Protective effects of β-Funaltrexamine against LPS-induced CCL2 expression and behavioral deficits

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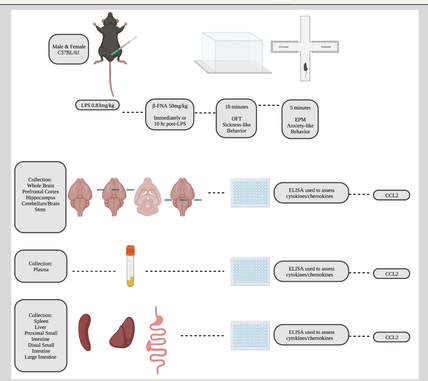
Introduction

Inflammation is present in both neurological and peripheral disorders. Specifically, inflammation is one of the common factors in diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), mood disorders which include anxiety and depression, and even inflammatory bowel disease (IBD). Thus, exploring potential treatments geared toward the assessment of inflammation is crucial to the continuation of treatment development. One pharmacological agent researched for its anti-inflammatory effects is β-funaltrexamine (β-FNA), a selective mu-opioid receptor antagonist. Preclinical studies using *in vitro* human astroglial cells showed that β-FNA inhibited inflammatory signaling, NF-κB signaling, and chemokine expression in a mechanism unrelated to MOR. Also, β-funaltrexamines neuroprotective effects were discovered in a preclinical model of lipopolysaccharide (LPS)-induced neuroinflammation and sickness-like behavior when administered before LPS[1]. This study determines the effects of β-FNA (50 mg/kg, i.p.) on LPS-induced (0.83 mg/kg, i.p.) sickness-like behavior using a 10 min open field test, and anxiety-like behavior, using a 5 min elevated plus maze in male and female C57BL/6J. It also assesses the effects on LPS-induced neuro and peripheral inflammation when β-FNA is administered immediately or 10 h post-LPS. Tissue collected included whole brain, hippocampus, prefrontal cortex, cerebellum/brain stem, spleen, liver, small intestine, large intestine, and plasma. Levels of inflammatory chemokine Monocyte Chemoattractant Protein-1 (MCP-1, also known as CCL2) was measured using an enzyme-linked immunosorbent assay (ELISA). Two-way analysis of variance revealed that at 24 hours, LPS increased chemokines, and β-FNA treatment was protective depending on the dosing schedule and had region-specific effects. Also, to our knowledge, this is the first time β-FNAs effect on female mice has been assessed. Differential effects of β-FNA were found between the whole brain vs. brain regions, central vs. peripheral, and sexes. This study provides insight into the inflammatory protection offered by β-FNA in both the central and peripheral systems and further knowledge of the potential therapeutic options for inflammatory disorders.

Methodology



Male and female C57BL/6J mice were administered LPS (i.p.) followed by treatment with β-FNA (i.p.) immediately or 10 h post-LPS. This was followed by behavioral testing for sickness-like and anxiety-like behavior with an open field test (OFT) and elevated plus maze (EPM). Collection of the brain, hippocampus, prefrontal cortex, cerebellum/brain stem, spleen, liver, proximal small intestine, distal small intestine, large intestine (colon), and plasma occurred at 24 h. Levels of inflammatory chemokine CCL2 in tissues were measured using an enzyme-linked immunosorbent assay (ELISA).

