Effect of *Lactobacillus farciminis* supplementation in preventing LPS-induced hippocampal neuroinflammation, in mice

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Background:

The discovery of adult neurogenesis in human dentate gyrus of the hippocampus opens new challenges on the role of a virtuous lifestyle (i.e. diet) aimed at counteracting/slowing the decay of new neurons production in the aged brain.
The use of probiotics could represent an intriguing approach for the prevention of specific diseases.

Lactobacillus farciminis Reuter (ATCC 29644) supplementation:

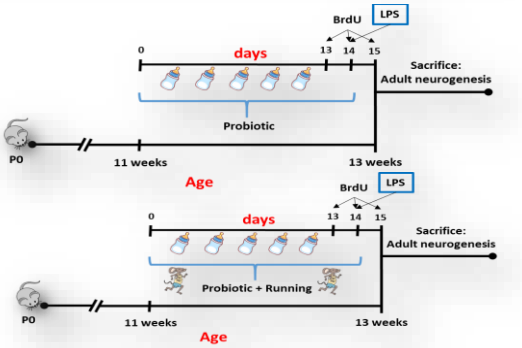
- Active on colonic inflammation, visceral pain and prevention of intestinal barrier impairment.
- Able to attenuate HPA response to an acute psychological stress in rats, and to decrease the associated neuroinflammation, through the prevention of stress-induced gut leakiness and LPS upload.
- Anti-inflammatory properties, through mechanisms principally linked to the release of nitric oxide in the colonic lumen.

Aims:

1st aim:
To study the potential **neuroprotective** effect of prolonged consumption of *Lactobacillus farciminis* (LF), in a mouse model of acute neuroinflammation.

2nd aim:
To evaluate whether the prolonged consumption of LF is able to provide an **additive effect** to the well-described **pro-neurogenic and anti-inflammatory** role exerted by **physical activity**, in the same model.

Experimental design and methods:



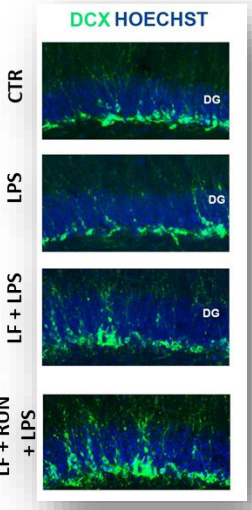
C57B6/J male mice (Charles River Laboratories) were divided in the following groups:

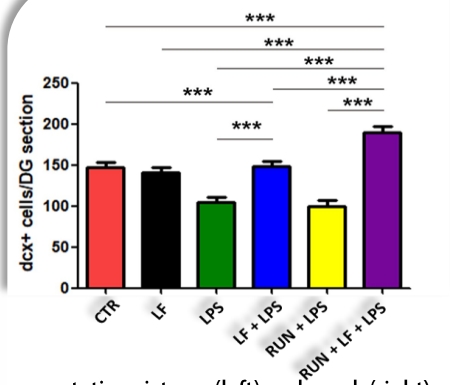
- **Control mice (CTR)**
- **Mice supplemented with LF (10^9 CFU/day, for 14 days in drinking water) (LF)**
- **Mice treated with LPS (0.1mg/kg, ip) (LPS)**
- **Mice supplemented with LF and injected the 14th day with LPS (LF + LPS)**
- **Running mice for 14 days and injected the 14th day with LPS (RUN +LPS)**
- **Running mice supplemented with LF and injected the 14th day with LPS (RUN + LF + LPS)**

Animals were sacrificed 24h after LPS injection

Results:

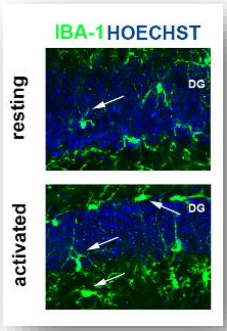
DCX+ cells in dentate gyrus (DG)

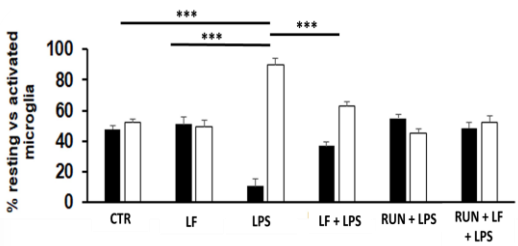




Representative pictures (left) and graph (right) show how a single injection of LPS induces a drastic reduction of hippocampal neural progenitors (DCX + cells). Treatment with LF. triggers a, yet to be identified, significant neuroprotective process that inhibits the anti-neurogenic action of LPS. It should be noted that in physiological situations LF has no pro-neurogenic role. Finally, the combined action of physical activity and administration of LF elicits a remarkable enhancement in neurogenesis resulting in an increase in DCX + cells beyond the control levels, despite the LPS-induced neuro-inflammatory state.

