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Association between the magnitude of the immune response and recurrent major depression disorder in humans and depression-like behavior in animals

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ASSOCIATION BETWEEN THE MAGNITUDE OF THE IMMUNE RESPONSE AND RECURRENT MAJOR DEPRESSION DISORDER IN HUMANS AND DEPRESSION-LIKE BEHAVIOR IN ANIMALS

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Abstract

There is a growing body of evidence supporting the association between inflammation and major depressive disorder (MDD). One plausible mechanism for this association is sensitization of the immune response, possibly due to prior exposure to stressors. To investigate the validity of this hypothesis, a series of three complimentary cross-species studies was conducted. Study 1 examined the associations between circulating levels of inflammatory markers and *in vitro* immune reactivity with women's history of recurrent MDD (rMDD) and their current symptoms of anhedonia. The potential moderating role of women's history of childhood abuse was also examined. Study 2 and 3 focused on animal models of the influence of adolescent stress on LPS-induced changes in adult anhedonic behavior (Study 2) and inflammatory gene expression in brain areas associated with reward processing and stress (Study 3). Although there was no evidence of increased circulating or stimulated levels of inflammation among women's history of rMDD in Study 1, current level of anhedonia was associated with increased stimulated levels of inflammatory markers. Results of Study 2 showed a marginally significant trend for the effect of adolescent stress exposure on anhedonia-like behavior in adult rats, such that rats subjected to an acute stressor in adolescence showed a decreased preference for palatable substance (sucrose) as adults. Finally, the results of Study 3 provided no support for altered neuroinflammatory response in the brain areas related to reward processing and stress based on the adolescent stress exposure. Overall, the findings highlight the importance for integration

of interdisciplinary methodology in psychological studies, yield initial support for the role of the immune system in anhedonia and provide important directions for future research.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

MDD = Major Depressive Disorder

rMDD = recurrent Major Depressive Disorder Depression and Inflammation

IL-1β = Interleukin-1β

NAc = Nucleus Accumbens

VTA = Ventral Tegmental Area

AMG = Amygdala

PVN = Paraventricular Nucleus of the Hypothalamu

Depression and Inflammation

Major depressive disorder (MDD) is the leading cause of disability worldwide ("WHO | Depression," 2016), with 10% of men and 20% of women in the U.S. experiencing at least one episode of MDD during their lives (Vos et al., 2012). Additionally, up to 60% of individuals who experience an initial MDD episode are expected to relapse following recovery and the risk of recurrence increases with each subsequent episode (Bulloch, Williams, Lavorato, & Patten, 2014; Solomon et al., 2000). Recurrent MDD (rMDD) is characterized by distinct genetic, neurobiological, and hormonal profiles compared to a single MDD episode (sMDD; Admon et al., 2014; Levinson et al., 2003), carries a greater risk for more adverse and chronic consequences,(Burcusa & Lacono, 2007; Lewinsohn, Allen, Seeley, & Gotlib, 1999), and is less responsive to antidepressant medication (Kaymaz, van Os, Loonen, & Nolen, 2008).

Although multiple neural mechanisms are likely to contribute to depression susceptibility later in life, a rapidly growing body of research suggests that immune-derived signaling factors, such as pro-inflammatory cytokines, play an important role in the pathogenesis of depression. For example, otherwise healthy individuals with MDD display elevated peripheral and cerebrospinal fluid (CSF) levels of inflammatory immune markers (e.g., IL-6, IL-1β, C-reactive protein; Dowlati et al., 2010; Ford & Erlinger, 2004; Levine et al., 1999; Liu, Ho, & Mak, 2012). In addition, individuals suffering from chronic and acute inflammatory conditions demonstrate increased levels of inflammatory

markers and depressive symptoms (Capuron et al., 2002; Musselman et al., 2001; Owen, Eccleston, Ferrier, & Young, 2001). Furthermore, up to 50% of patients undergoing cytokine immunotherapy develop MDD (Miller, Maletic, & Raison, 2009). Moreover, animals that were administered inflammatory cytokines demonstrate sickness behavior, which closely resembles the depressive phenotype in humans (Miller & Raison, 2016). Finally, higher levels or circulating inflammatory markers in childhood prospectively predicted greater risk of developing depression and psychopathology in adulthood (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014).

Several cytokines have emerged from previous research as strong candidates for playing a significant role in MDD, including IL-1β, IL-6, IL-1β, and IL-10 (Anisman, Ravindran, Griffiths, & Merali, 1999; Dowlati et al., 2010; Goshen & Yirmiya, 2009; Mesquita et al., 2008; Omrani et al., 2009). IL-6 has emerged as one of the strongest candidates from several meta-analyses that examined human studies of inflammatory processes and depression (Dowlati et al., 2010; Howren, Bryant, Lamkin, & Suls, 2009; Liu et al., 2012). Recent evidence suggests that one of the ways in which IL-6 impacts neurotransmission and subsequent behavior is by exerting direct regulatory control over serotonin transporter (SERT) activity, potentially linking this cytokine to one of the hypothesized mechanisms underlying the development and maintenance of depression Delgado (2000). Providing additional support for this link, there is evidence that IL-6 leads to decreases in mRNA and protein levels of SERT *in vitro* and *in vivo* (Kong et al., 2015). Conversely, the hippocampal tissue of mice lacking IL-6 expression (IL-6 KO) has been shown to contain increased levels of SERT, which corresponded to decreased

depression-like behaviors and blunted response to antidepressant medication (Kong et al., 2015).

Another cytokine that has been frequently implicated in MDD and depression-like behavior is IL-1β. An ample body of human and translational animal research suggests that MDD and depression-like behavior are frequently accompanied by increased levels of IL-1β (Michael Maes, Song, & Yirmiya, 2012). As with IL-6, IL-β has been shown to modulate SERT levels (Zhu, Blakely, & Hewlett, 2006), and mediate the effects of changes in SERT activity on subsequent depression-like behavior following exposure to a common pathogen, lipopolysaccharide (LPS), that it typically used in research to induce the immune response (Zhu et al., 2010).

Each of the cytokines reviewed thus far are proinflammatory cytokines and are associated with T helper 1 cells (Th1). In addition to these, there is evidence that Th2 cytokines, or anti-inflammatory cytokines, including IL-10, also play a role in MDD and depression-like behaviors, such that the circulating levels of IL-10 increase following anti-depressant treatment (Tavakoli-Ardakani, Mehrpooya, Mehdizadeh, Hajifathali, & Abdolahi, 2015). Providing further support for the role of IL-10 in MDD and depression-like behavior, Mesquita et al. (2008) showed that IL-10 knockout (KO) mice displayed increased depression-like behaviors, while mice that overexpressed IL-10 evidenced decreased depression-like behaviors. Moreover, systemic IL-10 administration has been shown to decrease depression-like behavior in rodents (Mesquita et al., 2008). Lastly, similarly to IL-6 and IL-1β, IL-10 has been shown to alter SERT levels. Specifically, high concentrations of IL-10 has been shown to induce SERT production *in vitro,* while low levels of IL-10 decrease SERT production (Latorre et al., 2013).

In addition to these well-established cytokines, there is growing evidence for the potential role of IL-33 in depression. IL-33 is a multifunctional ligand within the IL-1 superfamily (Baekkevold et al., 2003; Schmitz et al., 2005). By binding to the ST2 receptor, the ligand for which remained unknown until 2005, (Ali et al., 2007; Schmitz et al., 2005), IL-33 activates nuclear factor kappa B (NF-κB) and p38 mitogen-activated protein kinases (MAPK), culminating in altered expression of downstream pro-inflammatory cytokines, chemokines, and anti-inflammatory cytokines (Allakhverdi, Smith, Comeau, & Delespesse, 2007; Kakkar & Lee, 2008; Mirchandani, Salmond, & Liew, 2012; Moulin et al., 2007; Yasuoka et al., 2011). Thus, IL-33 appears to be embedded within key signaling pathways that are already known to link cytokine signaling with MDD. Additionally, previous research suggests the involvement of IL-33 in neuroinflammatory processes (Chapuis et al., 2009; Yu et al., 2012) and bipolar disorder (Barbosa et al., 2014). There is also preliminary evidence that IL-33 may increase risk for rMDD. Specifically, our laboratory previously examined whether naturally occurring variation in the *IL-33* and *IL-1β* genes would moderate the link between women's history of childhood abuse and their risk for MDD. We found that, among women with a history of childhood abuse, the protective *IL-33* CT haplotype was associated with their history of rMDD. We also found that circulating IL-33 and IL-1β levels were significantly higher among women with rMDD than among women with a single MDD episode (sMDD) and women with no history of depression. Additionally, we expanded the project to the CNS using rat brain tissue. We found that an acute stressor increased *IL-33* expression in the paraventricular nucleus of the hypothalamus (PVN) and, to a lesser extent, the rat analogue of the prefrontal cortex (PFC). In combination,

these results provide preliminary evidence for the role of IL-33 in stress response and risk for rMDD. Given the aforementioned findings, this proposal aims to focus on IL-6, IL-1β, IL-10, and IL-33 in the context of depression.

In addition to examining the association between circulating levels of inflammatory markers and depression diagnosis and symptoms, researchers developed a paradigm in which a stimulated *in vitro* cytokine response in peripheral blood mononuclear cells (PBMCs) to an immune stressor is measured. Early studies that employed this paradigm reported greater levels of induced IL-1β and IL-6 among psychiatric inpatients with MDD (Maes et al., 1993), compared to never depressed controls. However, interpretation of these findings is complicated by multiple factors that could impact immune functioning, including changes in usual diet, sleep schedule, and additional stress levels, which are all associated with inpatient hospitalization (Weizman et al., 1994). Similar studies conducted with outpatients with current MDD report increased production of inflammatory cytokines following an *in vitro* challenge with cortisol and dexamethasone (Heiser, Lanquillon, Krieg, & Vedder, 2008). To capture the potential association between history of rMDD and both ambient levels of peripheral inflammatory markers as well as the magnitude of a cytokine response to an immune challenge, we aimed to assess both circulating and stimulated levels of IL-1β, IL-6, IL-10 and IL-33.

In addition to examining the presence of depression generally, there is evidence that inflammation may play a key role in specific depressive symptoms suggesting the possible existence of different biological subtypes of MDD (Holtzheimer & Mayberg, 2011). Although only a small number of studies examined the effects of inflammation on

MDD features that go beyond somatic and vegetative symptoms (Slavich & Irwin, 2014), this growing body of literature suggests that inflammation could be particularly associated with symptoms of anhedonia. For instance, inflammation was shown to affect functional connectivity within reward neurocircuitry among patients with current MDD, such that higher circulating levels of CRP, IL-1β, IL-1RA, and IL-6 were associated with decreased connectivity in corticostriatal reward and motor neurocircuitry (Felger et al., 2015), suggesting that inflammation may play an important role in motor deficits and decreased motivation observed in depressed individuals. Moreover, an endotoxin-stimulated decrease in the activity of the ventral striatum, a key brain region involved in reward processing, mediated the link between increased circulating levels of inflammatory markers and depressed mood among adult participants with no history of chronic medical or psychiatric conditions (Eisenberger et al., 2010). Together, these finding point to an association between both circulating and induced inflammation levels and reward circuitry underlying anhedonia. Activity in the reward-processing network commonly referred to as the mesolimbic dopamine circuit that connects ventral tegmental area and nucleus accumbens, has been strongly linked to manifestations of anhedonia and depressive symptoms in previous research (Heshmati & Russo, 2015). However, these studies examined inflammation and depressive symptoms without isolating anhedonia symptoms, and whether the levels of inflammatory markers are associated specifically with current anhedonia symptoms remains unclear. This is important, as there is a growing recognition of the need to study dimensional constructs underlying core features of psychopathology as opposed to focusing on specific diagnoses (Carcone & Ruocco, 2017; Zalta & Shankman, 2016). Additionally the presence of anhedonia is hypothesized

to play a critical role in developing treatment resistant depression and is predictive of poor response to first-line antidepressant treatments in prospective clinical studies (Uher et al., 2012). Therefore, the project examined the association between circulating and stimulated cytokines and current levels of anhedonia among women.

Early Life Stress, Inflammation, and Depression

Stress is a well- known risk factor for depression (e.g., Hammen, 2005) and has been strongly linked to lasting changes in the inflammatory processes (Slavich & Irwin, 2014). For example, exposure to childhood adversity, including abuse, neglect, and low socioeconomic status, prospectively predicted greater levels of inflammation in adulthood (Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Raposa, Bower, Hammen, Najman, & Brennan, 2014; Slopen et al., 2010). In parallel, findings from rodent models of early life stress support the link between early life stress exposure and abnormal immune function, including immune over-activation assessed via hippocampal *IL-1β* expression, later on in life (Kanitz, Tuchscherer, Puppe, Tuchscherer, & Stabenow, 2004; Llorente et al., 2007; Stiller, Drugan, Hazi, & Kent, 2011). One of the biological pathways for the link between early life stress and MDD is through the sensitization of the immune responses (Anisman, Merali, & Hayley, 2003; Hymie Anisman, Merali, & Hayley, 2008; Michael Maes, Berk, et al., 2012). Intriguingly, exposure to early life stress may also impact the link between inflammation and depression. For example, depressed adults who also had a history of childhood abuse had greater levels of inflammatory markers compared to adults with no history of childhood abuse (Danese et al., 2008). In addition, early life stress moderated the magnitude of inflammatory responses to a laboratory-based stressor later in life, such that participants with a history of childhood abuse displayed greater levels of inflammatory markers following the stressor, compared to individuals with no history of abuse (Carpenter et al., 2010). Together, these findings provide

evidence for altered stress-induced immune responding among those exposed to early life stress. What remains unclear is whether the alterations in immune functioning persist after full remission of depression and could be detected among participants with a history of rMDD with no current depression diagnosis. Additionally, whether or not history of childhood abuse would moderate this association is unknown. To fill this gap, the current project will examine the association between cytokine reactivity following an immune challenge and rMDD history and the potential moderating effect of childhood abuse history on this link.

Previously described literature points to the link between early life stress and inflammation and connects inflammation to anhedonia. The next question then focuses on whether early life stress impacts anhedonia later in life. Intriguingly, findings from cross-sectional neuroimaging studies in humans show that history of childhood adversity was associated with greater current anhedonia symptoms, particularly among individuals who evidenced low levels of activation in the ventral striatum during a reward-focused task (Corral-Frías et al., 2015), suggesting that exposure to stress may have a prolonged affect on depressive symptoms. Animal studies, which generally allow assessment of causality, show that exposure to early life stress and chronic prenatal stress may lead to increased depression-like behaviors in adulthood (Ślusarczyk et al., 2015; Wohleb et al., 2012). Multiple paradigms have been developed to assess depression-like behaviors in studies utilizing animal models of depression, including those measuring animal's socialization, locomotion, spatial exploration, swimming, and preference for appetitive foods. Measuring preference for a sucrose solution, which is highly palatable to rodents, over

regular water is commonly used to assess anhedonia, a core symptom of depression, in animals (Der-Avakian & Markou, 2012).

Increase in depression-like behaviors, including anhedonia, can also be observed following an exposure to an immune challenge, such as a systemic administration of a known endotoxin, including LPS, to produce a robust immune response (Bison et al., 2009). Indeed, previous research suggests that LPS-stimulated anhedonia, expressed by a decrease in preference for sucrose, is one of the defining features of animal models of depression (De La Garza, 2005; Frenois et al., 2007). Although some previous studies found an increase in depression-like behaviors in mice following injections of LPS (Bison et al., 2009), others have failed to find any effects of LPS on depression-like behaviors (Deak et al., 2005). For instance, Deak et al. (2005) report no depressogenic effects of LPS administration on behavior during a subsequent forced swim test (FST) in rats. This could be partly due to differences in behavioral tests that were used to assess depression-like behaviors. To clarify previous findings, this project used a well-established measure of anhedonia-like behavior, the sucrose preference paradigm (O'Connor et al., 2009; Smolinsky, Bergner, LaPorte, & Kalueff, 2009). Additionally, the effect of the immune challenge in adult animals could be greatly enhanced by early life stress exposure. Therefore, building on previously mentioned findings regarding the impact of early life-stress on depression-like behaviors, we aimed to examine whether exposure to an adolescent stressor would impact behavioral responses, specifically sucrose preference, to an immune challenge in adulthood.

Findings from research focusing on biological sequalae of stress in the brain, typically conducted in rodents, suggests that exposure to stress leads to time and region-

dependent neuroinflammation. For example, exposure to an acute stressor via exogenous stress hormone administration generated a robust neuroinflammatory response in the key structures comprising the HPA axis in adult rats (Hueston & Deak, 2014). Similarly, chronic stress has been noted to lead to microglia activation and increase in inflammatory markers in the brain (for review, see Calcia et al., 2016). For example, repeatedly restrained adult rats evidenced increased number and activation in immune cell population (microglia) in stress-sensitive areas, including amygdala, paraventricular nucleus of the hypothalamus, nucleus accumbens, and hippocampus (Tynan et al., 2010). Another study that examined the time course of chronic stress-induced alterations in the immune system showed increased microglia activation following the first few days of stress exposure, which was followed by a decrease in a number of immune cells in the prefrontal cortex and hippocampus, suggesting that chronic stress could contribute to reduction in neurogenesis observed among depressed individuals (Kreisel et al., 2013). Stress exposure at an earlier age appears to have similar effects on the immune processes, as repeatedly socially defeated adolescent mice evidenced elevated and prolonged induction of *IL-1β* and *TNF-α* and microglia activation in the amygdala, paraventricular nucleus of the hypothalamus, prefrontal cortex, and hippocampus, following a subsequent immune challenge (Wohleb et al., 2012). Similarly, prenatal stress resulted in greater microglia activation in the rat prefrontal cortex and hippocampus, as well as increased levels of inflammatory markers, including IL-1β, IL-6, IL-18, TNF-α in microglia cultures. Moreover, these alterations in immune responses were associated with increase in depression-like behaviors, including decreased sucrose preference and prolonged

social withdrawal and among rodents subjected to stressors (Kreisel et al., 2013; Wohleb et al., 2012).

What remains unclear is whether early life stress affects neuroimmune responses to stress in brain regions associated with stress and anhedonia, a key symptom of depression, in adulthood. Given the previously mentioned findings from neuroimaging studies in humans linking peripheral inflammation with changes in connectivity in the brain regions underlying reward processing, animal studies showing the relation between early life stress and immune processes in the brain, we further extended the scope of our investigation to examine the role of early life stress in neuroinflammatory responses in the brain regions comprising a reward processing network to an immune challenge in adulthood.

Specific Aims of the Present Investigation

The overarching goal of the current study was to examine the association between inflammatory markers and depression. The project was comprised of a series of human and translational animal studies and employed interdisciplinary, cross-species assessment methods. This allowed us to capitalize on the strength of both human and animal research methodology to build a comprehensive investigation that examined research questions ranging from cross-sectional associations to prospective causality and underlying biological mechanisms (Figure 1).

In Study 1, we examined (a) the concentration of circulating cytokines and (b) levels of cytokines released from stimulated peripheral blood mononuclear cells (PBMCs) *in vitro*, which allowed us to overcome both technical and conceptual limitations associated with either technique alone. Thus, Study 1 focused on examining the association between women's history of rMDD and circulating and stimulated levels of cytokines (IL-1β, IL-6, IL-10, and IL-33). We hypothesized that women with a history of rMDD would evidence increased levels of both circulating and stimulated inflammatory markers compared to participants with no depression history. Additionally, Study 1 aimed to examine whether the link between history of rMDD and circulating and stimulated levels of cytokines was moderated by women's history of childhood abuse. We hypothesized that, among women with a history of rMDD, those who were also abused as children would evidence the highest levels of circulating and stimulated inflammatory markers. To begin to examine immune processes linked to different

depressive phenotypes, Study 1 also explored whether participants' current levels of anhedonia were associated with increased levels of circulating and stimulated cytokines (IL-1β, IL-6, IL-10, and IL-33). Exploratory analyses were also conducted to examine the potential association between anhedonia and other cytokines produced by monocytes and macrophages (e.g., Th-17 cytokines), which were selected due to their role in maintaining the balance between immunity and inflammation and involvement in the pathogenesis of multiple inflammatory and autoimmune conditions characterized by increased anhedonia (Guglani & Khader, 2010).

