

Peri-adolescent asthma: Acute impacts on innate immune response, corticosterone, and microglia in mice

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ABSTRACT

Asthma is highly comorbid with anxiety in youth. We investigated the hypothalamic-pituitary-adrenal (HPA) axis and microglia as mechanisms underlying asthma and anxiety comorbidity. We induced asthma symptoms in developing BALB/cJ mice with house dust mite (HDM) for airway inflammation and methacholine (MCH) for bronchoconstriction. On the last day of exposure, we analyzed samples at six timepoints. Lung *IL-5* and *IL-1 β* expression peaked 4 h after final HDM exposure. Circulating corticosterone was blunted in a sex- and treatment-specific temporal pattern. Hippocampal *IL-1 β* expression and microglial area were marginally increased 24 h after MCH exposure. These results provide a foundation for further work investigating asthma-anxiety mechanisms.

1. Introduction

Asthma is the most common chronic health condition that affects children and adolescents (Akinbami et al., 2016). Asthma is typically diagnosed in children in the first 2–5 years of life, and in youth, allergic asthma is the most prevalent form (60–90% of cases), with boys diagnosed more often and experiencing more severe symptoms than girls (Arathimos et al., 2017; Li et al., 1998; Patadia et al., 2014; Syamlal and Mazurek, 2008; Tai et al., 2009). Asthma symptom hallmarks, such as lung inflammation and bronchoconstriction, are symptoms that could affect important neurobiological development occurring in stress- and emotion-regulation brain areas during this time (Holder and Blaustein, 2014; Patadia et al., 2014; Spear, 2000). Children with asthma have a variety of other comorbidities, including internalizing disorders like anxiety and depression, which can contribute to worse asthma symptom burden and disease outcomes (Dudeney et al., 2017; Goodwin et al., 2003; Katon et al., 2007; Ortega et al., 2002; Richardson et al., 2006; Shams et al., 2018; Vila et al., 2000). Given that children with asthma

are three times as likely to develop an anxiety disorder compared to the general youth population (Dudeney et al., 2017), it is important to understand a mechanism for this comorbidity.

Hypothalamic-pituitary-axis (HPA) axis dysfunction is one potential mechanism that has been associated with many mental health conditions, particularly anxiety- and depression-type conditions (Adam et al., 2017; de Rooij et al., 2010; Dieleman et al., 2015; Feder et al., 2004). Low plasma adrenocorticotrophic hormone (ACTH), salivary and plasma cortisol, and salivary cortisol awakening response have been observed in patients with panic disorder and depression compared to controls (Petrowski et al., 2013; Stetler and Miller, 2005). Interestingly, children with asthma commonly develop adrenal insufficiency, which has been associated with lower morning baseline serum cortisol levels, cortisol awakening response, and serum cortisol reactivity in patients medicating with inhaled corticosteroids (Eid et al., 2002; Kroll et al., 2019; Sim et al., 2003). Compared to children without asthma, children with a history of asthma that were medicated with inhaled corticosteroids demonstrated long-term HPA dysfunction with lower cortisol levels in

Abbreviations: HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotrophic hormone; IL, interleukin; CD11b, cluster of differentiation molecule 11b; CD11c, cluster of differentiation molecule 11c; HDM, house dust mite; MCH, methacholine; Crhr1, corticotropin releasing hormone receptor 1; CON, control; AI, airway inflammation; BR, bronchoconstriction; USV, ultrasonic vocalization; P, postnatal day; Penh, enhanced pause; CORT, corticosterone; DG, dentate gyrus; ANOVA, analysis of variance; PBS, phosphate buffered saline.

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hair samples (Kamps et al., 2014). Although adrenal insufficiency is a known result of chronic steroid use, decreased salivary cortisol reactivity has been observed in children with asthma who were not treated with inhaled corticosteroids compared to healthy children, suggesting that adrenal insufficiency may be a feature of asthma itself and not simply an effect of inhaled corticosteroid treatments for asthma. (Buske-Kirschbaum et al., 2003; Caulfield and Cavigelli, 2020). Thus, because low cortisol is observed with anxiety and with asthma, HPA dysfunction represents an obvious potential mechanism that leads youth with asthma to develop anxiety later in life.

People with asthma have a distinct immune activation profile. Allergic asthma is characterized by increased T-helper-2-type activity with measurable increases in cytokines like interleukin (IL)-4, IL-5, and IL-13 (Fujita et al., 2012; Galli et al., 2008). IL-5 is a mediator of eosinophil recruitment and activity, which are a prominent kind of immune cell that infiltrate the lungs from the bone marrow in allergic asthma (Murdoch and Lloyd, 2010; Rothenberg and Hogan, 2006). This peripheral immune activity can affect immune function in the brain, especially during early development. Microglia, the immune cells of the brain that are derived from macrophage precursors produced in yolk sac, are critical for proper growth and maturation of the brain (Bilbo and Schwarz, 2012; Harry, 2013; Harry and Kraft, 2012; Lenz and McCarthy, 2015; McCarthy et al., 2015; Tremblay et al., 2011). In response to a variety of immune insults and injury, microglia transition from a surveying state with a small cell body and long, thin processes to a state with a large, activated amoeboid cell body (Harry and Kraft, 2008; Osborne et al., 2018; Schwarz et al., 2012). Many studies conducted with rodents indicate that microglial activation can be triggered by peripheral immune challenges and that this activation is associated with increased anxiety-like and depressive-like behaviors (Lehmann et al., 2016; Nemeth et al., 2014; Stein et al., 2017; Wohleb et al., 2012, 2013, 2015). Few studies have explored microglial activation in asthma and allergy, and little research explores microglial function in the context of asthma and anxiety (Klein et al., 2016; Vogel Ciernia et al., 2018). Thus, it is important to determine if chronic asthma symptoms affect microglial activation and if this is a mechanism by which asthma-associated anxiety develops.

Our lab uses a mouse model for asthma, where we induce symptoms of airway inflammation and bronchoconstriction to determine how these chronic symptoms, experienced during development, contribute to an anxiety-like phenotype in adulthood (Caulfield et al., 2017, 2018). Chronic exposure to house dust mite (HDM) leads to downregulation in serum corticosterone and lasting inflammation in the lungs that persists at least three months after final HDM exposure (Caulfield et al., 2017, 2018). On the other hand, repeated, acute bronchoconstriction events, stimulated with aerosolized methacholine (MCH), led to increased anxiety-like behavior and corticotropin-releasing hormone receptor 1 (*Crhr1*) gene expression in the hippocampus three weeks after final exposure to MCH (Caulfield et al., 2017). Pilot data from these initial studies revealed chronic MCH led to increased microglial activity (measured as gene expression of *CD11b* in the hippocampus) three weeks after final exposure. Thus, in the current study, we measured HPA- and microglial-related function to further investigate potential mechanisms underlying the asthma and anxiety comorbidity. To establish an acute timeline of central and peripheral responses to chronic HDM or MCH exposure during development, we measured circulating corticosterone concentration, expression of genes associated with inflammation in lungs (*IL-5*, *IL-1 β* , *CD11c*), and gene expression associated with stress and microglial activation in hippocampus (*Crhr1*, *IL-1 β* , *CD11b*) at multiple time points after final exposure. We also measured hippocampal microglia area and number 24 h after final HDM or MCH exposure. A priori, we hypothesized that mice exposed to HDM would have a heightened immune response and blunted circulating corticosterone concentration compared to unexposed mice, and that mice exposed to MCH would have increased *Crhr1* expression and microglial activity in the hippocampus compared to control and HDM-

treated groups.

