Asthma is a striking example of how an insufficient understanding of the fundamental mechanisms of mind-body relationships can hinder scientific and clinical progress. Affecting ~8% of the population,[1] asthma is characterized as a chronic inflammatory airway disease. Importantly, asthma is exacerbated by stress, shows high comorbidity with depression and anxiety, and may impact brain health long-term.[2] Although relationships between emotion-related brain activity and asthma have been documented,[2] ***how* inflammation in the body impacts the brain and *how* psychological processes like stress affect peripheral inflammation in asthma are unresolved**. Understanding these fundamental mechanisms that cut across organ systems will expand our understanding of and methods for examining chronic inflammatory disease processes with an integrative approach. These advancements are imperative for marginalized communities, who are disproportionately affected by asthma and the associated psychological burden.[1]

In the context of asthma, **neuroinflammation–through dysregulated glial activity–could result from chronic airway inflammation and contribute to observed mental health and neurocognitive comorbidities**.[2] These links have been demonstrated in animal models[2,3,4] and extended by pioneering human asthma research from our lab. We showed widespread deterioration in white matter integrity, correlated with biomarkers of neuroinflammation;[5] and accelerated dementia-related pathology in aging asthmatics.[6] Nonetheless, the basic mechanisms linking glial activation to chronic peripheral inflammation, depression, and neurodegeneration remain unknown.[3]

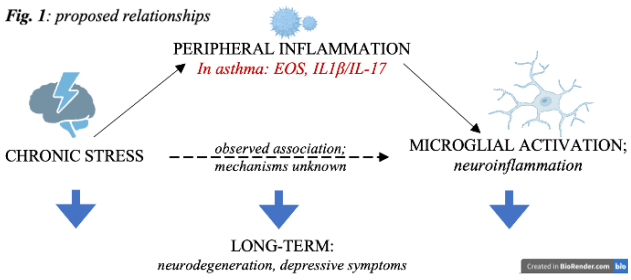

***Fig. 1**: proposed relationships*

Chronic stress is a psychological process that likely amplifies these relationships. Prolonged stress promotes systemic inflammation by simultaneously dampening anti-inflammatory signaling and upregulating proinflammatory signaling.[7] Notably, the IL-1β/IL-17 pathway has been associated with both psychological stress and peripheral inflammation,[3] including in asthma.[5,8] Supporting this association, during my first year of graduate school I found that amygdala reactivity to stress amplified airway mRNA expression of IL-23A, a cytokine in the IL-1β/IL-17 pathway, following an allergen challenge (*first-author manuscript in progress*). Furthermore, repeated stress exposure is thought to sensitize glial cell responses in the brain and peripheral inflammation.[9] Psychosocial stress has been associated with microglial activation in animal models,[10] altered brain structure and function in humans,[7] and increased risk of dementia-related decline in both animal models and humans[11] (Fig.1). **Despite evidence that chronic stress can promote inflammation and impair brain health, there remains a gap in our understanding of how chronic stress may contribute to glial dysfunction in the human brain in asthma**. No human research has directly tested my hypothesis that chronic stress amplifies the effect of airway inflammation on microglia activity in asthma.

My proposal will address these gaps in our understanding by investigating the impact of chronic stress on airway inflammation and microglial activation in response to an acute airway challenge in asthma. I will assess associations between baseline chronic stress, airway inflammation, and microglial activation following an allergen challenge. Though the focus of this proposal is on proximal mechanisms, the results of the proposed work will be foundational for future work examining the long-term consequences of asthma pathology on the brain. This represents the first comprehensive study of neurobiological mechanisms connecting chronic stress, acute inflammation, and neuroinflammation in asthma.

<u>Aim 1</u>: Determine the impact of chronic stress on airway inflammatory responses to an inhaled allergen challenge in asthma. I will measure airway inflammation using eosinophils, the canonical inflammatory cell in asthma, and IL-1β/IL-17 pathway biomarkers. Based on our prior work,[8] I predict that airway inflammatory responses will be amplified for those with higher levels of chronic stress.

<u>Aim 2</u>: Determine if inhaled allergen challenge increases glial activation and how chronic stress impacts this relationship. **Aim 2a**: I hypothesize that allergen challenge will increase glial activation relative to baseline, measured by positron emission tomography (PET), and that the challenge-related increase in glial activation will positively correlate with post-challenge airway inflammation (**Aim 2b**) and baseline chronic stress (**Aim 2c**). **Aim 2d**: I will test my hypothesis that challenge-related airway

inflammation mediates the relationship between chronic stress and post-challenge microglial activation.