Studies 2 and 3 sought to extend the results of Study 1 to an animal model of depression. The goal of Study 2 was to examine whether exposure to stressors in adolescence would affect anhedonia-like behavior following exposure to an *in vivo* immune challenge in adulthood. A well-established sucrose preference paradigm was used to index anhedonia-like behavior in rats. We hypothesized that rats exposed to stressors in adolescence would evidence greater levels of anhedonia-like behavior following an immune challenge. Additionally, we aimed to assess two distinct challenges during adolescence on later adhedonia-like behavior in adulthood. Specifically, we investigated the potential effect of the number of stress exposures (chronic vs. single) as well as variation in the stressor type (combination of restraint, forced swim, and acute footshock vs. acute footshock only). Although a single exposure to a severe stressor has been shown to induce depression-like phenotype in rodents (Chu et al., 2016), chronic stress paradigms are generally considered to have greater translational potential and the outcomes more closely resemble depressive symptoms in humans (Strekalova et al., 2011; Willner, 2017). Therefore, we included both types of stress paradigms to compare

the magnitude of their potential effect on adhedonia-like behavior. Specifically, one group of rats went through the Chronic Escalating Stress paradigm developed by Deak and colleagues (Doremus-Fitzwater, Paniccia, Gano, Vore, & Deak, 2018), which incorporates the use of several stressors that gradually increase in severity. The other group was subjected to a single exposure to a severe stressor (AFS).

Finally, Study 3 examined whether mRNA levels of *IL-1β*, *IL-6*, *IL-33*, and *IL-10* were modulated in brain areas underlying reward processing and stress reactivity (i.e., nucleus accumbens [NAc], ventral tegmental area [VTA], amygdala [AMG], and the paraventricular nucleus of the hypothalamus [PVN]) following an *in vivo* immune challenge in adult rats with and without a history of adolescent stress. We hypothesized that adult rats with a history of adolescent stress exposure would evidence higher cytokine gene (*IL-1β, IL-6, IL-10* and *IL-33*) expression in response to an LPS challenge in adulthood in the NAc, VTA, AMG, and PVN, compared to animals without a history of adolescent stress exposure or saline-injected rats. As in Study 2, we used two types of stress paradigms (chronic escalating stress vs. acute).

Study 1

This study investigated whether rMDD would be associated with potentiated immune reactivity. Based on our laboratory's earlier research suggesting that altered cytokine reactivity may be more strongly associated with rMDD than sMDD (Kudinova et al., 2017), we focused on immune reactivity of participants with a history of rMDD versus those with no lifetime depression. We collected biological samples from adult human participants in an ongoing study (R01 HD057066; PI: Gibb).

Method

Participants and Procedure. Participants were 39 adults recruited from the community as part of a larger study of depression and anxiety in children (Table 1). Participants were required to either have a history of recurrent MDD (rMDD; $n = 20$) or have no lifetime history of MDD ($n = 19$). The average age of participants was 36.81 years ($SD = 7.55$) and the majority were women (87.2%). In terms of race/ethnicity, the majority were Caucasian (76.3%) and the rest were African American (15.8%), Asian/Pacific Islander

(2.6 %), or from other racial/ethnic groups (5.3%). The median annual family income was between $45,001 and $50,000. Upon arrival at the laboratory, participants were asked to provide informed consent and then administered the SCID-I. Following this, participants completed questionnaires. Finally, peripheral blood samples, height, weight, and body temperature were collected. Blood samples of the majority of participants (66.7%) were collected between 3-7PM and the rest were collected between 9AM -2PM.

All participants were compensated $80 for their participation in the larger project, which was approved by the university's institutional review board.

Clinical diagnoses. The Structured Clinical Interview for DSM Disorders (SCID-I) (First, Spitzer, Gibbon, & Williams, 2002) was used to assess for lifetime histories of DSM-IV psychiatric disorders. The SCID-I is a widely used diagnostic interview with well-established psychometric properties (First et al., 2002). A subset of 20 SCID-I interviews was coded by a second interviewer and interrater reliability for diagnoses of MDD was excellent ($\kappa = 1.00$). Out of 39 participants, 20 women (51.3%) had a history of rMDD in this study. Distribution of lifetime occurrences of other diagnoses was a follows: Alcohol Use Disorder = 14 (35.9%), Post-Traumatic Stress Disorder (PTSD) = 9 (23.1%), Panic Disorder (PD) = 8 (20.5%), Social Phobia (SP) = 6 (15.4%), Substance Use Disorder = 6 (15.4%), Obsessive Compulsive Disorder (OCD) = 4 (10.3%), and Generalized Anxiety Disorder (GAD) = 4 (10.3%). Of those women, the distribution of participants who met criteria for current diagnoses was as follows: PD = 6 (15.4%), GAD = 4 (10.3%), SP = 3 (7.7%), OCD = 2 (5.1%), MDD = 2 (5.1%), and PTSD = 1 (5.1%).

Childhood abuse history. The Childhood Trauma Questionnaire (CTQ; Bernstein, Ahluvalia, Pogge, & Handelsman, 1997) was used to assess participants' histories of childhood emotional, physical, and sexual abuse. In this population sample, CTQ exhibited good to excellent internal consistency (emotional abuse $\alpha = .92$, physical abuse $\alpha = .84$, sexual abuse $\alpha = .91$). Using the established cutoffs on the CTQ (Bradley et al., 2014), moderate levels of abuse were defined as an emotional abuse (EA) subscale score greater than 12, a physical abuse (PA) subscale score greater than 9, and/or sexual abuse (SA) subscale score greater than 7. Of the women in our sample, 4 reported EA

only (10.3%), 1 reported PA only (2.6%), 6 reported SA only (15.4%), 2 reported EA and SA (5.1%), 3 reported PA and SA (7.7%), none reported EA and PA, and 4 reported all three types of abuse (10.3%). Consistent with prior research using the CTQ (Rebekah G. Bradley et al., 2008), women were coded as having a history of no or mild abuse (n = 19) or having a history of moderate to severe levels of abuse (n = 20). Thirteen (65%) participants with a history of CA also had a history of rMDD and 7 (35%) did not. Seven (36.8%) participants with no history of CA had a history of rMDD and 12 (63.2%) had no history of either CA or rMDD.

Anhedonia symptoms. Levels of anhedonia were assessed using the anhedonic depression subscale of the Mood and Anxiety Symptom Questionnaire (MASQ; Kendall et al., 2015). This is a well-established measure of anhedonia that demonstrated good reliability and validity in previous research (Bredemeier et al., 2010), with higher scores reflecting greater levels of symptomatology. In this sample, the MASQ-AD subscale exhibited excellent internal consistency ($\alpha = .92$).

Depressive symptoms. The Beck Depression Inventory-II (BDI-II) was used to assess women's levels of current depressive symptoms (Beck, Steer, & Brown, 1996). This measure demonstrated good reliability and validity in previous research (Arnau, Meagher, Norris, & Bramson, 2001) and showed good internal consistency in the current sample ($\alpha = .88$).

Circulating cytokine levels. Whole blood was collected via BD Vacutainer Blood Collection Sets into 4.0 mL tubes, coated with ethylenediaminetetraacetic acid (EDTA). Plasma was separated by centrifugation (1000 × g for 10 min at 4° C) and stored at –80°C. We used the Bio-Plex Pro Human Cytokine Panel (171-AA001M) and Bio-Plex

MAGPIX system to assay IL-1β, IL-6, IL-10, and IL-33 concentrations (Bio-Rad, Philadelphia, PA). MAGPIX system is a magnetic bead-based multi-analyte fluorescent detection system. Each sample was run in triplicate. The average intra- assay coefficients of variation were as follows: IL-1β = 5.0%, IL-6 = 5.4%, IL-10 = 3.8%, IL-33 = 4.2%. The average inter- assay coefficients of variation were as follows: IL-1β = 2.7%, IL-6 = 4.4%, IL-10 = 1.7%, IL-33 = 8.6%. The lower limits of quantification were as follows: IL-1β = 0.24 pg/mL, IL-6 = 1.65 pg/mL, IL-10 = 1.99 pg/mL, IL-33 = 4.18 pg/mL. Circulating levels of IL-1β for the majority of our participants (97.4%) were below the detection limit of our assay and we therefore excluded IL-1β from all subsequent analyses related to peripheral levels of inflammation. Values for the other three cytokines were not normally distributed and were log transformed.

Cytokine reactivity. We followed a well-established protocol for conducting an *in vitro* lipopolysaccharide (LPS) immune challenge (e.g., Schildberger, Rossmanith, Eichhorn, Strassl, & Weber, 2013). Whole blood was collected by certified phlebotomists using BD Vacutainer Blood Collection Sets into BD Vacutainer Cell Preparation silicon-coated 4.0 mL tubes with sodium citrate. PBMCs were isolated and stored using previously described methods (Mallone et al., 2011). Briefly, PBMCs were separated by centrifugation (1500 × *g* for 20 minutes at 23± 1ºC) and washed three times in phosphate buffered saline (PBS) suplemented with 10% fetal bovine serum (FBS, Fisher Scientific, Pittsburg, PA). The cells were re-suspended in RPM Media-1640 with 10% dimethyl sulfoxide sterile solution (DMSO, Sigma-Aldrich, St. Louis, MO) and 20% FBS and placed into a Coolcell® freezing container (Biocision, San Rafael, CA), which was kept in a –80 ºC freezer for 12h. After that, cryo vials with cells were transferred into liquid

nitrogen storage until furter testing. The average cell survival rate was > 98.00%, which is comparable or higher than the rates obtained in previous research (Mallone et al., 2011). On the day of the testing, a fraction of the cells from each sample (10 µl) were stained with Trypan Blue Stain solution (Thermo Fisher Scientific, Waltham, MA) and the live/dead cells were counted via a hemocytometer. Cells were then pelleted by centrifugation at 250 × g and re-suspended in 10% cell-culture grade Fetal Bovine Serum (Seradigm Life Sciences, Philadelphia, PA) and LPS-enriched RPMI 1640 media (Invitrogen, Waltham, MA) containing at 1×10^6 cells/mL and incubated for 24h at 37 ºC. LPS concentration (10 ng/mL) and duration were selected based on recommendations from previous research (Schildberger et al., 2013). After 24h, the cells were pelleted by centrifugation (2000 rpm for 5 min) and the supernatants removed and stored at –80°C. Levels of IL-1β, IL-6, IL-10, and IL-33 protein were subsequently measure using Bio-Plex Pro Human TH17 Cytokine assay (Bio-Rad, Philadelphia, PA). The average intra-assay coefficients of variation were as follows: IL-1β = 5.7%, IL-6 = 4.0%, IL-10 = 5.1%, IL-33 = 2.5%. The average inter- assay coefficients of variation were as follows: IL-1β = 3.8%, IL-6 = 3.0%, IL-10 = 2.5%, IL-33 = 3.3%. The lower limits of quantification were as follows: IL-1β = 0.24 pg/mL, IL-6 = 1.65 pg/mL, IL-10 = 1.99 pg/mL, IL-33 = 4.18 pg/mL. Values for all cytokines were not normally distributed and were log transformed. Levels of stimulated cytokines were not significantly associated with the levels of corresponding circulating cytokines (lowest $p = .81$).

Total Protein Concentration. Total plasma protein concentrations were assessed via bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, Waltham, MA). The lower limit of quantification was 20 ug/mL. The average inter and intra- assay

coefficients of variation were both below 1%. All circulating and stimulated cytokine values were normalized to the total protein levels, as recommended by previous research (Ber et al., 2014; Collins, An, Peller, & Bowser, 2015; Hepworth et al., 2012).

Lifetime smoking history. Participants' lifetime smoking history was assessed using the modified version of the Semi-Structured Assessment of the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994), which is a comprehensive psychiatric interview used to assess physical, psychological, social, and psychiatric manifestations of alcohol abuse or dependence and other psychiatric disorders in adults. Modifications were introduced to collect data on maternal smoking and drinking patterns during pregnancy (Knopik et al., 2005). Similarly to previous research (Kudinova et al., 2016), participants were dichotomized based on their smoking history into a group that smoked 100 or more cigarettes in their lifetime and those who smoked less.

Physiological Measures. To control for potential confounding effects of participants' Body Mass Index (BMI) and body temperature, participants' height and weight were measured for BMI calculation. Body temperature was measured by sliding the probe of an infrared thermometer across the participant's forehead (Exergen, Watertown, MA).

Results

Circulating cytokine levels. To examine whether circulating levels of IL-6, IL-10, and IL-33 were linked to participants' history of rMDD, we conducted a series of ANOVAs using circulating levels of each of the cytokines (IL-6, IL-10, and IL-33) as a dependent variable and rMDD history as an independent factor. There was no significant rMDD group differences for any of the three cytokines: IL-6, $F(1, 38) = 0.87$, $p = .36$, η^2

= .02, IL-10, $F(1, 38) = 0.33$, $p = .57$, $\eta^2 = .01$, or IL-33, $F(1, 38) = 0.16$, $p = .69$, $\eta^2 = .004$ (Figure 2).

Following this, to test whether women's history of CA moderated the relation between participants' history of rMDD and their circulating cytokine levels, we ran a series of ANOVAs using circulating levels of each cytokine (IL-6, IL-10, and IL-33) as a dependent variable and rMDD, CA, and the rMDD × CA interaction as independent factors. We found a significant main effect of CA history on circulating levels of IL-6, $F(1, 38) = 4.00$, $p = .05$, $\eta^2 = .10$, but not IL-10, $F(1, 38) = .02$, $p = .90$, $\eta^2 < .001$, or IL-33, $F(1, 38) = 0.50$, $p = .48$, $\eta^2 = .01$, with participants reporting a history of CA exhibiting greater levels of circulating IL-6 ($M = 0.03$ log pg/mg) compared to participants with no CA history ($M = 0.01$ log pg/mg; Figure 3). The rMDD × CA interaction was not significant for any of the cytokines: IL-6, $F(1, 38) = 0.49$, $p = .49$, $\eta^2 = .01$, IL-10, $F(1, 38) = 0.23$, $p = .63$, $\eta^2 = .01$, or IL-33, $F(1, 38) = 0.10$, $p = .75$, $\eta^2 = .003$. The results remained unchanged, for the most part, when we excluded participants with a current MDD diagnosis ($n = 2$), men ($n = 5$), or both ($n = 6$). Please see Study 1 supplementary data section for detail.

Stimulated cytokines. To test our hypotheses regarding cytokine reactivity, we again ran a series of ANOVAs with reactivity for each of the cytokines (IL-1β, IL-6, IL-10, and IL-33) as a dependent variable and rMDD history as an independent factor. We found no significant main effect of rMDD history on stimulated levels of IL-1β, $F(1, 38) = 0.24$, $p = .88$, $\eta^2 = .001$, IL-6, $F(1, 38) = 0.002$, $p = .96$, $\eta^2 < .001$, IL-10, $F(1, 38) = 0.77$, $p = .39$, $\eta^2 = .02$, or IL-33, $F(1, 38) = 2.19$, $p = .15$, $\eta^2 = .06$ (Figure 4).

Following this, to test whether women's history of CA moderated the relation between participants' history of rMDD and stimulated cytokine levels, we ran a series of ANOVAs using immune reactivity for each of the cytokines (IL-1β, IL-6, IL-10, and IL-33) as a dependent variable and rMDD, CA, and the rMDD × CA interaction as independent factors. We found no significant main effect of CA history on stimulated levels of IL-1β, $F(1, 38) = 0.54$, $p = .47$, $\eta^2 = .02$, IL-6, $F(1, 38) = 1.34$, $p = .25$, $\eta^2 = .04$, IL-10, $F(1, 38) = 0.03$, $p = .86$, $\eta^2 = .001$, or IL-33, $F(1, 38) = 0.002$, $p = .96$, $\eta^2 < .001$. In addition, the rMDD × CA interaction was not significant for any of the cytokines: IL-1β, $F(1, 38) = 0.15$, $p = .70$, $\eta^2 = .004$, IL-6, $F(1, 38) = 0.90$, $p = .35$, $\eta^2 = .02$, IL-10, $F(1, 38) = 0.50$, $p = .48$, $\eta^2 = .01$, or IL-33, $F(1, 38) = 1.23$, $p = .27$, $\eta^2 = .03$. Again, the results remained unchanged, when we excluded participants with a current MDD diagnosis ($n = 2$), men ($n = 5$), or both ($n = 6$). Please see Study 1 supplementary data section for detail.

Exploratory analyses. To examine whether levels of anhedonia, assessed using MASQ –AD subscale, were associated with increased circulating levels of cytokines, we conducted exploratory correlations analyses using all circulating ($n = 4$; IL-6, IL-10, IL-33, and TNFα) and stimulated ($n = 14$; IL-1β, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL31, IL-33,TNFα, IFNγ, and sCDL40) cytokines for which the data was available in the multiplex array. There was no significant association between current anhedonia and circulating levels of IL-6, $r(37) = –.11$, $p = .51$, IL-10, $r(37) = –.01$, $p = .96$, or IL-33, $r(37) = –.13$, $p = .45$.

To examine whether levels of anhedonia were associated with increased stimulated levels of cytokines, we conducted similar analyses. We found that higher

levels of anhedonia symptoms were associated with increased stimulated levels of IL-33, $r(37) = .46$, $p = .006$ (Figure 5), but not IL-1β, $r(37) = .17$, $p = .31$, IL-6, $r(37) = .14$, $p = .38$, or IL-10, $r(37) = .23$, $p = .15$. Notably, the association between anhedonia and stimulated levels of IL-33 was maintained when we statistically controlled for participants' age, $r(36) = 0.40$, $p = .38$, sex, $r(36) = 0.44$, $p = .006$, ethnicity (Caucasians vs. others), $r(39) = 0.45$, $p = 0.005$, income, $r(36) = 0.45$, $p = .01$, body mass index (BMI), $r(36) = 0.45$, $p = .005$, body temperature, $r(36) = 0.44$, $p = .006$, time of sample collection $r(36) = 0.46$, $p = .004$, or lifetime smoking, $r(36) = 0.41$, $p = .01$.

We conducted similar analysis to examine the association between current anhedonia symptoms and stimulated cytokine levels among individuals with no current MDD diagnosis ($n = 37$). The results remained unchanged and we found that higher levels of anhedonia symptoms were associated with increased stimulated levels of IL-33, $r(35) = .38$, $p = .02$, but not IL-1β, $r(35) = .11$, $p = .53$, IL-6, $r(35) = .09$, $p = .59$, or IL-10, $r(35) = .22$, $p = .19$. The results were maintained when we conducted similar analyses among women only ($n = 34$): IL-33, $r(33) = .47$, $p = .005$, IL-1β, $r(33) = .21$, $p = .24$, IL-6, $r(33) = .21$, $p = .23$, and IL-10, $r(33) = .27$, $p = .13$. Lastly, the results were also maintained when we excluded both individuals with a current MDD diagnosis and men ($n = 33$): IL-33, $r(32) = .42$, $p = .01$, IL-1β, $r(32) = .13$, $p = .48$, IL-6, $r(32) = .14$, $p = .45$, or IL-10, $r(32) = .26$, $p = .14$. Intriguingly, exploratory analyses showed significant association between anhedonia symptoms and other cytokines, including IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-31, IFNγ, and sCDL40. Full results can be found in the Study 1 supplementary data section.

