## Introduction

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. The Bhatnagar Lab uses the resident-intruder paradigm of social defeat to stress rats. Here, a male Sprague Dawley rat (intruder) is removed from its homecage and placed in the homecage of a retired male breeder Long Evans rat (resident) for up to 15 minutes. Following defeat, which is signified by the intruder assuming a supine position, a mesh barrier is installed between the resident and intruder. The intruder remains in the cage of the resident for the remainder of the 30 minute trial[1]. Female defeat is similar, except a lactating female is used as a resident. In this paradigm, vulnerable and resilient rats can be identified because vulnerable rats display short defeat latencies, depression- and anxiety-like behavior. Rats that display longer defeat latencies and resist submission behave similarly to non-stressed controls.

One factor that promotes stress vulnerability is increased inflammatory processes (Raison). 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)[2]. 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 ($S1PR3^{GR-/GR-}$ 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 in the mPFC are unknown.

## Methods

In this study, we explored the S1PR3 signaling pathway in wildtype (WT) rats, wherein stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis triggers increased plasma corticosterone, leading to binding with cytoplasmic glucocorticoid receptors (GRs). This binding initiates a cascade of events, including translocation of activated GRs to the nucleus and subsequent binding to glucocorticoid response elements (GREs) near target genes such as S1PR3, thereby modulating gene expression and influencing inflammatory processes. To elucidate the specific role of the GRE near the S1PR3 gene, S1PR3GR-/GR- rats were generated via targeted knockout techniques,

preventing GR-induced upregulation of S1PR3 expression. Both WT and S1PR3GR-/GR- rats were randomly assigned to either a social defeat stress paradigm or a novel cage control condition for 7 days. During each episode of social stress, rats were introduced into the home cage of an aggressive conspecific, while those in the control group were housed individually in a novel cage environment. Social interaction behavior, indicative of social anxiety-like behavior, was assessed using the social interaction paradigm. Following the stress paradigm, rats were euthanized, and brain sections of the medial prefrontal cortex (mPFC) were collected and analyzed using standard immunohistochemistry procedures. Microglia were identified using IBA1 staining, aiming to investigate neuroinflammatory processes associated with stress-induced alterations in social behavior.

## Results

The results indicate that the presence of glucocorticoid receptor (GR)-induced S1PR3 is crucial for stress-mediated alterations in microglia density, particularly in male rats. In the infralimbic cortex (IL) of male wildtype (WT) rats, exposure to social defeat stress led to a significant reduction in microglia density, suggesting a stress-induced modulation of microglial activity. However, this reduction was not observed in male S1PR3GR-/GR- rats, indicating the necessity of GR-induced S1PR3 expression for stress-induced changes in microglia density. Intriguingly, defeated male S1PR3GR-/GR- rats exhibited an unexpected increase in microglia density in the IL compared to defeated male WT rats, suggesting a potential compensatory mechanism in the absence of GR-induced S1PR3. Conversely, in female rats, neither stress exposure nor the presence of GR-induced S1PR3 significantly altered microglia density in the medial prefrontal cortex (mPFC). This suggests a sex-specific response to stress and GR-induced S1PR3 expression, with females showing resilience to stress-induced alterations in microglial activity. Moreover, while modest sex-mediated effects were observed in the mPFC of WT and S1PR3GR-/GR- rats, no significant differences were detected in microglia density between male and female rats across genotypes. Overall, these findings highlight the complex interplay between stress, GR-induced S1PR3 expression, and sex in modulating microglial activity within the mPFC, underscoring the importance of considering these factors in understanding neuroinflammatory responses to stress.

## Conclusion

In conclusion, our study sheds light on the intricate mechanisms underlying stress-induced alterations in social behavior and neuroinflammation, with implications for potential

therapeutic interventions. We found that inhibiting medial prefrontal cortex (mPFC)-projecting locus coeruleus (LC) neurons increases social interaction behavior, indicating a role for these neurons in social functioning. Moreover, our results demonstrate that glucocorticoid receptor (GR)-induced S1PR3 expression contributes to stress-mediated decreases in microglia density specifically in the male infralimbic cortex (IL), corroborating our earlier findings on stress-related changes in peripheral monocytes. Interestingly, we observed that GR-induced S1PR3 reduced inflammation selectively in males. This may be attributed to females releasing higher levels of pro-inflammatory NE compared to males[3]. The significant interaction between genotype and sex in defeated rats suggests sex-dependent effects of S1PR3 in the context of stress. Our study underscores the pivotal role of stress-induced S1PR3 in mitigating inflammation in the male brain and highlights the potential of targeting S1PR3 for treating stress-related disorders. Moving forward, future investigations will delve deeper into the molecular mechanisms underlying these effects, assessing cytokine expression and exploring additional cellular functions regulated by S1PR3. Furthermore, upcoming projects will explore the efficacy of anti-inflammatory drugs in preventing stress vulnerability in S1PR3-deficient rats, offering promising avenues for therapeutic development in stress-related conditions.

## References


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