NEURAL ACTIVITY DURING FACIAL MEMORY RETRIEVAL IN PATIENTS WITH ASTHMA: ASSOCIATIONS WITH PERIPHERAL AIRWAY INFLAMMATION AND HPA-AXIS ACTIVITY

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Neural Activity During Facial Memory Retrieval in Patients with Asthma: Associations with Peripheral Airway Inflammation and HPA-Axis Activity

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Accumulating research indicates that individuals with asthma are at an increased risk for mild cognitive impairment; however, neural contributions to these detected neurocognitive deficits are yet to be elucidated. Recent neuroimaging studies in asthma observe volume reductions in the hippocampus, the primary neural region involved in memory, as well as alterations in the hippocampal chemical composition indicative of neuronal integrity. These studies highlight how both neural structure and chemical composition may be associated with detected neurocognitive deficits in asthma. However, to the best of our knowledge, no study has examined indicators of dynamic neural activity captured by blood oxygenated level dependent (BOLD) signal change through functional magnetic resonance imaging (*f*MRI) in patients with asthma during a neurocognitive task. Thus, the present study utilized *f*MRI to examine group differences in hippocampal signal change of neurocognitively intact individuals with and without asthma during a memory retrieval task, and their association with markers of peripheral physiology relevant to neurocognitive processes.

While no group difference was observed in hippocampal signal change, whole-brain analyses revealed that individuals with asthma demonstrated greater signal change in the right inferior frontal cortex (IFC) when engaged in a memory retrieval task. Behaviorally, no group

differences were observed for total accuracy, but those with asthma were less accurate identifying previously observed faces.

These findings corroborate previous studies suggesting that individuals with asthma perform poorer on some, but not all, behavioral tasks of neurocognition. Further, imaging analyses suggest that even younger adults with well controlled asthma may be working harder than those without asthma by recruiting additional areas of the right inferior frontal cortex (IFC) during memory retrieval. The present study continues to underscore the importance of studying and monitoring neurocognition in individuals with asthma.

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Introduction

Accumulating research supports the idea that individuals with asthma are at an increased risk for neurocognitive difficulties (Irani et al., 2017; Caldara-Alvarado et al., 2013). Despite decades of behavioral observations of these neurocognitive differences (Dunleavy et al., 1980; 1981), there are only a handful of studies examining the brain in asthma (Davenport et al., 2000; VonLeupoldt et al., 2009; 2011; Rosenkranz et al., 2016; Ritz et al., 2019) and fewer with the intention of better understanding potential contributions to neurocognition (Brown et al., 2004; Carlson et al., 2017; Kroll et al., 2018; Bian et al., 2018). Here, the present understanding of neurocognition in asthma from behavioral, neural, and animal perspectives are discussed, with particular emphasis on the hippocampus - the primary neural region involved in memory and learning. Next, the potential roles of peripheral physiology (e.g. airway inflammation, endocrine response) and treatment related factors (e.g. asthma control, commonly utilized medications) in neurocognition and neural processes are discussed. These constructs will be considered in light of the present study which examined 1) hippocampal blood oxygen level dependent (BOLD) signal change, as an indirect indicator of neural activity, in patients with and without asthma during a memory task and 2) whether hippocampal signal during a memory task was associated with disease relevant peripheral physiology or treatment related factors.

Asthma

Asthma is a chronic inflammatory disease, characterized by hyperresponsiveness of the airways, which impacts up to 300 million people worldwide (GINA, 2020). Asthma is one of the few chronic diseases which occurs throughout the lifespan impacting both children and adults. The proportion of adults with asthma continues to increase globally (GINA, 2020). While mortality rates due to this chronic respiratory disease have declined, there is currently no cure for

asthma (GINA, 2020). Furthermore, in the US asthma disproportionally impacts those of lower socioeconomic status and racial minorities (CDC, 2019). Asthma is a largely heterogeneous disease, with patients experiencing varying degrees of chronic symptoms and intermittent acute exacerbations. Asthma disease processes consists of both airway inflammation and narrowing due to smooth muscle spasm and bronchoconstriction. These disease processes can additionally be exacerbated by environmental allergens or chemicals, postural changes including physical exertion, and even strong emotions (Ritz et al., 2010). Common physiological presentations of acute asthma exacerbation include symptoms of chest tightness, wheezing, cough, shortness of breath, and chest pain, and by definition vary over time and in intensity, together with variable expiratory airflow limitation (GINA, 2020), while chronic disease process can go unnoticed if not interfering with daily activities. As such, asthma control and consistent medication use is highly variable with national surveys estimating over 60% of adult patients have uncontrolled disease (CDC, 2019). Patients with asthma are treated not with a curative intent, but rather with the aim of achieving asthma control and preventing acute disease exacerbation. This is achieved, depending on disease severity, with a combination of various daily medications (e.g. corticosteroids, long acting bronchodilators) and self-administered quick-relief rescue medication. In the contexts of severe life-threatening exacerbations, asthma is often acutely managed with systemic steroids to achieve sufficient disease control. In addition to the staggering health, psychological, and economic burden of managing this chronic disease, accumulating research and meta-analytic studies suggest that individuals with asthma are at an increased risk for mild neurocognitive impairment (Caldera-Alvarado et al., 2013, Irani et al., 2017).

Importance of Neurocognition in Asthma

When cognitive performance is compromised in asthma, there are observed negative impacts on treatment adherence (O'Connor et al., 2005; Skloot et al., 2015) and disease control (Ray et al., 2015). Poorer neurocognitive performance is additionally associated in some samples with underreporting of severe asthma symptoms in children (Koinis-Mitchell et al., 2009), and even mortality (Belia et al., 2007). Furthermore, both fluid and crystalized cognitive abilities have independently observed associations with medication behavior in patients with asthma (O'Connor et al., 2015). While it is unclear the directional influence of this associations, poorer neurocognition leading to poorer disease control vs. poorer disease control contributing to neurocognitive difficulties, this body of research underscores the relevance of studying and incorporating measures of neurocognition in patient care to support both respiratory and brain health.

Mild Cognitive Impairment in Asthma

As early as the 1980's, behavioral studies document discrepancies in neurocognitive performance in children with asthma compared to peers without asthma, particularly in children with severe presentation of the disease (Dunleavy et al., 1980; 1981). As neuropsychological testing in patients with asthma has progressed beyond measuring only one domain typical for the context of an academic environment (e.g. attention or executive function) to include testing across multiple neurocognitive domains (e.g. processing speed or various components of memory) in both children and adults, a growing body of research highlights an increased potential for mild neurocognitive deficits in patients with asthma across the lifespan.

In both large domestic and international samples, elevated rates of neurocognitive impairment (ranging from mild cognitive impairment (MCI) to dementia) are observed in

patients with asthma (Chen et al., 2017; Caldara-Alvarado et al., 2013; Lavielle et al., 2015; Irani et al., 2017). Studies with the highest estimates observe a 78% increased rate of MCI in otherwise healthy individuals with asthma over the age of 55, while controlling for self-rated health status, depression, and anxiety (Caldara-Alvarado et al., 2013).

In attempts to consolidate this literature across adults and children, Irani et al. (2017) categorized neurocognitive tasks into eight domains: global intellect, academic achievement, attention, processing speed, executive function, language, learning and memory, and visuospatial/visuoperceptual abilities. Through meta-analysis of 15 studies, they concluded that significant reductions in neurocognitive performance exist in asthma patients across *all* cognitive domains with small to medium effect sizes (pooled effect size of -0.33) (Irani et al., 2017). Strongest effects were identified in the broadest neurocognitive domains including: academic achievement (-0.61), executive functioning (-0.45), and attention (-0.45). Smaller, but still statistically significant effects, were identified in visuospatial functioning (-0.33), processing speed (-0.31), and learning and memory tasks (-0.20). The pooled effect was moderated by age (stronger deficits observed in younger patients, but no overall difference between child and adult studies), asthma severity (greater deficits with increased severity), gender (stronger deficits observed in males), minority background (stronger deficits observed in minority populations), and socioeconomic status (SES) (lower performance in those with annual household income < $15,000), emphasizing the relevance of the environmental and psychosocial context to neurocognitive performance in patients with asthma. While categorization between childhood and adult asthma samples did not statistically moderate their findings, the highest rates of MCI in asthma patients are observed in samples of older adults (Caldara-Alvarado et al., 2013).