Additionally, to examine whether the association between anhedonia symptoms and stimulated cytokine levels were moderated by participants' history of rMDD, we conducted a series of ANOVAs using stimulated cytokine levels as a dependent variable, and rMDD history, anhedonia symptoms, and their interaction as independent variable. We found a significant main effect of rMDD history on stimulated levels of IL-1β, $F(1, 38) = 8.99$, $p = .005$, $\eta^2 = .20$, and a significant rMDD × anhedonia interaction, $F(1, 38) = 8.75$, $p = .006$, $\eta^2 = .20$, but no significant main effect of anhedonia on stimulated levels of IL-1β, $F(1, 38) = 1.79$, $p = .19$, $\eta^2 = .05$. To examine the form of this interaction, we ran correlation analysis separately among individuals with and without rMDD history. We found that anhedonia was significantly associated with stimulated IL-1β levels among individuals with rMDD history, $r(18) = .54$, $p = .02$, but not among individuals with no rMDD history, $r(17) = –.31$, $p = .20$.

Similarly, for stimulated IL-6 levels, we found a significant main effect of rMDD history, $F(1, 38) = 8.02$, $p = .008$, $\eta^2 = .19$, and a significant rMDD × anhedonia interaction, $F(1, 38) = 7.73$, $p = .009$, $\eta^2 = .18$, but no significant main effect of anhedonia on stimulated levels of IL-1β, $F(1, 38) = 1.43$, $p = .24$, $\eta^2 = .04$. As before, we ran correlation analysis separately among individuals with and without rMDD history. We found that anhedonia was significantly associated with stimulated IL-6 levels among individuals with rMDD history, $r(18) = .58$, $p = .007$, but not among individuals with no rMDD history, $r(17) = –.25$, $p = .31$.

For stimulated levels of we found no significant main effect of rMDD history, $F(1, 38) = 0.001$, $p = .98$, $\eta^2 < .001$, anhedonia, $F(1, 38) = 1.27$, $p = .27$, $\eta^2 = .04$, or rMDD × anhedonia interaction, $F(1, 38) < 0.001$, $p = 1.00$, $\eta^2 < .001$.

Lastly, for IL-33, we found a significant main effect of anhedonia, $F(1, 38) = 6.35$, $p = .02$, $\eta^2 = .15$, but not rMDD history, $F(1, 38) = 1.91$, $p = .18$, $\eta^2 = .05$, or rMDD × anhedonia interaction, $F(1, 38) = 2.03$, $p = .16$, $\eta^2 = .06$.

In summary, the results of this first study provided no support for the association between participants' history of rMDD and circulating or stimulated levels of IL-1β, IL-6, IL-10, or IL-33. Additionally, there was no evidence supporting the hypothesis that a history of CA would moderate the relation between rMDD and circulating or stimulated levels of IL-1β, IL-6, IL-10, or IL-33. However, the results of exploratory analyses suggest a link between participants' current levels of anhedonia and stimulated, but not circulating, levels of IL-33, and other T-helper 17 cytokines that were included in the array, such as IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-31, IFNγ, and sCDL40, highlighting the association between stimulated, but not circulating cytokine levels and anhedonia symptoms. Intriguingly, rMDD moderated the association between anhedonia and stimulated IL-1β and IL-6 levels, such that greater levels of anhedonia were associated with higher stimulated IL-1β and IL-6 levels only among individuals with a history of rMDD.

Study 2

Building on the reported association between inflammation and anhedonia symptoms in humans, Study 2 focused on investigating the influence of adolescent stress exposure on anhedonia-like behavior in response to an *in-vivo* immune challenge in adult rats. Additionally, we aimed to test whether different type of stressors could affect the magnitude of the behavioral response to an immune challenge in adulthood and employed chronic escalating stress (CES) and acute stress paradigms (AFS). We hypothesized that animals exposed to adolescent stressors receive would exhibit greater levels of anhedonia, assessed via sucrose preference test, following an LPS challenge in adulthood compared to animals with no history of adolescent stress exposure or vehicle-injected animals.

Method

Subjects and Procedure. Figure 6 illustrates the timeline of the present study. Specifically, 32 female Sprague–Dawley rats, which were bred on site using pathogen-free breeders originally derived from Harlan (Envigo), were randomly assigned to one of four conditions: (i) chronic escalating stress (CES) + adult LPS challenge, (ii) acute footshock only (AFS) + adult LPS challenge, (iii) no adolescent stress + adult LPS challenge, and (iv) no adolescent stress or adult LPS challenge. Rats were allowed to develop normally until P21, at which point animals were weaned and housed with age and sex-matched partners (non litter-mates), 2 per cage, under standard conditions. Following a 6-day acclimation to colony conditions (P27), rats were handled

by researchers for 2–3 min to familiarize them with human contact prior to experimentation. Rats in the CES condition went through the adolescent stress challenge procedure described in detail below from P28-P39, whereas animals in the AFS condition were stressed for one day only (P39). Animals were treated in accordance with Public Health Service (PHS) policy and all experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Binghamton University.

Adolescent stress paradigm. These studies used a novel model of Chronic Escalating Distress involving an 11-day procedure culminating in footshock exposure on the last day, which has recently been shown to produce robust neuroimmune changes and altered alcohol sensitivity (Doremus-Fitzwater et al., 2018). Rats assigned to the escalating chronic stress paradigm were first subjected to 5 days of whole body restraint lasting 60 minutes (P28). Starting at P33, the 60-minutes of restraint was preceded by a 30-minute forced swim each day for 5 days. Finally, on P39, rats were subjected a 120-minute foot shock. The footshock chamber measured 30.5 (L) × 26.5 (W) × 33 (H) cm (Habitest Chamber, Model H10-11R-TC-SF, Coulbourn Instruments, Allentown, PA). The sidewalls of the chamber were constructed of stainless steel except the front doors that were constructed of clear Plexiglas. The chambers were adapted to deliver scrambled shocks through the grid floor (18 bars spaced 1.1 cm apart with a diameter of 4.0 mm) from a shock generator (LABLINC Model H01-01, and Precision Animal Shocker Model H13-15, Coulbourn Instruments, Allentown, PA). The sound-attenuating chambers were illuminated by a 20-W white light bulb and background noise was provided by individual ventilation fans. Animals were exposed to inescapable footshocks (1.0 mA, 5 s each, 90 s variable intertribal interval [ITI]) for 120 min (approximately 80 shocks). Timeline for

this CES procedure is depicted in 6. Animals in the AFS condition remained undisturbed in their home cages from P28 until P39, when they were subjected to the footshock procedure, identical to that in the CES condition.

In vivo LPS challenge. Rats remained undisturbed in their home cages until P70, at which point all animals (n = 24) except those in the no adolescent stress/no adult LPS challenge condition were administered an intraperitoneal (i.p.) injection of LPS. The LPS (L6529, Sigma- Aldrich, St. Louis, MO) solution was diluted to 1.0 mg/mL using sterile endotoxin-free isotonic saline and stored in frozen aliquots at −20°C until use. On the day of experimentation, an aliquot of LPS was thawed and mixed fresh daily to the working concentration of 100 μg/mL LPS and delivered on a 1 mL/kg basis, also in pyrogen-free physiological saline. The dose of LPS was selected on the basis of its reported ability to induce depression-like behaviors in rats (Bison et al., 2009). Immediately following injection of LPS, the animals were returned to their home cages where they remained undisturbed for 0.5h. The rest of the animals were administered (n = 8) sterile endotoxin-free isotonic saline.

Sucrose preference time course. Given that sucrose is a palatable solution for rats, its decreased consumption was used as an index of anhedonic symptoms. Initially, all animals were granted free access to a 1% sucrose solution, in addition to regular food and water, for 4 days for habituation (P66-P70; Bison et al., 2009). On P70, all animals were presented with water and a 1% sucrose solution in graduated bottles with fitted sipper-tubes in their home cages, in addition to regular food. Specifically, Plexiglas dividers were installed in each cage to separate animals and ensure that each animal has access to their own bottles of sucrose solution and water. Total cumulative fluid

consumption (mL) defined as the sum of water and sucrose solution intake, was assessed 30 minutes, 1h, 2h, 4h, 8, and 16h later. The timing was chosen based on previous research (Bison et al., 2009). A sucrose solution preference score was calculated as the percentage of sucrose solution intake (%= sucrose solution intake × 100/total fluid consumption).

Results

First, to examine the potential effect of group on rats' body weight, we ran an ANOVA using weight as a dependent variable and group as an independent factor. We found no significant effect of group on animals weight at P66, which was the beginning of sucrose habituation procedure, $F(3,31) = .24$, $p = .87$, $\eta^2 = .02$. To examine the effect of adolescent stress exposure on anhedonia-like behavior (sucrose preference) in adulthood, we conducted a 4 (Group: AFS+LPS, CES+LPS, NS+LPS, and NS+Veh) × 6 (Time: 0.5, 1, 2, 4, 8, and 16h) repeated measures ANOVA with sucrose preference serving as the dependent variable. Sucrose preference was calculated using sucrose and water consumption data normalized by individual animal's weight. The values were then winsorized to normalize their distribution. We found a significant main effect of time on animal's sucrose preference, $F(5,135) = 4.41$, $p = .001$, $\eta^2 = .14$, with animals generally exhibiting a linear increase in sucrose preference over time. The main effect of group on sucrose preference was a nonsignificant trend, $F(5,135) = 2.36$, $p = .09$, $\eta^2 = .21$, and the group × time interaction was nonsignificant, $F(5,135) = 1.13$, $p = .33$, $\eta^2 = .11$ (Figure 8). Sucrose and water consumption analyses and time course during the sucrose preference test can be found in the supplementary section (Supplementary Figure 1). There was no support for the effect of adolescent stress exposure and *in-vivo* immune

challenge in adulthood on adult levels of anhedonia-like behavior in rats. However, the marginally significant trend found in this study suggests that conducting similar studies using fewer stress conditions and/or increased group sample sizes is warranted.

Study 3

Whereas Study 2 examined the potential impact of early life stress on the behavioral response to an immune challenge in adulthood, the current study focused on the impact of early life stress on the neurobiological processes potentially underlying that behavioral response. Translational studies as such frequently use animals injected with the cytokine inducer LPS (Kujawa et al., 2015) and describe an LPS-stimulated increase in cytokine gene expression (Erickson & Banks, 2011; Rey, Randolf, Wildmann, Besedovsky, & Jessop, 2009). Given that inflammation may be linked to increased levels of psychopathology in many, but not all individuals (Slavich & Irwin, 2014), identification of factors that may potentiate immune responses and specific biological processes underlying this process is critical for improving prevention effort and developing targeted interventions. Exposure to early life stress is emerging as one such factor in a rapidly growing body of literature (Muscatell, Slavich, Monroe, & Gotlib, 2009; Slavich & Irwin, 2014). For example, a stressful early life environment has been associated with increased immune reactivity in adolescence (Miller & Chen, 2010). Additionally, findings from a prospective study suggest that cumulative stress exposure in childhood was related to greater circulating levels of inflammation in adolescence (Slopen, Kubzansky, McLaughlin, & Koenen, 2013). Findings from animal translational research are similar and suggest that adolescent stress exposure may magnify peripheral immune response to a predatory stressor in adulthood (Barnum, Pace, Hu, Neigh, & Tansey, 2012). What remains unclear is whether adolescent stress exposure leads to a

potentiated neuroimmune response specifically to immune challenges in adulthood in the brain areas related to reward processing and stress and whether this sensitization was related to a specific type of stressors. Therefore, Study 3 focused on examining the influence of adolescent stress exposure on cytokine gene expression in brain regions associated with reward and emotion processing in response to an *in vivo* LPS challenge in adult rats. Additionally, to examine the potential effect of different stressor characteristics, including length and stressor variability, we employed two types of stressors: Chronic Escalating Stress (CES) and Acute Footshock (AFS) that were used in Study 2. We hypothesized that animals in the CES and AFS groups would evidence the greatest expression of inflammatory genes (*IL-1β, IL-6, IL-10, IL- 33*) in the VTA, AMG, NAc, and PVN in response to an LPS challenge in adulthood, compared to rats that had no exposure to an adolescent stressor.

Method

Subjects and Procedure. The subjects and procedure were similar to those used in Study 2 (Figure 9). Briefly, 32 female Sprague–Dawley rats, bred on site using pathogen-free breeders were randomly assigned to one of the four conditions: CES +LPS, AFS+LPS, NS+LPS, and NS+Veh. Rats were allowed to develop normally until P21, at which point animals were weaned and housed with age and sex-matched partners (non litter-mates) under standard conditions. Rats were handled by researchers (P27) for 2–3 min to familiarize them with human contact prior to experimentation. Rats in the CES went through the adolescent stress challenge procedure described in detail below from P28-P39, whereas animals in the AFS condition were stressed on one day only (P39). Animals were treated in accordance with Public Health Service (PHS) policy and all experimental

protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Binghamton University.

Adolescent stress paradigm. The procedure was identical to that used in Study 2. Briefly, rats that were assigned to the escalating chronic stress paradigm were first subjected to 5 days of whole body restraint lasting 60 minutes (P28). At P33, 60-minute restrain was preceded by a 30-minute forced swim for 5 days. On P39 rats were exposed to inescapable footshocks for 120 min (approximately 80 shocks). Timeline for this CES procedure is depicted in Figure 7. Animals in the AFS condition remained undisturbed in their home cages from P28 until P39, when they were subjected to the footshock procedure, identical to that in the CES condition.

In vivo LPS challenge. This procedure was also identical to that described in Study 2. Specifically, on P70, all animals (n = 24) except for those in the NS + Veh condition were administered an i.p. injection of 100 μg/mL LPS and delivered on a 1 mL/kg basis in pyrogen-free physiological saline. Immediately following injection of LPS, the animals were returned to their home cages where they remained undisturbed for 1h.

Cytokine gene expression. All RT-PCR was conducted using previously described procedures (Hueston & Deak, 2014). Rats were sacrificed via rapid decapitation (unanesthesized) 1h following LPS/Saline injections. Brains were removed and stored at −80°C. The VTA, AMG, NAc, and PVN were identified using a rat brain atlas (Watson & Paxinos, 2005), punched using biopsy punches, and stored at –80 °C until the time of RNA extraction. Tissue samples were homogenized in Trizol RNA reagent (Invitrogen) using a TissueLyser and 5 mm stainless steel beads (Qiagen,

Germantown, MD). Total RNA was then extracted via RNeasy mini columns (Qiagen, Germantown, MD) following manufacturer's instructions. RNA yield and purity were evaluated using a Nanodrop system (Thermo Fisher Scientific, Waltham, MA) and cDNA was synthesized using a QuantiTect reverse transcription kit (Qiagen, Germantown, MD). All RT-PCR was run using a CFX384 real-time PCR detection system (Bio-Rad, Philadelphia, PA). Relative gene expression was quantified using the $2^{-\Delta\Delta CT}$ method. Primer sequences for all targets run can be found in Table 2. Results were normalized to GAPDH as a housekeeper gene. Significant group differences in GAPDH expression were observed in the AMG, $F(3,31) = 3.14$, $p = .04$, $\eta^2 = .25$ (Supplementary Figure 2). Thus, non-normalized data was used in all analyses of cytokine gene expression for that region. There were no significant group differences in GAPDH expression in any of the other brain regions (lowest $p = .27$). Values > 2 *SDs* away from the mean were classified as outliers and excluded from the analysis. Two outliers identified in the expression of GAPDH in the NAc also produced extreme values in the expression of several cytokines in other regions and were therefore excluded from all subsequent analyses.

Results

To test the hypotheses from Study 3, we conducted a series of 4 (region: NAc, VTA, AMG, and PVN) × 4 (group: CES+LPS, AFS+LPS, NS+LPS, and NS+Veh) repeated measures ANOVAs separately for *IL-1β, IL-6*, *IL-10,* and *IL-33.* For *IL-1β*, we found a significant main effect of region, $F(3,78) = 3.48$, $p = .02$, $\eta^2 = .12$, but no significant effect of group, $F(3, 26) = 1.19$, $p = .33$, $\eta^2 = .12$, or group × region interaction, $F(9,78) = 1.18$, $p = .32$, $\eta^2 = .12$. Post hoc analysis revealed that *IL-1β*

expression was significantly higher in the NAc compared to the VTA, $p = .02$, and AMG, $p < .001$, with no differences between any other groups (lowest $p = .08$).

For *IL-6,* we found a significant main effect of region, $F(3,66) = 4.64$, $p = .005$, $\eta^2 = .17$, but no significant effect of group, $F(3, 26) = 0.90$, $p = .46$, $\eta^2 = .11$, or region or group × region integration, $F(9,66) = 0.72$, $p = .69$, $\eta^2 = .09$. Post hoc analysis revealed that *IL-6* expression was significantly lower in the NAc compared to the VTA, $p = .05$, and PVN, $p = .004$, with no differences between any other groups (lowest $p = .08$).

For *IL-10,* we found a significant main effect of region, $F(3,72) = 3.39$, $p = .02$, $\eta^2 = .12$, but no significant effect of group, $F(3, 24) = 1.06$, $p = .40$, $\eta^2 = .12$, or region or group × region integration, $F(9,66) = 0.72$, $p = .69$, $\eta^2 = .09$. Post hoc analysis revealed that *IL-10* expression was significantly lower in the AMG compared to the NAc, $p = .03$, VTA, $p = .01$, and PVN, $p = .02$, with no differences between any other groups (lowest $p = .38$).

For *IL-33,* we found a significant main effect of region, $F(3,78) = 3.27$, $p = .03$, $\eta^2 = .11$, but no significant effect of group, $F(3, 26) = 0.36$, $p = .78$, $\eta^2 = .04$, or region or group × region integration, $F(9,78) = 1.09$, $p = .38$, $\eta^2 = .11$. Post hoc analysis revealed that *IL-33* expression was significantly lower in the AMG compared to the NAc, $p = .009$, VTA, $p = .004$, and PVN, $p = .03$, with no differences between any other groups (lowest $p = .31$). The results are depicted in Figure 10.

We found no support for differences in the expression of *IL-1β, IL-6, IL-10,* or *IL-33* in the brain areas associated with reward processing and stress based on animal's history of early life stress. Lack of a significant increase in the cytokine expression among animals who had no stress exposure in adolescence but went through an immune

challenge in adulthood suggests that the timing between the LPS injection and tissue collection might have been insufficient for an expected neuroinflammatory response to develop.

Discussion

The current investigation aimed to answer a line of research questions related to the role of stress and inflammation in depression and anhedonia. The project consisted of a series of complimentary human and animal studies and integrated multiple modes of assessment (circulating levels, cytokine reactivity, gene expression) to examine the following research areas: (1) the link between history of recurrent major depressive disorder (rMDD) and inflammatory markers and how this may be moderated by history of childhood abuse in women, (2) the association between inflammatory markers and current anhedonia symptoms in the same sample of women, (3) the effect of early life stress on anhedonia-like behavior in adult rats, and (4) effect of early life stress on inflammatory gene expression in brain areas related to stress and reward processing in adult rats. The use of both human and animal research methodologies allowed us to extend the scope of our investigation from using *in vitro* techniques in humans to *in-vivo* immune challenge in rats to examining the causal effects of early life stress on immune responses, as well as to expand the investigation from peripheral markers of inflammation in humans to potential brain mechanisms in rats.

