Investigation of the NF-κB Pathway in a Inflammation-Based Depression Model in Female Subjects

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Background

Depression is a chronic disorder that has low favorable pharmacological treatment outcomes. Disease models are becoming increasingly relevant in understanding the complexities that underlie the behavioral & morphological changes behind depression.

- Since the macrophage theory of depression was proposed by Ronald S. Smith in 1991 (Smith, 1991), there has been a strong body of evidence indicating that neuroinflammation is critical to the pathogenesis of depression
- Evidence from prior studies using the LPS-induced depressive-like model argues that microglia may have a larger role than what is currently understood.

Trans factor NF-κB has recently come to the spotlight due to its involvement in the inducible expression of these pro-inflammatory cytokines.

- NF-κB is heavily involved both directly and indirectly in the manifestation of disease, specific processes including recruiting B lymphocytes, T cell differentiation, and cytokine regulation (Liu et. al., 2017).

Women account for a significantly larger portion of depression diagnoses. However, due to biological research's long-standing history of sex bias and sex omission (Will, et. al., 2017), there is little research in general on female subjects

Methods

Figure 1: Brain Region of Interests *XXXXX*

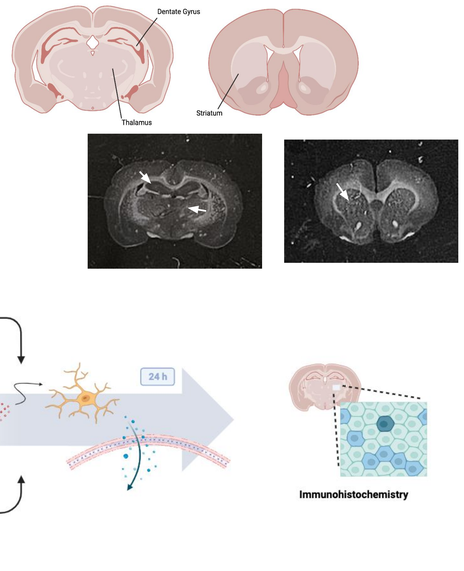

Figure 2: Experimental Procedure *Female Sprague Dawley rats were injected with either LPS or saline and then sacrificed via transcardial perfusion 24h following injections and IHC stained for IBA-1 and NF-κB .*

This study aimed to:

- Determine NF-κB and microglial activation following an LPS-induced depression-like inflammation paradigm in female Sprague-Dawley rats
- Contextualized this relationship in the context of depression pathogenesis, by studying one major brain region and two minor regions involved in serotonin (5-HT) signaling: the thalamus (TH), and the dentate gyrus (DG) and the striatum (STr)

Here we show that there is a potential connection between serotonin signaling and alternative NF-κB signaling in female subjects.

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Results

Microglia Activation is Increased in a LPS-Based Depression Model in Female Subjects

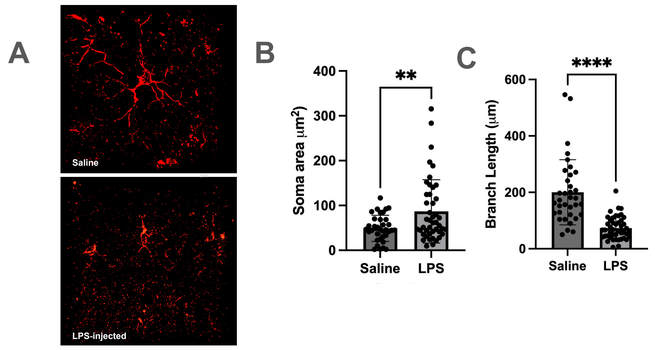

Figure 2: Morphological Characterization of Female Sprague Dawley Rats in Response to LPS-Injection: A) Samples images taken from hippocampal microglia cells (IBA-1 positive), LPS-injected—Left and Saline-injected—Right, taken at 63x using confocal microscopy B) Comparison of soma size between LPS and saline control conditions(N = 16, 8 LPS microglia, 8 Saline) Significance key: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.; C) Comparison of total branch length per microglial cell between LPS and saline control conditions(N = 16, 8 LPS microglia, 8 Saline)

Microglia Morphology in the Thalamus is Significantly Different from Other Brain Regions