2. Materials and methods

2.1. Study design

As described previously, a longitudinal study was used to induce asthma symptoms over the course of mouse development (Caulfield et al., 2017, 2018, 2020). There were three experimental groups: airway inflammation (AI, $n = 69$; exposed to HDM), bronchoconstriction (BR, $n = 67$; exposed to MCH), and procedural control (CON, $n = 63$; exposed to saline). The AI group received intranasal house dust mite extract (HDM) administered three times per week and aerosolized saline in a plethysmograph one time per week (control for procedures required to administer methacholine to BR group). The BR group received intranasal saline three times per week (procedural control) and aerosolized methacholine (MCH) in the plethysmograph once per week. The CON group received intranasal saline three times per week and aerosolized saline once per week (Fig. 1). To document the acute physiological responses to AI or BR, we measured circulating corticosterone and gene expression in the lungs at six different time points after final exposure to chronic HDM/MCH/saline: immediate (within 5 min of exposure, $n = 33$), 1 h ($n = 31$), 2 h ($n = 33$), 4 h ($n = 34$), 8 h ($n = 32$), and 24 h after exposure ($n = 36$). Hippocampal gene expression was examined immediately and after 4 and 24 h. Each time point had 15–17 males and females evenly distributed across all three experimental groups (5–7 males and females per experimental group per time point). Hippocampal microglia number and area were analyzed after 24 h in a subset of mice ($n = 13$). All tests and procedures were approved by the Pennsylvania State University IACUC committee and abided by the National Institute of Health guide for care and use of animals.

2.2. Mouse breeding and housing

BALB/cJ breeders were obtained from Jackson Laboratories (Bar Harbor, ME) and were bred in a Pennsylvania State University vivarium (37 litters across 10 cohorts, 3–12 pups per litter, 95 male and 104 female offspring). The vivarium was maintained at $72 \pm 2^\circ\text{F}$, and mice had ad libitum access to food and water throughout the experiments. Lights were maintained on a 12:12 h light:dark cycle, with lights OFF at 10:00 h EDT. All procedures began at 10:00 h EDT. To control for natural variation in pre-manipulation anxiety-prone phenotypes in pups, ultrasonic vocalizations (USV) were recorded during postnatal days (P)3–5 at 65 Hz using the “Isolation” method (2 min/day), and then high- vs. low-calling pups were evenly-distributed among all experimental groups (Branchi et al., 2001; Brunelli et al., 1997; Caulfield et al., 2017, 2018; Dichter et al., 1996; Hahn and Lavooy, 2005). Pups were identified with non-toxic Sharpie® markings until they received unique ear punches on P9–10. After the first aerosolized MCH/saline exposure on P21, mice were weaned into same-sex groups with 2–3 littermates/group, and experimental conditions were balanced within each group as best as possible. Mice were sacrificed on P56 to collect samples at one of six times after final exposure to HDM, or MCH, or saline (immediate, 1, 2, 4, 8, or 24 h; Fig. 1).

2.3. Allergen administration

Allergen was administered as previously described (Caulfield et al., 2017, 2018, 2020; Saglani et al., 2009). House dust mite extract (*Dermatophagoides pteronyssinus*, HDM; Greer Labs, NC, USA) diluted in saline was given intranasally three times per week. Mice in the AI group received 10 μg protein in 10 μL saline from P7–16 and 15 μg protein in 15 μL saline under brief isoflurane anesthesia from P17–56. Mice in the CON and BR groups received an equivalent volume of saline on the same schedule (Caulfield et al., 2017, 2018, 2020; Saglani et al., 2009).

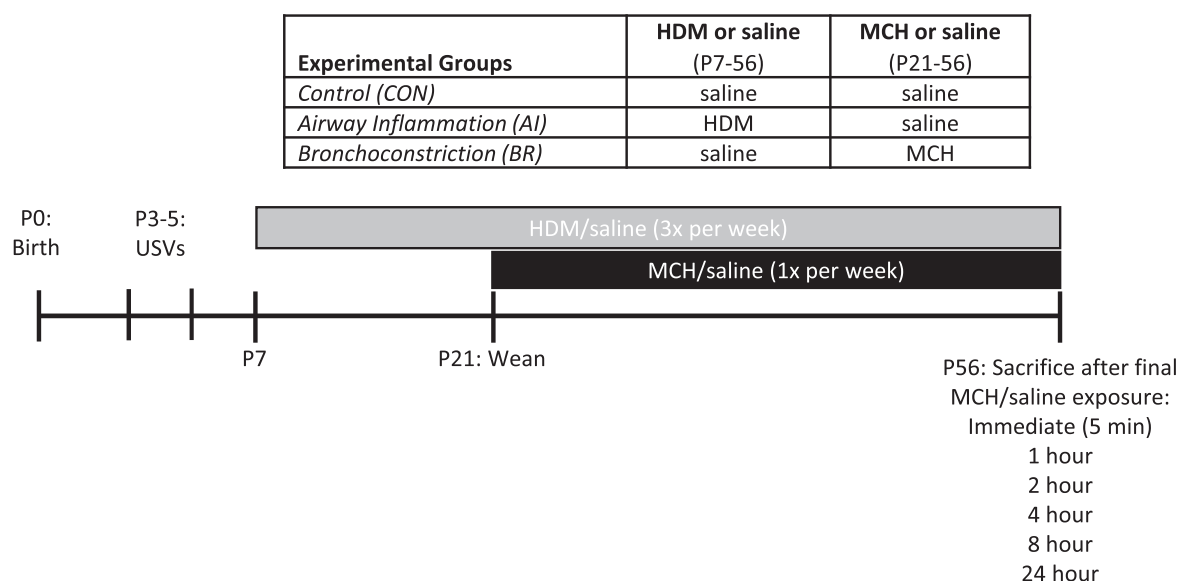


Fig. 1. Experimental design & timeline: Three experimental groups were used: *Control* (CON; intranasal saline plus aerosolized saline), *Airway Inflammation* (AI; intranasal house dust mite extract and aerosolized saline), and *Bronchoconstriction* (BR; intranasal saline and aerosolized methacholine). Mice were born and ultrasonic vocalizations (USVs) were measured on postnatal days (P)3–5. House dust mite extract (HDM) or saline was intranasally administered three times per week during P7–56, and aerosolized methacholine (MCH) or saline was administered once a week during P21–56 (immediately after HDM/saline administration). On P56, fecal samples were collected immediately after final MCH/saline exposure, and then mice were sacrificed at one of the following times (immediate – within 5 min, 1, 2, 4, 8, or 24 h) to collect lung, blood, and brain samples for hormone and gene expression analyses. A subset of brains collected at 24 h were stained to examine microglia in the hippocampus.

2.4. Bronchoconstriction

Procedures to induce bronchoconstriction were conducted as previously described (Caulfield et al., 2017, 2018). Once a week from P21–56, mice were placed in a whole-body plethysmograph holding chamber (7.5 × 7 cm, Data Sciences International, MN, USA). They were given 3 min to acclimate to the chamber, 3 min to measure baseline breathing, and then five 3 min phases of exposure to aerosolized substances. The first phase was a saline control. In the BR group, the final four phases involved increasing doses of MCH (6.25, 12.5, 25, 50 ng/mL in 100 μ L saline) to elicit labored breathing. Mice in the CON and AI groups received aerosolized saline as a control for the last four doses. To verify and estimate the extent of bronchoconstriction, enhanced pause (Penh) was recorded (Hamelmann et al., 1997) for each dose at each age using FinePointe software. Behavioral measures were also recorded during each phase (active, sit still, hunched, labored breathing, drool, gape). In prior studies we have shown that this method produces significant labored breathing in BALB/cJ mice (Caulfield et al., 2017, 2018).

2.5. Sample collection timeline

In order to measure acute neuroimmune and neuroendocrine responses to asthma symptoms in the mouse model, we collected samples at six different times following final exposure to HDM or MCH: immediate, 1, 2, 4, 8, or 24 h from the end of substance exposure. For ‘immediate’ samples, mice were sacrificed within 5 min from the end of exposure without returning to the home cage. For the later samples, mice were returned to the colony room after substance exposure and then were retrieved individually at the designated sample collection time.

2.6. Corticosterone analyses

Fecal samples were collected during the final MCH/saline exposure on P56 and were stored at -80°C until corticosterone (CORT) metabolites were extracted and measured. CORT metabolites in excreted feces

reflect concentrations of CORT in circulation 4 h prior to fecal collection (Touma et al., 2003). Upon euthanasia, a blood sample was immediately collected onto ice via cardiac puncture and centrifuged at 4°C for 15 min at 12,000g. Serum was collected and stored at -80°C . CORT metabolites and circulating CORT were measured from fecal extracts and serum respectively using a commercial [^{125}I] radioimmunoassay kit (MP Biomedicals, Solon, OH, USA) following manufacturer guidelines (Caruso et al., 2018; Caruso et al., 2017b; Caulfield et al., 2017, 2018, 2020; Cavigelli et al., 2018). Intra- and inter-assay coefficients of variation were 9.82 and 10.06 (for low control) and 11.45 and 8.20 (for high control).