Results

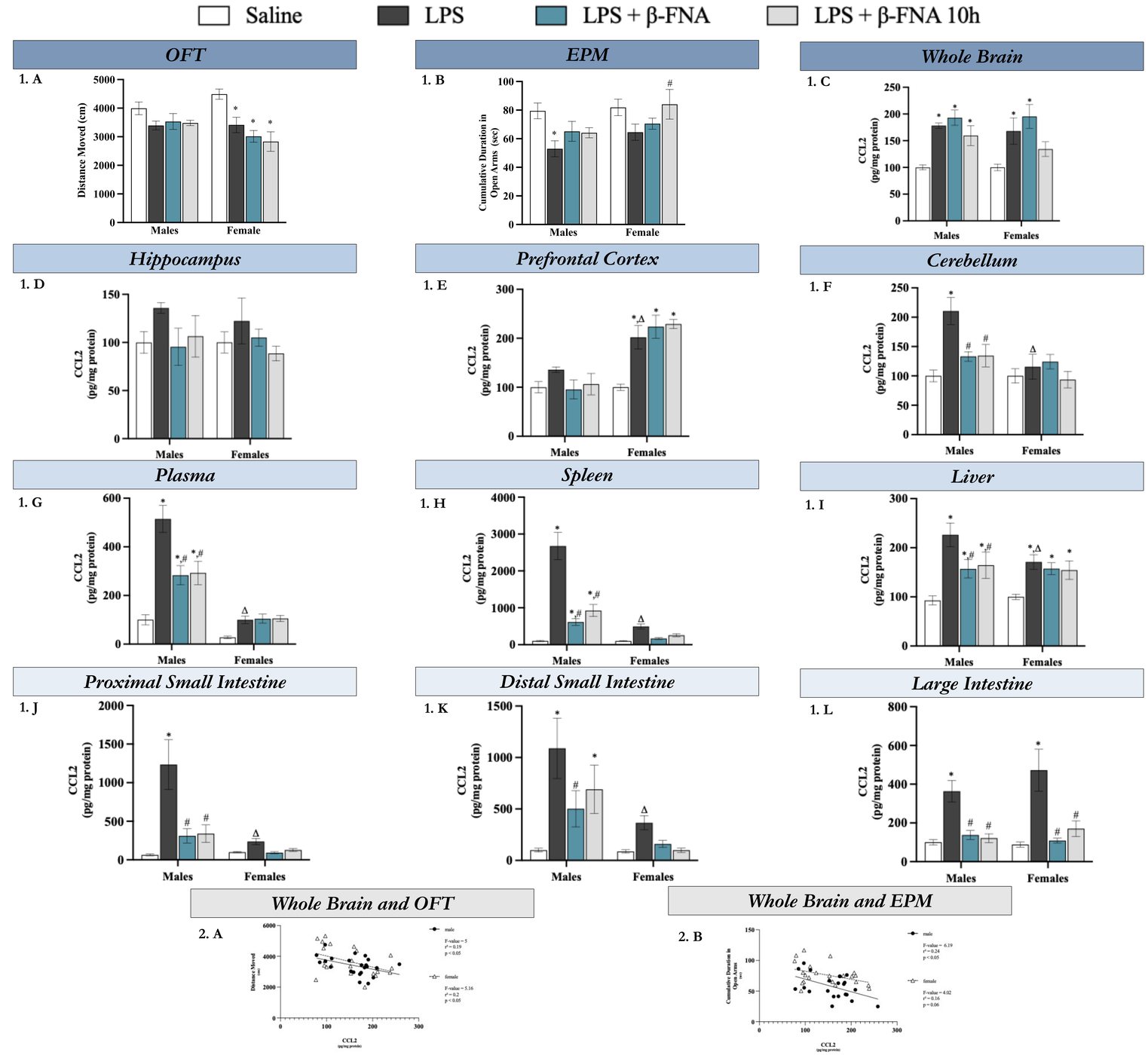


Fig 1. Effect of β-FNA on LPS-induced behavioral deficits and chemokine CCL2. Mice were administered LPS (0.83 mg/kg; i.p.) followed by β-FNA treatment (50 mg/kg; i.p.) immediately or 10 h post-LPS injection. At 24 h after LPS administration a 5-minute OFT (**A**) and 10-minute EPM (**B**) was conducted followed by tissue collection. CCL2 levels in whole brain (**C**), hippocampus (**D**), prefrontal cortex (**E**), cerebellum (**F**), plasma (**G**), spleen (**H**), liver (**I**), proximal small intestine (**J**), distal small intestine (**K**), and large intestine (colon) (**L**) were measured by ELISA. Data reported as mean ± SEM (n=6-12) and analyzed by two-way ANOVA, and Fisher's LSD. *p < 0.05 vs. saline group; # p<0.05 vs. LPS group; Δ p<0.05 vs. males LPS.

Fig. 2. Whole brain levels of CCL2 were differentially correlated with measures of LPS-induced sickness and anxiety-like behavior in C57BL/6J mice. Mice were administered LPS (0.83 mg/kg; i.p.) followed by β-FNA treatment (50 mg/kg; i.p.) immediately or 10 h post-LPS injection (n = 24/sex). Chemokine expression (per ELISA) were assessed at 24 h post-treatment. Linear regression analysis across β-FNA doses was used to assess the correlation of (**A)** OFT- distance moved and (**B**) EPM-cumulative duration in open arm with CCL2 levels in the whole brain. Linear regression statistics and symbols are provided in figure.

Conclusions

Behavioral

-Mice showed evidence of sickness-like behavior and β-FNA was not protective at this specific time point for females.
-EPM is a more accurate measure used to assess anxiety-like behavior. The data suggest a trend towards anxiety-like behavior in males. β-FNA shows some level of protection in males. In females there is anxiety-like behavior, however, not to the level of significance.

Brain/Brian Regions

-In the whole brain, there was no protection regardless of sex, except for females in the LPS + β-FNA 10 h group, which were protected by β-FNA.
-In the hippocampus, there was no difference between groups, regardless of sex and timing of treatment.
-The prefrontal cortex showed a similar trend to that of the hippocampus, with males showing no significant difference between groups; however, females had a pronounced expression of CCL2 in the LPS group and no protection by β-FNA regardless of treatment timing.
-The cerebellum showed protection with β-FNA in males but no difference between groups in females. Males also showed a greater elevation of CCL2 in the LPS group compared to females.

Peripheral

-In the plasma, there was protection in males with the groups that received β-FNA immediately and 10 h after LPS, this protection was not seen in females.
-In the spleen, no level of protection was seen in females regardless of treatment. Males showed protection by β-FNA at both time points.
-Protection was observed in male livers when β-FNA was administered immediately and 10 h after LPS. Females showed no protection by β-FNA.
-In the proximal small intestine, males showed protection by β-FNA and had a higher elevation of CCL2 in the LPS group than females. Females had no differences between groups.
-In the distal small intestine, β-FNA was protective in males only when administered immediately after LPS. Females showed no significant differences.
-The large intestine showed protection in the males and females regardless of the timing of the treatment.

Correlations

-Levels of CCL2 in the whole brain were all negatively correlated with distance moved and cumulative duration in open arms in males and females, except for females in the EPM with a p = 0.06.

Future Directions

-Further assess temporal effects of β-FNA on neuroimmune signaling.
-Further assess β-FNA effects on female mice.

References

[1]Davis, R. L., Stevens, C. W., & Curtis, J. T. (2017). The opioid antagonist, β-funaltrexamine, inhibits lipopolysaccharide-induced neuroinflammation and reduces sickness behavior in mice. *Physiology & behavior*, *173*, 52-60.
Methods image created with BioRender.com

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