Examining the Effects of Stress on Microglia Density in the Medial Prefrontal Cortex of Male and Female S1PR3$^{GR-/GR-}$ Rats

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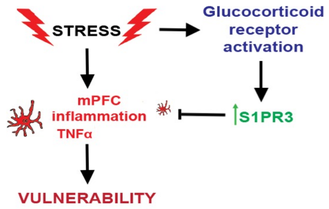
1. Background

Chronic or repeated exposure to stress in the form of trauma or major life events such as bereavement, prolonged conflict, or low socioeconomic status can promote the onset or development of stress-related psychiatric disorders in some, but not all, individuals. Thus, some individuals are more vulnerable to the adverse effects of stress while others are more resilient. One factor that promotes stress vulnerability is increased inflammatory processes[1]. We are also beginning to understand factors that promote stress resilience. The Bhatnagar Lab recently reported that a G-protein coupled receptor called sphingosine-1-phosphate receptor 3 (S1PR3) promotes stress resilience by mitigating stress-induced inflammation in the medial prefrontal cortex (mPFC). S1PR3 mRNA was also reduced in the blood of PTSD patients. In resilient rats, S1PR3 is increased by glucocorticoid receptors (GRs). To study the importance of GR-induced S1PR3, the Bhatnagar Lab developed a rat line in which the GR binding site near the S1PR3 gene is deleted (S1PR3GR-/GR- rats). These rats display anxiety-like behavior as assessed by the social interaction paradigm and increased peripheral inflammation as assessed by increased lymphocytes. However, effects of inflammatory processes in the mPFC are unknown.

2. Hypothesis

- Stress-induced inflammatory processes, which is driven by norepinephrine (NE) and other factors, contributing to maladaptive behavior
- GR-induced S1PR3 mitigates stress-induced inflammation in mPFC



3. Preliminary Data: S1PR3 promote resilient behavior by mitigating stress-induced inflammation

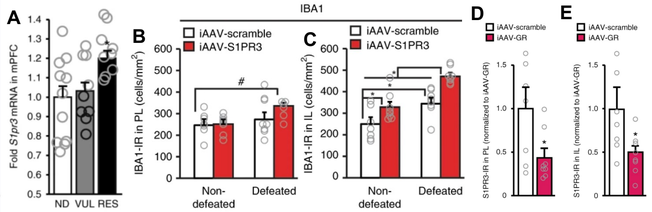


Figure 3. S1PR3 is an important contributor to resilient behavior in rats exposed to stress. A) Rats that are resilient to stress exhibit increased levels of S1PR3 in mPFC. **B)** Neither stress nor S1PR3 knockdown in the mPFC alone affect microglia densities in the prelimbic cortex (PL). **C)** S1PR3 knockdown exacerbates stress-induced microglia density in the infralimbic cortex (IL). GR knockdown reduces expression of S1PR3 in both the **D)** PL and **E)** IL. Thus, GRs drive S1PR3 expression in the mPFC.

4. Preliminary Data: Peripheral inflammation and social anxiety are increased in S1PR3$^{GR-/GR-}$ rats.

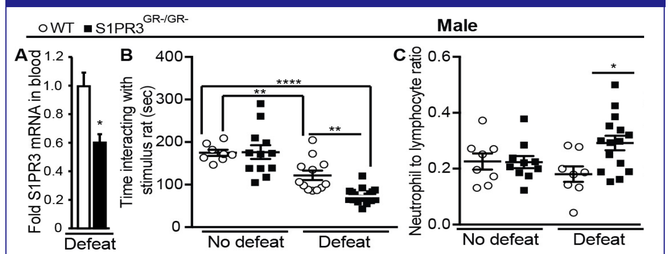
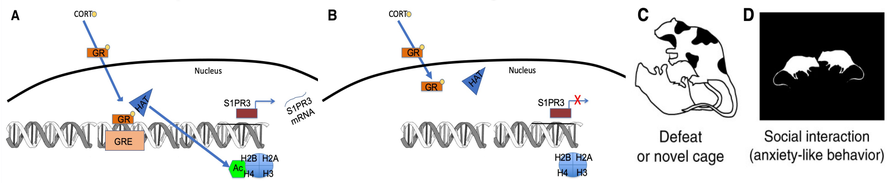