Specifically, Study 1 examined whether a history of rMDD was associated with greater circulating and stimulated levels of inflammatory markers (IL-1β, IL-6, IL-10, or IL-33) in adult women and whether this relation was moderated by their history of childhood abuse. Additionally, this study examined the association between stimulated

levels of inflammatory markers and current symptoms of anhedonia. We found no support for the first two aims and some support for the latter one. Specifically, participants' history of rMDD was not related to either circulating or stimulated levels of any of the cytokines examined, which contradicts our hypothesis of increased inflammation being a trait-like marker of depression, independent of current depression. Furthermore, women's retrospectively reported history of childhood abuse did not moderate the link between rMDD and circulating or stimulated cytokine levels, which is inconsistent with previous research (Danese et al., 2008). One difference between the two studies is that Danese et al. (2008) examined the moderating effect of early life stress among individuals with a current depression diagnosis, whereas we investigated those with a history of MDD, which could have impact the results. Therefore, future research could concentrate on the moderating role of childhood abuse in the context of inflammation during a current depressive episode. Another limitation of this study, particularly for the moderation analyses, is the sample size and future research using more participants is needed. We should also note that, in the current study, women with a history of childhood abuse evidenced higher circulating levels of IL-6 than women with no history of childhood abuse. This is consistent with previous meta-analysis showing that history of childhood trauma was associated with greater circulating levels of inflammatory markers, including IL-6 (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016).

As noted above, a secondary aim of Study 1 was to examine the association between anhedonia and inflamamtion. Although levels of anhedonia were not significantly related to circulating levels of any of the cytokines examined, we found that

higher stimulated levels of IL-33 and eight other Th17 cytokines produced by monocytes and macrophages (IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IFNγ, and sCDL40) were associated with higher levels of anhedonia in women, suggesting the potential involvement of inflammatory processes in anhedonia, a key symptom of depression. Moreover, we found that participants' history of rMDD moderated the association between current anhedonia and induced levels of IL-1β and IL-6, such that greater levels of anhedonia were associated with greater levels of IL-1β and IL-6 only among participants who also had a history of rMDD. This is consistent with a growing body of research focusing on the role of inflammation in anhedonia. Despite the growing recognition of the need to study dimensional constructs underlying core features of psychopathology, including MDD, in addition to focusing on specific diagnoses (Carcone & Ruocco, 2017; Zalta & Shankman, 2016), research examining depression beyond vegetative and somatic manifestations of depression is scarce (Slavich & Irwin, 2014). However, this growing body of literature highlights the role of inflammation in anhedonia. For example, increased circulating levels of CRP, IL-1β, IL-1RA, and IL-6 were found to be associated with decreased connectivity in corticostriatal reward and motor neurocircuitry (Felger et al., 2015) underlying anhedonia symptoms (Heshmati & Russo, 2015). Moreover, previous research points out that as a trans-diagnostic symptom of many psychiatric and medical conditions, anhedonia may be impacted by common immune mechanisms that impact reward processing, including TH-17 mediated immunity (Contreras et al., 2016; Escalona & Fawcett, 2017; Swardfager, Rosenblat, Benlamri, & McIntyre, 2016). The current findings, therefore, provide promising evidence that supports and extends this research to stimulated, but not circulating levels of cytokines

and points to new targets among inflammatory markers. However, because the analyses of those cytokines were exploratory, conclusions must remain tentative pending replication. This said, the results do suggest the utility of exploring the role of more novel inflammatory markers beyond those most commonly researched in this context and testing the moderating role of rMDD.

The main aim of Study 2 was to examine the role of adolescent stress exposure on behavioral response to an immune challenge in adult rats. We were particularly interested in anhedonia-like behavior and employed a well-established sucrose preference paradigm that is commonly used to index anhedonia in rodents (Der-Avakian & Markou, 2012). We also compared two different types of stressors, chronic escalating stress (CES) and acute footshock (AFS) to examine the potential effects of stressor characteristics. In addition, rather than only examining the effect of an immune challenge on anhedonia during a single time point, we assessed sucrose preference at 6 different timepoints: 0.5, 1, 2, 4, 8, and 16 hours following systemic LPS administration. We found a marginally significant trend for stress condition differences in sucrose preference. Specifically, rats in the AFS group generally evidenced decreased preference for sucrose compared to animals that were not exposed to stress as adolescents (whether they received a vehicle injection or LPS challenge in adulthood). This trend is consistent with previous research suggesting that an exposure to a severe stressor (24-h restraint) may induce a lasting depression-like phenotype, including decreased glucose uptake in the brain and hippocampal neurogenesis in mice (Chu et al., 2016). When sucrose consumption vs. preference was examined, there was a significant time × group interaction, such that animals that were not subjected to adolescent stress and were administered saline instead

of LPS in adulthood (NS+Veh) evidenced significantly higher sucrose consumption compared to rats in all other groups (NS+LPS, CES+LPS, and AFS+LPS) 8 hours following the immune challenge. There are several possible reasons why marginally significant vs. significant results were obtained with sucrose preference including lack of power to detect significant differences in animal behavior. Indeed, this study evidenced an effect size that was medium in magnitude (η^2 = .21), supporting the effect of group assignment on sucrose preference and encouraging of future research using larger sample sizes. A second potential reason for our overall lack of significant results may have been due to the developmental timing of the stressor. Our timing (P28) allowed us to avoid the stress hyporesponsivity phase described by previous research, during which animals evidence blunted corticosteroid response to stressors (Levine, 2001). Although no studies, of which we know, compared the long-term impact of post-natal vs. adolescent stress on adult functioning, there is evidence that adolescent rats display severely blunted neuroimmune responses, compared to adult animals (Doremus-Fitzwater, Gano, Paniccia, & Deak, 2015). Thus, future research may consider this aspect when studying the effect of early life stress on behavioral responses to an immune challenge in adult rodents.

Building from the previous two studies, Study 3 focused on the role of early life stress in neuroinflammatory response to an immune challenge later on in life. Specifically, the main aim of Study 3 was to examine the effect of adolescence stress exposure on inflammatory gene expression in brain areas underlying reward processes and stress, including the nucleus accumbens (NAc), ventral-tegmental area (VTA), amygdala (AMG), and paraventricular nucleus of the hypothalamus (PVN). Similar to Study 2, we used two types of adolescent stress, CES and AFS, and an identical

procedure for the immune challenge in adulthood. We found no significant group differences in *IL-1β, IL-6, IL-10,* or *IL-33* gene expression in any of the brain areas we examined. The reason for this may lie in an insufficient time (1h) between an LPS administration and tissue collection. Specifically, we did not observe the expected increase in inflammatory markers among animals who were not exposes to an adolescent stressor but were administered LPS as adults, suggesting that the brain samples were collected before the immune response fully unfolded. For example, LPS *in-vivo* challenge has been shown to increase *IL-1β* and *IL-6* expression in the PVN and AMG of adult rats 3 hours following the injection (Doremus-Fitzwater et al., 2015). Moreover, a study that examined a range of LPS doses (1, 5, 15, 50, 125, and 250 μg/kg) reported an increase in circulating IL-1β, IL-6, IL-10 levels 2 hours post injection starting at 5 μg/kg (Bison et al., 2009). In a separate study that used a much higher dose of LPS (2mg/kg), levels of circulating inflammatory markers (IL-1β) increased 30 minutes following an injection, while IL-1β hippocampal gene expression significantly increased 24h following an immune challenge and peaked at day 7 (Fu et al., 2014). Future research examining the time course for the LPS-induced neuroinflammation in the brain areas related to anhedonia is needed to inform studies in this area.

The primary strength of this study was the integration of human and animal methodologies that allowed us to examine a range of research questions related to the interplay between stress, inflammation, and depression. Additional strengths included (i) the use of structured clinical interviews to obtain women's diagnostic histories in Study 1, (ii) the focus on both circulating and stimulated levels of inflammatory markers in Study 1, (iii) the assessment of change in anhedonia-like behavior over time, as opposed

to only a single assessment, in Study 2, and (iv) the focus on cytokine gene expression in several specific brain regions related to anhedonia in Srudy 3. Despite these strengths, there are limitations that provide directions for future research. For example, the cross-sectional design of Study 1 precludes us from making any causal conclusions and future research utilizing prospective design is needed to determine whether higher levels of immune reactivity are a cause, consequence, or only a correlate of anhedonia symptoms. Additionally, estrous phase was not monitored in Studies 2 and 3, which may have impacted the results. Estrous cycle is known to affect animal behaviors, including food intake (Asarian & Geary, 2006) as well as inflammatory response to stress (Arakawa, Arakawa, Hueston, & Deak, 2014). Therefore, including estrous cycle data is recommended for future research using female rodents. Additionally, we did not assess for where women were in their menstrual cycle, which has been shown to affect peripheral inflammation levels (Bertone-Johnson et al., 2014) and, therefore, could have impacted the results. Moreover, no information about participant's habitual dietary intake or exercise was collected. Given that previous research describes the impact of dietary composition (Galland, 2010) and exercise routine (Kelley & Kelley, 2006) on systemic inflammatory markers, future studies that include these data in the analyses are warranted.

In summary, this investigation found no support for our hypotheses regarding the association between a history of rMDD and circulating or stimulated levels of cytokines, which may be due to study limitations or lack of a robust relation between inflammation and history of depression, which could be more evident among currently depressed individuals. Additionally, we found no support for the moderation of this association by a

history of early life stress. There was a significant relation between a history of childhood abuse and circulating levels of IL-6, which is consistent with previous research highlighting the link between early life-stress and inflammation in adulthood, corroborating existing evidence for the lasting effects of early life stress on immune processes. Additionally, there was evidence of an association between stimulated levels of inflammatory markers and current symptoms of anhedonia, which was maintained when we statistically controlled for potential confounders, including relevant demographic and physiological variables. This finding is in line with a small, but growing body of literature supporting the role of inflammation in anhedonia and extends previous research by showing the link between levels of cytokines released in response to an *in vitro* immune challenge with a known endotoxin and andhedonia. Pending replication and longitudinal research, these findings could lead to more detailed understanding of this trans –diagnostic phenomenon and inform treatment development. We extended this line of research questions to examining the causal relationship between early life stress and the andhedonia in adulthood following an *in-vivo* immune challenge using an animal sample. We found a marginally significant trend for the effect of early life stress on increased in andhedonia-like behavior in rats following an immune challenge with a known endotoxin. Additionally, we expanded the scope of our investigation to examining the effect of early life stress on neuroinflammation in the brain areas underlying stress and anhedonia processes following an immune challenge in adulthood. We found no support for increased expression of inflammatory genes in those areas following an immune induction in adulthood among animals that were stressed as adolescents. This investigation demonstrates the value of cross-species interdisciplinary

design in psychopathology research that is currently underutilized, yet much needed in the field. Indeed, researchers in our field commented on how psychological science is uniquely positioned due to its natural overlap with many other disciplines, to benefit from the interdisciplinary effort (Cacioppo, 2007). Additionally, pending future replication, the findings related to the association of current anhedonia symptoms and anhedonia-like behavior highlight the emerging research initiative to focus on processes that manifest in various forms of psychopathology, rather than solely diagnosis-based design.

Table 1. Descriptive statistics for participants in Study 1

	No rMDD History (n = 19)	History of rMDD (n = 20)	$r_{effect\ size}$
Parent Age (*M, SD*)	38.68 (8.29)	35.03 (6.47)	– .25
Parent Ethnicity (% Caucasian)	78.9	73.7	– .01
Sex (% female)	89.5	85.0	– .01
Annual Family Income	45,001-50,000	25,001-30,000	– .26
BDI-II (*M, SD*)	5.71(6.83)	10.43 (9.50)	.66**
MASQ AD (*M, SD*)	52.83 (13.17)	68.81 (12.38)	.54**
CTQ (% with a history of moderate or severe abuse)	36.8	65.0	.28
BMI (*M, SD*)	31.88 (5.12)	36.10 (8.58)	.29
Body Temperature (*M, SD*)	98.22 (.59)	98.20 (.56)	– .02
Lifetime cigarette smoking (% who smoked >100)	42.1	57.9	.28

Note. BDI-II = Beck Depression Inventory-II, rMDD = Recurrent Major Depressive Disorder, MASQ AD = Mood and Anxiety Symptom Questionnaire- Anhedonia subscale, MASQ AA = Mood and Anxiety Symptom Questionnaire- Anxious Arousal subscale, BMI = Body Mass Index
*$p < .05$. **$p < .01$

Table 2. Primer sequences used in Study 3.

Name:	Sequence:
GAPDH Forward	5' GTG CCA GCC TCG TCT CAT AG 3'
GAPDH Reverse	5' AGA GAA GGC AGC CCT GGT AA 3'
IL-1β Forward	5' CAG CTT TCG ACA GTG AGG AGA 3'
IL-1β Reverse	5' TGT CGA GAT GCT GCT GCT GTG AG 3'
IL-6 Forward	5' TAG TCC TTC CTA CCC CAA CTT CC 3'
IL-6 Reverse	5' TTG GTC CTT AGC CAC TCC TTC 3'
IL-10 Forward	5' TGC GAC GCT GTC ATC GAT TT 3'
IL-10 Reverse	5' TGG CCT TGT AGA CAC CTT TGT 3'
IL-33 Forward	5' CCT GAG CAC ATA CAA CGA CCA 3'
IL-33 Reverse	5' TTC TTC CCA TCC ACA CCG TC 3'

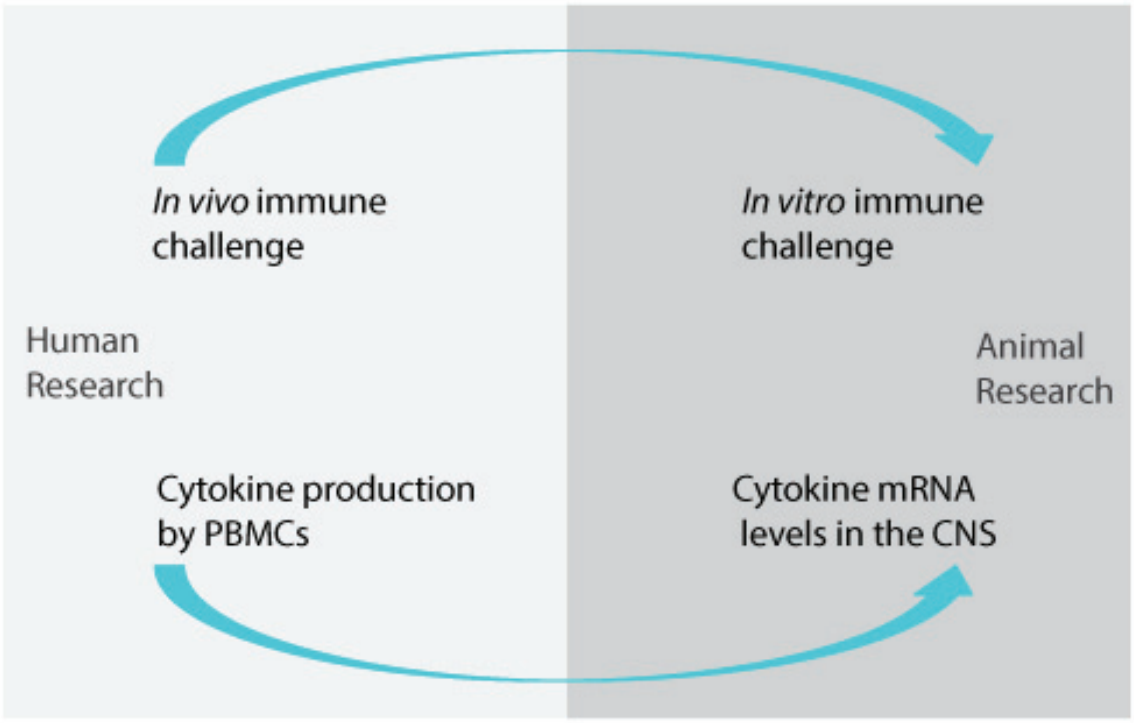


Figure 1. Schematic of the parallels between human and animal research questions in this project. *In vitro* and in-vivo challenges = Lipopolysaccharide (LPS) treatment/injections. Combining human and animal research allowed us to parallel an *in vitro* immune challenge in humans with an *in-vivo* challenge in rats and extend the scope of our investigation from assessing PBMC cytokine production to mRNA levels in the bran regions of interest.

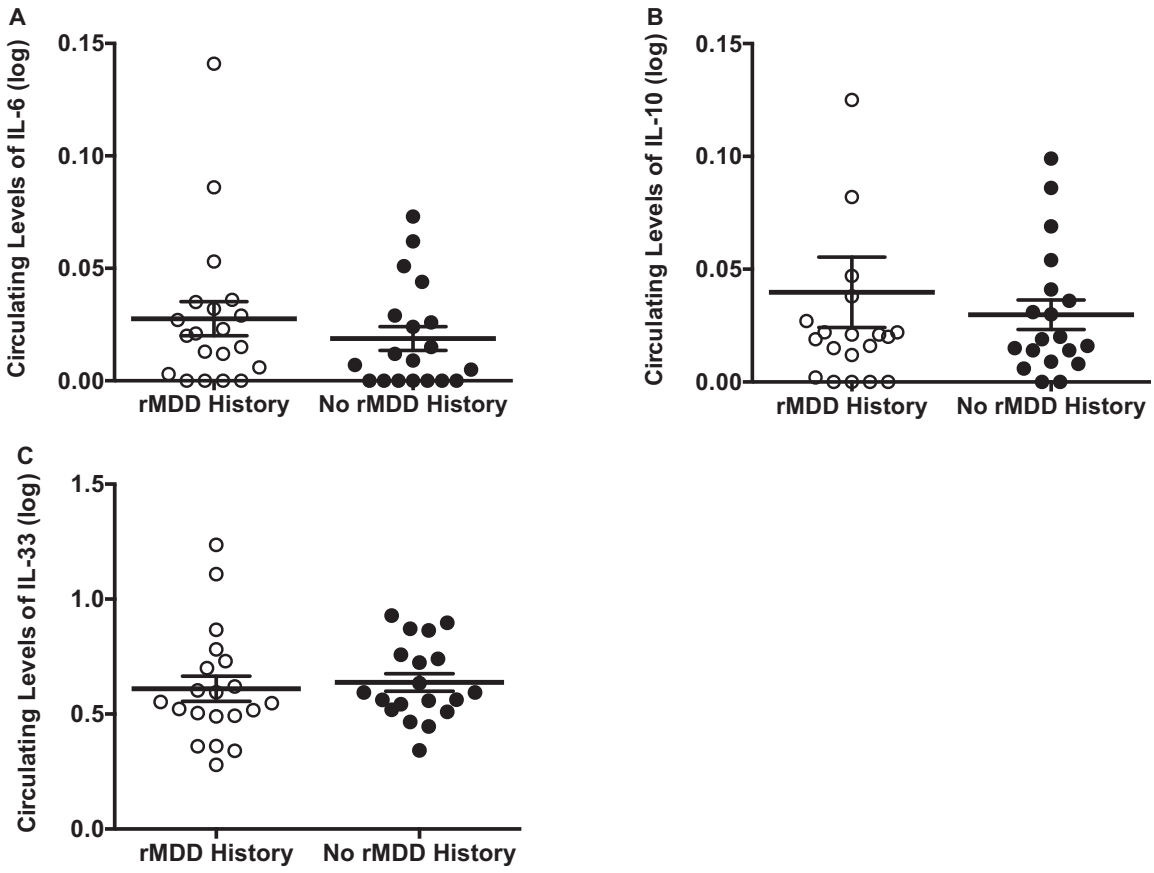


Figure 2. Differences in circulating levels of cytokines based on participants' history of rMDD. No significant differences in circulating levels of IL-6 (A), IL-10 (B) or IL-33 (C) were detected based on participants' history of rMDD. Bars represent the mean and standard error of the mean (SEM).