As cross-sectional studies supporting neurocognitive impairment in asthma accumulate, a handful of studies in younger patients with less severe asthma do not find this association (Cohen et al., 2016) and several prospective medication manipulation studies suggest a likely impermanence of neurocognitive impairment. In one sample of young adults who at baseline had poorer neurocognitive performance on some but not all measures compared to healthy controls, poorer scores on domains where reductions were observed (e.g. focused attention, concentration, and processing capacity captured by the color-word chart of a Stroop Task and all components of the Paced Auditory Serial Addition Test), were associated with greater circadian variation in lung function (e.g. peak-flow variability, clinically utilized as an indicator of airway hyperresponsiveness or asthma control) (Weersnick et al., 1997). When peak-flow variability was successfully treated over a 6-week period (regardless of the type of asthma medication administered), neurocognitive performance subsequently improved to levels consistent with non-asthmatic controls (Weersink et al., 1997). Some improvement was likely due to test-retest effects, as neurocognitive improvement was observed for both asthma patients and controls; however, this early study highlights three key points: 1) baseline group differences in neurocognition are observed between patients with asthma and controls; 2) neurocognition in patients with asthma is associated with disease related variables including disease control; and most importantly 3) neurocognition in patients with asthma is malleable and potentially ripe for intervention by more aggressive disease treatment or other interventions. Indeed, meta-analytic synthesis identifies disease severity as a moderator of neurocognitive deficits in asthma (Irani et al., 2017).

Corroborating these findings, Bozek and colleagues stratified 359 older adults (>65 years) with uncontrolled asthma, across three levels of neurocognitive functioning (i.e. ranging

from good cognition, mild cognitive impairment (MCI), to dementia) and treated them with asthma medication for one year. The patients' documented improvement in asthma control coincided with significant improvements in neurocognitive performance on the Mini Mental Status Exam (MMSE) (Bozek et al., 2010). Patients with baseline scores indicative of dementia demonstrated the greatest raw improvement (mean MMSE pre=18.2, post=21.9), although all patients saw improvements on the 30 point neurocognitive screener: MCI (mean MMSE pre=25.4, post=27.2), and good cognition (mean MMS pre=28.1, post=29.7) (Bozek et al., 2010).

Despite decades of accumulating research identifying neurocognitive deficits in individuals with asthma, the mechanisms or neurophysiological underpinnings for the increased risk of MCI in patients with asthma are, at this point, only speculative. Hypothesis for putative contributing factors to neurocognitive deficits in patients with asthma, based on other models of chronic respiratory disease, preclinical work, and animal models, include: intermittent hypoxia (Alberi et al., 2013), iatrogenic consequence of pharmacological treatment of disease (Dunleavy et al., 1981; Brown et al.,2009; 2013), cumulative consequences of chronic inflammatory disease processes (Lavielle et al., 2015), vulnerability to oxidative stress (Dozor, 2010), and/or comorbid conditions with known impact on neurocognition (e.g. sleep difficulty, psychiatric diagnoses, metabolic syndrome). Many of the proposed factors have established influences on neural circuits and central nervous system processes relevant to neurocognitive performance – particularly those of the hippocampus, the primary neural region for memory. Thus, the hippocampus is one of the first structures considered in efforts to examine neural correlates of neurocognition in asthma.

Neural Correlates of Neurocognition in Asthma

A nascent literature has examined neural contributions to neurocognitive performance in asthma, largely studying the hippocampus, the primary neural region involved in memory with an equally significant role in signal integration (Fan et al., 2017). “Declarative” or “explicit” memory are all terms used to describe hippocampal dependent memory referring to an ability to remember specific events and facts through direct efforts to access memories through conscious recollection. “Episodic” memory further specifies the ability to recall specific personal experiences which occur in a unique spatial and temporal context compared to “semantic memory”, which refers to accumulated knowledge about the world not depending on any specific event (Eichbaum 2017). Observations of neurocognitive deficits in asthma are quite broad spanning multiple domains; however, in the same Dallas Heart Study sample, used by Caldara-Alvarado and colleagues to document the greatest observed increased risk (78%) for MCI in participants with asthma (Caldara-Alvarado et al., 2013), one of the most commonly missed items on the Montreal Cognitive Assessment (MoCA) by all participants, not just those with asthma, was delayed free recall, an episodic memory task, missed by 56% of individuals tested (Rossetti et al., 2011). Following hippocampal damage, episodic memory is frequently impaired even when semantic memory can remain relatively intact (Eichbaum, 2017), highlighting importance of understanding episodic memory and the hippocampus in patients with asthma.

Hippocampus in Animal Models of Asthma. In murine models of asthma, chronic airway inflammation and labored breathing is demonstrated to impact the hippocampus, specifically with increased markers of neuroinflammation (Cd11b protein), neuronal loss, and damaged synaptic structure (Xia et al 2014; Guo 2013; Caulfield et al., 2017). In conjunction with neural consequences of experimentally induced chronic airway inflammation, additional

studies observe behavioral deficits (e.g. longer time spent on a plus maze task) used as a model for cognitive performance (Guo et al., 2013). Allergen induced inflammatory challenges are additionally demonstrated to influence the CNS, observing post-allergen challenge increases neuronal nitric oxide synthase (nNOS) in the hippocampus (Chen et al., 2008; Basso et al., 2003). Animal models highlight the influence of allergic sensitization and/or asthma disease processes on the hippocampus, yet the extent it may generalize to human physiology provides important targets for further exploration in human models.

Neuroimaging of Hippocampus in Asthma. Only a handful of studies have examined the hippocampus in patients with asthma, to generate initial understanding. In a large study of middle-aged adults (n=1,287), the 10.8% who had an asthma diagnosis had, on average, smaller hippocampal volumes compared to aged matched controls without asthma (Carlson et al., 2017). Interestingly, these findings were driven by males. In addition, chemical metabolites indicative of neuronal integrity (as observed through markers of metabolic activity including N-acetyl aspartate and creatine) have been observed to be lower in the left hippocampus of a sample of young adults with asthma compared to age and gender matched healthy controls (Kroll et al., 2018); however, gray matter volume differences were not observed in this region (Ritz et al., 2019). This could potentially indicate a developmental trajectory of neural deterioration observed in the metabolically active hippocampi, which precedes the gross structural and behavioral deficits observed in middle to older aged asthma samples (Carlson et al., 2016; Caldara-Alvarado et al., 2013). Broad reductions in white matter integrity in a sample of middle-aged adults have additionally been associated with measures of executive function in one asthma sample; however, no findings were specific to the hippocampal areas (Bian et al., 2018).

Role of Peripheral Physiology in Asthma

Neural memory processes; however, do not occur in isolation. Indeed, Bian and colleagues observed that the broad white matter abnormalities in asthma patients, were associated with longer duration of asthma and poorer asthma control (Bian et al., 2018). As disease control and severity are significant moderators of neurocognition (Irani et al., 2017), further investigation into peripheral physiology of the asthma disease process which may contribute to neurocognition and neural memory circuits is warranted.

Peripheral inflammation. Asthma is at its core a disease characterized by inflammation and airway hyperreactivity; however, it is multifactorial in nature with no single biomarker indicating its presence. Th2 cells have been identified as the primary culprits of allergic airway inflammatory responses in asthma, yet there is evidence for a predominantly Th1 subtype (Holgate et al., 2015). Oxidative stress may influence both Th1 and Th2 immune responses and activate additional pro-inflammatory cytokines through NF-kB prolonging the inflammatory state (Dozor, 2010). Despite the known inflammatory disease processes of asthma, it was not previously understood that biological processes in the periphery could influence the central nervous system, primarily due to the role of the blood-brain barrier (BBB). However, recent research has identified several mechanisms, by which systemic inflammation crosses the BBB, and can subsequently influence neural chemistry (Di Benedetto et al., 2017). Beyond the Th2 driven eosinophilic model of asthma, several authors propose a Th1 driven systemic inflammatory influence on the CNS (Lavielle et al., 2015; Alberi et al., 2013). Emerging evidence of "inflammaging" highlights the relationship between low-grade chronic peripheral Th1 mediated inflammation and the development of age-related diseases (Di Benedetto et al., 2017), which may be an important contribution to neurocognitive impairment observed in asthma. Another possibility is that peripheral pro-inflammatory cytokines indirectly influence

CNS chemical profile through stimulation of the afferent vagal nerve or directly cross the BBB through non-disruptive (e.g., peripheral cytokines binding to endothelial cell receptors modulating BBB function) or disruptive changes (e.g. morphological change in tight-junctions and endothelial cell damage influenced by nitric oxide) (Varatharaj & Galea, 2017). As pro-inflammatory cytokine receptors are observed in high concentration on the hippocampus and prefrontal cortex (Rosano, Marsland, & Gianaros, 2011), examining peripheral inflammatory processes may be of particular importance to memory processes in asthma.

In human neuroimaging studies, fractional exhaled nitric oxide (FeNO), clinically used as a marker of airway inflammation, is associated with neural glucose metabolism and BOLD response during tasks of emotion (Rosenkranz et al., 2016; 2017; Ritz et al., 2019); however, the extent to FeNO may be associated with neural activity during memory retrieval in patients with asthma, to the best of our knowledge, has not been studied. While elevated fractional exhaled nitric oxide captures only one component of a complex inflammatory response in asthma, it may be an initial noninvasive methodology to capture a more non-specific airway inflammatory response on neurocognition and the hippocampus.