2.7. Gene expression

After cardiac blood collection, mice were perfused of remaining blood with 10–12 mL cold saline. Tissues were dissected (lungs, hippocampus), stored at -80°C , and processed as previously described (Caulfield et al., 2017, 2018, 2020). Briefly, RNA was isolated from tissue using TRIzolTM (Invitrogen, USA) following manufacturer guidelines, and concentrations were determined with a NanoDropTM spectrophotometer (Thermo Fisher Scientific, USA). cDNA was reverse transcribed from RNA using High-Capacity cDNA Reverse Transcription kits (Applied Biosystems, USA). Quantitative real time PCR (qRT-PCR) was used to measure the relative abundance of genes using the standard $2^{-\Delta\Delta\text{CT}}$ method, and expression was calculated relative to the average male control at the immediate collection time. Primers used for PCR were determined from NCBI GenBank and included: beta-actin (forward: 5'-GCCCTGAGGCTCTTTCC-3', reverse: 5'-TGCCACAGGATTCCATACCC-3'), interleukin 5 (*IL-5*; forward: 5'-ACAGACATGCACCATGCGCA-3', reverse: 5'-TGGGTTCATCTCCAGCACT-3'), interleukin-1 β (*IL-1 β* ; forward: 5'-CCAAAAGATGAAGGGCTGCT-3', reverse: 5'-TCATCTGGACAGCCAGGTC-3'), corticotropin releasing hormone receptor 1 (*Crhr1*; forward: 5'-GTGCCTCCCATGTTTGGAT-3', reverse: 5'-TCGTGTGAAGCCTTGGGTTT-3'), cluster of differentiation molecule 11b (*CD11b*; forward: 5'-CTTGAGGAACCGTGCCAAAG-3', reverse: 5'-TGCTGATACCGAGGTGCTCC-3'), and cluster of

differentiation molecule 11c (*CD11c*; forward: 5'-GCCGACA-CATTTCACATGG-3', reverse: 5'-TGGAGCACACTGTGTCGAA-3').

2.8. Microglia visualization

One half of the brain from 13 mice (1 cohort, 5 male and 8 female offspring) was collected 24 h after symptom exposure to visualize hippocampal microglia, and methods were adapted from those previously described (Chen et al., 2019; Zou et al., 2015). Briefly, brains were immediately collected into 4% paraformaldehyde and stored at 4 °C overnight. Then, tissue was cut into 40 µm sections using a vibratome (Leica). Brain sections were permeabilized in 2% Triton X-100 in phosphate buffered saline (PBS) for 40 min, followed by 1 h of incubation in blocking buffer (5% normal donkey serum and 0.1% Triton X-100 in PBS). Primary antibodies (Rabbit anti Iba-1, 019–19,741, Wako, Japan, Guinea pig anti NeuN, ABN90, Millipore, USA) were added to the blocking buffer with brain sections and incubated overnight at 4 °C. Primary antibodies were washed off with PBS, and then brain sections were protected from light and incubated with secondary antibodies (Donkey anti Alexa 488 and Cy3, 1:800, Molecular Probes, USA) for 1 h at room temperature. Slices were washed in PBS, mounted on a glass slide, and stored at 4 °C. Hippocampal images were obtained at 20× magnification using a Zeiss LSM 510 confocal microscope (Carl Zeiss, USA), and images were analyzed using ImageJ v2.0.0. Four sections in the hippocampus (at least one slice each of dorsal and ventral hippocampus) were analyzed per mouse brain. Images were collected of CA1, CA3, and dentate gyrus (DG) for each slice. Percent area of microglia stained (Iba-1) in the image frame was calculated using ImageJ. Microglia within the image frame were also manually counted. For each region, we also calculated percent area per cell counted (percent area/count). Experimenters were blind to sex and experimental condition of each animal until after coding was completed.

2.9. Statistical analyses

Statistical analyses were conducted with Statistical Package for the Social Sciences (SPSS, version 26). To determine temporal responses to HDM and MCH exposure, we used omnibus ANOVAs with experimental group (CON, AI, BR), time (immediate, 1, 2, 4, 8, 24 h), and sex (male, female) as factors for each outcome variable (CORT, and lung/hippocampal gene expression). If an omnibus ANOVA indicated a main effect of time or an experimental group × time interaction, to better analyze individual experimental group temporal patterns, we followed up with separate ANOVAs at each time point (experimental group and sex as factors). If main effects or interactions with sex were evident ($p < 0.10$), males and females were analyzed separately. Results with an alpha of $p < 0.05$ are reported as significant effects. For main effects of experimental condition or sex, pairwise comparisons are indicated on figures and in legends, and for effects of time, pairwise comparisons are reported only in figure legends. All data figures were made with Graphpad Prism 8.4.3.

A subset of mice was analyzed for lung gene expression ($n = 44$ –48 per experimental group). For serum CORT analyses, time to collect blood from mouse cage disturbance was examined as a possible covariate, and it was only included in analyses for the 8 h time (8 h: $p < 0.05$, all other times: $p > 0.296$). A subset of fecal samples was analyzed to measure CORT metabolites, with some samples combined to achieve extractable weight ($n = 21$ –29 samples per experimental group at analysis).

For microglia immunohistochemistry, we used a repeated measures ANOVA with the four slices collected per region as the repeated measure per mouse. We report between-subject statistics. Because of the small sample size ($n = 13$ mice, 5 males, 8 females), experimental group was the only factor used in these analyses.

To achieve a normal distribution for parametric analyses, some outcome variables were natural log-transformed. These included Penh

(all ages), serum CORT (all times measured), fecal CORT, gene expression in lung (all times for *IL-5*, *IL-1β*, *CD11c*), gene expression in hippocampus (immediate, 4, and 24 h for *Crhr1*, *CD11b*, *IL-1β*). Untransformed means are shown in graphs.

To control for significant systematic cohort effects, cohort mean for the outcome variable was included as a factor in ANOVAs, and if $p < 0.10$, it was included as a covariate. This was the case for P35 labored breathing counts and Penh, gene expression in the lungs (all times for *IL-5*), and gene expression in the hippocampus (immediate, 4, and 24 h for *Crhr1*, *CD11b*, *IL-1β*). In these cases, estimated marginal means were shown in graphs.

3. Results

3.1. Methacholine behavior and Penh

At all ages, labored breathing (counts and mean Penh) was significantly higher in the BR group compared to CON and AI groups. At P42 and P56, there was evidence that AI mice had more labored breathing than CON mice (all statistics in Table 1).

3.2. Lung gene expression

IL-5 and *IL-1β* gene expression in lungs peaked at 4 h after HDM exposure (AI) and remained significantly elevated at 8 h compared to non-allergen-exposed mice (CON, BR), whereas *CD11c* expression was chronically elevated in AI vs. CON and BR mice.

