than the Milk Fat animals following the 3rd swim test. Further, the DHEA/CORT ratio (a marker of emotional resilience) was time dependent with the DHEA/CORT ratio significantly higher in the MD rats than in the Milk Fat rats on the last day.



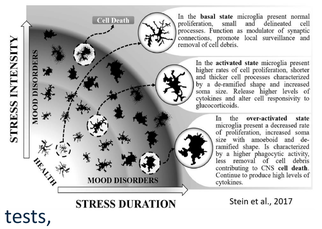
Discussion and Future Directions:

- These results provide further evidence that probiotics influence anxiety-like responses in rodent models; however, this effect doesn't appear to be maintained by the endocrine markers (DHEA and CORT).
- Acute stress may not induce gut microbiota dysbiosis as intensely as chronic stress
- Milk Fat may act as a prebiotic that lowers stress-induced inflammation

Future Studies:

Continuing analysis of microglia activation states and immune function, behavioral tests, and PCR analysis of colonization of the intestines.

- Effects of probiotics on maternal separation model of depression



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