Hypothalamic Pituitary Adrenal (HPA) Axis. An equally significant peripheral system, with innervations to the central nervous system, relevant to asthma is the hypothalamic pituitary adrenal (HPA) axis. The HPA-axis serves an essential role in learning and memory, by both consolidating learning and readying the body during the day for alertness and concentration (Schwab et al., 2012). The HPA-axis is regulated by negative feedback, largely from glucocorticoid end products, making it a natural point of intersection with many of the corticosteroid medications used to treat patients with asthma. As individuals with asthma, have demonstrated an overall dampening of HPA-axis activity (Buske-Kirschbaum et al., 2003;

Miysaka et al., 2018), this may be due to a biological process or as a direct consequence of medication treatment. A robust literature on HPA-axis in psychological processes identifies endocrine influence on neural regions important to memory, in particular the hippocampus (Wingfield & Wolf, 2014), and cognitive performance (McEwen & Sapolsky, 1995; McEwen, 2000). Sustained elevation of circulating glucocorticoids may lead to CNS consequences and can impede memory retrieval and overall cognitive performance, while acute elevation of cortisol can strengthen working memory (McEwen et al., 2012). In children with asthma, cortisol change in response to an ACTH stimulation test has been associated with stronger working memory performance on tasks of attention (additionally conceptualized as arousal) (Annett et al., 2005). Further interactions of the anti-inflammatory hormone cortisol and peripheral inflammation are observed in asthma (Ritz et al., 2011; 2015; Kroll et al., 2019), suggesting the potential for asthmatics with a blunted HPA-axis response to have exaggerated or prolonged immune responses. The extent to which the cortisol awakening response (CAR), which is purported to support the body in readying itself for any threats that may come during the day (Stadler et al., 2016) may contribute to neurocognition or neural processes in asthma has yet to be established.

Disease Relevant Factors. Multiple components of asthma pathology and treatment with observed neural sequelae are hypothesized as potential mechanisms contributing to cognitive performance deficits in asthma. The strongest moderators include disease control and disease severity (Irani et al., 2017), which encompass a range of behavioral, psychological, biological, and treatment related mechanistic hypotheses for the impact of asthma on the central nervous system (Caulfield et al., 2017). It is also plausible that disease processes may exert cumulative effects on the brain over time. While many of these facets of asthma are proposed as mechanisms or moderators of neurocognition in asthma (e.g. disease control, disease severity, and disease

duration), none have been formally examined in relation to neural activity during memory retrieval.

Treatment Related Factors. The influence of asthma medication on the central nervous system is understudied and availability of systematic medication data is limited, yet emerging evidence supports the notion that asthma medication can indeed cross the BBB to either the detriment or benefit of CNS. Two types of commonly prescribed medications, oral and inhaled corticosteroids, and their potential impact on the CNS regarding relevant to neurocognition is discussed.

Oral corticosteroids are effective in accelerating disease control and could be expected to mitigate the influence of atopic asthma disease processes on the CNS; however, they exert a far-reaching systemic influence beyond the airways, which ultimately negatively influence the CNS and can lead to alterations in both cognitive performance, particularly with long-term use (Judd et al., 2014; Brown et al., 2007, 2009). Longer use and dosages at supraphysiological levels are associated with reduced hippocampal volume, reduced hippocampal N-acetyl aspartate (NAA) metabolite ratios, poorer declarative memory, and increases in both depressive and manic symptoms (Brown et al., 2002; 2004; 2008; Bender et al., 2002). Long-term oral corticosteroid treatment has additionally been observed to contribute to stunted growth and HPA-axis suppression in children (Aljebab et al., 2017), and more recent findings have shown that even short-term oral corticosteroid administration can lead to hippocampal volume reductions over days (Brown et al., 2015).

Inhaled corticosteroids. Compared to oral corticosteroids, inhaled corticosteroids (ICS) have a more targeted influence on the airways and are recommended for long-term asthma control, due to limited side effects (NHLBI/NAEPP, 2007). In a small sample of asthma patients,

ICS use was associated with higher levels of hippocampal metabolites indicative of neuronal integrity and cellular energy (Kroll et al., 2018), which coincides with animal models demonstrating inhaled budesonide as a protective treatment against chronic asthma-induced neuroinflammation (Xia et al., 2015). On the other hand, ICS in high doses can contribute to HPA axis suppression (for review see Dahl, 2006), although adrenal suppression has also been observed prior to ICS treatment in children, and improvements in HPA axis function has coincided with asthma remission after ICS treatment (Priftis et al., 2008). ICS may reach systemic circulation by deposition in the pulmonary airways or in the mouth, where it is swallowed. National guidelines therefore recommend rinsing mouth after ICS use to minimize potential systemic influence (NHLBI/NAEPP, 2007). It has also been proposed that long-term corticosteroid medication may influence dyspnea perception (von Leupoldt et al., 2007) through neural plasticity in the PAG or insular cortex (von Leupoldt et al., 2011). Thus, beyond their influence on the airways, ICS appear to have a broader impact on the CNS. Alternatively, ICS could exert their influence through the enhancement of asthma control, making their proper use protective against the potentially stronger negative consequences of poor asthma control. Neuroimaging studies will be essential to further examine these possibilities.

Summary and Aims of the Present Study

In efforts to continue to explore neural contributions to cognitive performance in asthma patients, the present study addresses the following aims: 1) compare memory retrieval accuracy between individuals with and without asthma; 2) compare BOLD % signal change in bilateral hippocampi during memory retrieval task between individuals with and without asthma; and 3) examine if signal change in hippocampus is associated with airway inflammation, HPA-axis activity as captured by CAR, and disease related variables which might impact neurocognitive

performance including: asthma control, asthma severity, and disease duration. As no *f*MRI imaging studies have been done with memory retrieval previously in patients with asthma, we will additionally explore Aims 2 and 3 utilizing a data driven whole brain analysis approach.

As previous studies observe poorer performance on both global neurocognitive tasks and in specific domains of learning and memory in patients with asthma (Irani et al., 2017), we hypothesize that individuals with asthma will have less accurate performance than control participants on all components of the memory task. During the memory task, we further hypothesize that the BOLD signal in the hippocampus will be reduced in participants with asthma compared to those without asthma. As high concentration of proinflammatory cytokine receptors are observed in the hippocampus (Rosano et al., 2011), we predict that BOLD signal change in the hippocampus will be negatively associated with markers of airway inflammation (FeNO). As cortisol awakening response plays a role in memory consolidation and general alertness during the day and the hippocampus has been demonstrated to be sensitive to cortisol (McEwen, 2015), we predict that BOLD signal change in the hippocampus will be positively associated with cortisol awakening response. Finally, as poorer disease control and longer disease duration (Ritz et al., 2019a, Ritz et al., 2019b) have been associated with CNS alterations in asthma across a variety of regions (VonLeupoldt et al., 2011), we hypothesis that larger BOLD signal change in the hippocampus will be associated with poorer asthma control, greater disease severity, and longer disease duration.

Methods

Design Overview

Participants were recruited and screened for eligibility over the phone then scheduled for two study sessions, ~1 week apart. During the first session, participants completed

questionnaires and physiological measurements capturing asthma control. Between the two sessions, participants complete three time points of saliva sampling at home and returned their samples to the lab. On the second session, participants went through a behavioral memory encoding task then a MR Imaging protocol, where blood oxygen level-dependent (BOLD) *f*MRI was used to capture signal change during a memory retrieval task.

Participants

Data for this study were obtained as part of a larger multimethod project on asthma and emotion conducted between February 2014 and March 2017. Forty participants were screened for health and psychological contraindications and enrolled in the study: twenty with a physician's diagnosis of asthma and twenty age- and gender matched control participants with no history of asthma or other respiratory disease.

Participants were identified using both passive recruitment on two academic campuses and neighboring establishments (e.g. posting flyers in coffee shops, restaurants with community boards) and active recruitment by calling participants with asthma who participated in previous studies and consented to be re-contacted in the future to participate in research.

All participants completed a phone screen and were eligible if they met the following criteria: over age 18, confirmed diagnosis of asthma from participant's physician, access to a rescue inhaler, and spoke English as their first language. Participants were ineligible if they endorsed the following: current clinically significant levels of depression; current or recent history (within 1 year) of substance related disorders including alcohol abuse (>20 drinks per week and/or 5 or more drinks on one occasion), recreational drug use, or tobacco use; any lifetime experience of a manic episode or psychosis; claustrophobia; cardiovascular disease; neurological disorders, including dementia or developmental delay; any lung disease other than

asthma; or the use of corticosteroids (oral or injected) in the previous 3 months. All participants were additionally screened for the presence of orthopedic circumstances, metallic inserts (braces or orthodonture), or other conditions (e.g. pregnancy, recent tattoo) contraindicated for MR imaging.