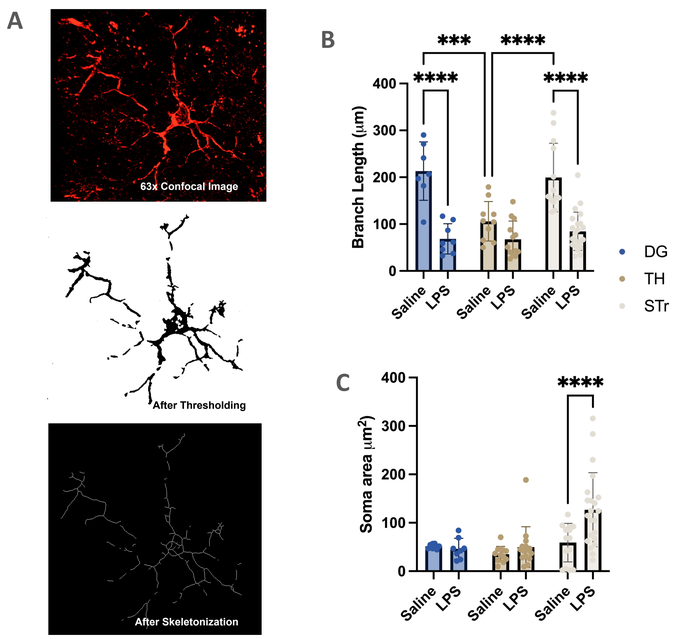

Figure 3: Analysis of LPS-induced Microglial Activation Across Brain Regions: A) Soma size and process length skeletonization of 63x image of hippocampal microglia (IBA-1 positive) taken with confocal microscope; B) Comparison of soma size between LPS and saline control conditions across all brain regions (N=16, 8 LPS, 8 control, outliers omitted); C) Comparison of total branch length per microglial cell between LPS and saline control conditions across all brain regions. Significance key: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Future Implications

NF-κB Expression is Increased in a LPS-Based Depression Model in Female Subjects

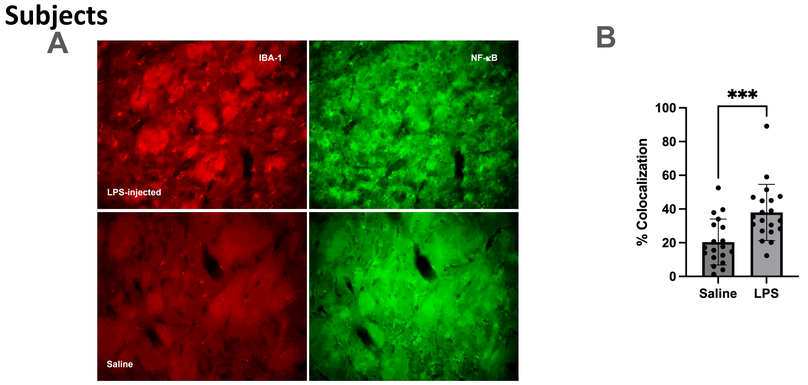

Figure 4: Change of NF-κB Expression in Female Sprague Dawley Rats in Response to LPS-Injection A) NF-κB —Left and IBA-1—Right, sample images taken from the striatum of dual-stained female Sprague Dawley rats, Bottom—saline, control condition, Top—LPS condition, taken at 20x using widefield microscopy; B) Comparison of % global colocalization (N=14, 7 LPS, 7 control, outliers omitted) Significance key: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

NF-κB and IBA-1 Colocalization Increased Following LPS-Injection Specifically in the Thalamus

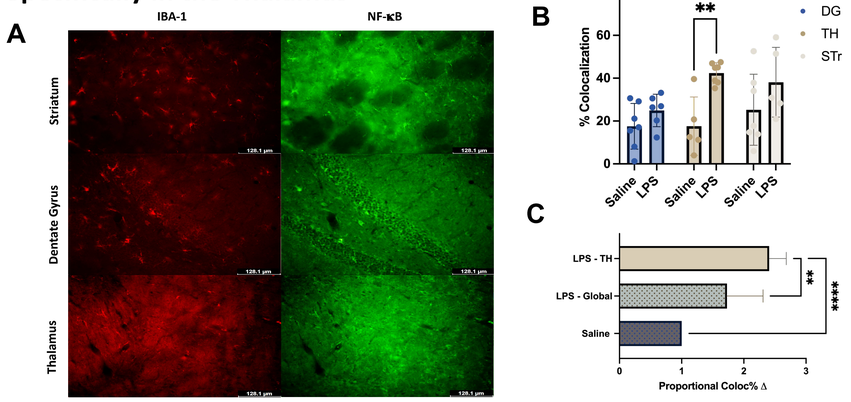

Figure 5: Analysis of NF-κB Expression in Response to an LPS-Injection Across Brain Regions: A) LPS injected female Sprague-Dawley rat brain tissue in the Striatum and Dentate Gyrus; top--Striatum, bottom--Dentate Gyrus, from left to right: 63x confocal microscopy imaging for IBA-1, NF-κB , IBA-1, and NF-κB merged, colocalization as an isolated characterization, and all images merged B) Comparison of % colocalization of IBA-1 and NF-κB in the Dentate Gyrus, Striatum, and Thalamus (N=14, 7 LPS, 7 control, outliers omitted); Significance key: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. C) Comparison of proportional increase in % colocalization of IBA-1 and NF-κB in the Thalamus versus Global

Conclusions

1. Through global analysis, it was suggested that LPS-induced inflammation results in a significant increase in microglia activation and NF-κB levels, however when compared by region, only the thalamus was found to have a unique robust change.
2. Inconsistent with the suggested relationship from the microglial morphology change, the opposite effect was seen in the NF-κB colocalization analysis of the thalamus.
   - Microglial studies with LPS and NF-κB indicate that NF-κB 's transcriptional binding sites are fairly diverse and non-region-specific (Sun, 2017).
   - This may suggest that in female subjects in the presence of serotonin, NF-κB increasingly binds in an alternative inflammatory pathway resulting in a protective effect through anti-inflammatory cytokine release
3. Overall, there is still much that is unclear about depression in female subjects, however, these results act as a framework for further investigation and reveal some insight into how 5-HT-based antidepressants may work in terms of macrophage activity.

References
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