Chronic administration of probiotic L. rhamnosus increases anxiety-like behavior in group-housed male Long Evans rats

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Introduction

- 6.2% of Americans aged 18 and older had an alcohol (ethanol) use disorder as of 2015. It is estimated that 88,000 people die from alcohol-related causes each year, making alcohol the third leading cause of preventable death in the United States (NIAAA, 2017).
- Early life stress is correlated with increased incidence of anxiety and alcohol use disorders (Keyes et al., 2011).
- The present study was done to investigate probiotics as a potential protective factor against the development of anxiety-like behavior and increased ethanol intake.

Methods

Subjects

16 male Long Evans rats arrived PND 21

Figure 1. Housing Conditions

![Housing Conditions](image)

Figure 1. SI, socially isolated; GH, group housed; Slp, socially isolated probiotic; GHp, group housed probiotic.

Probiotics

The probiotic *L. rhamnosus* was administered daily M-F for the duration of the 6-week housing protocol. A standardized dose was placed on a small amount of peanut butter for administration.

Behavioral Tests for Anxiety-like Behavior

- In the elevated plus maze, increased time on the open arms indicates decreased anxiety-like behavior (Pellow et al., 1985).
- In the light/ dark box, increased time in the light box indicates decreased anxiety-like behavior (Slawecki, 2005).

Ethanol

- A two-bottle choice intermittent access ethanol drinking paradigm was used for the final 4 weeks.
- The rats had access to 20% ethanol on MWF for 24 hours. Total intake and preference were measured.

Results

Figure 3. No significant differences in fecal sample *L. rhamnosus* content

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Being Compared</th>
<th>GH vs Slp p-value</th>
<th>GH vs SI p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3wk to before feeding</td>
<td>0.1953</td>
<td>0.1996</td>
<td></td>
</tr>
<tr>
<td>6wk to before feeding</td>
<td>0.1642</td>
<td>0.2063</td>
<td></td>
</tr>
<tr>
<td>6wk to before feeding</td>
<td>0.0906</td>
<td>0.2478</td>
<td></td>
</tr>
<tr>
<td>3wk to 6wk</td>
<td>0.1360</td>
<td>0.0968</td>
<td></td>
</tr>
<tr>
<td>3wk to 5wk after feeding</td>
<td>0.0550</td>
<td>0.0993</td>
<td></td>
</tr>
<tr>
<td>6wk to 5wk after feeding</td>
<td>0.1732</td>
<td>0.1507</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. No significant differences in light box duration

Data were analyzed with a 2-way ANOVA. It was hypothesized that Slp rats would have less anxiety-like behavior than SI rats and that GHp rats would have less anxiety-like behavior than GH rats. There were no significant differences.

Figure 5. SI rats and GHp rats have significantly greater anxiety-like behavior than GH rats

Data were analyzed with a 2-way ANOVA which showed a main effect of housing. Uncorrected Fisher’s LSD post hoc testing indicated significant differences between the GHp and GH groups as well as the GH and SI groups. It was hypothesized that Slp rats would have less anxiety-like behavior than SI rats and that GHp rats would have less anxiety-like behavior than GH rats. This hypothesis was not supported.

Conclusions

- Our EPM results were consistent with dozens of other studies with a significant difference in the open arm time between the group-housed and socially isolated groups.
- Our novel finding suggests that in group-housed animals, probiotics increase anxiety-like behavior, which is the opposite of what we had hypothesized.
- The project is being replicated to further investigate the effect of probiotics on anxiety-like behavior.
- Future studies will need to be done to investigate how the gut microbiota influences the central nervous system to alter behavior.

Acknowledgements

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