Participants with severe and uncontrolled asthma according to the National Heart, Lung, and Blood Institute's Guidelines for the Diagnosis and Management of Asthma (NHLBI) were additionally excluded due to potential safety concerns inside of the scanner; however, no asthma exacerbations were experienced by any participant in the scanning environment (NHLBI, 2007). On the day of each study visit, spirometry was conducted according to NHLBI guidelines in order to assure that the participant's forced expiratory volume (FEV_1) was greater than seventy percent of their predicted value to ensure participant safety. No recruited participants were excluded due to spirometry or asthma severity.

Participants with asthma brought their rescue medication with them to both sessions in case of emergency; however, no participant required use of rescue medications. Participants were additionally asked to refrain from using their rescue medication on the day of a study session and could reschedule their session if they needed to use their medication that day. No participants rescheduled their sessions due to medication use.

Assessment Procedure

The Institutional Review Boards of both the University of Texas Southwestern Medical Center (UTSW) and Southern Methodist University (SMU) reviewed and approved the procedures used in the study. Written informed consent was obtained by all participants. Each participant completed two study sessions, approximately 1 week apart. Participants were

compensated $100 for taking part in both sessions and students could alternatively elect to receive course credit.

Behavioral Session. At the first session conducted at SMU, participants completed self-report questionnaires, provided physiological measures of airway inflammation and lung function, and underwent brief neurocognitive screening. Participants were additionally instructed on how to complete take home saliva samples.

At home. On the evening before and the morning of the second session participants completed three at-home salivary cortisol measures: right before they went to bed; immediately upon awakening; and 30 minutes after awakening. Participants returned saliva samples to lab on the day of the imaging session.

MR Imaging Session. At the second session conducted at UTSW Advanced Imaging Research Center, participants completed self-report questionnaires, physiological markers of airway inflammation and lung function, and a behavioral emotion identification task to encode facial stimuli (see Figure 1). Participants then underwent a neuroimaging MR protocol including: structural scan, passive viewing of emotional videos, the memory retrieval task of interest for the present study, and a spectroscopy scan. The order of scans was consistent for all participants. For additional details on the structural scans see Ritz et al., 2019b, passive viewing of emotional videos Ritz et al., 2019a and spectroscopy scans see Kroll et al., 2018.

Measures

Sample Characterization. Participants completed a demographic survey that assesses age, sex, race, ethnicity, height, weight, and handedness. As an index of socioeconomic status, participants additionally reported their years of completed education.

Asthma History. An ad-hoc measure of current symptoms, history, medication use, allergies, and family history was completed by each participant with asthma.

Neurocognitive Screener. Neurocognitive function was assessed with the brief cognitive screening tool, Montreal Cognitive Assessment (MoCA) designed to detect mild cognitive impairment (MCI) (Nasreddine et al, 2005). This 10-minute screening tool has demonstrated adequate test re-test reliability and internal consistency, yielding a Cronbach alpha of .83 with both individual tasks and composite scores reliably discriminating between patients with mild cognitive impairment and normal controls while controlling for effects of age and education (Nasreddine et al, 2005). Individuals are scored on a 30-point scale, with those scoring above 26 "extremely unlikely to meet clinical and neuropsychological criteria for MCI even after extensive evaluation" (Nasreddine et al, 2005). Subsequent large studies determined mean MoCA total scores in a population without cognitive decline at 23.36, indicating that individuals scoring above 23 may lack any cognitive impairment (Rossetti et al, 2011). The MoCA demonstrates very good to good specificity for identifying normal controls (87%) and excellent sensitivity in identifying MCI (90%) with sensitivity calculated as the percentage of normal controls who scored at or above the cut off score of 26 (Nasreddine et al, 2005). In comparison to the Mini Mental Status Examination (MMSE), the MoCA has demonstrated superior sensitivity to detecting MCI (Nasreddine et al, 2005) and was selected for the present study due to the brevity of administration and used to characterize the sample and ensure no presence of moderate to severe neurocognitive deficits. The MoCA was scored by two trained raters (graduate student and undergraduate research assistant) to ensure accuracy.

Forced Expiratory Volume in one second (FEV_1). Along internationally accepted guidelines, the best of three trials of the forced expiratory maneuver into a handheld spirometer

(*AM2*, Jaeger/Toennies, Höchberg, Germany) was used to obtain the forced expiratory volume in one second (FEV_1), and the percentage of predicted FEV_1 (FEV_1 %) based off of height, weight, and sex was scored (Quanjer et al., 1993). Participant scoring less than 70 FEV_1 % predicted, indicative of severe or uncontrolled asthma (NHLBI, 2007), was a part of the exclusion criteria at each session; however, no participants scored less than 70% of their predicted value at any time during their participation. FEV_1 % predicted is used to characterize the sample and is additionally incorporated into the Asthma Control Questionnaire (ACQ).

Questionnaires Participants (with and without asthma) completed a battery of questionnaires at the two in-person study sessions.

Asthma Control Questionnaire (ACQ). Asthma control over the previous week was measured by the ACQ. This brief 7-item instrument has demonstrated high reliability (ICC=0.90) and adequate validity with other measures of general health status (SF-36) and asthma quality of life questionnaire (AQLQ) (Juniper et al, 1999). The ACQ consists of 7 multiple choice items reflecting participants' experience of asthma symptoms (e.g., "How much of the time did you wheeze?", "How much shortness of breath did you experience because of your asthma?"), symptom interference (e.g., "How often were you woken by your asthma during the night?", "How bad were your asthma symptoms when you woke up in the morning?", "How limited were you in your activities?") and medication use ("How many puffs of a short-acting bronchodilator have you used each day?"). Participants ranked items from a 0 – 6 scale, with 0 indicating the absence of symptoms and 6 indicating most severe ranking of symptoms

Asthma Control Test (ACT). Asthma control over the past four weeks was measured by the ACT at the first session. This 5 item self-report instrument has demonstrated good internal consistency (α=.84) and moderate correlation with specialist ratings (Nathan et al, 2004). The

ACT consists of 5 multiple choice questions, reflecting the participants' experience of asthma symptoms (e.g. "How often have you had shortness of breath?"), daytime interference ("How much of the time did your asthma keep you from getting as much done at work, school, or at home?"), nighttime interference ("How often did your asthma symptoms wake you up at night or earlier than usual in the morning?"), medication use ("How often have you had to use your rescue inhaler or nebulizer medication?"), and perception of asthma control ("How would you rate your asthma control during the past 4 weeks?"). All items are equally weighted with total scores ranging from 5 (poor control) to 25 (total control) (Nathan et al, 2004). Both the ACT and ACQ have been designated as core instruments for National Institutes of Health initiated clinical research trials in adults (Jia et al, 2013).

Physiological Assessments.

Fractional Exhaled Nitric Oxide (FeNO). The fraction of nitric oxide in exhaled breath in ppb, FeNO, is clinically used as an indicator of airway inflammation. Nitric oxide is produced throughout the body and serves in various anti-inflammatory roles. As such, a variety of immune cells activated in allergic asthma produce large amounts of nitric oxide making it useful as a biomarker of peripheral airway inflammation (Barnes, Dweik, & Gelb, 2010; Dweik et al., 2011). As diet and physical exertion are known to impact FeNO, all participants were instructed to refrain from eating an hour before their session and rinsed their mouth with water before capturing FeNO. FeNO was measured as participants kept a steady exhale of 10 seconds into a handheld electrochemical analyzer (*Niox Mino; Aerocrine*, Solna, Sweden).

Cortisol Awakening Response (CAR). Participants provided samples of saliva by cotton swab (*Salivette,* Sarstedt, Germany*)* at three time points on the day before and say of their second assessment: bedtime, awakening, and thirty minutes after awakening. Participants recorded the

time of saliva sampling directly on the *Salivette* and stored their samples in their home freezer until samples to the imaging session with an ice pack to prevent any sample denaturing due to an additional freeze-thaw cycle. *Salivettes* were frozen until the completion of data collection. While longer storage times of earlier collected may potentially present additional variability, salivary cortisol is stable for years at both -80 ºC and -60 ºC (Stadler et al., 2016). Saliva samples were analyzed at The University of Texas Southwestern Medical Center's, Biosciences Core in one batch to minimize any variability due to extraction procedures. *Salivettes* were spun at 1600xg for 3 minutes to obtain saliva for analyses. Standards and quality controls (QCs) were spiked with phosphate-buffered saline (PBS). 100 microliters of samples, standards, and QCs were precipitated with 200ul of crash containing: methanol, formic acid (0.15% final 0.1%) and D4 Cortisol (*Sigma-Aldrich*, final 10ng). The tubes were incubated at room temperature for 10 minutes and centrifuged at 13,200 rpm for 5 minutes at 4℃ in a standard micro-centrifuge. Aliquots of ~250 microliters supernatant were transferred to HPLC vials and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). Values from the three analytical runs were averaged, with an average CV% calculated. Any samples with values ≤.025 were identified as below the detection limit and recorded as .025 ng/ml. CAR was calculated from two time points as the absolute difference between awakening and thirty minutes after awakening (Stadler et al., 2016). For additional procedures see Kroll et al., 2019.