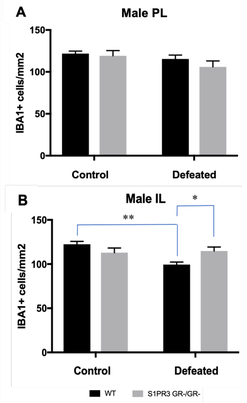
Figure 4. Rats with the GR binding site near the S1PR3 gene exhibit stress-induced behavior and peripheral inflammation. A. Compared to defeated WT controls, S1PR3$^{GR-/GR-}$ rats exhibited reduced S1PR3 mRNA in the blood following social defeat stress. **B.** S1PR3$^{GR-/GR-}$ rats displayed reduced time interacting with the stimulus rat, a measure of social anxiety, in the social interaction paradigm. **C**. S1PR3$^{GR-/GR-}$ rats showed an increase in the neutrophil to lymphocyte ratio, which is a biomarker for depression. Thus, GR-induced S1PR3 mitigates stress-induced anxiety and peripheral inflammation.

5. Methods



Rat model and methods used in this project. A) S1PR3 signaling pathway in the wildtype rats. Increased plasma corticosterone following stress-induced HPA axis activation binds to the glucocorticoid receptor in the cytoplasm. GRs bind to glucocorticoid response elements (GREs) to reduce inflammatory processes by altering gene expression, including increasing S1PR3 expression. **B)** The GRE near the S1PR3 gene is knocked out in S1PR3$^{GR-/GR-}$ rats, preventing GR-induced S1PR3 expression. **C)** Both WT and S1PR3$^{GR-/GR-}$ rats were randomly assigned to either a social defeat or novel cage control for 7 days. During each episode of social stress, a rat was placed into the home cage of a retired breeder Long-Evans resident. **D)** Time of social interaction, which was used as a marker for social anxiety-like behavior, was measured using the social interaction paradigm. Brain sections of the mPFC were collected and analyzed after standard immunohistochemistry procedure. IBA1 was used to identify microglia.

6. GR-induced S1PR3 is necessary for stress-mediated reductions in microglia density in males



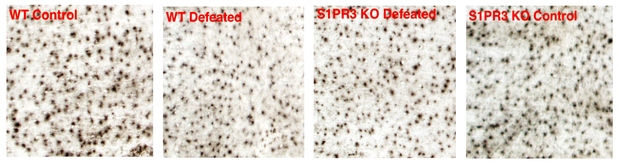


Figure 6. Social defeat reduced microglia density in the Infralimbic Cortex (IL) of the mPFC in WT males , but not S1PR3$^{GR-/GR-}$ rats. A) In the prefrontal cortex (PL), neither stress nor genotype affected microglia density. **B)** In the IL of male WT, but not S1PR3GR-/GR- rats, microglia density was reduced by defeat (p = 0.0024). Defeated male S1PR3$^{GR-/GR-}$ rats display increased microglia density in the IL compared to defeated male WT rats (p = 0.0452).*p < 0.05, **p < 0.01 **C)** Images of microglia density in ILs

(PL, Interaction $F_{1,48}$ = 0.3064, p = 0.5825; Stress $F_{1,48}$ = 2.474, p = 0.1223; Genotype $F_{1,48}$ = 0.9593, p = 0.3323. IL, Interaction $F_{1,44}$ = 8.482, p = 0.0056; Stress $F_{1,44}$ = 6.044, p = 0.0180; Genotype $F_{1,44}$ = 0.4763, p = 0.4937) Control WT male n = 13; Control S1PR3$^{GR-/GR-}$ male n = 9; Defeated WT male n = 12; Defeated S1PR3$^{GR-/GR-}$ male n = 18.

7. Neither stress nor GR-induced S1PR3 alter microglia density in the mPFC of females

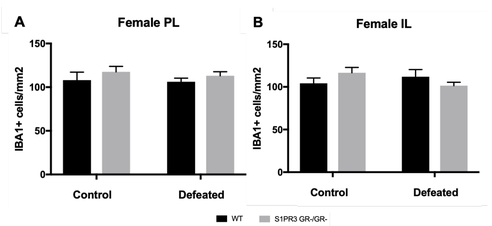


Figure 7. Neither social defeat nor genotype altered microglia density in the prefrontal cortex (PL) or the infralimbic cortex (IL) of the mPFC in female rats. In both the **A)** PL and **B)** IL, neither stress nor genotype affected microglia density. No significant differences were observed. (PL, Interaction $F_{1,18}$ = 0.05228, p = 0.8217; Stress $F_{1,18}$ = 0.3113, p = 0.5837; Genotype $F_{1,18}$ = 1.982, p = 0.1762. IL, Interaction $F_{1,44}$ = 2.871, p = 0.1043; Stress $F_{1,44}$ = 0.3051, p = 0.5863; Genotype $F_{1,44}$ = 0.0298, p = 0.8862). Control WT female n = 3; Control S1PR3$^{GR-/GR-}$ female n = 6; Defeated WT female n = 6; Defeated S1PR3$^{GR-/GR-}$ female n = 11.