IL-5, *IL-1β*, and *CD11c* expression in lung tissue differed among experimental groups – HDM-exposed mice (AI) had greater expression than CON and BR mice (main effect of experimental group: *IL-5* – $F_{2,92} = 43.2$, $p < 0.001$, Fig. 2A; *IL-1β* – $F_{2,81} = 18.4$, $p < 0.001$, Fig. 2B; *CD11c* – $F_{2,93} = 347.3$, $p < 0.001$, Fig. 2C). Across all experimental groups, immune-related gene expression changed during the 24 h period after HDM/MCH/saline exposure, with greatest *IL-5* expression at 2 and 4 h, greatest *IL-1β* expression at 4 and 8 h, and greatest *CD11c* expression at 2 h (main effect of time: *IL-5* – $F_{5,92} = 5.2$, $p < 0.001$, *IL-1β* – $F_{5,81} = 12.6$, $p < 0.001$, *CD11c* – $F_{5,93} = 2.7$, $p < 0.05$). For *IL-5* and *IL-1β*, these temporal profiles differed between experimental groups (interaction of experimental group and time: *IL-5* – $F_{10,92} = 2.2$, $p < 0.05$, *IL-1β* – $F_{10,81} = 4.7$, $p < 0.001$). Specifically, when time points were analyzed separately, AI, compared to CON and BR, had greater *IL-5* and *IL-1β* expression at the immediate, 4, and 8 h time points, greater *IL-5* expression at 24 h, and greater *CD11c* expression at all time points (main effect of experimental group: *IL-5* immediate – $F_{2,14} = 7.4$, $p < 0.01$, 4 h – $F_{2,15} = 55.8$, $p < 0.001$, 8 h – $F_{2,16} = 29.7$, $p < 0.001$, 24 h – $F_{2,10} = 9.3$, $p < 0.01$; *IL-1β* immediate – $F_{2,14} = 6.5$, $p < 0.01$, 4 h – $F_{2,16} = 19.8$, $p < 0.001$, 8 h – $F_{2,11} = 20.7$, $p < 0.001$; *CD11c* immediate – $F_{2,15} = 207.6$, $p < 0.001$, 1 h – $F_{2,17} = 144.7$, $p < 0.001$, 2 h – $F_{2,17} = 62.0$, $p < 0.001$, 4 h – $F_{2,15} = 107.2$, $p < 0.001$, 8 h – $F_{2,17} = 58.7$, $p < 0.001$, 24 h – $F_{2,15} = 10.3$, $p < 0.01$; pairwise comparisons reported in Fig. 2 legend).

Overall, females expressed more *IL-5*, *IL-1β*, and *CD11c* than males (main effect of sex: *IL-5* – $F_{1,92} = 4.9$, $p < 0.05$; *IL-1β* – $F_{1,81} = 7.4$, $p < 0.01$; *CD11c* – $F_{1,93} = 8.1$, $p < 0.01$). Only *CD11c* showed a sex by experimental condition interaction, where HDM-treated (AI) females had greater *CD11c* expression than HDM-treated (AI) males (interaction of experimental group and sex: $F_{2,93} = 7.5$, $p < 0.001$, Fig. 2C). When analyzed separately by sex, both sexes showed greater *IL-5*, *IL-1β*, and *CD11c* expression in HDM-treated (AI) mice compared to both control (CON) and MCH-treated (BR) mice (main effect of experimental group: *IL-5* – females – $F_{2,47} = 19.3$, $p < 0.001$, males – $F_{2,44} = 22.9$, $p < 0.001$; *IL-1β* – females – $F_{2,43} = 13.0$, $p < 0.001$, males – $F_{2,38} = 6.3$, $p < 0.01$; *CD11c* – females – $F_{2,48} = 413.9$, $p < 0.001$, males – $F_{2,45} = 86.6$, $p < 0.001$). Overall, female peak *IL-5* expression was at 2, 4, and 8 h, peak *IL-1β* expression at 4 and 8 h, and peak *CD11c* expression at 1 and 2 h (main effect of time: *IL-5* – females – $F_{5,47} = 3.2$, $p < 0.05$, *IL-1β* – females – $F_{5,43} = 7.3$, $p < 0.001$, *CD11c* – females – $F_{5,48} = 4.6$, $p < 0.01$, Figs. 2A,

Table 1

Methacholine labored breathing counts and Penh: At all methacholine administration ages, labored breathing counts and mean enhanced pause (Penh) were greater in the BR compared to CON and AI groups. Main effects of experimental group are reported with *F* statistic, degrees of freedom, and *p* values. Symbols represent significant differences between groups based on pairwise comparisons: ^Ω BR vs. CON *p* < 0.001, [†] BR vs. AI *p* < 0.001, ^{\$} AI vs. CON *p* < 0.05.

Age	Labored Breathing Count				Mean Penh			
	Main effect of Experimental Group	Mean (StDev)			Main effect of Experimental Group	Mean (StDev)		
		CON	AI	BR		CON	AI	BR
P21	$F_{2,191} = 339.4, p < 0.001$	0.014 (0.119)	0.103 (0.414)	2.053 $^{\Omega \dagger}$ (0.728)	$F_{2,162} = 19.0, p < 0.001$	0.645 (0.499)	0.608 (0.412)	1.139 $^{\Omega \dagger}$ (0.733)
	$F_{2,193} = 491.7, p < 0.001$	0.000 (0.000)	0.026 (0.226)	2.325 $^{\Omega \dagger}$ (0.802)	$F_{2,178} = 43.3, p < 0.001$	0.665 (0.512)	0.730 (0.530)	1.517 $^{\Omega \dagger}$ (1.008)
P28	$F_{2,191} = 461.9, p < 0.001$	0.016 (0.126)	0.176 (0.487)	2.881 $^{\Omega \dagger}$ (1.453)	$F_{2,185} = 40.5, p < 0.001$	0.727 (0.691)	0.808 (0.684)	1.678 $^{\Omega \dagger}$ (0.914)
P35	$F_{2,193} = 330.5, p < 0.001$	0.069 (0.484)	0.282 (0.719)	2.896 $^{\Omega \dagger}$ (0.912)	$F_{2,184} = 47.6, p < 0.001$	0.686 (0.528)	1.008 ^{\$} (1.182)	1.682 $^{\Omega \dagger}$ (0.828)
P42	$F_{2,193} = 455.4, p < 0.001$	0.097 (0.609)	0.115 (0.483)	2.974 $^{\Omega \dagger}$ (0.858)	$F_{2,193} = 653.4, p < 0.001$	0.741 (0.500)	0.833 (0.526)	1.704 $^{\Omega \dagger}$ (0.856)
	$F_{2,193} = 411.9, p < 0.001$	0.000 (0.000)	0.218 ^{\$} (0.638)	2.805 $^{\Omega \dagger}$ (0.828)	$F_{2,184} = 69.6, p < 0.001$	0.750 (0.528)	0.812 (0.574)	1.790 $^{\Omega \dagger}$ (1.071)
P49								
P56								

B,C), whereas male expression only changed over time for *IL-1β* with a peak at 4 and 8 h, particularly in HDM-treated (AI) males (main effect of time: *IL-1β* – males – $F_{5,38} = 6.3, p < 0.001$; interaction of experimental group and time: *IL-1β* – males – $F_{10,38} = 3.5, p < 0.01$). No other significant main or interaction effects were observed for lung *CD11c*, *IL-5*, or *IL-1β* expression ($F_s < 2.6, p > 0.108$).

3.3. Corticosterone

3.3.1. Serum corticosterone

Allergen exposure during development dampened circulating CORT concentrations in both males and females, although the time at which this occurred differed by sex. As expected, females had higher serum CORT concentrations than males at most times.

Circulating CORT concentrations differed among experimental groups and across time: HDM-treated (AI) mice had lower levels than control (CON) mice, and across all conditions, CORT concentrations peaked immediately and were lowest at 8 h (main effect of experimental group: $F_{2,161} = 4.0, p < 0.05$; main effect of time: $F_{5,161} = 68.3, p < 0.001$; Fig. 3A). The temporal CORT pattern was marginally different among experimental groups (interaction of experimental group and time: $F_{10,161} = 1.8, p = 0.059$). When time points were analyzed separately, AI mice had lower CORT than BR mice and marginally lower than CON mice immediately after exposure, and this was driven by females (main effect of experimental group: $F_{2,27} = 4.2, p < 0.05$, females – $F_{2,14} = 4.8, p < 0.05$, males – $F_{2,16} = 0.4$, ns). One hour after exposure, AI mice had lower CORT than BR mice, and this was more pronounced in males (main effect of experimental group: $F_{2,25} = 3.5, p < 0.05$, females – $F_{2,13} = 1.7$, ns, males – $F_{2,12} = 2.9, p < 0.10$). Four hours after exposure, CON mice had marginally higher CORT than AI and BR mice, and these effects were driven by males (main effect of experimental group: $F_{2,28} = 2.6, p < 0.10$, females – $F_{2,14} = 0.9$, ns, males – $F_{2,14} = 4.9, p < 0.05$).