Methods: I will collect data as part of an NIA-funded study at UW-Madison (1RF1AG082215). Participants ages 30-75y with mild asthma (n=50) will undergo inhaled allergen challenge to model the inflammatory effects of an asthma exacerbation in a controlled setting. To quantify microglial activation, [^{18}F]-FEPPA PET scans will be acquired on a dual PET/MR scanner at baseline and 24h after allergen challenge. I will be directly involved in collecting and processing neuroimaging data. Allergen challenge-related immune activation will be quantified by eosinophils and a composite measure of IL-1β/IL-17 inflammation in induced sputum. In collaboration with Dr. George Slavich at UCLA, I will assess chronic stress using the self-report stress severity subscore from the Stress and Adversity Inventory.[12]

Analyses: To examine the effects of baseline chronic stress on airway inflammatory responses (**Aim 1**), I will use linear mixed models to regress airway inflammation outcomes on time (pre- and post-challenge), baseline chronic stress, and their interaction. Next, I will test whether allergen challenge increases glial activation (**Aim 2a**) by measuring changes in [^{18}F]-FEPPA binding in the brain from pre- to post-challenge. I will perform a one-sample *t*-test on pre- minus post-challenge difference images (pre-post), using non-parametric permutation tests in FMRIB Software Library. To determine whether challenge-related glial activation is associated with challenge-induced airway inflammation (**Aim 2b**) and baseline chronic stress (**Aim 2c**), I will regress pre-post PET signal on pre-post inflammatory outcomes and baseline chronic stress. Finally, if conditions for mediation are met, I will use multilevel mediation analyses to determine if airway inflammation mediates the relationship between chronic stress and glial activation following allergen challenge (**Aim 2d**).

Alternative Outcomes: [^{18}F]-FEPPA binds to a mitochondrial protein whose expression increases in microglia during neuroinflammation,[13] yet some relevant changes in glial activity and neuroinflammatory processes are not reflected with this marker. If I find no challenge-related changes in [^{18}F]-FEPPA binding, I will perform exploratory analyses using magnetic resonance data that can provide additional indices of inflammation in the brain, including changes in brain microstructure, blood-brain permeability, and intracellular neuronal and glial metabolites.

Intellectual Merit. This work will have impacts across the fields of neuroscience, immunology, and psychology. Chronic stress may catalyze neuroinflammation through its effect on peripheral inflammation. In asthma, stress intensifies airway inflammatory responses.[8] If airway inflammation contributes to glial activation, the chronicity of inflammation across many years with asthma may lead to glial cell dysfunction and eventual neurodegeneration. Therefore, this work represents a foundational first step in identifying common neurobiological pathways underlying the increased risk of long-term brain health decline in populations with chronic inflammation and/or chronic stress. Crucially, the **scope of our work's impact will be amplified as this basic knowledge is translated to other inflammatory conditions**.

Broader Impacts. Given the immense economic and health burden of asthma and neurodegenerative diseases, particularly for disadvantaged and low-income populations,[1] understanding underlying mechanisms may mitigate costs and improve quality of life through optimized prevention and treatment.

In the short-term, this research will broaden participation in science by expanding undergraduate research opportunities. I am prioritizing training and mentoring underrepresented students in data collection, neuroimaging processing and analysis, and statistics, to foster equitable early research involvement. To disseminate this project's results, I will seek out interdisciplinary opportunities like the Psychoneuroimmunology Research Society conference, as well as conferences in each discipline (e.g., Society for Neuroscience; American Academy of Allergy, Asthma, and Immunology). I will also share my results at local venues such as the Re-Connectome Symposium held by the Neuroscience Training Program at UW-Madison. This outreach supports key NSF directives through interdisciplinary dialogue.

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[1]CDC (2023). [2]Caulfield, *Brain Behav Immun-Health* (2021). [3]Beurel et al., *Neuron* (2020). [4]Kanaya et al., *J Neuroinflammation* (2022). [5]Rosenkranz, et al., *Biol Psychol* (2022). [6] Nair et al., *Brain Commun* (2023). [7]Ravi et al., *BJPsych Adv* (2021). [8]Rosenkranz et al., *Brain Behav Immun* (2016). [9]Bisht et al., *Neurobiol Stress* (2018). [10]Calcia et al., *Psychopharmacology (Berl)* (2016). [11]Peña-Bautista et al., *Clinica Chimica Acta* (2020). [12]Slavich & Shields, *Psychosom Med* (2018). [13]Venneti et al., *Prog Neurobiol* (2006).