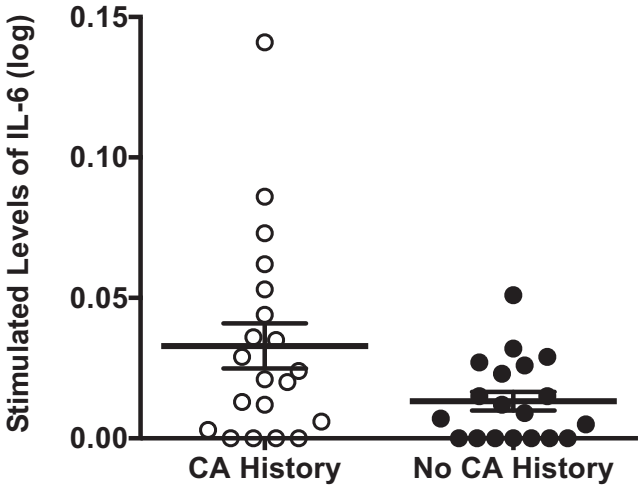


Figure 3. Differences in stimulated levels of IL-6 based on participants' history of CA. Parcipants with a history of CA evidenced higher stimulated levels of IL-6. Bars represent the mean and standard error of the mean (SEM).

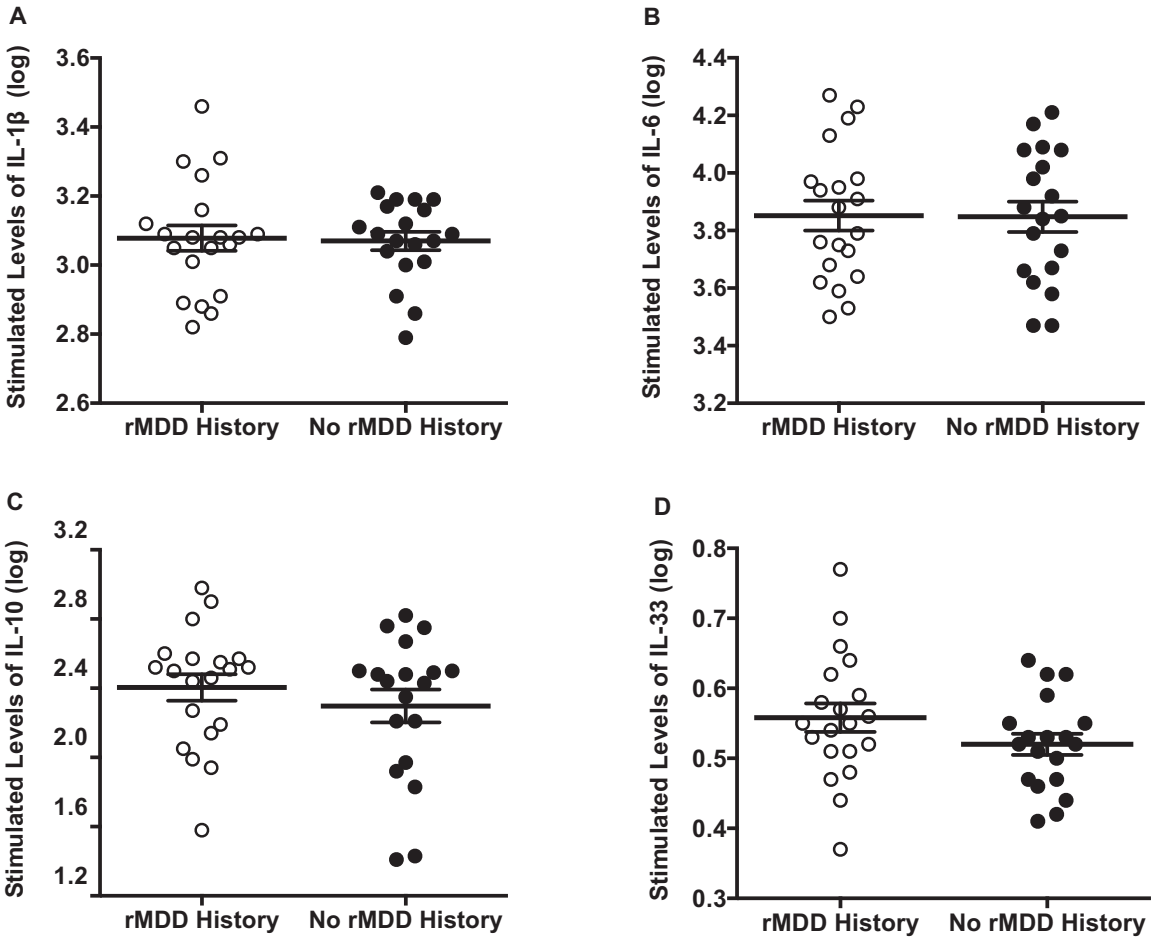


Figure 4. Differences in stimulated levels of cytokines based on participants' history of rMDD. No significant differences in stimulated levels of IL-1β (A), IL-6 (B), IL-10 (C) or IL-33 (D) were detected based on participants' history of rMDD. Bars represent the mean and standard error of the mean (SEM).

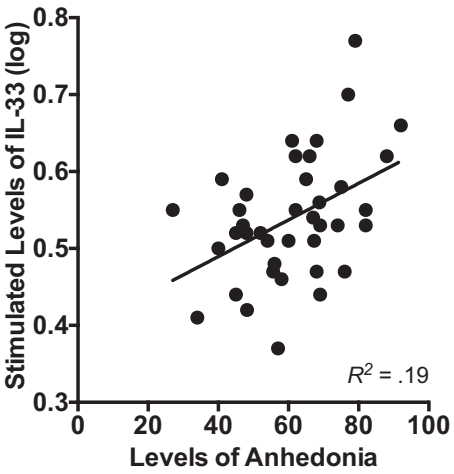


Figure 5. Levels of anhedonia and IL-33. Greater levels of current anhedonia symptoms were significantly associated with higher stimulated levels of IL-33.

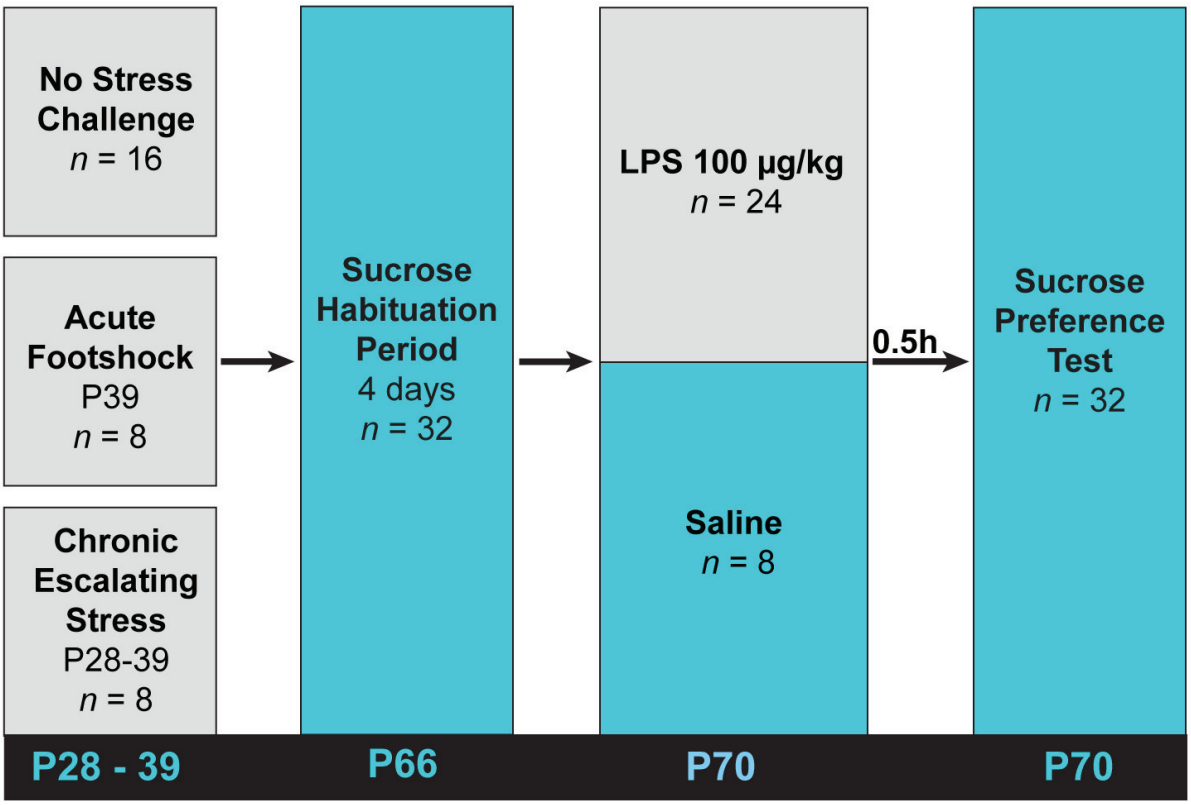


Figure 6. Schematic of the experimental design and timeline for Study 2.

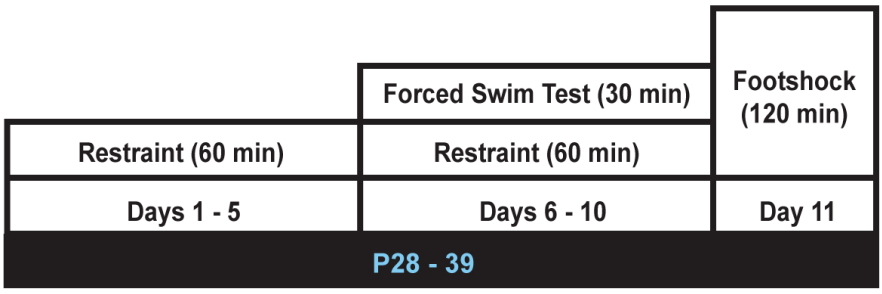


Figure 7. Time course and stressors for the chronic escalating stress (CES) paradigmused in Studies 2 and 3. Animals in the acute footshock condition (AFS) remained undisturbed in their home cages until P39, on which they went through a single session of footshock.

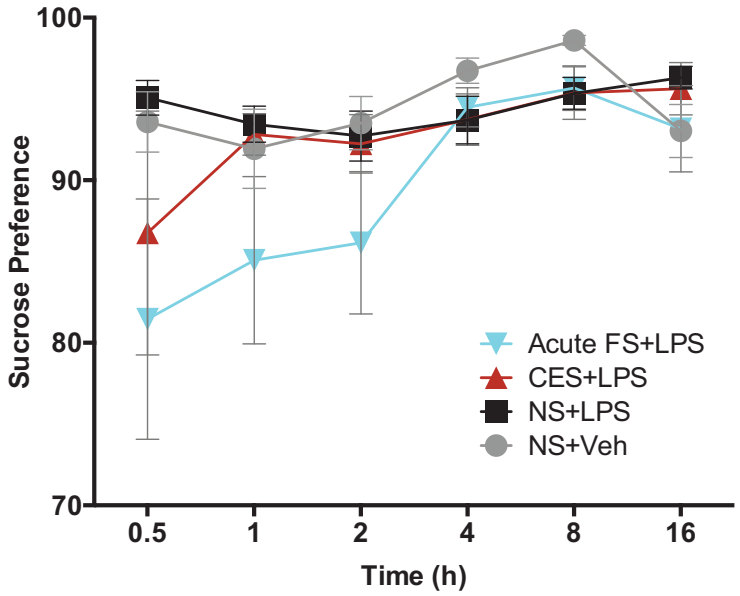


Figure 8. Sucrose preference over time. There was a marginally significant trend for the between-subject effect of group on sucrose preference. Bars represent the mean and standard error of the mean (SEM).

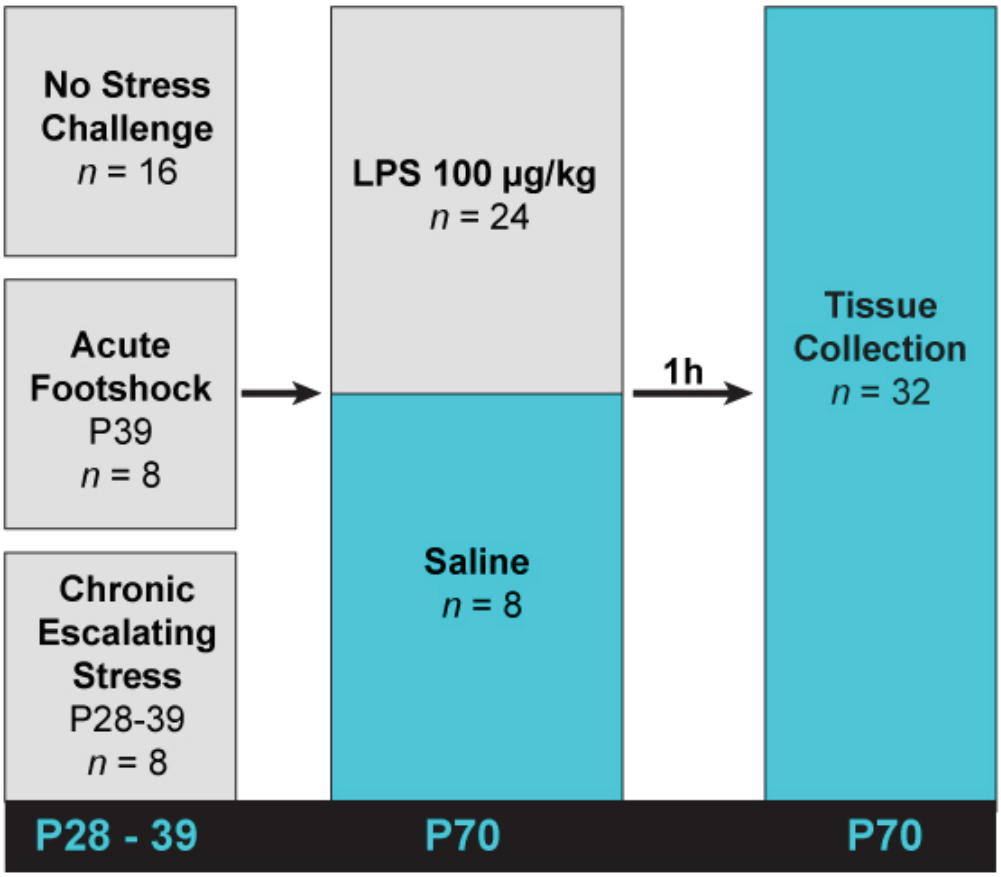


Figure 9. Schematic of the experimental design and timeline for Study 3.

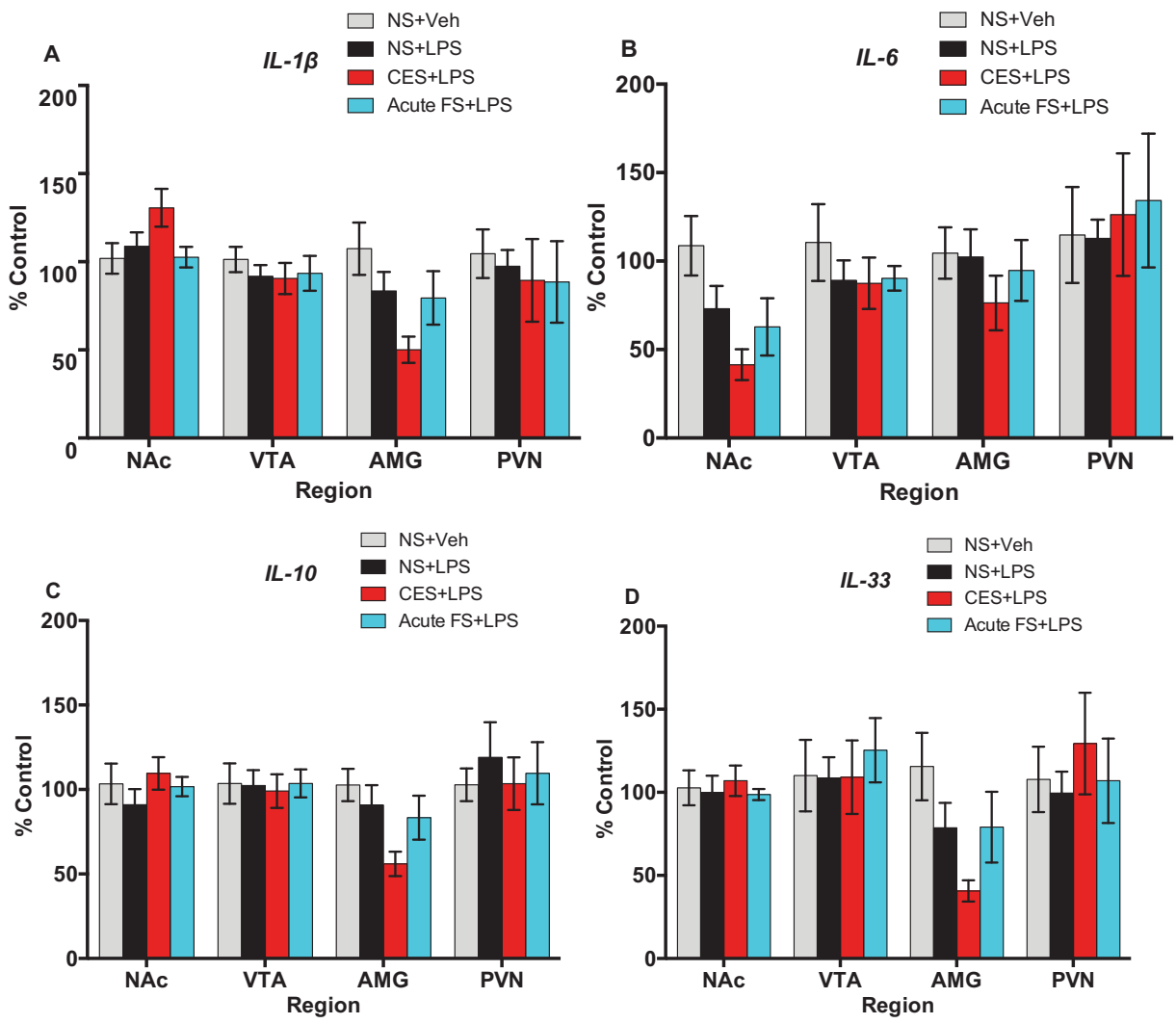


Figure 10. Expression of *IL-1β* (A), *IL-6* (B), *IL-10* (C), and *IL-33* (D) in the NAc, VTA, AMG and PVN. There were no significant main effects of group or group × region interaction.

Appendix A

Study 1 Supplementary data

Circulating cytokines. We conducted a series of ANOVAs using circulating levels of each of the cytokines (IL-6, IL-10, and IL-33) as a dependent variable and rMDD history as an independent factor among participants with no current MDD diagnosis (n = 37) only and the results were maintained. Specifically, there was no significant rMDD group differences for any of the three cytokines: IL-6, $F(1, 36) = 0.81$, $p = .37$, $\eta^2 = .02$, IL-10, $F(1, 36) = 0.24$, $p = .62$, $\eta^2 = .01$, or IL-33, $F(1, 36) = 0.15$, $p = .70$, $\eta^2 = .004$. We also conducted the same analyses among women only (n = 34) and the results remained unchanged: IL-6, $F(1, 33) = 0.28$, $p = .60$, $\eta^2 = .01$, IL-10, $F(1, 33) = 0.56$, $p = .46$, $\eta^2 = .02$, or IL-33, $F(1, 33) = 0.27$, $p = .61$, $\eta^2 = .008$. Lastly, we excluded both individuals with a current MDD diagnosis and men (n = 33) and obtained similar results: IL-6, $F(1, 32) = 0.30$, $p = .59$, $\eta^2 = .01$, IL-10, $F(1, 32) = 0.37$, $p = .55$, $\eta^2 = .01$, or IL-33, $F(1, 32) = 0.52$, $p = .47$, $\eta^2 = .02$.