Females produced more CORT than males, and the magnitude of this sex effect changed across time (main effect of sex: $F_{1,161} = 30.4, p < 0.001$; interaction of time and sex: $F_{5,161} = 4.9, p < 0.001$). In particular, females produced more CORT than males at 2, 4, 8, and 24 h (main effect of sex: 2 h – $F_{1,27} = 14.9, p < 0.001$, 4 h – $F_{1,28} = 9.3, p < 0.01$, 8 h – $F_{1,24} = 12.6, p < 0.01$, 24 h – $F_{1,29} = 21.4, p < 0.001$, Fig. 3A). Within time points, there were significant interactions of sex and experimental group at the immediate and 4 h time points (interaction of experimental group and sex: immediate – $F_{2,27} = 4.4, p < 0.05$, 4 h – $F_{2,28} = 3.8, p < 0.05$). When sexes were analyzed separately at each time point, HDM-exposed (AI) females had lower circulating CORT than CON and BR females immediately and at 24 h (main effect of experimental group in females: immediate – $F_{2,14} = 4.8, p < 0.05$, 24 h – $F_{2,15} = 7.5, p < 0.01$), whereas

MCH-exposed (BR) males had lower CORT than CON males at 4 h (main effect of experimental group in males: 4 h – $F_{2,14} = 4.9, p < 0.05$). No other significant effects were observed at any collection times ($F_s < 2.4, p > 0.112$).

3.3.2. Fecal corticosterone

Concentration of fecal corticosterone metabolites was quantified as nanograms of metabolite per gram of feces excreted at the time of HDM/MCH/saline exposure on P56 only. Females had more concentrated CORT metabolites in feces than males (main effect of sex: $F_{1,66} = 375.1, p < 0.001$, Fig. 3B). In females, BR mice produced more CORT than CON and AI mice (main effect of experimental group: $F_{2,23} = 5.1, p < 0.05$, Fig. 3B). No other significant effects were observed ($F_s < 1.9, p > 0.157$).

3.4. Hippocampal gene expression

In hippocampus, we measured gene expression associated with HPA function (*Crhr1*, Fig. 4A), microglial activity (*CD11b*, Fig. 4B; *IL-1β*, Fig. 4C), and proinflammatory response (*IL-1β*). Expression of immune-related genes (*CD11b* and *IL-1β*) were only affected by time (*CD11b* – $F_{2,85} = 3.6, p < 0.05$; *IL-1β* – $F_{2,85} = 6.8, p < 0.01$). *CD11b* expression was greatest immediately after substance exposure, and *IL-1β* expression was greatest immediately after exposure and at 4 h. When *IL-1β* expression was analyzed separately at each time point, males showed significantly more *IL-1β* expression than females at the immediate time point (main effect of sex: $F_{1,27} = 6.3, p < 0.05$, not shown in figure). There were no other significant group, sex, or interaction effects observed for *Crhr1*, *CD11b*, or *IL-1β* ($F_s < 2.3, p > 0.138$).

3.5. Microglia assessment

On P56, we examined percent area and count of microglia in the CA1, CA3, and DG regions of the hippocampus 24 h after HDM/MCH/saline exposure (Fig. 5A). In the CA1, AI mice had marginally less area represented by microglia compared to BR mice (experimental group pairwise comparisons: AI vs. BR, $p < 0.10$, Fig. 5B). There were no other differences among experimental groups in any region for both percent area and count ($F_s < 1.9, p > 0.206$; percent area: Fig. 5B, count: Fig. 5C). There were no differences in percent area per cell counted among experimental groups ($F_s < 1.6, p > 0.254$).

4. Discussion

In the present study, we explored HPA function and microglia as potential mechanisms related to comorbidity of asthma and anxiety in youth. We used an established model of peri-adolescent asthma to

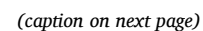


Fig. 2. Inflammatory cytokine gene expression in lung tissue: Lung (A) *IL-5*, (B) *IL-1 β* , and (C) *CD11c* expression were elevated in the AI group compared to both CON and BR groups. Compared to CON and BR, AI had greater expression of *IL-5* and *IL-1 β* immediately and at 4 and 8 h after final HDM/saline exposure, greater *IL-5* expression at 24 h, and greater *CD11c* expression at all time points. Females expressed more of each gene than males, and *CD11c* expression was greater in AI females compared to AI males. Pairwise comparisons of experimental groups at significant time points: *IL-5*: 0 h – AI>CON & BR ($p < 0.01$), 4 & 8 h – AI>CON & BR ($p < 0.001$), 24 h – AI>CON ($p < 0.01$), AI>BR ($p < 0.05$); *IL-1 β* : 0 h – AI>CON ($p < 0.01$), AI>BR ($p < 0.05$), 4 & 8 h – AI>CON & BR ($p < 0.001$); *CD11c*: 0, 1, 2, 4, 8 h – AI>CON & BR ($p < 0.001$), 24 h – AI>CON & BR ($p < 0.01$). Female pairwise comparisons: Experimental group – *IL-5*: AI>CON & BR ($p < 0.001$), BR > CON ($p < 0.05$); *IL-1 β* : AI>CON & BR ($p < 0.001$); *CD11c*: AI>CON & BR ($p < 0.001$), CON>BR ($p < 0.05$); Time – *IL-5*: 2 h > 1 & 24 h ($p < 0.01$), 2 h > immediate ($p < 0.05$), 4 & 8 h > 24 h ($p < 0.05$); *IL-1 β* : 4 & 8 h > immediate & 1 h ($p < 0.001$), 4 & 8 h > 2 h ($p < 0.05$), 2 & 24 h > immediate ($p < 0.05$); *CD11c*: 2 h > 4 & 8 h ($p < 0.001$), 2 h > immediate & 24 h ($p < 0.01$), 1 h > 4 & 8 h ($p < 0.05$). Male pairwise comparisons: Experimental group – *IL-5*, *IL-1 β* , and *CD11c*: AI>CON & BR ($p < 0.001$); Time – *IL-1 β* : 4 h > immediate ($p < 0.001$), 4 h > 24 h ($p < 0.01$), 4 & 8 h > 1 & 2 h ($p < 0.01$), 8 h > immediate ($p < 0.01$), 8 h > 24 h ($p < 0.05$). Experimental group x time: *IL-1 β* : 4 h – AI>CON ($p < 0.01$), AI>BR ($p < 0.05$), 8 h – AI>CON & BR ($p < 0.01$). Statistics – main effect of sex: *** $p < 0.001$; experimental group pairwise comparisons: AI vs. CON: \$\$\$ $p < 0.001$; AI vs. BR: ††† $p < 0.001$; CON vs. BR: Ω $p < 0.05$.

induce chronic airway inflammation with exposure to house dust mite (HDM) every other day and acute bronchoconstriction with weekly methacholine (MCH) exposure (Caulfield et al., 2017, 2018, 2020; Saglani et al., 2009). Previously, we have shown anxiety-related effects three weeks after final exposure to MCH (Caulfield et al., 2017). To establish an acute timeline of peripheral and central responses to these allergic asthma-inducing agents, in the current study, we measured lung gene expression, serum CORT, and hippocampal gene expression at multiple different times within 24 h after final HDM/MCH/saline exposure.