8. Sex-mediated effects in WT and S1PR3$^{GR-/GR-}$ rats are modest

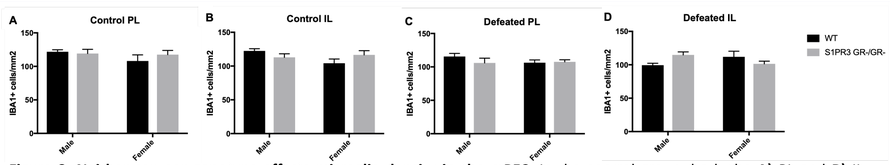


Figure 8. Neither sex nor genotype affects microglia density in the mPFC. In the control group, both the **A)** PL and **B)** IL, neither sex nor genotype affected microglia density. **C)** In PL of the defeated group, neither sex not genotype affected microglia density. **D)** In the IL of the defeated group, we observed a significant interaction for genotype and sex on microglia density (Interaction $F_{1,40}$ = 6.73, p = 0.0132).

(PL Control, Interaction $F_{1,27}$ = 0.9892, p = 0.3288; Sex $F_{1,27}$ = 1.576, p = 0.2201; Genotype $F_{1,27}$ = 0.3145, p = 0.5796. IL Control, Interaction $F_{1,26}$ = 3.679, p = 0.0662; Sex $F_{1,26}$ = 1.618, p = 0.2476; Genotype $F_{1,26}$ = 0.06179, p = 0.8056. PL Defeated, Interaction $F_{1,44}$ = 0.692, p = 0.4100; Sex $F_{1,44}$ = 0.3451, p = 0.5599; Genotype $F_{1,44}$ = 0.3866, p = 0.5373. IL Defeated, Interaction $F_{1,40}$ = 6.73, p = 0.0132; Sex $F_{1,40}$ = 0.00757, p = 0.9311; Genotype $F_{1,40}$ = 0.2544, p = 0.6168) Control WT male n = 13; Control S1PR3$^{GR-/GR-}$ male n = 9; Control WT female n = 3; Control S1PR3$^{GR-/GR-}$ female n = 6; Defeated WT male n = 12; Defeated S1PR3$^{GR-/GR-}$ male n = 18; Defeated WT female n = 6; Defeated S1PR3$^{GR-/GR-}$ female n = 11.

9. Discussions and Conclusions

- Inhibiting mPFC-projecting LC neurons increases social interacting behavior.
- GR-induced S1PR3 causes stress-mediated decreases in microglia density in the male IL.
- This finding in the IL is consistent with our preliminary data demonstrating that GR-induced S1PR3 is necessary for mitigating stress-induced inflammation.
- GR-induced S1PR3 reduced inflammation in males but not females. This may be attributed to females releasing higher levels of pro-inflammatory NE compared to males[3].
- Due to the significant interaction between genotype and sex in defeated rats, we can conclude certain effects of S1PR3 in the context of stress vary depending on sex.

10. Implications and Future Directions

- This study shown that stress-induced S1PR3 plays a key role in decreasing inflammation in the male brain. Future application of drugs or gene therapies that relates to the processes for increasing the expression of S1PR3 may one day be used to treat stress-related disorders.
- This is supported by the fact that S1PR3 mitigates stress-induced maladaptive behavior as shown in the preliminary studies.
- Future work will assess cytokine expression to determine whether defeated male S1PR3$^{GR-/GR-}$ rats express higher levels of inflammatory cytokines in the mPFC compared to defeated WT controls.
- We will also determine whether other cell functions regulated by S1PR3, such as modulating neuronal activity, are altered in S1PR3$^{GR-/GR-}$ rats and contribute to stress vulnerability.
- Future projects will determine whether treatment with anti-inflammatory drugs prevents stress vulnerability in S1PR3$^{GR-/GR-}$ rats.

11. References

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