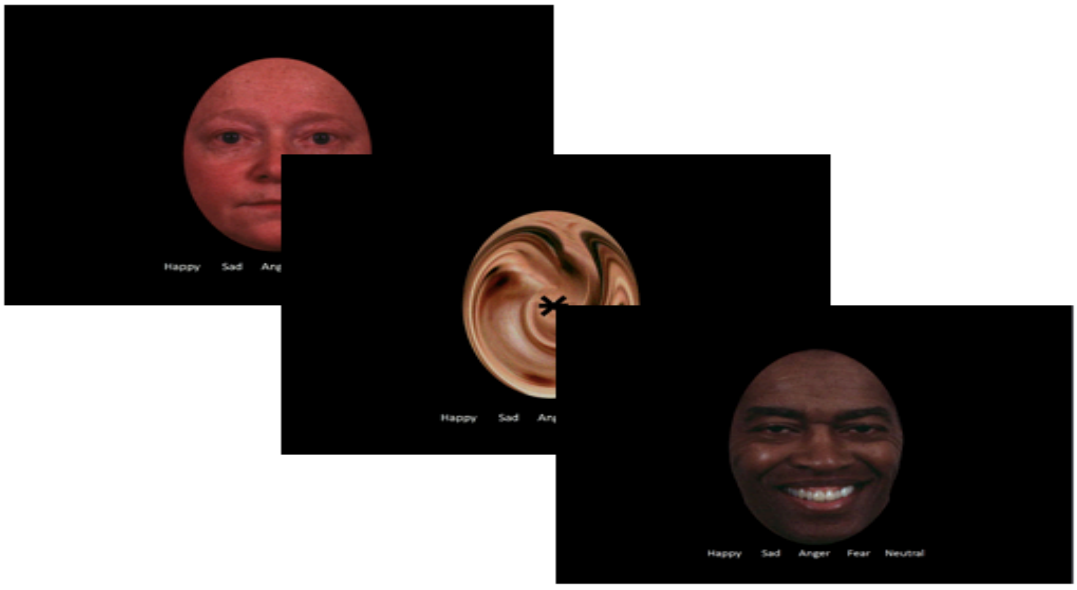
MR Imaging Session. The MR imaging session consisted of four separate components: 1) questionnaire data as described above, 2) physiological measures of FeNO and spirometry as described above, 3) memory encoding behavioral task, and 4) MR scanning protocol.

Memory Encoding Behavioral Task. Prior to entry into the scanning environment, participants were presented by computer with a standardized 60 item facial encoding task.

Participants viewed each face with five choices of emotion (i.e. happy, sad, anger, fear, or neutral) presented below the face. In order to ensure participants were attending and encoding facial stimuli, participants verbalized their response for the experimenter, who recorded it manually. Stimuli were colored faces from previous neuroimaging studies of emotion identification originally selected from a larger database (Gur et al. 2002) and have previously been used utilized in neuroimaging studies of emotion (Wolf et al., 2011; Satterwhaite et al., 2011). Images in this task are not associated with elicitation of arousal (Britton, et al., 2006), and they were not intended to elicit any change in the participant's affective state. Faces were displayed for 5.5 seconds, separated by a jittered inter-stimulus interval (ISI) between 500 milliseconds to 18.5 seconds where a complex crosshair was displayed (see Figure 1). Each emotion was displayed 12 times, with no face displayed more than once. Total number of items accurately identified was calculated for each participant and used for analyses.

Figure 1.

Behavioral Memory encoding task



Memory encoding task was presented to all participants by computer to encode facial stimuli prior to entering the scanning environment. To ensure appropriate attention, participants verbally responded with their selection of the emotion that best described each face out of the following emotions: happy, sad, anger, fear, or neutral. A total of 60 images (50% male, 12 of each emotion) were presented in a standardized order for 5.5 seconds, separated by a variable interval between 500 milliseconds to 18.5 seconds where a complex crosshair (center) was displayed.

MR Image Acquisition. Participants were placed in a light helmet with a mirror to both allow participant to view stimuli presented on screen placed behind the participant and to reduce artifact associated with head movement. BOLD *f*MRI was acquired with a 3 Tesla (3T) Philips Achieva scanner equipped with a 32-channel head coil at UTSW Advanced Imaging Research Center. Functional images were acquired using an Echo Planar Imaging (EPI) sequence with the following parameters: repetition time (TR)=1500 ms, echo time (TE)=30 ms, voxel size = 3.4 x 3.4 x 4.0 mm, flip angle=70°, field of view (FOV)=220 x 220 x 116 mm, matrix size=64 x 64, 39 axial slices and 292 volumes per run; in some instances, field of view placement excluded the most dorsal regions of the brain. Five dummy volumes were discarded at the beginning of the scan to allow for T1 stabilization. For anatomical reference and to aid spatial normalization to standard atlas space, a high resolution anatomical image was acquired for each participant using magnetization-prepared rapid gradient-echo (MPRAGE) sequence: TR/TE/TI=8.2/3.8/873 ms, voxel size=1.0 × 1.0 × 1.0 mm, flip angle=12°; FOV=256 × 256 mm, 160 sagittal slices.

fMRI Memory Retrieval Task. As the last task of the MR protocol, participants completed a facial memory retrieval task (Figure 2). In the retrieval task, participants were presented with faces from the encoding task or new emotional faces taken from the same database (Gur et al., 2002). Participants made a binary decision if the image was "New," in that they had not seen the face before or, "Old," in that they had seen the face in the previous task. Participants viewed the task from *f*MRI stimulus presentation helmet controlled using

Presentation software (Neurobehavioral Systems, Albany, CA) and responded using their right hand on the button box. A total of 60 faces in an event-related design was presented with an inter-stimulus interval between 500 milliseconds to 18.5 seconds. Each emotion was displayed 12 times, with no face displayed more than once. Participants were not informed ahead of time that they would be asked to remember the faces presented during the memory encoding task, completed approximately 60 minutes prior to entry in the scanner.

Figure 2.

*f*MRI Memory Retrieval Task

Memory retrieval task was presented to all participants while they were in the scanner. Participants responded using a button box placed in their dominant hand to indicate if a face was either old (presented in the encoding task) or new (had not seen the face previously). A total of 60 images (50% new, 50% male, 12 of each emotion) were presented in a standardized order for 2.5 seconds, separated by a variable interval between 500 milliseconds to 18.5 seconds where a complex crosshair was displayed.

Data Analysis

Statistical analyses were conducted using SPSS 26.0 (IBM Corp., 2019) and FSL (Woolrich et al., 2004). An alpha level of 0.05 was used as the significance criterion for hypothesis tests. Behavioral and peripheral data were screened for normality (skew cutoff=1, kurtosis cutoff=3), univariate outliers, and impossible values. As previously demonstrated, FeNO tends to have a skewed distribution (Ritz et al., 2019b) and was therefore log transformed and used in analyses. Independent sample *t*-tests and χ^2 tests were used to compare groups on demographics and sample characteristics (e.g. lung function, BMI, neurocognitive screener). As previously published from a larger number of subjects from this sample (Ritz et al., 2019; Kroll et al., 2019), we hypothesized that participants with asthma will have greater levels of FeNO and a lower cortisol awakening response (CAR) compared to those without asthma (Ritz et al., 2019; Kroll et al., 2019).

Behavioral analyses

Independent sample t-tests were used to compare group differences in memory task accuracy. Spearman rank order correlations were used to examine task accuracy with physiological assessments for all participants (e.g. lnFeNO), and with disease control, severity and duration in participants with asthma. As inhaled corticosteroid (ICS) use likely influences the HPA axis by negative feedback, thereby dampening endogenous cortisol levels (Stadler et al., 2016), all correlations examining association with CAR utilized Pearson partial correlations, to control for presence of a current inhaled corticosteroid prescription (yes/no). As individuals with asthma have previously observed reductions in neurocognitive domains of attention, learning & memory, and processing speed (Irani et al., 2017), we hypothesized that participants with asthma would score poorer on the memory retrieval task. As greater CAR is associated with greater

episodic memory (Ennis et al., 2016) and peripheral inflammation may contribute to neurocognitive deficits (Bradburn et al., 2018), we further hypothesized that poorer task accuracy would be correlated with lower CAR and higher lnFeNO. In individuals with asthma, we hypothesized that poorer task accuracy will be associated with poorer disease control, longer disease duration, and greater disease severity.