Asthma and allergy are characterized by heightened T_H2 immune activation, and allergic asthma is characterized by airway inflammation with eosinophil infiltration in the lungs (Greenfeder et al., 2001; Rothenberg and Hogan, 2006). In previous studies with this model, we have demonstrated that chronic exposure to HDM during development led to significant eosinophilic inflammation and *IL-5* gene expression three weeks and three months after the last HDM exposure (Caulfield et al., 2017, 2018, 2020). *IL-5* is a major mediator of eosinophilic activation (Murdoch and Lloyd, 2010). In the current study, we examined allergen-stimulated changes in lung *IL-5*, *IL-1 β* , and *CD11c* gene expression at an earlier age to estimate acute allergic and proinflammatory responses during development. Mice chronically exposed to allergen during development (AI group) had significantly increased *IL-5* and *IL-1 β* gene expression in the lungs immediately and at 4 and 8 h after allergen exposure, and *IL-5* expression was still significantly increased 24 h after allergen exposure. This represented both an accentuated and prolonged allergic and proinflammatory response in allergen-exposed mice. Given that mice were chronically exposed to the allergen, they may demonstrate an early- and late-phase immune response as is observed in humans with chronic asthma and allergies (Bianco et al., 1989; López et al., 2010; O'Sullivan et al., 1998). In patients with allergic rhinitis, this acute early- and late-phase response has been documented with increased symptoms (sneezing, nasal obstruction, and itching) within 15 min and then again 6 h after allergen (HDM) exposure (López et al., 2010). Another study revealed that asthma patients exposed to allergen produced measurable urinary markers of early- and late-phase immune responses, including 9 α ,11 β -PGF $_2$, methyl-histamine, and LTE $_4$ (O'Sullivan et al., 1998). In the present study, the immediate peak, that would represent the “early” response, may be due to a time of day effect or may reflect chronically-elevated *IL-5* and *IL-1 β* expression in mice chronically-exposed to allergen. We did not measure inflammatory markers in other peripheral organs or other media to determine whether this response was systemic beyond the lungs, nor did we use other metrics to understand how this gene expression related to other molecular function. Human airway smooth muscle cells exposed to serum from asthma patients in vitro had increased *IL-5* and *IL-1 β* protein and DNA levels in the tissue at 3, 6, and 24 h after initial serum exposure (Hakonarson et al., 1999). Studies with animals have demonstrated increased levels of cytokines (e.g. *IL-4* *IL-5*, *IL-13*) and immune cells (e.g. eosinophils, neutrophils, lymphocytes) in bronchoalveolar lavage fluid within the first few days after final sensitization with an allergen (Antunes et al., 2010; Joachim et al., 2003; Nogueira et al., 1999; Wakahara et al., 2008). Together, the results of the present study

demonstrate that exposure to allergen, but not bronchoconstriction with methacholine, induces an acute allergic response in the lungs with a modest increase observed in less than one hour followed by a larger secondary peak 4 to 8 h after exposure.

To further investigate asthma-related HPA axis regulation, we measured acute temporal changes in circulating glucocorticoids and basal excreted glucocorticoid levels following final allergen and bronchoconstriction challenges. Prior studies indicate that stress, anxiety, and worsened asthma symptoms are related to one another (Theoharides et al., 2012). For example, in children exposed to persistent maternal distress in early life, those with asthma have significantly decreased plasma cortisol compared to children without asthma (Dreger et al., 2010). Further, baseline and reactivity levels of cortisol in youth with asthma predicts neuropsychological functioning in subsequent years (Dinces et al., 2019). In the present study, we examined CORT in mouse serum at six different times after final allergen or bronchoconstriction exposure. Results showed decreased CORT levels in response to allergen at the same times that allergic immune-related gene expression was greatest in the lungs. In females exposed to allergen (vs. saline), serum CORT was lower immediately and 24 h later, whereas in males, allergen exposure led to decreased circulating CORT 1 and 4 h later. Females had more concentrated circulating and excreted CORT than males, and females that experienced repeated bronchoconstriction excreted particularly high CORT compared to allergen-exposed females. Though we observed acute group differences in CORT after allergen/bronchoconstriction, we did not observe acute changes in hippocampal gene expression of *Crhr1*. Previously with this mouse model, we demonstrated that *Crhr1* gene expression in the hippocampus was elevated three weeks after final MCH exposure (Caulfield et al., 2017). Corticotropin releasing hormone (CRH) is produced by the hypothalamus as part of the HPA axis and binds to *Crhr1*, and *Crhr1* knockout in the forebrain has been associated with decreased anxiety-like behavior and disrupted signaling in limbic regions like the amygdala and hippocampus (McEwen, 2006; Müller et al., 2003; Refojo et al., 2011; Zaloga and Marik, 2001). Regions like the hippocampus and prefrontal cortex are important for HPA negative feedback, and decreased glucocorticoid receptor protein in these regions after chronic stress has also been associated with increased anxiety-like behavior (Chiba et al., 2012; Romeo, 2010). Thus, alterations in genes associated with HPA function are associated with changes in anxiety-like behaviors. The results from the current and prior studies suggest that peri-adolescent asthma may have an acute effect on HPA function without acute alterations to stress-related gene expression in the hippocampus, and that changes in hippocampal gene expression may only occur after a longer delay. Together, the results from the developmental asthma mouse model indicate dysregulation of the HPA axis, particularly in females. Given these results, and the fact that children with asthma also have altered HPA regulation, further research is needed to determine specific HPA mechanisms and whether these underlie asthma-related anxiety and/or sex-differences in this susceptibility.

In addition to the HPA axis, we investigated microglia after exposure to chronic asthma symptoms during development. Microglia are the immune cells of the brain that become activated in response to an