Following this, we ran a series of ANOVAs using circulating levels of each cytokine (IL-6, IL-10, and IL-33) as a dependent variable and rMDD, CA, and the rMDD × CA interaction as independent factors among participants with no current MDD diagnosis (n = 37). We found a significant main effect of CA history on circulating levels of IL-6, $F(1, 36) = 4.16$, $p = .05$, $\eta^2 = .11$, but not IL-10, $F(1, 36) = .001$, $p = .97$, $\eta^2 < .001$, or IL-33, $F(1, 36) = 0.09$, $p = .76$, $\eta^2 = .003$, with participants reporting a history of CA exhibiting greater levels of circulating IL-6 (M = 0.03 log pg/mg) compared to participants with no CA history (M = 0.01 log pg/mg). The rMDD × CA interaction was

not significant for any of the cytokines: IL-6, $F(1, 38) = 0.26$, $p = .61$, $\eta^2 = .008$, IL-10, $F(1, 38) = 0.09$, $p = .76$, $\eta^2 = .003$, or IL-33, $F(1, 38) = 1.16$, $p = .28$, $\eta^2 = .03$.

We then conducted the same analyses among women only ($n = 34$). We found no significant main effect of CA history on circulating levels of IL-6, $F(1, 33) = 3.09$, $p = .09$, $\eta^2 = .09$, IL-10, $F(1, 33) = .03$, $p = .87$, $\eta^2 = .001$, or IL-33, $F(1, 33) = 0.29$, $p = .86$, $\eta^2 = .01$. The rMDD × CA interaction was not significant for any of the cytokines: IL-6, $F(1, 33) = 0.24$, $p = .63$, $\eta^2 = .008$, IL-10, $F(1, 33) = 0.22$, $p = .64$, $\eta^2 = .007$, or IL-33, $F(1, 33) = 0.69$, $p = .79$, $\eta^2 = .002$.

Lastly, we conducted the same analyses excluding individuals with a current MDD diagnosis and men ($n = 33$). We found no significant main effect of CA history on circulating levels of IL-6, $F(1, 32) = 3.12$, $p = .09$, $\eta^2 = .10$, IL-10, $F(1, 32) = .03$, $p = .87$, $\eta^2 = .001$, or IL-33, $F(1, 32) = 0.03$, $p = .86$, $\eta^2 = .01$. The rMDD × CA interaction was not significant for any of the cytokines: IL-6, $F(1, 32) = 0.18$, $p = .67$, $\eta^2 = .006$, IL-10, $F(1, 33) = 0.22$, $p = .64$, $\eta^2 = .007$, or IL-33, $F(1, 32) = 0.07$, $p = .79$, $\eta^2 = .002$.

Stimulated cytokines. We conducted a series of ANOVAs using stimulated levels of each of the cytokines (IL-1β, IL-6, IL-10, and IL-33) as a dependent variable and rMDD history as an independent factor among participants with no current MDD diagnosis ($n = 37$) and the results were maintained. Specifically, there was no significant rMDD group differences for any of the cytokines: IL-1β, $F(1, 36) = 0.008$, $p = .93$, $\eta^2 < .001$, IL-6, $F(1, 36) = 0.001$, $p = .97$, $\eta^2 < .001$, IL-10, $F(1, 36) = 0.55$, $p = .46$, $\eta^2 = .02$, or IL-33, $F(1, 36) = 1.15$, $p = .29$, $\eta^2 = .03$. We also conducted the same analyses among women only ($n = 34$) and the results remained unchanged: IL-1β, $F(1, 33) = 0.26$, $p = .62$, $\eta^2 = .008$, IL-6, $F(1, 33) = 0.38$, $p = .54$, $\eta^2 = .01$, IL-10, $F(1, 33) = 1.11$, $p = .30$, $\eta^2 =$

.03, or IL-33, $F(1, 33) = 2.50$, $p = .12$, $\eta^2 = .07$. Lastly, we excluded both individuals with a current MDD diagnosis and men ($n = 33$) and obtained similar results: IL-1β, $F(1, 32) = 0.06$, $p = .81$, $\eta^2 = .002$, IL-6, $F(1, 32) = 0.14$, $p = .71$, $\eta^2 = .004$, IL-10, $F(1, 32) = 0.98$, $p = .33$, $\eta^2 = .03$, or IL-33, $F(1, 32) = 1.83$, $p = .19$, $\eta^2 = .06$.

Following this, we ran a series of ANOVAs using stimulated levels of each cytokine (IL-1β, IL-6, IL-10, and IL-33) as a dependent variable and rMDD, CA, and the rMDD × CA interaction as independent factors among participants with no current MDD diagnosis ($n = 37$). We found no significant main effect of CA history on stimulated levels of IL-1β, $F(1, 36) = 0.07$, $p = .79$, $\eta^2 = .002$, IL-6, $F(1, 36) = .41$, $p = .52$, $\eta^2 = .01$, IL-10, $F(1, 36) = 0.03$, $p = .86$, $\eta^2 = .001$, or IL-33, $F(1, 36) = 0.002$, $p = .97$, $\eta^2 < .001$. In addition, the rMDD × CA interaction was not significant for any of the cytokines: IL-1β, $F(1, 36) = 0.71$, $p = .41$, $\eta^2 = .02$, IL-6, $F(1, 36) = 2.16$, $p = .15$, $\eta^2 = .06$, IL-10, $F(1, 36) = 0.47$, $p = .49$, $\eta^2 = .01$, or IL-33, $F(1, 36) = 1.16$, $p = .29$, $\eta^2 = .03$.

We then conducted the same analyses among women only ($n = 34$). We found no significant main effect of CA history on stimulated levels of IL-1β, $F(1, 33) = 0.36$, $p = .55$, $\eta^2 = .01$, IL-6, $F(1, 33) = 1.32$, $p = .26$, $\eta^2 = .04$, IL-10, $F(1, 33) = 0.01$, $p = .92$, $\eta^2 < .001$, or IL-33, $F(1, 33) = 0.18$, $p = .68$, $\eta^2 <= .006$. In addition, the rMDD × CA interaction was not significant for any of the cytokines: IL-1β, $F(1, 33) = 0.51$, $p = .48$, $\eta^2 = .02$, IL-6, $F(1, 33) = 2.27$, $p = .14$, $\eta^2 = .07$, IL-10, $F(1, 33) = 0.46$, $p = .50$, $\eta^2 = .01$, or IL-33, $F(1, 33) = 0.52$, $p = .48$, $\eta^2 = .02$.

Lastly, we conducted the same analyses excluding individuals with a current MDD diagnosis and men ($n = 33$). Again, the results remained unchanged, as we found no significant main effect of CA history on stimulated levels of IL-1β, $F(1, 32) = 0.17$, p

= .69, η^2 = .006, IL-6, $F(1, 32) = 0.92$, $p = .03$, η^2 = .04, IL-10, $F(1, 32) = 0.04$, $p = .95$, $\eta^2 < .001$, or IL-33, $F(1, 32) = 0.06$, $p = .81$, $\eta^2 = .002$. In addition, the rMDD × CA interaction was not significant for any of the cytokines: IL-1β, $F(1, 32) = 0.86$, $p = .36$, η^2 = .03, IL-6, $F(1, 32) = 2.98$, $p = .09$, $\eta^2 = .09$, IL-10, $F(1, 32) = 0.49$, $p = .50$, $\eta^2 = .02$, or IL-33, $F(1, 32) = 0.80$, $p = .38$, $\eta^2 = .03$.

Exploratory analyses. By way of testing the exploratory aim (1d), we examined whether levels of anhedonia, assessed using MASQ –AD subscale, were associated with increased circulating levels of cytokines. Specifically, we conducted exploratory correlations analyses using other circulating ($n = 1$) and stimulated ($n = 10$) cytokines on the panel in addition to IL-1β, IL-6, IL-10, and IL-33. All of the circulating IFNγ levels were below the detection limit and were thus excluded from the analyses. The percentages of the values for other circulating cytokine levels that were below the detection limit of our assay were as follows: IL-4 (33.3%), IL17A (69.2%), IL17F (97.4%), IL-21 (94.9%), IL-22 (74.4%), IL-23 (94.9%), IL-25 (82.1%), IL-31 (89.7%), and sCD40L (97.4%). These cytokines were, therefore, excluded from our analyses as well. We found no significant associations between current anhedonia symptoms and circulating levels of the remaining TNFα, $r(39) = 0.005$, $p = .97$.

In relation to stimulated cytokine levels, we excluded IL-4 from the analyses due to high percented of values that were below the detection limit (97.4%). We found significant associations between anhedonia and stimulated levels of IL-17A, $r(37) = .39$, $p = .01$, IL-17F, $r(37) = .36$, $p = .02$, IL-21, $r(37) = 0.44$, $p = .005$, IL-22, $r(37) = .33$, p = .04, IL-23, $r(37) = .33$, $p = .04$, IL-25, $r(37) = .40$, $p = .01$, IL-31, $r(37) = .38$, $p = .02$, IFNγ, $r(37) = .33$, $p = .04$, and sCDL40 cells, $r(37) = .43$, $p = .007$ (Supplementary

Figure 1), but not TNFα, $r(39) = .25$, $p = .13$. Notably, the results remained largely unchanged when we statistically controlled for participants' age, sex, ethnicity, income, body mass index (BMI), body temperature, time of sample collection, or lifetime smoking (Supplementary Table 1).

We conducted similar analyses among to examine the association between current anhedonia levels and stimulated Th-17 cytokine levels among participants with no current MDD diagnosis ($n = 37$). We found significant associations between anhedonia and stimulated levels of IL-17A, $r(35) = .34$, $p = .04$, IL-21, $r(35) = 0.38$, $p = .02$, IL-25, $r(35) = .33$, $p = .05$, IL-31, $r(35) = .33$, $p = .04$, $p = .04$, and sCDL40 cells, $r(35) = .37$, $p = .02$, but not IL-17F, $r(35) = .29$, $p = .08$, IL-22, $r(35) = .29$, $p = .09$, IL-23, $r(35) = .29$, $p = .08$, IFNγ, $r(35) = .24$, $p = .16$, or TNFα, $r(35) = .19$, $p = .26$.

We then conducted the same analyses among women only ($n = 34$). We found significant associations between anhedonia and stimulated levels of IL-17A, $r(33) = .41$, $p = .02$, IL-17F, $r(33) = .39$, $p = .02$, IL-21, $r(33) = 0.48$, $p = .004$, IL-22, $r(33) = .36$, $p = .04$, IL-23, $r(33) = .39$, $p = .02$, IL-25, $r(33) = .44$, $p = .009$, IL-31, $r(33) = .42$, $p = .01$, IFNγ, $r(33) = .41$, $p = .02$, and sCDL40 cells, $r(33) = .46$, $p = .007$, but not TNFα, $r(33) = .29$, $p = .10$.

Lastly, we conducted the same analyses excluding individuals with a current MDD diagnosis and men ($n = 33$). We found significant associations between anhedonia and stimulated levels of IL-17A, $r(32) = .37$, $p = .03$, IL-21, $r(32) = 0.43$, $p = .01$, IL-23, $r(32) = .35$, $p = .05$, IL-25, $r(32) = .37$, $p = .03$, IL-31, $r(32) = .38$, $p = .03$, $p = .04$, and sCDL40 cells, $r(32) = .41$, $p = .02$, but not IL-17F, $r(32) = .33$, $p = .06$, IL-22, $r(32) = .32$, $p = .07$, IFNγ, $r(32) = .33$, $p = .06$, or TNFα, $r(32) = .21$, $p = .23$.

Additionally, we found significant associations between current depressive symptoms and stimulated levels of IL-17A, $r(37) = .33$, $p = .04$, IL-17F, $r(37) = .31$, $p = .05$, IL-21, $r(37) = .36$, $p = .02$, IL-25, $r(37) = .34$, $p = .04$, IL-31, $r(37) = .38$, $p = .02$, IFNγ, $r(37) = .31$, $p = .05$, and sCDL40 cells, $r(37) = .34$, $p = .03$, but not IL-22, $r(37) = .23$, $p = .13$, IL-23, $r(37) = .31$, $p = .06$, or TNFα, $r(37) = .26$, $p = .11$.

Study 2 Supplementary data

To examine the potential impact of adolescent life stress on sucrose and water consumption separately, as opposed to sucrose preference, we conducted a 6 (time: 0.5h, 1h, 2h, 4h, 8h, and 16h) × 4 (group: CES+LPS, AFS+LPS, NS+LPS, and NS+Veh) repeated measures ANOVA separately for sucrose and water consumption values normalized by body weight. We found a significant main effect of time, $F(5,140) = 139.89$, $p < .001$, $\eta^2 = .83$ and time × group interaction, $F(15,140) = 3.24$, $p < .001$, $\eta^2 = .26$, on sucrose consumption. There was no significant between-subject effect of group, $F(3,28) = 2.12$, $p = .12$, $\eta^2 = .18$. Post hoc analysis revealed that *IL-6* expression was significantly lower in the NAc compared to the VTA, $p = .12$ and PVN, $p = .004$, with no differences between any other groups (lowest $p = .08$). When we broke this interaction down to examine its form and conducted separate analyses for each timepoint, we found that group effect on sucrose consumption was significant 8h following LPS administration, $F(3,31) = 3.52$, $p = .03$, $\eta^2 = .27$, but not at any other timepoint (lowest $p = .14$).

Post hoc analysis showed that 8h following an immune challenge, rats in the NS+Veh consumed significantly more sucrose compared to animals in NS+LPS, $p = .01$,

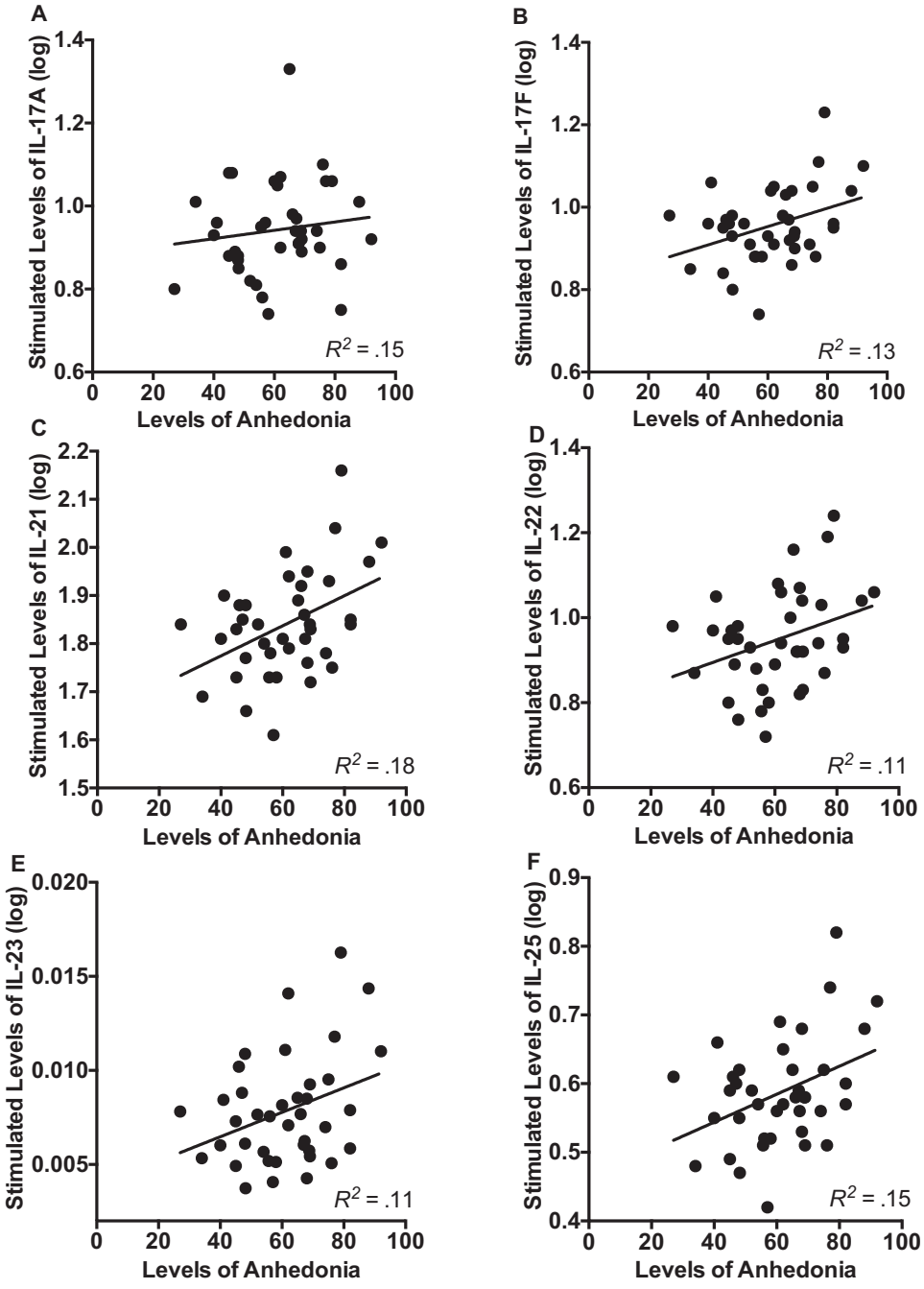
CES+LPS, $p = .03$, or AFS+LPS, $p = .01$, groups (Supplementary Figure 2). There were no significant differences between any other groups, (lowest $p = .70$).

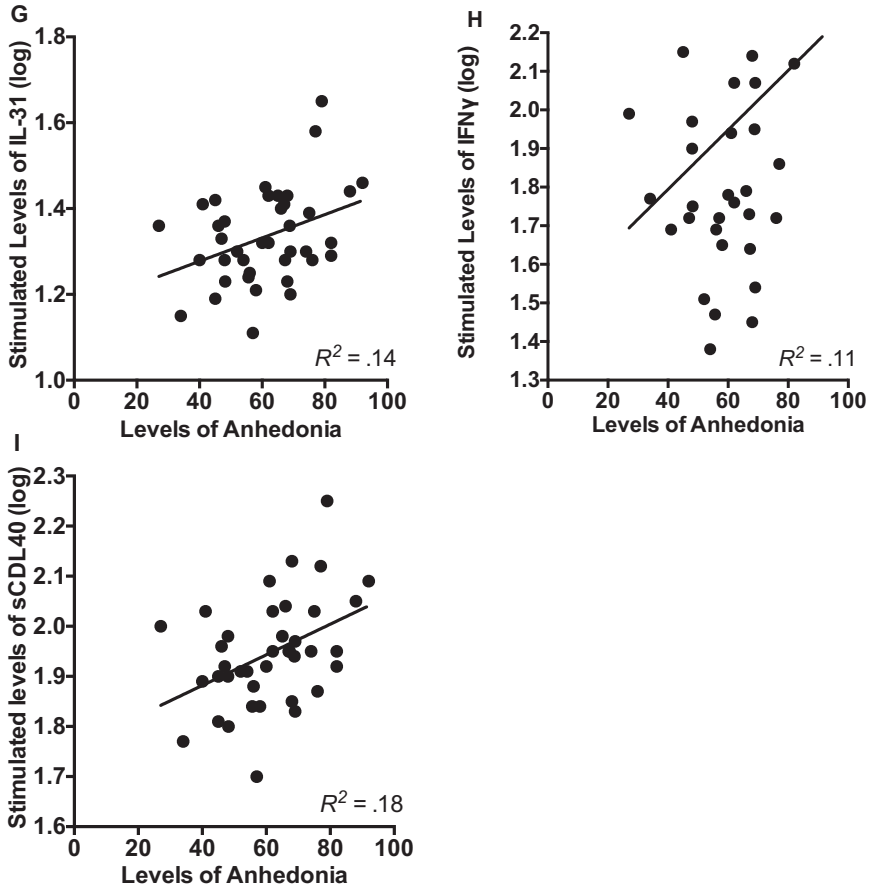
We conducted similar analysis using water consumption as a dependent variable. We found a significant main effect of time, $F(5,140) = 26.85$, $p < .001$, $\eta^2 = .49$ and a significant time × group interaction, $F(15,140) = 1.88$, $p = .03$, $\eta^2 = .17$. There was no significant between-subject effect of group, $F(15,140) = 0.92$, $p = .44$, $\eta^2 = .09$. When we conducted separate analyses for each timepoint, we found that no significant group effect on water consumption at any of the six timeponts (lowest $p = .18$).