MR Image Analysis

*f*MRI data were preprocessed and analyzed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fMRIb.ox.ac.uk/fsl). Registration to high resolution structural and/or standard space images was carried out using FLIRT, with images coregistered to participant space and normalized into T1 Montreal Neurologic Institute (MNI) space using trilinear interpolation (Jenkinson 2002). The following pre-statistics processing was applied: motion correction using MCFLIRT with 12-df affine transformation using the median image in each time series as the template and by including FSL's motion outliers as a regressor of no interest to account for any variance due to motion, TR double gamma (Jenkinson 2002); slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using BET with robust brain center estimation (Smith 2002); spatial smoothing using a Gaussian kernel of FWHM 8.0mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich et al., 2001). All events were modeled in the GLM after convolution with a double-gamma hemodynamic response function. The time series model included viewing of old and new faces. Due to the previously observed associations of corticosteroid use and hippocampus (Brown et al., 2004;

Judd et al., 2014), current inhaled corticosteroid (ICS) prescription (y/n) was coded 0, 1 then mean centered and used as a nuisance regressor and separately examined for potential of an independent positive and negative impact. Mixed-effects analyses using FLAME (FMRIB's local analyses of mixed effects) were performed to conduct one-sample t-tests on subject-level whole-brain contrasts.

An event-related first-level model was specified, in which events were modeled with a custom three column format and convolved with a double-gamma hemodynamic response function. Each component of the memory retrieval task (old, new, old-new, new-old, all faces) was modeled separately.

To examine between-group differences of participants with and without asthma, all first-level contrasts were entered into an independent sample *t*-test random-effects analysis. Regions of interest (ROI) for the left and right hippocampus were then defined as with the Harvard-Oxford Subcortical Map Atlas in FSL, and binary unweighted anatomical masks were generated using FSLeyes. ROI analyses were then performed using FSL's *featquery*. Significant clusters of activation within each ROI were identified based on a statistical threshold of z=2.0, p<.05, and the mean percent signal change across all voxels was extracted for each participant and entered into SPSS for correlational analyses with other behaviors and physiological measures of interest.

Independent sample t-tests were used to compare activity in bilateral hippocampi during memory retrieval task between groups (asthma vs. control): 1) viewing of old stimuli; 2) viewing of new stimuli; 3) contrast of old > new stimuli and 4) contrast of new > old stimuli. We hypothesized that bilateral hippocampi would demonstrate greater activity during all components of the memory task in participants with asthma compared to those without asthma.

Correlation analyses

Spearman rank order correlations were used to examine associations among hippocampal % signal change during memory task with behavioral performance on task (e.g. accurate old, accurate new, total accuracy), peripheral physiology (FeNO), and for individuals with asthma, asthma control (ACT, ACQ), asthma duration, and asthma severity. As inhaled corticosteroid use likely influences the HPA axis by negative feedback dampening endogenous cortisol levels (Stadler et al., 2016), Pearson partial correlations, controlling for current ICS prescription (yes/no), were used to examine any association with CAR. We hypothesized that in individuals with asthma, greater hippocampal signal change will be associated behavioral task accuracy, lower CAR, higher FeNO, poorer asthma control, longer asthma duration, and greater asthma severity. We did not make any a priori hypothesis for left vs. right hippocampus or viewing old vs. new stimuli.

Exploratory Whole-Brain Analyses

While the hippocampus is the primary neural region associated with memory, memory retrieval is an integrative process influenced by many neural areas and the modulation of memory retrieval is not yet fully understood (Roozendaal, et al, 2009). As such, exploratory voxelwise analyses with the conservative family-wise error (FWE) correction of $p(\text{FWE})<0.0001$ were conducted for the specific contrasts and analyses outlined above to identify any group differences in neural activity outside of hippocampus. As clusters did not survive correction at the most stringent threshold ($z=3.1$ $p<.0001$), analyses were run at corrected level of $z=2.0$, $p<.05$). Clusters that passed the thresholding, were then used in an exploratory fashion to generate a functionally defined ROI for the contrast asthma>controls of a general task effect of seeing all faces. A binary unweighted ROI mask was generated using *fslmaths*, which was then used with FSL's *featquery* to extract % signal change during the overall task and input into

SPSS. Spearman rank order correlation were utilized to examine associations between signal change during task with task accuracy, peripheral physiology (FeNO), asthma control (ACT, ACQ), asthma duration, and asthma severity. Pearson partial correlations, controlling for ICS, were used for examining an association with CAR.

Results

Of the 40 participants enrolled, all participants completed the entire procedure. At the *f*MRI preprocessing stage, data were excluded due to motion and data abnormality. Of participants with usable MRI data, task performance data was unavailable for one participant with asthma due to error with button box. All results are reported for those with available imaging data.

Sample Characterization

Final analyses were based on 32 participants, 15 with asthma and 17 controls, who were young to middle aged with no obvious neurocognitive deficits. Those with asthma had mostly well-controlled disease. No group differences were observed on demographic variables. (Table 1).

Table 1

Sample Characteristics and Group Comparisons

	Asthma (n=15)		Control (n=17)		
	Mean	SD	Mean	SD	*p*
Age, yrs.	25.13	9.25	25.06	9.56	0.98
Sex					
% Male	53.33		56.25		0.88
BMI	23.81	3.96	25.40	5.09	0.34
MoCA (0-30) [a]	27.88	2.19	27.40	2.64	0.59
Education, yrs.	14.90	2.83	15.13	3.43	0.57
Race (%)					0.52
White	53.33		52.94		
Black	0.00		29.41		

Asian	6.66		0.00		
Multiracial	20.00		11.76		
Other	20.00		5.88		
Ethnicity (%)					0.74
Non-Hispanic/Latinx	80.00		76.47		
Hispanic/Latinx	20.00		23.53		
FeNO (ppb)	60.84	57.66	20.0	13.90	0.003**
FEV_1 % Predicted	95.04	11.17	100.00	20.00	0.54
Asthma Severity [b] (%)					
Intermittent	20.00		N/A		
Mild, Persistent	46.67		N/A		
Moderate, Persistent	26.67		N/A		
Severe, Persistent	6.67		N/A		
Asthma Control					
ACT (5-25)	20.27	3.86			
ACQ (0-6)	0.97	0.56			
Inhaled Corticosteroids (%)	26.66		N/A		

Fractional Exhaled Nitric Oxide (FeNO), Forced Expiratory Volume in One Second (FEV_1), Montreal Cognitive Assessment (MoCA); ACT, Asthma Control Test; ACQ, Asthma Control Questionnaire. Note, independent samples (two-tailed) t-test found no statistically significant difference between groups on demographic variables. Ranges of questionnaire scores are provided in parentheses after each measure. ACT is scored with higher values indicating better control and ACQ is scored with lower values indicating better control. [a]MoCA scores <23 indicative of mild cognitive impairment, Rosetti et al., 2011, [b]NIH/NAEPP (2007) severity and medication step therapy guidelines, $^{*}p < .05$, $^{**}p < .01$

Task Performance

Task accuracy for all participants was low (mean 55% accuracy), with no difference between old (mean 55%) or new faces (mean 55%). While there was no group difference in overall task accuracy, individuals with asthma identified old faces (mean 49.5%) less accurately than participants without asthma (mean 59.7%), $t(30)=-2.59$, $p=.015$ (Table 2).

Table 2

Group Comparisons between Task Performance and Cortisol Awakening Response

	Asthma (n=14)	Control (n=17)

	Mean	SD	Mean	SD	*t*	*p*
Accurate, All (0-60)	33.14	4.44	33.41	5.04	-0.16	0.88
Accurate, Old (0-30)	14.93	3.20	17.82	3.00	-2.59	0.02*
Accurate, New (0-30)	18.21	4.92	15.24	5.09	1.64	0.11
	Asthma (n=14)		Control (n=16)			
CAR	1.02	1.32	2.96	2.39	-2.44	.021*

Ranges of possible scores are provided in parentheses after each measure. Cortisol Awakening Response (CAR), *$p < .05$

Group Differences in Peripheral Physiology

As expected, results of the independent sample t-test indicated that individuals with asthma had lower CAR compared to those without asthma, $t(28)=-2.47$, $p=0.021$ (Table 2). For individuals with asthma, FeNO was on average high (mean >60 ppb) and, as anticipated, significantly greater in individuals with asthma, $t(28)=3.19$, $p=.003$ (Table 1).

Associations Among Task Performance and Peripheral Physiology

For all participants, Spearman nonparametric analyses revealed that total task accuracy was neither significantly correlated with lnFeNO $rs(27)= -0.182$, $p= 0.36$, nor with CAR $rs(29)= 0.033$, $p= 0.87$. Spearman correlations examining associations among each type of stimuli (old faces accuracy, new faces) and lnFeNO and CAR did not reveal significant findings. For individuals with asthma, task accuracy was not significantly correlated with lnFeNO, CAR, asthma disease severity, duration, or control (Table 3).