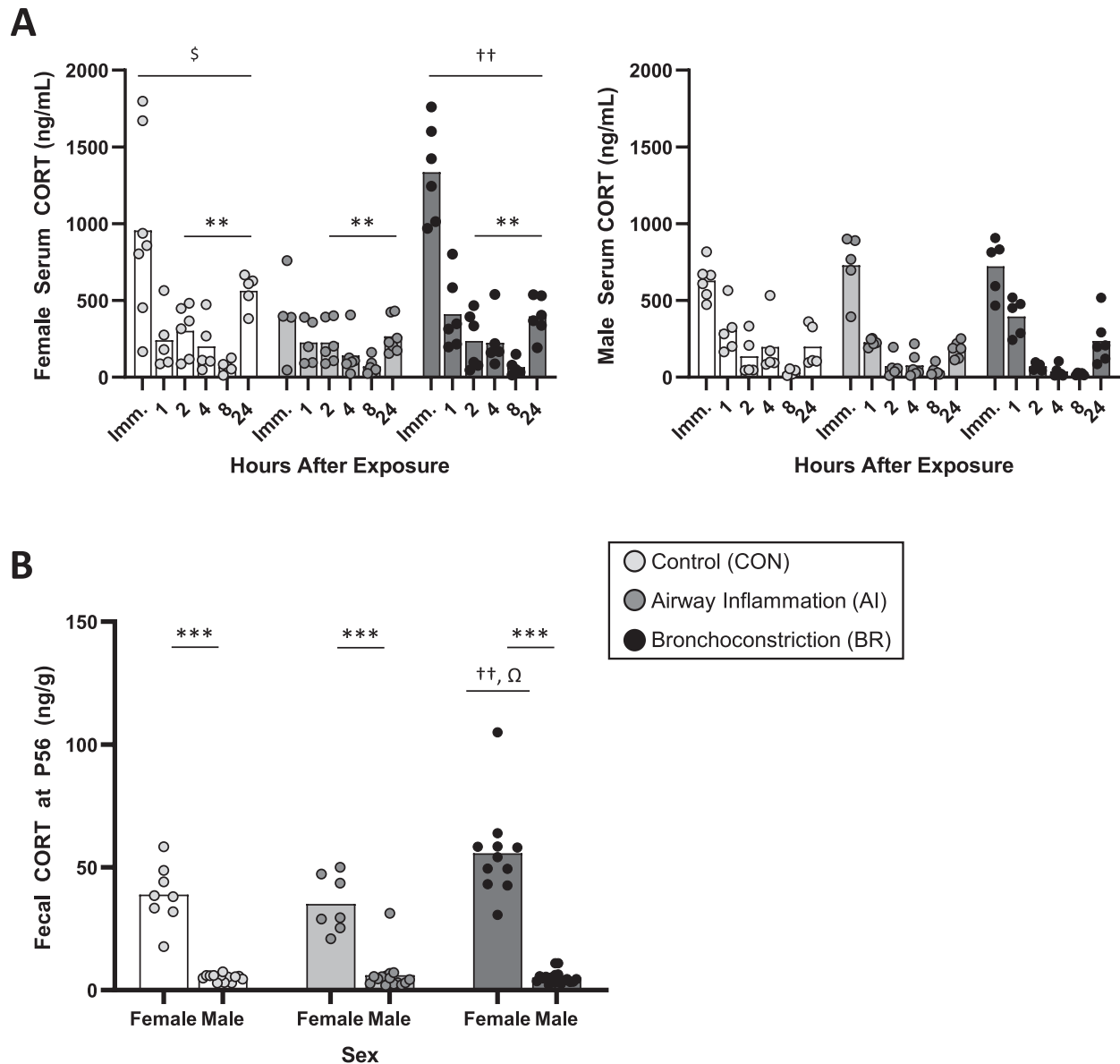


Fig. 3. Adult serum and fecal corticosterone (CORT): (A) Circulating CORT concentrations were lower in the AI vs. CON and BR groups, and this effect was primarily at the immediate time point in females (left panel) and at 1 h in males (right panel). At 24 h, AI females also had lower CORT than CON females, and at 4 h, BR males had lower CORT than CON males. Overall, females have greater CORT concentrations than males at 2, 4, 8, and 24 h. (B) On P56, CORT metabolites in feces were significantly higher in BR females vs. CON and AI females. Pairwise comparisons of experimental groups at significant time points: Serum CORT: 0 h – AI < BR ($p < 0.01$), AI < CON ($p < 0.10$), 1 h – AI < BR ($p < 0.05$), BR > CON ($p < 0.10$), 4 h – CON > AI & BR ($p < 0.10$). Female pairwise comparisons: Experimental group – Serum CORT: AI < CON ($p < 0.05$), AI < BR ($p < 0.01$), Fecal CORT: AI < BR ($p < 0.01$), CON < BR ($p < 0.05$). Time – Serum CORT: immediate > 1, 2, 4 & 8 h ($p < 0.001$), 1, 2, 4 & 24 h > 8 h ($p < 0.001$), 24 h > 4 h ($p < 0.001$), immediate > 24 h ($p < 0.05$), 24 h > 2 h ($p < 0.05$); Experimental group x time – Serum CORT: immediate AI < BR ($p < 0.01$), 24 h AI < CON ($p < 0.01$). Male pairwise comparisons: Time – Serum CORT: immediate > 1, 2, 4, 8 & 24 h ($p < 0.001$), 1 h > 2, 4 & 8 h ($p < 0.001$), 1, 2, 4 & 24 h > 8 h ($p < 0.001$), 24 h > 2, 4 & 8 h ($p < 0.001$), 1 h > 24 h ($p < 0.05$); Experimental group x time – Serum CORT: 1 h BR > AI ($p < 0.05$); 4 h CON > BR ($p < 0.01$). Statistics – main effect of sex, $F > M$: serum 2, 4, 8 and 24 h: $^{**}p < 0.01$, fecal CORT: $^{***}p < 0.001$; experimental group pairwise comparisons: AI vs. BR: $^{\dagger}p < 0.01$; CON vs. AI: $^{\S}p < 0.05$; CON vs. BR: $^{\Omega}p < 0.05$.

immune challenge; early exposure to such challenges can have lasting impacts on later-life cell morphology and behavior (Harry and Kraft, 2008; Nemeth et al., 2014; Osborne et al., 2017; Schwarz et al., 2012; Wohleb et al., 2012). Microglia have receptors for CRH, thus there is a signaling mechanism by which microglia can be activated by stressors, immune or otherwise (Kritas et al., 2014). Specific markers like cluster of differentiation molecule 11b (CD11b) can indicate microglial activity, and cytokines like IL-1 β are produced by activated microglia (Akiyama and McGeer, 1990; Bilbo and Schwarz, 2012; Osborne et al., 2017, 2018; Williamson et al., 2011; Yang et al., 2010). Preliminary data from an

initial study that established the developmental allergic asthma mouse model indicated that three weeks after final exposure to MCH, there was increased hippocampal expression of *CD11b* in the same group that demonstrated increased anxiety-like behavior and hippocampal *Crhr1* gene expression (Caulfield et al., 2017). In the present study, we examined acute changes in *Crhr1*, *CD11b*, and *IL-1 β* gene expression in the hippocampus. While there were no experimental group differences in expression of these genes, *CD11b* and *IL-1 β* expression changed over time, with the highest expression immediately after allergen/bronchoconstriction/vehicle exposure compared to 24 h later. These temporal

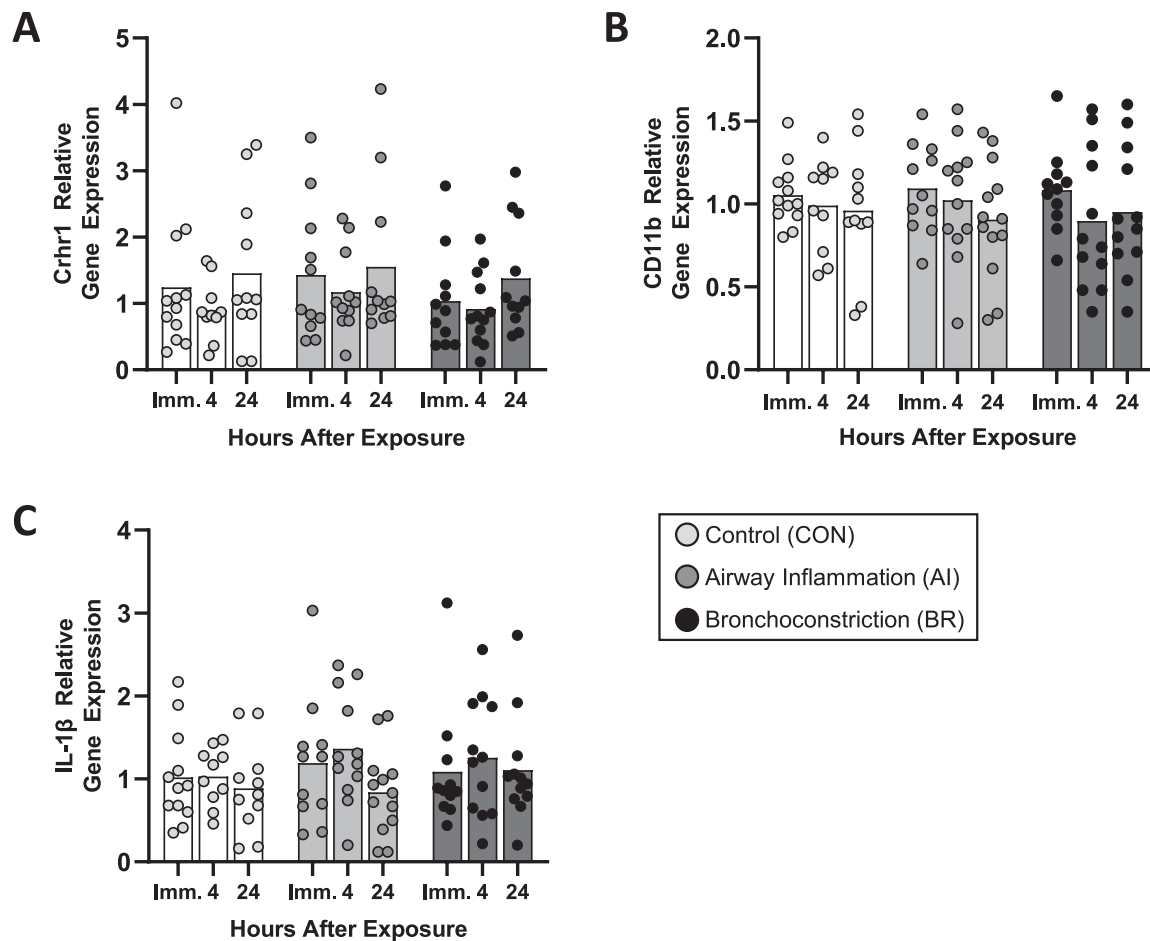


Fig. 4. Hippocampal gene expression: Hippocampal (A) *Crhr1*, (B) *CD11b*, and (C) *IL-1β* gene expression did not differ between experimental groups at any time point (immediate, 4, or 24 h after HDM/MCH/saline exposure). Hippocampal gene expression changed over time, with greater expression immediately compared to 24 h later. Pairwise comparisons: Time – *CD11b*: immediate > 4 & 24 h ($p < 0.05$); *IL-1β*: immediate & 4 h > 24 h ($p < 0.01$).

patterns were no different across experimental groups. A marginal increase in hippocampal CA1 percent area of microglia was evident in BR mice compared to AI mice. These results suggest that bronchoconstriction may modestly increase acute microglial activation more than allergen exposure, and this finding could be strengthened with increased power. While other research suggests that HDM may contribute to increased microglia size in the subfornical organ compared to control mice (Lewkowich et al., 2020), this is the first study to suggest that bronchoconstriction (induced by MCH) during development can affect microglia in the hippocampus. These findings suggest that acute changes in microglia may be detectable within 24 h after exposure to bronchoconstriction; this is the same group that showed adult anxiety-like behavior in a prior study. Changes in microglia may continue to occur beyond the timepoints examined in the current study and may influence anxiety-like development later in life. Thus, microglial function should continue to be examined as a possible mechanism for asthma-related anxiety.