Supplementary Table 1. Results of the partial correlation analysis (*r*-values) between participants' current anhedonia levels and stimulated cytokines, controlling for various demographic and physiological factors.

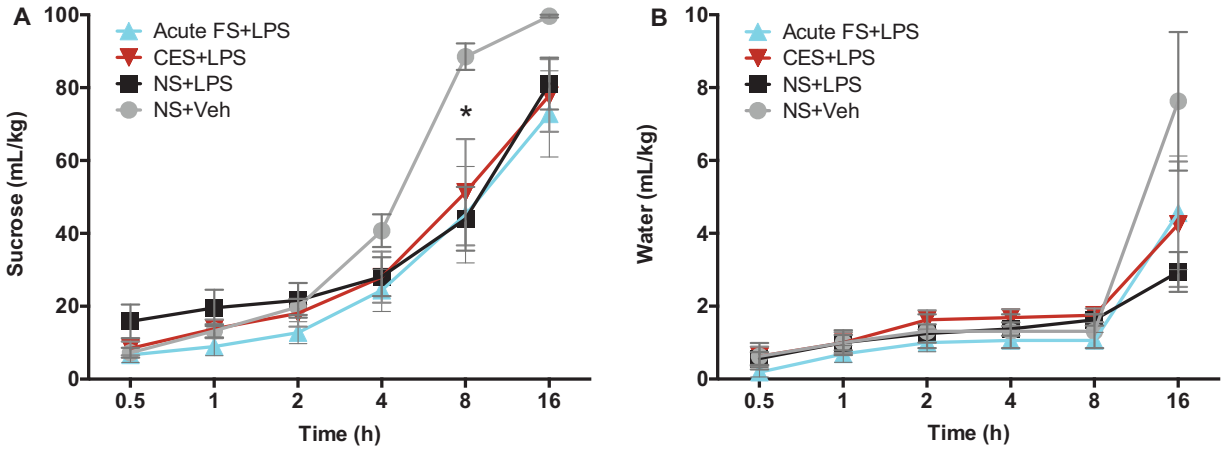
	Current Anhedonia Levels							
Controlling for:	Age	Sex	Income	Ethnicity	BMI	BT	TOD	Smoking
Cytokine:								
IL-17A	.35*	.38*	.35*	.39*	.40**	.39*	.41**	.36*
IL-17F	.32*	.36*	.34*	.37*	.38*	.36*	.40**	.34*
IL-21	.39*	.44**	.40**	.45**	.45**	.44**	.47**	.44**
IL-22	.31	.33*	.28	.32*	.35*	.33*	.37*	.29
IL-23	.26	.33*	.30	.33*	.34*	.33*	.34*	.37*
IL-25	.36*	.40*	.38*	.42**	.42**	.40**	.43**	.39*
IL-31	.36*	.38*	.34*	.40**	.39*	.38*	.42**	.36*
IFNγ	.30	.33*	.29	.31*	.33*	.33*	.32*	.31
sCDL40	.41**	.43**	.41**	.44**	.44**	.43**	.46**	.38*

Note. *$p < .05$. **$p < .01$ Degrees of freedom for all analyses = 36
BMI = Body Mass Index; BT = body temperature; TOD = time of day

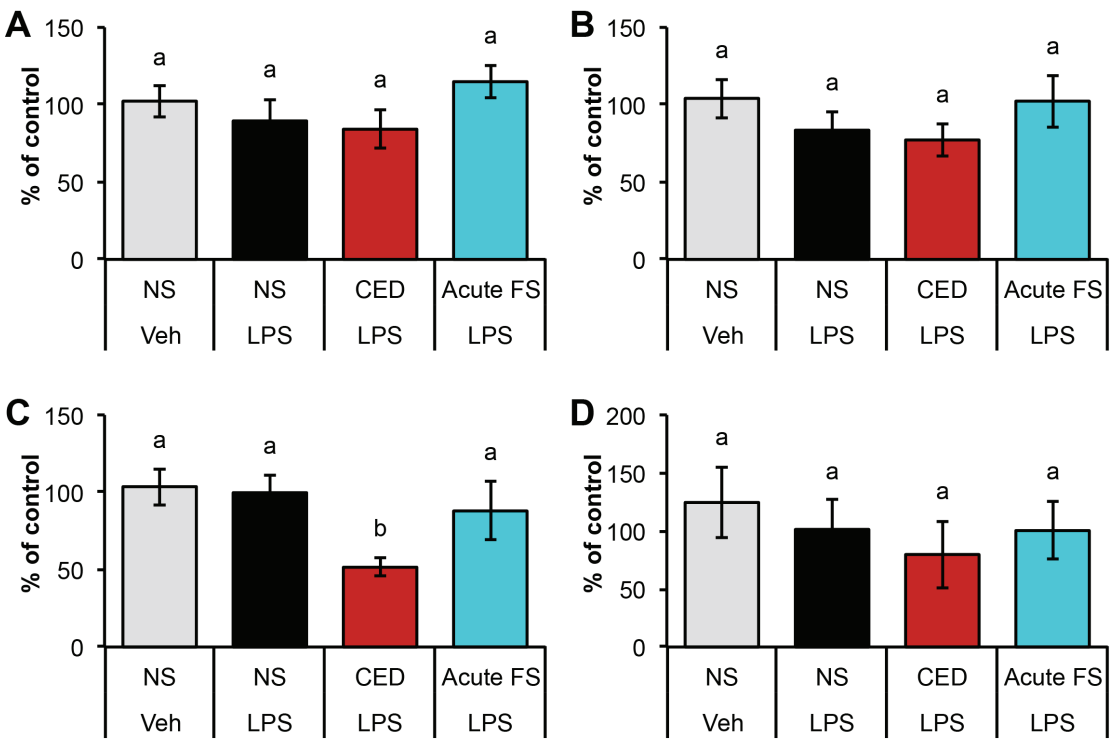

A
Stimulated Levels of IL-17A (log)
1.4
1.2
1.0
0.8
0.6
R^2 = .15
0
20
40
60
80
100
Levels of Anhedonia
B
Stimulated Levels of IL-17F (log)
1.4
1.2
1.0
0.8
0.6
R^2 = .13
0
20
40
60
80
100
Levels of Anhedonia
C
Stimulated Levels of IL-21 (log)
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
R^2 = .18
0
20
40
60
80
100
Levels of Anhedonia
D
Stimulated Levels of IL-22 (log)
1.4
1.2
1.0
0.8
0.6
R^2 = .11
0
20
40
60
80
100
Levels of Anhedonia
E
Stimulated Levels of IL-23 (log)
0.020
0.015
0.010
0.005
R^2 = .11
0
20
40
60
80
100
Levels of Anhedonia
F
Stimulated Levels of IL-25 (log)
0.9
0.8
0.7
0.6
0.5
0.4
R^2 = .15
0
20
40
60
80
100
Levels of Anhedonia



Supplementary Figure 1. Levels of anhedonia symptoms and stimulated cytokines. Levels of current anhedonia were significantly associated with stimulated levels of IL 17A (A), IL-17F (B), IL-21(C), IL-22 (D), IL-23 (E), IL-25 (F), IL-31 (G), IFNγ (H), and sCD40L (I) cells.



Supplementary Figure 2. Sucrose (A) and water (B) consumption time course during the sucrose preference test. Rats in the NS+Veh group consumed significantly more sucrose compared to animals in any other group 8h following the systemic administration of the LPS. There were no significant group differences in water consumption at any of the timepoints.



Supplementary Figure 3. GAPDH gene expression in the NAc (A), VTA (B), AMG (C), and PVN (D). There was a significant effect of group on GAPDH in the AMG. Thus, we used non-normalized data for all analysis for that region. There was no significant effect of group in any of the other brain regions.

References

Admon, R., Holsen, L. M., Aizley, H., Remington, A., Whitfield-Gabrieli, S., Goldstein, J. M., & Pizzagalli, D. a. (2014). Striatal Hypersensitivity During Stress in Remitted Individuals with Recurrent Depression. *Biological Psychiatry*, (49), 67–76. http://doi.org/10.1016/j.biopsych.2014.09.019

Ali, S., Huber, M., Kollewe, C., Bischoff, S. C., Falk, W., & Martin, M. U. (2007). IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 18660–18665. http://doi.org/10.1073/pnas.0705939104

Allakhverdi, Z., Smith, D. E., Comeau, M. R., & Delespesse, G. (2007). Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *Journal of Immunology (Baltimore, Md. : 1950)*, *179*, 2051–2054. http://doi.org/179/4/2051 [pii]

Anisman, H., Merali, Z., & Hayley, S. (2003). Sensitization associated with stressors and cytokine treatments. *Brain, Behavior, and Immunity*, *17*(2), 86–93. http://doi.org/10.1016/S0889-1591(02)00100-9

Anisman, H., Merali, Z., & Hayley, S. (2008). Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. *Progress in Neurobiology*, *85*(1), 1–74. http://doi.org/10.1016/j.pneurobio.2008.01.004

Anisman, H., Ravindran, A. V, Griffiths, J., & Merali, Z. (1999). Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Molecular Psychiatry*, *4*(2), 182–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10208451

Arakawa, K., Arakawa, H., Hueston, C. M., & Deak, T. (2014). Effects of the Estrous Cycle and Ovarian Hormones on Central Expression of Interleukin-1 Evoked by Stress in Female Rats. *Neuroendocrinology*, *100*(2–3), 162–177.

Arnau, R. C., Meagher, M. W., Norris, M. P., & Bramson, R. (2001). Psychometric evaluation of the Beck Depression Inventory-II with primary care medical patients. *Health Psychology : Official Journal of the Division of Health Psychology, American Psychological Association*, *20*(2), 112–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11315728

Asarian, L., & Geary, N. (2006, July). Modulation of appetite by gonadal steroid hormones. *Philosophical Transactions of the Royal Society B: Biological Sciences*. London. http://doi.org/10.1098/rstb.2006.1860

Baekkevold, E. S., Roussigné, M., Yamanaka, T., Johansen, F.-E., Jahnsen, F. L., Amalric, F., … Girard, J.-P. (2003). Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *The American Journal of Pathology*, *163*(1), 69–79. http://doi.org/10.1016/S0002-9440(10)63631-0

Barbosa, I. G., Morato, I. B., de Miranda, A. S., Bauer, M. E., Soares, J. C., & Teixeira, A. L. (2014). A preliminary report of increased plasma levels of IL-33 in bipolar disorder: Further evidence of pro-inflammatory status. *Journal of Affective Disorders*, *157*, 41–44. http://doi.org/10.1016/j.jad.2013.12.042

Barnum, C. J., Pace, T. W. W., Hu, F., Neigh, G. N., & Tansey, M. G. (2012). Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge. *Journal of Neuroinflammation*. http://doi.org/10.1186/1742-2094-9-9

Baumeister, D., Akhtar, R., Ciufolini, S., Pariante, C. M., & Mondelli, V. (2016, May). Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Molecular Psychiatry*. http://doi.org/10.1038/mp.2015.67

Beck, A., Steer, R., & Brown, G. (1996). *Manual for the Beck Depression Inventory-II.* San Antonio, TX: Psychological Corporation.

Ber, Y., Shiloh, R., Gilad, Y., Degani, N., Bialik, S., & Kimchi, A. (2014). DAPK2 is a novel regulator of mTORC1 activity and autophagy. *Cell Death And Differentiation*, *22*, 465. Retrieved from http://dx.doi.org/10.1038/cdd.2014.177

Bernstein, D. P., Ahluvalia, T., Pogge, D., & Handelsman, L. (1997). Validity of the Childhood Trauma Questionnaire in an adolescent psychiatric population. *Journal of the American Academy of Child and Adolescent Psychiatry*, *36*(3), 340–8. http://doi.org/10.1097/00004583-199703000-00012

Bertone-Johnson, E. R., Ronnenberg, A. G., Houghton, S. C., Nobles, C., Zagarins, S. E., Takashima-Uebelhoer, B. B., … Whitcomb, B. W. (2014). Association of inflammation markers with menstrual symptom severity and premenstrual syndrome in young women. *Human Reproduction*, *29*(9), 1987–1994. http://doi.org/10.1093/humrep/deu170

Bison, S., Carboni, L., Arban, R., Bate, S., Gerrard, P. a, & Razzoli, M. (2009). Differential behavioral, physiological, and hormonal sensitivity to LPS challenge in rats. *International Journal of Interferon, Cytokine and Mediator Research*, *1*, 1–14.

Bradley, R. G., Binder, E. B., Epstein, M. P., Tang, Y., Nair, H. ., & Liu, W. (2014). Influence of Child Abuse on Adult Depression. *Archives of General Psychiatry*, *65*(2), 190–200.

Bradley, R. G., Binder, E. B., Epstein, M. P., Tang, Y., Nair, H. P., Liu, W., … Ressler, K. J. (2008). Influence of Child Abuse on Adult Depression. *Archives of General Psychiatry*, *65*(2), 190. http://doi.org/10.1001/archgenpsychiatry.2007.26

Bredemeier, K., Spielberg, J. M., Silton, R. L., Berenbaum, H., Heller, W., & Miller, G. A. (2010, September). Screening for depressive disorders using the MASQ anhedonic depression scale: A receiver-operator characteristic analysis. *Psychological Assessment*. http://doi.org/10.1037/a0019915

Bucholz, K. K., Cadoret, R., Cloninger, C. R., Dinwiddie, S. H., Hesselbrock, V. M., Nurnberger, J. I., … Schuckit, M. A. (1994). A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *Journal of Studies on Alcohol*, *55*(2), 149–58. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8189735

Bulloch, A., Williams, J., Lavorato, D., & Patten, S. (2014). Recurrence of major depressive episodes is strongly dependent on the number of previous episodes. *Depression and Anxiety*, *31*(1), 72–76. http://doi.org/10.1002/da.22173

Burcusa, S., & Lacono, W. (2007). Risk for Recurrence in Depression. *Clinical*

Psychology Review, *27*(8), 959–985. Retrieved from http://www.sciencedirect.com/science/article/pii/S027273580700058X

Cacioppo, J. T. (2007). Better Interdisciplinary Research Through Psychological Science. *APS Observer*, *20*(10). Retrieved from https://www.psychologicalscience.org/observer/better-interdisciplinary-research-through-psychological-science

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology*. Berlin/Heidelberg. http://doi.org/10.1007/s00213-016-4218-9

Capuron, L., Ravaud, a, Neveu, P. J., Miller, a H., Maes, M., & Dantzer, R. (2002). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Molecular Psychiatry*, *7*(5), 468–73. http://doi.org/10.1038/sj.mp.4000995

Carcone, D., & Ruocco, A. C. (2017). Six Years of Research on the National Institute of Mental Health's Research Domain Criteria (RDoC) Initiative: A Systematic Review . *Frontiers in Cellular Neuroscience* . Retrieved from https://www.frontiersin.org/article/10.3389/fncel.2017.00046

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., Lee, J. K., Anderson, G. M., & Price, L. H. (2010). Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(13), 2617–23.

Chapuis, J., Hot, D., Hansmannel, F., Kerdraon, O., Ferreira, S., Hubans, C., … Lambert, J. C. (2009). Transcriptomic and genetic studies identify IL-33 as a candidate gene for Alzheimer's disease. *Molecular Psychiatry*, *14*(11), 1004–16. http://doi.org/10.1038/mp.2009.10

Chu, X., Zhou, Y., Hu, Z., Lou, J., Song, W., Li, J., … Li, W. (2016). 24-hour-restraint stress induces long-term depressive-like phenotypes in mice. *Scientific Reports*, *6*, 32935. Retrieved from http://dx.doi.org/10.1038/srep32935

Collins, M. A., An, J., Peller, D., & Bowser, R. (2015). Total protein is an effective loading control for cerebrospinal fluid western blots. *Journal of Neuroscience Methods*, *251*, 72–82. http://doi.org/https://doi.org/10.1016/j.jneumeth.2015.05.011

Contreras, F., Prado, C., González, H., Franz, D., Osorio-Barrios, F., Osorio, F., … Pacheco, R. (2016). Dopamine Receptor D3 Signaling on CD4+ T Cells Favors Th1- and Th17-Mediated Immunity. *The Journal of Immunology*, *196*(10), 4143–4149. http://doi.org/10.4049/jimmunol.1502420

Corral-Frías, N. S., Nikolova, Y. S., Michalski, L. J., Baranger, D. A. A., Hariri, A. R., & Bogdan, R. (2015, September). Stress-related anhedonia is associated with ventral striatum reactivity to reward and transdiagnostic psychiatric symptomatology. *Psychological Medicine*. http://doi.org/10.1017/S0033291715000525

Danese, A., Moffitt, T. E., Pariante, C. M., Ambler, A., Poulton, R., & Caspi, A. (2008). Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Archives of General Psychiatry*, *65*(4), 409–15. http://doi.org/10.1001/archpsyc.65.4.409

Danese, A., Pariante, C. M., Caspi, A., Taylor, A., & Poulton, R. (2007). Childhood maltreatment predicts adult inflammation in a life-course study. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(4), 1319–24. http://doi.org/10.1073/pnas.0610362104

De La Garza, R. (2005). Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia. *Neuroscience & Biobehavioral Reviews*, *29*(4), 761–770. http://doi.org/https://doi.org/10.1016/j.neubiorev.2005.03.016

Deak, T., Bellamy, C., D'Agostino, L. G., Rosanoff, M., McElderry, N. K., & Bordner, K. a. (2005). Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lipopolysaccharide. *Behavioural Brain Research*, *160*(1), 125–34. http://doi.org/10.1016/j.bbr.2004.11.024

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *The Journal of Clinical Psychiatry*, *61 Suppl 6*, 7–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10775018

Der-Avakian, A., & Markou, A. (2012, January). The Neurobiology of Anhedonia and Other Reward-Related Deficits. *Trends in Neurosciences*. http://doi.org/10.1016/j.tins.2011.11.005

Doremus-Fitzwater, T. L., Gano, A., Paniccia, J. E., & Deak, T. (2015, September). Male adolescent rats display blunted cytokine responses in the CNS after acute ethanol or lipopolysaccharide exposure. *Physiology & Behavior*. http://doi.org/10.1016/j.physbeh.2015.02.032

Doremus-Fitzwater, T. L., Paniccia, J. E., Gano, A., Vore, A. S., & Deak, T. (2018). Differential effects of acute versus chronic stress on ethanol sensitivity: Evidence for interactions on both behavioral and neuroimmune outcomes. *Brain, Behavior, and Immunity*, *70*, 141–156. http://doi.org/https://doi.org/10.1016/j.bbi.2018.02.009

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological Psychiatry*, *67*(5), 446–57. http://doi.org/10.1016/j.biopsych.2009.09.033

Eisenberger, N. I., Berkman, E. T., Inagaki, T. K., Rameson, L. T., Mashal, N. M., & Irwin, M. R. (2010). Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biological Psychiatry*, *68*(8), 748–54. http://doi.org/10.1016/j.biopsych.2010.06.010

Erickson, M. A., & Banks, W. A. (2011, November). Cytokine and Chemokine Responses in Serum and Brain After Single and Repeated Injections of Lipopolysaccharide: Multiplex Quantification with Path Analysis. *Brain, Behavior, and Immunity*. http://doi.org/10.1016/j.bbi.2011.06.006

Escalona, R., & Fawcett, J. (2017, January). Pramipexole in Treatment Resistant-Depression, Possible Role of Inflammatory Cytokines. *Neuropsychopharmacology*. http://doi.org/10.1038/npp.2016.217

Felger, J. C., Li, Z., Haroon, E., Woolwine, B. J., Jung, M. Y., Hu, X., & Miller, A. H. (2015). Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry in depression. *Molecular Psychiatry*. http://doi.org/10.1038/mp.2015.168

First, M., Spitzer, R., Gibbon, M., & Williams, J. (2002). *Structured clinical interview for DSM-IV-TR axis I disorders, research version, non-patient edition. (SCID-I/NP)*. New York: Biometrics Research, New York State Psychiatric Institute.

