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# Modulatory Effects of GABA(B) Receptor Facilitation in a Model of Chronic Inflammation

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## INTRODUCTION

Inflammation within the brain (neuroinflammation) has been associated with a number of neurodegenerative diseases, including Alzheimer's disease (AD) (Solito et al., 2012). Within the brain, inflammation is defined broadly as prolonged activation of the brain's immune cells, known as glial cells. Excessive activation of glial cells within the brains of AD patients is a hallmark of the disease, however the mechanism by which this contributes to disease pathology is relatively unclear (Jo et al., 2014). Recently, studies have shown that glial cells, known as astrocytes, are able to synthesize and release the inhibitory neurotransmitter GABA (Charles et al., 2003). Further, microglia cells, the primary immunocompetent cells of the brain, have been shown to be GABAceptive cells, which express GABA (B) type receptors (Kuhn et al., 2004). Early characterizations of AD first described alterations in astrocyte location and activation in the disease and interestingly, differences in the total abundance of GABA within the brains of AD patients have recently been reported. Combined, these data provide support for the hypothesis that astrocytes regulate microglia activity through the release of GABA acting at GABA(B) type receptors. The activation of GABA(B) on microglia may serve to reduce the activation status of these microglia, thereby reducing the number of pro-inflammatory cytokines present within the brain (Streit et al., 2002). In the present study, we examined the effects of the GABA(B) agonist baclofen on chronic inflammation in rodents administered lipopolysaccharide (LPS). LPS is a bacterial endotoxin derived from the cell wall of gram-negative bacteria and is capable of mounting an immune response through the activation of toll-like receptor 4 (TLR4). Our data indicate that the administration of baclofen initially attenuated the pyrogenic effects of LPS administration, though this effect was lost after two weeks of injections. The administration of baclofen also rescued deficits in spatial learning and memory seen in animals chronically administered LPS. Furthermore, a significant increase in the total abundance of AB oligomers, believed to play a role in the pathology of AD, was seen in the brains of animals chronically administered LPS. Together, these data provide evidence that the modulation of GABA(B) receptor function altered the immune response evoked by activation of TLR4. These data also provide support for a potential role of GABA(B) in modulating aberrant immune activity seen in AD populations.

## EXPERIMENTAL PROCEDURE

### Subjects:

30 male Sprague Dawley rodents (n=30) were used in this experiment. Subjects were divided into three groups (n=10/group) and randomly assigned to one of three treatment schedules: Saline-Saline (control), LPS-Saline (LPS), or LPS-Baclofen (LPS-Bac). All animals were individually housed in a standard animal facility with a 12-12 hour light, dark cycle with food and water available ad libitum throughout the course of the experiment. All procedures were performed in accordance with the institutional Animal Care and Use Committee and NIH guidelines.

### Drug Administration:

As part of a larger experiment, stereotaxic surgeries were performed on all animals under aseptic conditions and ketamine/dexametomidine anesthesia. 8μl of artificial cerebrospinal fluid (ACSF) was slowly infused into each lateral ventricle using the stereotaxic coordinates 0.7mm posterior, 1.4mm lateral to Bregma, and 3.5 mm ventral to the surface of the skull. Following a recovery period of one week after surgeries, animals received intraperitoneal (i.p.) injections of LPS (0.5mg/mL/kg) or saline (1mL/kg) twice a week for seven weeks for a total of 14 injections. Four hours following LPS or Saline injections, animals received i.p. injections of baclofen (1mg/mL/kg) or saline (1mL/kg) resulting in the three treatment groups: Saline-Saline (control), LPS-Saline (LPS), and LPS-Baclofen (LPS-Bac).

### Temperatures:

To ensure an immune response was evoked by the administration of LPS, rectal temperatures were tracked prior to drug administration and at 1, 2, and 3-day intervals post injection throughout the course of the experiment. Following the last injection, animals were given three weeks for temperatures to return to baseline prior to behavioral testing.

### Morris Water Task:

The Morris water task was conducted in a white circular tank, 1.8 m in diameter, 75 cm in height, and 4.7 mm in thickness. To mask the hidden platform, each morning the water was made opaque with the addition of white non-toxic paint. For each subject, a 10cm x 10cm square platform was placed in the center of one of the four quadrants of the tank, 1.5 cm below the water. At the start of each trial, rats were placed into the maze and given sixty seconds to find the hidden platform located below the surface of the water. If after sixty seconds the animal was unsuccessful in locating the hidden platform, a trained experimenter guided the animal to the hidden platform. Once reaching the platform, animals were given twenty seconds to orient themselves to the distal spatial cues. Each animal performed four trials for each day of experimental testing. Twenty-four hours after the control group reached an average latency of ten seconds, a single probe trial was conducted in which the platform was removed from the maze and selective search behavior was recorded for each animal. Following the probe trial, two days of visible training in which the hidden platform was replaced with a visible platform were conducted in order to detect any visual or motor deficits.

### SDS Page/Western Blot:

Tissue was prepared for western blotting as previously described (Sabbagh et al., 2013). For each sample, 20 micrograms of protein were loaded at 10% SDS-PAGE gels and proteins were separated by molecular weight through electrophoresis. Proteins were then transferred to nitrocellulose membranes and blocked in a solution of 5% milk to prevent non-specific antibody binding. Following blocking, membranes were incubated overnight in primary antibody (rabbit Aβ, 1:1000, Cell Signaling; mouse β-actin, 1:20000, ProteinTech) at 4°C. Following overnight incubation, membranes were washed and placed into secondary antibody solution (IR dye anti-rabbit 800, 1:5000, Li-Cor; IR dye anti-mouse 680, 1:10000, Li-Cor) for 2 hours. Membranes were imaged using the Odyssey CLx Infrared Imaging System and band intensity was obtained for each target of interest. Each sample was run in duplicate with β-actin to normalize protein levels.