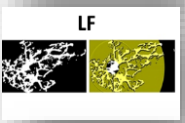
Microglial cell activation

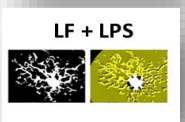




Our data show that in the LPS mice group there is a high percentage of activated microglia (representative image on the left), as hallmark of brain dysfunction. Consumption of LF restores the % between resting vs activated, as shown in the graph, (on the right).

Microglial cell activation: sholl analysis

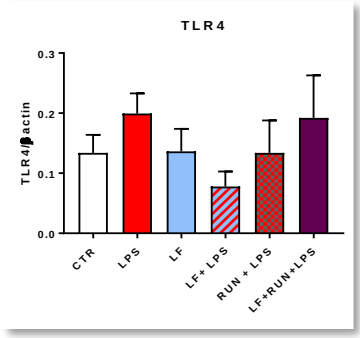


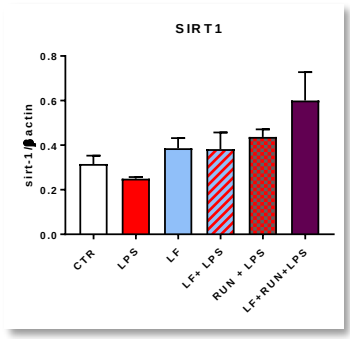


Evaluation of the morphological transformation of a microglial cell from a resting form to an activated form following neuro-inflammatory processes, through the analysis of:

1) Increased soma area
2) Decrease in branching
3) Shortening of extensions in the arborization.

Colonic expression of TLR4 and SIRT1





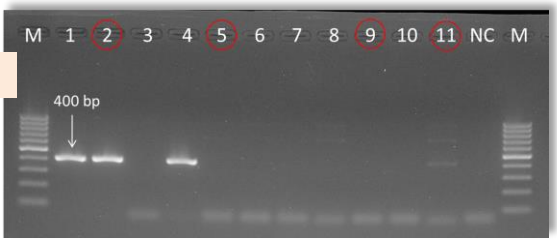
- Western blott analysis of Toll like receptor 4 (TLR4) and Sirtuin 1 (SIRT1) in colonic samples from mice of each experimental group. Preliminary results show an expected increase of TLR4 expression induced by LPS. Interesting, consumption of LF prevents its upregulation.
- LPS decreases SIRT1 colonic expression . Both LF and running prevent LPS-induced SIRT1 down regulation. Interesting, the combination of LF consumption and physical exercise has a synergic positive effect on SIRT1 expression.

Summary:

Preliminary results of our study show that:

- Prolonged consumption of LF is able to prevent decline in cognitive processes, by limiting the onset of neuroinflammation of the hippocampus, and by favoring the genesis of new neurons in adult mice.
- Prolonged consumption of LF in conjunction with physical activity provides an additive effect on the hippocampal neurogenesis.

Microbiological control of Lactobacillus farciminis culture

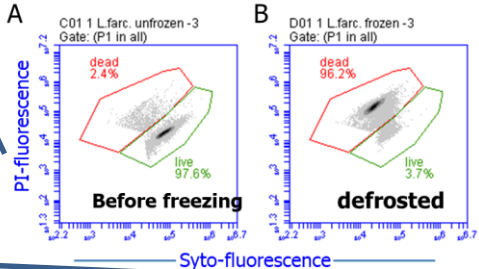


Primers: 23S/p7 + Lf

Detection of *L. farciminis* in fecal samples

M = DNA ladder 100 bp
1 = putative *L. farciminis* culture
2 = colony 1 from streaking
3 = *L. plantarum*
4 = putative *L. farciminis* in saline
5 = colony 1p from fecal sample (T0 10-3)
6 = colony 2g from fecal sample (T0; 10-3)
7 = colony 3g from fecal sample (T0; 10-4)
8 = colony 4g from fecal sample (T0; 10-4)
9 = colony 5g from fecal sample (T7; 10-3)
10 = colony 6g from fecal sample (T7; 10-3)
11 = colony 8g from fecal sample (T7; 10-4)
NC = negative control

Overnight culture of *L. farciminis* ATCC 29644 was washed and suspended in saline solution (NaCl 9 g/l). Live and dead cell quantification of *L. farciminis* was carried out by flow cytometry before and after freezing (panel A and B).



General considerations:

In our experimental condition, the pro-neurogenic and anti-inflammatory effects induced by LF consumption could be due to a very low concentration of live organisms. Moreover, metabolites or bacterial component released by LF could be responsible for the beneficial effects observed.