Table 3

Spearman Rank order Correlations among Task Performance, lnFeNO, CAR, and Asthma Duration, Control and Severity for Individuals with Asthma (n=15)

	lnFeNO	CAR[a]	Asthma Duration	Asthma Severity	ACT	ACQ
Task Accuracy (All faces)	-0.205	-0.184	-0.155	0.015	0.055	-0.415

Task Accuracy (Old faces)	-0.099	0.171	-0.192	0.206	0.126	-0.200
Task Accuracy (New faces)	-0.100	-0.326	-0.050	-0.335	0.080	-0.412
lnFeNO	--	0.332	0.315	-0.118	-0.283	0.329
CAR[a]		--	0.327	-0.077	0.015	0.129
Asthma Duration			--	0.197	-0.163	0.068
Asthma Severity				--	-0.364	0.439
ACT					--	-0.800**
ACQ						--

Natural Log of Fractional Exhaled Nitric Oxide (lnFeNO), Cortisol Awakening Response (CAR), Asthma Control Test (ACT), Asthma Control Questionnaire (ACQ), Note: ACT is scored with higher values indicating better control and ACQ is scored with lower values indicating better control. [a]Pearson partial correlations were used for CAR to control for ICS use, *$p < .05$, **$p < .01$

Task Response

The examination of all trials (both old and new faces) revealed significant BOLD signal change, as an indirect marker of neural activation, for the task in distributed regions including bilateral frontal, occipital, and thalamic, consistent with studies examining face recognition (Satterwhaite et al., 2009). No clusters survived thresholding at $z = 2.0$, $p < .05$ for old > new or new < old contrasts, indicating no statistically significant difference in signal change when participants viewed old compared to new faces during the memory task.

Hippocampal Region of Interest Analyses

No differences in signal change were observed between individuals with or without asthma in either hippocampi when viewing old or new stimuli (Table 4).

Table 4

Group Comparisons in Hippocampal % Signal Change

	Asthma (n=15)		Control (n=17)			
	Mean	SD	Mean	SD	*t*	*p*
LHC Average (Old)	0.083	0.155	0.068	0.121	0.32	0.76
LHC Average (New)	-0.198	0.104	-0.891	0.149	1.51	0.14
RHC Average (Old)	0.076	0.171	0.844	0.123	-0.15	0.88
RHC Average (New)	-0.013	-0.115	-0.731	0.155	1.23	0.23

Left Hippocampus (LHC), Right Hippocampus (RHC)

Behaviorally, Spearman correlations revealed that participants with asthma who demonstrated greater % signal change in the right hippocampus (RHC) when viewing new faces were less accurate on the total task accuracy for all faces, $r(14)= -0.538$, $p= 0.047$. These associations were neither observed bilaterally, nor when viewing but significant associations were not revealed while viewing old faces or with the left hippocampus (LHC).

Table 5

Spearman Correlations among Hippocampal % Signal Change and Task Accuracy in patients with asthma (n=15)

	Task Accuracy (all faces)	Task Accuracy (old faces)	Task Accuracy (new faces)
LHC Average (Old)	-0.011	-0.100	0.165
LHC Average (New)	0.049	0.062	0.002
RHC Average (Old)	0.169	0.175	0.085
RHC Average (New)	-0.538*	-0.182	-0.343

Left Hippocampus % Signal Change (LHC), Right Hippocampus % Signal Change (RHC), *$p <$ .05

For participants with asthma, there was a trend level only association in the hypothesized direction observing that less % signal change in the left hippocampus when viewing old stimuli

approached significant association with greater asthma severity, $rs(14)= -0.454$, $p= 0.089$ (Table 6).

Table 6

Spearman Correlations among Hippocampal % Signal Change, Asthma Control, Disease Duration and Asthma Severity (n=15)

	Asthma Control					
	ACT	ACQ	lnFeNO	CAR[a]	Disease Duration	Asthma Severity
LHC Average (Old)	-0.188	0.009	-0.220	0.105	0.064	-0.454^{+}
LHC Average (New)	0.165	-0.097	0.016	0.595	0.177	0.147
RHC Average (Old)	0.248	-0.253	0.104	0.344	-0.035	-0.250
RHC Average (New)	-0.217	0.291	-0.319	0.269	0.454^{+}	0.204

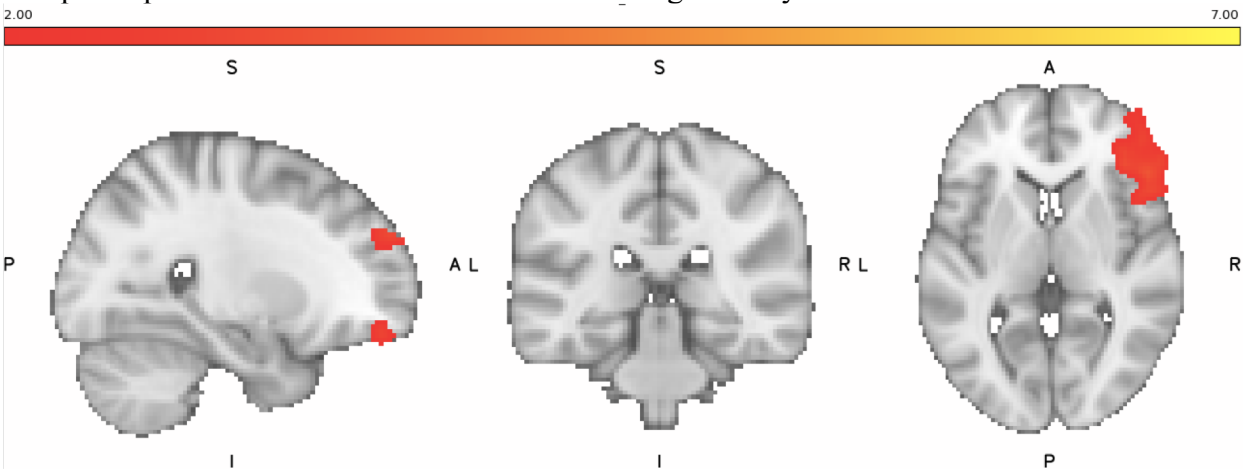
Left Hippocampus % Signal Change (LHC), Right Hippocampus % Signal Change (RHC), Asthma Control Test (ACT), Asthma Control Questionnaire (ACQ), Note: ACT is scored with higher values indicating better control and ACQ is scored with lower values indicating better control. [a]Pearson partial correlations were used for CAR to control for ICS use, $*p < .05$, $^{+}p < .10$

Exploratory Whole-Brain Analyses

In order to examine brain regions beyond those selected in the a priori analyses, we performed a whole-brain analyses of the asthma > control and control > asthma contrasts when viewing old faces, new faces, old > new, new > old faces, and all faces. While there was no difference between viewing old and new faces, the R. Inferior Frontal Cortex (IFC) showed significantly greater BOLD response in those with asthma compared to controls when viewing both old and new faces at a Z threshold of 2.00 and a $p<0.05$ (Figure 3, Table 6).

Figure 3

Unique response of R. Inferior Frontal Cortex during memory task for Asthma > Control contrast



Presented in Neurological view. Note: Colored bar indicates z thresholds ranging from 2.00-7.00. A, anterior, P, posterior, S, superior, I, inferior, L, left, R, right.

Table 6

Regions of significant whole-brain analysis % signal change for group level comparison (contrast: asthma > control)

				MNI		
Region	Cluster Size, mm^3	Z Max	p	x	y	z
R. Inferior Frontal Cortex						
Old Faces	2259	4.89	0.046	32	58	28
New Faces	2949	3.49	0.014	48	32	0
All Faces	4242	4.11	0.001	34	58	28
Old Faces > New Faces	0					
New Faces > Old Faces	0					

Z maps thresholded at family-wise error of $p<0.05$ (cluster corrected, $z=2.0$).
R, right, MNI, Montreal Neurologic Institute.

In patients with asthma, Spearman nonparametric correlations did not reveal any associations among signal change in this cluster defined ROI or overall behavioral performance on total task accuracy $rs(14)=-0.131$, $p=0.655$, task accuracy for old faces $rs(14)=-0.153$, $p=-.601$, or task accuracy for new faces $rs(14)=-0.016$, $p=.958$. Spearman nonparametric

correlations additionally did not reveal any associations among signal change and lnFeNO, *rs*(14)=-.071, *p*=0.817. Pearson partial correlations, controlling for ICS, did not reveal any association among signal change in cluster defined ROI and CAR *r*(11)=-0.254, *p*=0.401. Spearman correlations did reveal a significant association between signal change in cluster defined ROI and asthma duration, *rs*(14)=-0.521, *p*=0.047, suggesting that the longer a patient has suffered from asthma, the smaller the signal change in the right inferior frontal cortex when engaged in a memory task.