## RESULTS

### Administration of baclofen attenuated the pyrogenic effects of chronic LPS.

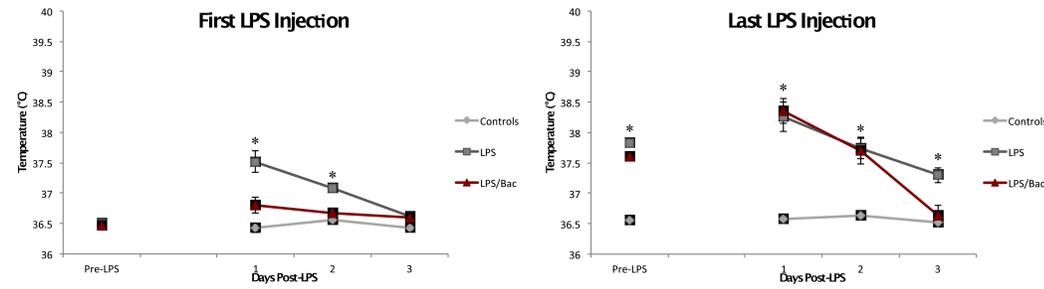


Figure 1. Mean temperatures immediately prior to injections and at 1, 2, and 3 post-injection day intervals. (\*p < 0.05 compared to controls). Error bars denoted as ± Standard Error of Mean (SEM).

### Administration of baclofen rescued LPS-induced deficits in learning and memory in the Morris water task. No differences were seen in visible performance, speed or percent thigmotaxis between animals.

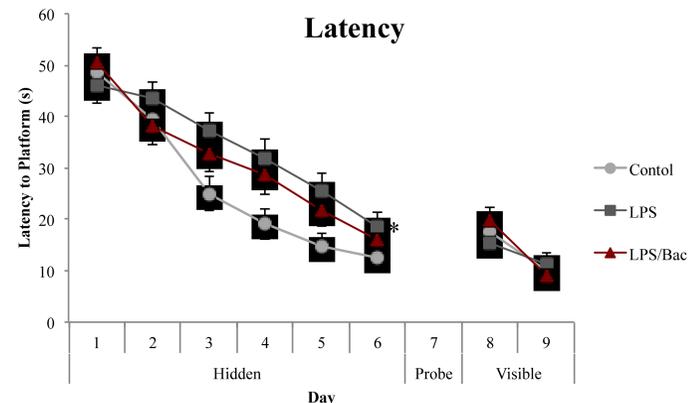
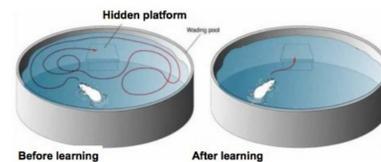


Figure 2. Mean latency, swim speed, and percent thigmotaxis (±SEM) for animals in the Morris water task. (\*p < 0.05 compared to controls)

### In the Morris water task probe trial, all groups spent significantly more time in the target quadrant. Only controls showed a significant increase in the number of annulus crossings.

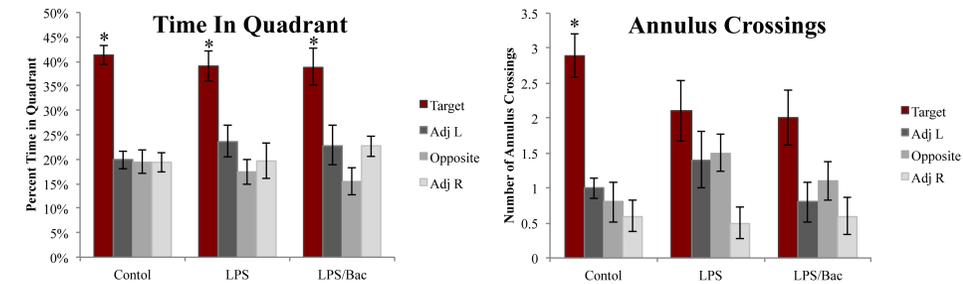


Figure 3. Mean proportion of time in quadrant and average number of annulus crossings (±SEM) during the probe trial. (\*p < 0.05 compared to controls)

### Total Aβ oligomeric protein levels were significantly elevated in animals administered LPS. No differences were seen between LPS and LPS-Bac animals.

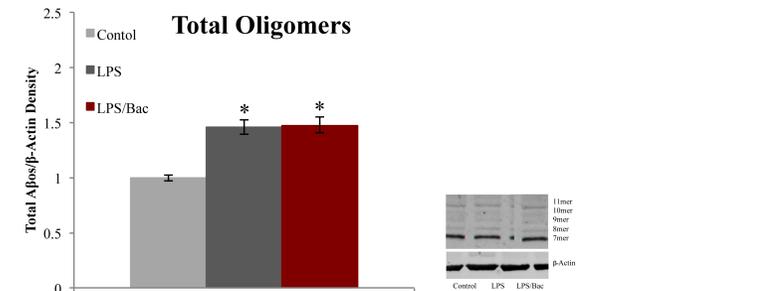


Figure 4. Protein levels proportion to control (±SEM) in hippocampal tissue collected from animals. (\* p < 0.05) Representative Western Blot image.

## CONCLUSION

- ☒ Baclofen attenuated the pyrogenic response evoked through the administration of LPS .
- ☒ Baclofen rescued learning and memory deficits seen in animals administered LPS.
- ☒ LPS and LPS-Bac animals show a significant increase in total Aβ oligomeric protein levels.
- ☒ Combined, these data provide support for the role of the GABA (B) receptor in modulating chronic neuroinflammation.

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