Ford, D. E., & Erlinger, T. P. (2004). Depression and C-Reactive Protein in US Adults.

Archives of Internal Medicine, *164*, 1010–1014.

Frenois, F., Moreau, M., Connor, J. O., Lawson, M., Micon, C., Lestage, J., … Castanon, N. (2007, June). Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology*. http://doi.org/10.1016/j.psyneuen.2007.03.005

Fu, H. Q., Yang, T., Xiao, W., Fan, L., Wu, Y., Terrando, N., & Wang, T. L. (2014). Prolonged Neuroinflammation after Lipopolysaccharide Exposure in Aged Rats. *PLoS ONE*, *9*(8), e106331. http://doi.org/10.1371/journal.pone.0106331

Galland, L. (2010). Diet and Inflammation. *Nutrition in Clinical Practice*, *25*(6), 634–640. http://doi.org/10.1177/0884533610385703

Goshen, I., & Yirmiya, R. (2009). Interleukin-1 (IL-1): a central regulator of stress responses. *Frontiers in Neuroendocrinology*, *30*(1), 30–45. http://doi.org/10.1016/j.yfrne.2008.10.001

Guglani, L., & Khader, S. A. (2010, March). Th17 cytokines in mucosal immunity and inflammation. *Current Opinion in HIV and AIDS*. http://doi.org/10.1097/COH.0b013e328335c2f6

Hammen, C. (2005). Stress and depression. *Annual Review of Clinical Psychology*, *1*, 293–319. http://doi.org/10.1146/annurev.clinpsy.1.102803.143938

Heiser, P., Lanquillon, S., Krieg, J.-C., & Vedder, H. (2008). Differential modulation of cytokine production in major depressive disorder by cortisol and dexamethasone. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology*, *18*(12), 860–70. http://doi.org/10.1016/j.euroneuro.2008.07.003

Hepworth, M. R., Daniłowicz-Luebert, E., Rausch, S., Metz, M., Klotz, C., Maurer, M., & Hartmann, S. (2012, April). Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proceedings of the National Academy of Sciences of the United States of America*. http://doi.org/10.1073/pnas.1112268109

Heshmati, M., & Russo, S. J. (2015, September). Anhedonia and the brain reward circuitry in depression. *Current Behavioral Neuroscience Reports*. http://doi.org/10.1007/s40473-015-0044-3

Holtzheimer, P. E., & Mayberg, H. S. (2011). Stuck in a rut: rethinking depression and its treatment. *Trends in Neurosciences*, *34*(1), 1–9. http://doi.org/10.1016/j.tins.2010.10.004

Howren, M., Bryant, M., Lamkin, D., & Suls, J. (2009). Associations of Depression With C-Reactive Protein, IL-1, and IL-6: A Meta-Analysis. *Psychological Medicine*, *71*(2), 171–186.

Hueston, C. M., & Deak, T. (2014). The inflamed axis: The interaction between stress, hormones, and the expression of inflammatory-related genes within key structures comprising the hypothalamic-pituitary-adrenal axis. *Physiology and Behavior*, *124*, 77–91. http://doi.org/10.1016/j.physbeh.2013.10.035

Kakkar, R., & Lee, R. T. (2008). The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nature Reviews. Drug Discovery*, *7*(10), 827–40. http://doi.org/10.1038/nrd2660

Kanitz, E., Tuchscherer, M., Puppe, B., Tuchscherer, A., & Stabenow, B. (2004). Consequences of repeated early isolation in domestic piglets (Sus scrofa) on their behavioural, neuroendocrine, and immunological responses. *Brain, Behavior, and Immunity*, *18*(1), 35–45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14651945

Kaymaz, N., van Os, J., Loonen, A. J. M., & Nolen, W. . (2008). Evidence That Patients With Single Versus Recurrent Depressive Episodes Are Differentially Sensitive to Treatment Discontinuation: A Meta-Analysis of Placebo-Controlled Randomized Trials. *Journal of Clinical Psychiatry*, *69*(9), 1424–1437. Retrieved from http://ectweb.pbworks.com/f/Evidence+That+Patients+With+Single+Versus+Recurrent.pdf

Kelley, G. A., & Kelley, K. S. (2006). Effects of aerobic exercise on C-reactive protein, body composition, and maximum oxygen consumption in adults: a meta-analysis of randomized controlled trials. *Metabolism - Clinical and Experimental*, *55*(11), 1500–1507. http://doi.org/10.1016/j.metabol.2006.06.021

Kendall, A. D., Zinbarg, R. E., Bobova, L., Mineka, S., Revelle, W., Prenoveau, J. M., & Craske, M. G. (2015). Measuring Positive Emotion With the Mood and Anxiety Symptom Questionnaire: Psychometric Properties of the Anhedonic Depression Scale. *Assessment*, *23*(1), 86–95. http://doi.org/10.1177/1073191115569528

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., & Jones, P. B. (2014, October). Association of Serum Interleukin 6 and C-Reactive Protein in Childhood With Depression and Psychosis in Young Adult Life: A Population-Based Longitudinal Study. *JAMA Psychiatry*. http://doi.org/10.1001/jamapsychiatry.2014.1332

Knopik, V. S., Sparrow, E. P., Madden, P. A. F., Bucholz, K. K., Hudziak, J. J., Reich, W., … Heath, A. C. (2005). Contributions of parental alcoholism, prenatal substance exposure, and genetic transmission to child ADHD risk: a female twin study. *Psychological Medicine*, *35*(5), 625–35. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15918339

Kong, E., Sucic, S., Monje, F. J., Savalli, G., Diao, W., Khan, D., … Pollak, D. D. (2015). STAT3 controls IL6-dependent regulation of serotonin transporter function and depression-like behavior. *Scientific Reports*, *5*, 9009. http://doi.org/10.1038/srep09009

Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V, … Yirmiya, R. (2013). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Molecular Psychiatry*, *19*, 699. Retrieved from http://dx.doi.org/10.1038/mp.2013.155

Kudinova, A. Y., Deak, T., Hueston, C. M., McGeary, J. E., Knopik, V., Palmer, R., & Gibb, B. E. (2016). Cross-species evidence for the role of interleukin-33 in depression risk. *Journal of Abnormal Psychology*, *125*, 482–94.

Kujawa, A., Hajcak, G., Danzig, A. P., Black, S. R., Bromet, E. J., Carlson, G. A., … Klein, D. N. (2015). Neural Reactivity to Emotional Stimuli Prospectively Predicts the Impact of a Natural Disaster on Psychiatric Symptoms in Children. *Biological Psychiatry*. http://doi.org/10.1016/j.biopsych.2015.09.008

Latorre, E., Mendoza, C., Matheus, N., Castro, M., Grasa, L., Mesonero, J. E., & Alcalde, A. I. (2013). IL-10 modulates serotonin transporter activity and molecular expression in intestinal epithelial cells. *Cytokine*, *61*(3), 778–84.

Levine, J., Barak, Y., Chengappa, K. N. R., Rapoport, A., Rebey, M., & Barak, V. (1999). Cerebrospinal Cytokine Levels in Patients with Acute Depression. *Neuropsychobiology*, *40*(4), 171–176. Retrieved from http://www.karger.com/DOI/10.1159/000026615

Levine, S. (2001). Primary social relationships influence the development of the hypothalamic–pituitary–adrenal axis in the rat. *Physiology & Behavior*, *73*(3), 255–260. http://doi.org/https://doi.org/10.1016/S0031-9384(01)00496-6

Levinson, D. F., Zubenko, G. S., Crowe, R. R., DePaulo, R. J., Scheftner, W. S., Weissman, M. M., … Chellis, J. (2003). Genetics of recurrent early-onset depression (GenRED): design and preliminary clinical characteristics of a repository sample for genetic linkage studies. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *119B*(1), 118–130. http://doi.org/10.1002/ajmg.b.20009

Lewinsohn, P. M., Allen, N. B., Seeley, J. R., & Gotlib, I. H. (1999). First onset versus recurrence of depression: differential processes of psychosocial risk. *Journal of Abnormal Psychology*, *108*(3), 483–489. http://doi.org/10.1037/0021-843X.108.3.483

Liu, Y., Ho, R. C.-M., & Mak, A. (2012). Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *Journal of Affective Disorders*, *139*(3), 230–9. http://doi.org/10.1016/j.jad.2011.08.003

Llorente, R., Arranz, L., Marco, E.-M., Moreno, E., Puerto, M., Guaza, C., … Viveros, M.-P. (2007). Early maternal deprivation and neonatal single administration with a cannabinoid agonist induce long-term sex-dependent psychoimmunoendocrine effects in adolescent rats. *Psychoneuroendocrinology*, *32*(6), 636–650.

Maes, M., Berk, M., Goehler, L., Song, C., Anderson, G., Gałecki, P., & Leonard, B. (2012). Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Medicine*, *10*(1), 66. http://doi.org/10.1186/1741-7015-10-66

Maes, M., Scharpé, S., Meltzer, H. Y., Bosmans, E., Suy, E., Calabrese, J., & Cosyns, P. (1993). Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pituitary-adrenal axis in severe depression. *Psychiatry*

Research, *49*(1), 11–27.

Maes, M., Song, C., & Yirmiya, R. (2012). Targeting IL-1 in depression. *Expert Opinion on Therapeutic Targets*, *16*(11), 1097–112. http://doi.org/10.1517/14728222.2012.718331

Mallone, R., Mannering, S. I., Brooks-Worrell, B. M., Durinovic-Belló, I., Cilio, C. M., Wong, F. S., & Schloot, N. C. (2011). Isolation And Preservation Of Peripheral Blood Mononuclear cells for analysis of islet antigen-reactive T cell responses: Position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society. *Clinical and Experimental Immunology*, *163*(1), 33–49. http://doi.org/10.1111/j.1365-2249.2010.04272.x

Mesquita, A. R., Correia-Neves, M., Roque, S., Castro, A. G., Vieira, P., Pedrosa, J., … Sousa, N. (2008). IL-10 modulates depressive-like behavior. *Journal of Psychiatric Research*, *43*(2), 89–97. http://doi.org/10.1016/j.jpsychires.2008.02.004

Miller, A., Maletic, V., & Raison, C. (2009). Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. *Biological Psychiatry*, *65*(9), 732–741. http://doi.org/10.1016/j.biopsych.2008.11.029.Inflammation

Miller, A., & Raison, C. (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature Reviews Immunology*, *16*(1), 22–34. http://doi.org/10.1038/nri.2015.5

Miller, G. E., & Chen, E. (2010). Harsh family climate in early life presages the emergence of a proinflammatory phenotype in adolescence. *Psychological Science*, *21*(6), 848–56. http://doi.org/10.1177/0956797610370161

Mirchandani, A. S., Salmond, R. J., & Liew, F. Y. (2012). Interleukin-33 and the function of innate lymphoid cells. *Trends in Immunology*, *33*(8), 389–396.

Moulin, D., Donzé, O., Talabot-Ayer, D., Mézin, F., Palmer, G., & Gabay, C. (2007). Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine*, *40*(3), 216–25. http://doi.org/10.1016/j.cyto.2007.09.013

Muscatell, K. A., Slavich, G. M., Monroe, S. M., & Gotlib, I. H. (2009). Stressful life

events, chronic difficulties, and the symptoms of clinical depression. *The Journal of Nervous and Mental Disease*, *197*(3), 154–60. http://doi.org/10.1097/NMD.0b013e318199f77b

Musselman, D. L., Miller, a H., Porter, M. R., Manatunga, a, Gao, F., Penna, S., … Nemeroff, C. B. (2001). Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. *The American Journal of Psychiatry*, *158*(8), 1252–7.

O'Connor, J. C., Lawson, M. a, André, C., Moreau, M., Lestage, J., Castanon, N., … Dantzer, R. (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Molecular Psychiatry*, *14*(5), 511–22. http://doi.org/10.1038/sj.mp.4002148

Omrani, M. D., Bushehri, B., Bagheri, M., Salari-Lak, S., Alipour, A., Anoshae, M.-R., & Massomi, R. (2009). Role of IL-10 -1082, IFN-gamma +874, and TNF-alpha -308 genes polymorphisms in suicidal behavior. *Archives of Suicide Research : Official Journal of the International Academy for Suicide Research*, *13*(4), 330–9. http://doi.org/10.1080/13811110903266418

Owen, B. M., Eccleston, D., Ferrier, I. N., & Young, A. H. (2001). Raised levels of plasma interleukin-1beta in major and postviral depression. *Acta Psychiatrica Scandinavica*, *103*, 226–228.

Raposa, E. B., Bower, J. E., Hammen, C. L., Najman, J. M., & Brennan, P. A. (2014, June). A Developmental Pathway from Early Life Stress to Inflammation: The Role of Negative Health Behaviors. *Psychological Science*. http://doi.org/10.1177/0956797614530570

Rey, A. del, Randolf, A., Wildmann, J., Besedovsky, H. O., & Jessop, D. S. (2009, August). Re-exposure to endotoxin induces differential cytokine gene expression in the rat hypothalamus and spleen. *Brain, Behavior, and Immunity*. http://doi.org/10.1016/j.bbi.2009.02.009

Schildberger, A., Rossmanith, E., Eichhorn, T., Strassl, K., & Weber, V. (2013). Monocytes, peripheral blood mononuclear cells, and THP-1 cells exhibit different cytokine expression patterns following stimulation with lipopolysaccharide. *Mediators of Inflammation*, *2013*, 697972. http://doi.org/10.1155/2013/697972

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., … Kastelein, R. a. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*, *23*(5), 479–90. http://doi.org/10.1016/j.immuni.2005.09.015

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychological Bulletin*, *140*(3), 774–815. http://doi.org/10.1037/a0035302

Slopen, N., Kubzansky, L. D., McLaughlin, K. A., & Koenen, K. C. (2013, February). Childhood adversity and inflammatory processes in youth: A prospective study. *Psychoneuroendocrinology*. http://doi.org/10.1016/j.psyneuen.2012.05.013

Slopen, N., Lewis, T. T., Gruenewald, T. L., Mujahid, M. S., Ryff, C. D., Albert, M. A., & Williams, D. R. (2010, September). Early life Adversity and Inflammation in African Americans and Whites in the Midlife in the United States Survey. *Psychosomatic Medicine*. http://doi.org/10.1097/PSY.0b013e3181e9c16f

Ślusarczyk, J., Trojan, E., Głombik, K., Budziszewska, B., Kubera, M., Lasoń, W., … Basta-Kaim, A. (2015). Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. *Frontiers in Cellular Neuroscience*. http://doi.org/10.3389/fncel.2015.00082

Smolinsky, A., Bergner, C., LaPorte, J., & Kalueff, A. (2009). Analysis of Grooming Behavior and Its Utility in Studying Animal Stress, Anxiety, and Depression. In T. D. Gould (Ed.), *Mood and Anxiety Related Phenotypes in Mice* (Vol. 42). Totowa, NJ: Humana Press. http://doi.org/10.1007/978-1-60761-303-9

Solomon, D. a, Keller, M. B., Leon, a C., Mueller, T. I., Lavori, P. W., Shea, M. T., … Endicott, J. (2000). Multiple recurrences of major depressive disorder. *The American Journal of Psychiatry*, *157*(2), 229–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10671391

Stiller, A. L., Drugan, R. C., Hazi, A., & Kent, S. P. (2011). Stress resilience and vulnerability: The association with rearing conditions, endocrine function, immunology, and anxious behavior. *Psychoneuroendocrinology*, *36*(9), 1383–1395. http://doi.org/10.1016/J.PSYNEUEN.2011.03.012

Strekalova, T., Couch, Y., Kholod, N., Boyks, M., Malin, D., Leprince, P., & Steinbusch, H. M. W. (2011). Update in the methodology of the chronic stress paradigm: internal control matters. *Behavioral and Brain Functions : BBF*. http://doi.org/10.1186/1744-9081-7-9

Swardfager, W., Rosenblat, J. D., Benlamri, M., & McIntyre, R. S. (2016). Mapping inflammation onto mood: Inflammatory mediators of anhedonia. *Neuroscience & Biobehavioral Reviews*, *64*, 148–166. http://doi.org/https://doi.org/10.1016/j.neubiorev.2016.02.017

Tavakoli-Ardakani, M., Mehrpooya, M., Mehdizadeh, M., Hajifathali, A., & Abdolahi, A. (2015). Association between Interlukin-6 (IL-6), Interlukin-10 (IL-10) and depression in patients undergoing Hematopoietic stem cell transplantation. *International Journal of Hematology-Oncology and Stem Cell Research*, *9*(2), 80–7.

Tynan, R. J., Naicker, S., Hinwood, M., Nalivaiko, E., Buller, K. M., Pow, D. V., … Walker, F. R. (2010). Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions. *Brain, Behavior, and Immunity*, *24*(7), 1058–1068. http://doi.org/10.1016/j.bbi.2010.02.001

Uher, R., Perlis, R. H., Henigsberg, N., Zobel, A., Rietschel, M., Mors, O., … McGuffin, P. (2012). Depression symptom dimensions as predictors of antidepressant treatment outcome: replicable evidence for interest-activity symptoms. *Psychological Medicine*, *42*(5), 967–980. http://doi.org/10.1017/S0033291711001905

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., … Moradi-Lakeh, M. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, *380*(9859), 2163–2196. http://doi.org/10.1016/S0140-6736(12)61729-2

Watson, G. H., & Paxinos, C. (2005). *The Rat Brain in Stereotaxic Coordinates*. Burlington, MA: Elsevier Academic Press.

Weizman, R., Laor, N., Podliszewski, E., Notti, I., Djaldetti, M., & Bessler, H. (1994). Cytokine production in major depressed patients before and after clomipramine treatment. *Biological Psychiatry*, *35*(1), 42–47. http://doi.org/10.1016/0006-3223(94)91166-5

WHO | Depression. (2016). Retrieved from http://www.who.int/mediacentre/factsheets/fs369/en/

Willner, P. (2017). Reliability of the chronic mild stress model of depression: A user survey. *Neurobiology of Stress*, *6*, 68–77. http://doi.org/https://doi.org/10.1016/j.ynstr.2016.08.001

Wohleb, E. S., Fenn, A. M., Pacenta, A. M., Powell, N. D., Sheridan, J. F., & Godbout, J. P. (2012, September). Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice. *Psychoneuroendocrinology*. http://doi.org/10.1016/j.psyneuen.2012.02.003

Yasuoka, S., Kawanokuchi, J., Parajuli, B., Jin, S., Doi, Y., Noda, M., … Suzumura, A. (2011). Production and functions of IL-33 in the central nervous system. *Brain Research*, *1385*, 8–17. http://doi.org/10.1016/j.brainres.2011.02.045

Yu, J.-T., Song, J.-H., Wang, N.-D., Wu, Z.-C., Zhang, Q., Zhang, N., … Tan, L. (2012). Implication of IL-33 gene polymorphism in Chinese patients with Alzheimer's disease. *Neurobiology of Aging*, *33*(5), 1014.e11-4. http://doi.org/10.1016/j.neurobiolaging.2010.07.003

Zalta, A. K., & Shankman, S. A. (2016, March). Conducting Psychopathology Prevention Research in the RDoC Era. *Clinical Psychology : A Publication of the Division of Clinical Psychology of the American Psychological Association*. http://doi.org/10.1111/cpsp.12144

Zhu, C.-B., Blakely, R. D., & Hewlett, W. a. (2006). The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology*, *31*(10), 2121–31. http://doi.org/10.1038/sj.npp.1301029

Zhu, C.-B., Lindler, K. M., Owens, A. W., Daws, L. C., Blakely, R. D., & Hewlett, W. A. (2010). Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(13), 2510–20. http://doi.org/10.1038/npp.2010.116