The current findings highlight sex differences resulting from chronic allergen and bronchoconstriction exposure throughout development. We observed that males and females exhibit the same magnitude of change in *IL-5* and *IL-1β* expression in the acute response to allergen challenge, whereas females had a greater *CD11c* expression response to allergen than did males. These findings suggest that acute allergic and proinflammatory responses do not differ by sex, but that females invoke a stronger dendritic signaling response than males. This is surprising given numerous studies which indicate that female allergic inflammation and immune responses are more robust and more persistent than

male responses (Antunes et al., 2010; Blacqui re et al., 2010; Caulfield et al., 2017, 2018, 2020). In addition to acute lung function, sex differences in glucocorticoid production are well-established (Bale and Epperson, 2015; Caruso et al., 2017; Caulfield et al., 2017, 2020; Henderson et al., 2017). In the current study, females did not show greater CORT production than males until 2 h after exposure to allergen or bronchoconstriction. These results suggest that males and females had a similar acute stress response to the handling procedures required to induce asthma symptoms. We did not have enough statistical power to analyze immunohistochemistry data separately for males and females, so future work on microglia and asthma should be powered to allow for analyses of sex differences. Sex differences in microglia number and morphology have been reported in the hippocampus (CA1, CA3, and DG) over the course of normal development, where females exhibit more inactive/surveying microglia in adulthood compared to males (Schwarz et al., 2012). However, aged female mice also have more hippocampal microglial and inflammatory genes compared to aged male mice (Mangold et al., 2017). Increased activity of microglia is associated with anxiety-like behavior in other rodent models, and in humans, internalizing disorders are more common in females compared to males, even within adolescent asthma patients (Alt mus, 2006; Jalnapurkar et al., 2018; Kessler et al., 1994; Lehmann et al., 2016; Machluf et al., 2020; Wohleb et al., 2012, 2013, 2015). Together current and previous findings suggest that asthma impacts females more strongly than males, and the HPA axis is more persistently dysregulated by asthma symptoms in females compared to males.

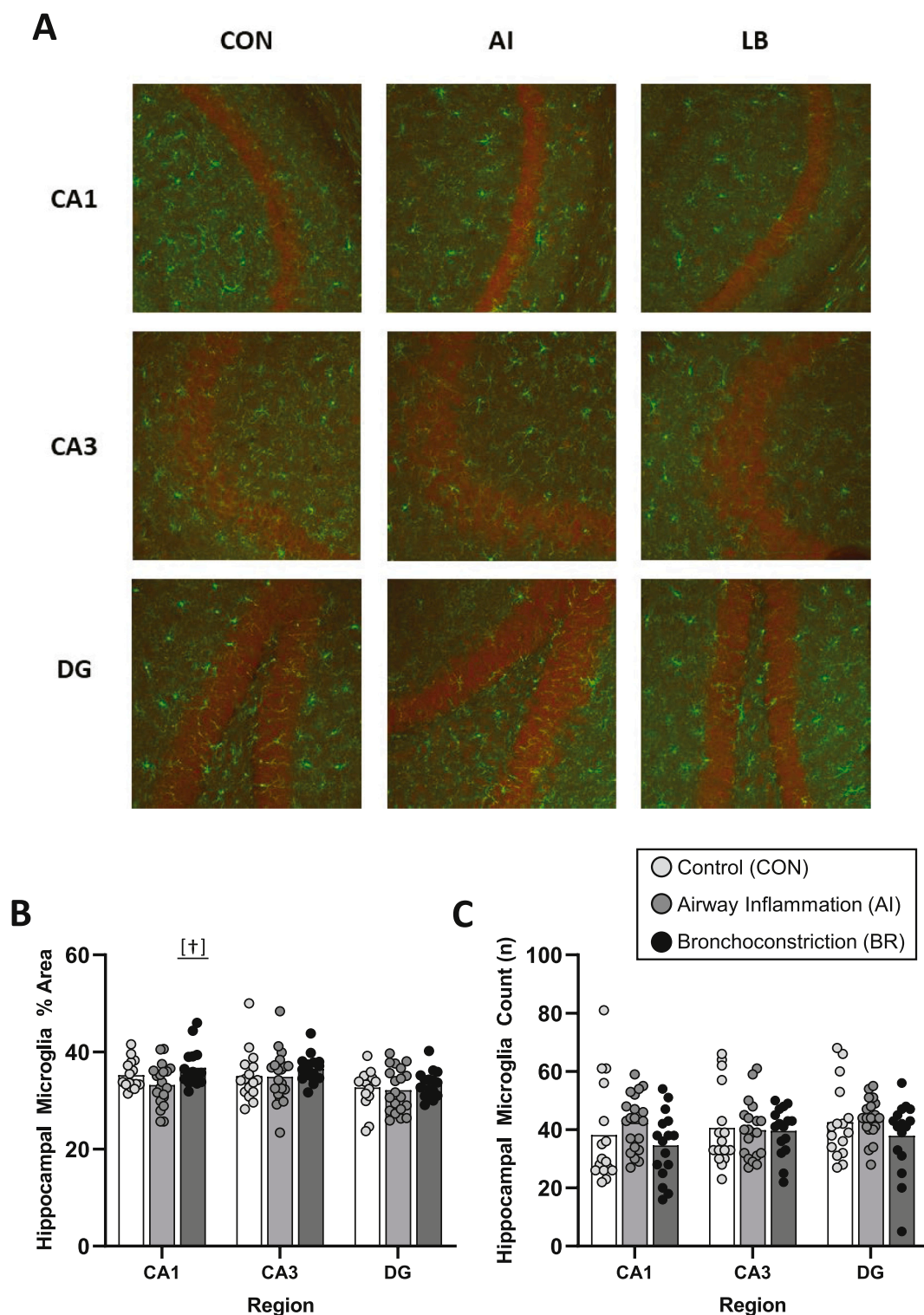


Fig. 5. Microglia immunohistochemistry: (A) Representative images of hippocampal CA1, CA3, and DG subregions with microglia stained with Iba1 (green) and neurons stained with NeuN (red). (B) Percent area of microglia was marginally lower in the CA1 for AI mice compared to BR mice. (C) No significant differences were observed for microglia count. Statistics – experimental group pairwise comparisons comparing within the same hippocampus region: AI vs. BR: $^{†}p < 0.10$.

4.1. Conclusion

To examine potential mechanisms that predispose young individuals with asthma to develop anxiety, the current study explored acute hypothalamic-pituitary-adrenal and microglia changes after airway inflammation and bronchoconstriction stimulation. Airway

inflammation was induced with inhaled allergen exposure (chronic house dust mite extract), and bronchoconstriction was induced with inhaled muscarinic receptor agonist (repeated exposure to methacholine). Peripheral and central responses during the 24 h after final allergen/methacholine exposure indicated that allergen exposure led to a prolonged acute increase in lung immune gene expression that peaked

after four hours with a blunted immediate corticosterone response that mimics responses seen in human asthma. We did not see comparable acute changes in the hippocampal gene expression related to HPA function or microglial activity; these central processes may require a longer period to show significant alterations. These results further support the role of HPA and microglial mechanisms involved in asthma-related mental health in youth.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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