Table 7

Spearman Correlations among functionally defined ROI % Signal Change, Asthma Control, Disease Duration and Asthma Severity (n=15)

	Asthma Control			
	ACT	ACQ	Disease Duration	Asthma Severity
R. Inferior Frontal Cortex				
All Faces	0.127	-0.084	-0.521*	-0.391

Asthma Control Test (ACT), Asthma Control Questionnaire (ACQ) Note: ACT is scored with higher values indicating better control and ACQ is scored with lower values indicating better control. *$p < .05$,

Discussion

The primary goal of the present study was to compare individuals with asthma to those without asthma at both behavioral and neural levels during a memory retrieval task, in efforts to elucidate potential neural contributions to the increasingly identified neurocognitive deficits in patients with asthma. It was hypothesized that individuals with asthma would have less accurate behavioral performance on the memory task than controls, and that signal change in the hippocampus would be reduced in participants with asthma during the memory task. To test these predictions, high resolution *f*MRI was utilized to assess BOLD signal change as individuals

completed a facial memory task. Behavioral results largely confirmed previous findings, as individuals with asthma had poorer performance on some, but not all measures of memory retrieval task. On a neural level and contrary to our prediction, no group differences in hippocampal signal change were observed. In efforts to study the brain-behavior relationship in asthma, we additionally compared hippocampal signal change with task accuracy and measures of peripheral physiology and disease processes with previously observed or purported influences on neurocognition. Exploratory whole brain analyses revealed that during memory task, those with asthma had greater signal change in the right inferior frontal cortex (IFC) compared to those without asthma. The implications and limitations of these findings are discussed below.

First, a generalized inspection of the within subject imaging data revealed that all groups demonstrated significant and broad signal change in regions of the occipital, thalamic and bilateral frontal regions while completing the facial memory task. These findings are consistent with studies examining facial recognition and demonstrate that participants were appropriately engaged in the task. (Satterwhaite et al., 2009, Wolf et al., 2011). Behaviorally, the task was quite difficult with average accuracy of 55%, providing sufficient opportunity for variability of behavioral measures.

Second, as is previously observed in dozens of studies (Irani et al., 2017), individuals with asthma performed poorer than healthy controls on some, but not all, behavioral measures of neurocognition. Specifically, individuals with asthma performed poorer on accurately identifying old faces, a task which captures one's ability not only to retrieve but also successfully encode a memory. Interestingly, participants with asthma were similarly accurate at correctly identifying if a face was new or old as their age and gender matched control groups. This may indicate that individuals with asthma have more subtle neurocognitive differences in in learning and memory

which only emerge during challenging tasks, but they are similarly successful at a slightly different neurocognitive task of selectivity. Indeed, behavioral studies do observe significant reductions in learning and memory in patients with asthma but with smaller effect sizes compared to domains of executive function or attention (Irani et al., 2017). Contrary to expectation, performance on this task was not associated with any markers of peripheral physiology or asthma disease. As this was a single task and markers of peripheral physiology (e.g. FeNO and CAR) used in the present study are non-invasive and likely several biological pathways removed from direct influences on neurocognition or neural circuitry, the null results in this young sample with generally well-controlled asthma were not surprising. Overall, behavioral findings are consistent with previous literature in that patients with asthma inconsistently demonstrate poorer performance on tasks for neurocognition. It may be important for future studies to ensure sufficient challenge in learning and memory tasks, to capture more subtle group differences that may emerge prior to the onset of clinically relevant neurocognitive deficits.

Third, the primary aim of this study was to compare signal change in bilateral hippocampi in individuals with and without asthma, where we anticipated that less signal change would be observed in patients with asthma compared to controls during the memory task. While the memory task demonstrated sufficient difficulty as outlined above, no group differences in hippocampal signal were observed during the task. These null findings may be most parsimoniously attributed to lack of neural activity differences during a memory task in this young and well controlled sample, not presenting with any obvious neurocognitive difficulties or complaints. Although group differences have been observed in the hippocampal metabolic profile of younger individuals with asthma (Kroll et al., 2018) and volume reductions are

observed in middle aged individuals with asthma (Carlson et al., 2016), it may be that any consequences of this disease has yet to impact a related but distinct process of hippocampal neural activity captured with the BOLD signal.

Within the asthma group, ROI analyses of the right hippocampus (RHC) revealed that greater signal change while viewing new faces was associated with poorer accuracy on the memory task of all faces. Although statistically significant, this correlational finding provides only tentative support for a relation between greater RHC activity and accuracy and should be cautiously interpreted. One potential interpretation could be that the greater the effort spent attempting to discriminate from new faces results in less total accuracy.

Fourth, exploratory whole brain analyses revealed that individuals with asthma have greater signal response during the memory task generally, in a cluster likely representing the right inferior frontal cortex (IFC). The MNI standardized z-max coordinates entered in to Neurosynth.org, a meta-analytic tool to compare signal changes across studies, identified studies observing signal increases in the similar peaks in response to viewing emotional faces (Loughead et al., 2008). Consistent with our results, there appears to be a greater prevalence of studies observing signal changes in the right vs. left IFC. Both of these concepts are consistent with the type of stimuli presented in the current study. While exploratory in nature, this finding for the first time suggests that individuals with asthma may be working harder than those without asthma by recruiting additional areas to engage with the same task. Correlational findings with asthma disease processes suggest that, less signal change in the functionally defined ROI of the right IFC during the memory task was associated with a longer disease duration. Again, these findings should be interpreted cautiously; however, it suggests potential for differential neural activity over the course of disease. For example, cumulative effects might contribute to a

decrease in activation in some regions or may alternatively indicate influence of learning or efficiency of neural response.

Taken together, these findings are largely consistent with behavioral studies observing neurocognitive performance differences in individuals with asthma on some but not all tasks and suggest that individuals with asthma may be working harder at a neural level than those without asthma to achieve the same overall task accuracy.

Limitations

The present study has several limitations. Most notably, the smaller sample size recruited and available for final analyses limits the generalizability of these findings and the study was likely underpowered to detect more subtle pre-clinical differences in neural signal. Furthermore, this was a relatively young and well-educated sample. A majority of those presenting with asthma had well controlled disease, which likely restricted the range of our variables and limited opportunities to detect group differences or associations. Further, many of the factors proposed to impact neurocognition and neural circuitry that coincide with poor disease control in asthma may not be relevant for this sample (e.g. comorbid medical conditions or chronic use of systemic steroids to achieve asthma control).

Second, the specifics of study design may have limited our ability to detect a more subtle difference neural hippocampal signal if one does exist. Encoding rather than retrieval may yield greater hippocampal signal change (Zeineh et al., 2003), and the technical challenges of imaging the hippocampus with the present design, may obscure more detailed responses from hippocampal subregions. The functional voxel size of, 3.4 x 3.4 x 4.0 mm, likely averages together the anatomically and functionally heterogenous sub-regions of the hippocampus, around

1 x 1 x 1 mm. Additionally, given the nature of the task, there is most certainly an affective component contributing to the memory process.

Despite these limitations of the study, our ability to observe any group differences with the whole brain analyses in individual this young of age without neurocognitive difficulties, suggests that even in fairly well-controlled patients with asthma, pre-clinical differences in neural activity during memory tasks might be present.

Future Directions

Future studies are needed with variability in the known risk factors for poorer neurocognition in asthma: low socioeconomic status, poor asthma control, and racial minorities (Irani et al., 2017). As age is a risk factor for poorer neurocognition generally, extending this study with a lifespan approach to examine an asthma by aging interaction hypothesis is warranted.

Future technical directions may include extracting a "flat-map" of the hippocampus, and look at changes in the CA1-CA3, CA4 or Dendate Gyrus, and Subiculum substructures, which have been demonstrated to be differentially sensitive to both encoding and memory retrieval of facial stimuli (Zeineh et al., 2003).

Most importantly, prospective studies are needed, particularly surrounding manipulation of asthma control to elucidate the brain-behavior relationships observed in asthma. These relations are likely bidirectional and multifactorial. As these studies have been safely conducted (Weersnick et al., 1997; , incorporating a neuroimaging component to treatment intervention studies would provide substantial clarity to this field and further identify opportunities and targets for intervention.

Conclusion

In conclusion, the present study corroborates previous findings that individuals with asthma may have poorer behavioral memory retrieval for some, but not all memory tasks. In addition, when asked to make a memory decision, individuals with asthma tended to recruit additional areas of the right inferior frontal cortex (RIFC) compared to age and gender matched controls, suggesting that they may be working harder when completing the same type of memory task and achieving the same total accuracy as healthy controls. These findings provide novel insights into neural contributions to neurocognition in asthma and continue to underscore the importance of studying the neural processes in asthma and monitoring for neurocognitive difficulties in individuals with asthma, even in young patients with well controlled disease.

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