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Associations Between Peripheral Immune Markers, Neural Response to Stress, and Depressive
Symptoms During Adolescence: The Role of Daily Stressors, Affect, and Sleep Habits

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Psychology

by

Jessica Phuong Uy

2020

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ABSTRACT OF THE DISSERTATION

Associations Between Peripheral Immune Markers, Neural Response to Stress, and Depressive Symptoms During Adolescence: The Role of Daily Stressors, Affect, and Sleep Habits

by

Jessica Phuong Uy

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2020

Professor Adriana Galván, Chair

Adolescence is characterized by marked development in neurobiological, neuroendocrine, and psychosocial domains that are posited to contribute to the increased onset and prevalence of depression and other psychiatric disorders. Emerging research also suggests an association between inflammation and affective symptoms in adolescents; however, the neural correlates that link immune functioning and affective symptoms in adolescents have been relatively understudied. The current dissertation utilized a multi-method approach (daily diary, fMRI, venipuncture samples) to investigate the role of inflammation in modulating neural function of stress/affective circuitry in a sample of adolescents (14-15 years). Results revealed that negative affect and poor sleep (short sleep duration and high sleep variability) moderated the associations between peripheral inflammatory markers and neural activation in stress-related circuitry. Specifically, among adolescents who reported high negative affect and short sleep

duration, greater levels of the pro-inflammatory tumor necrosis factor-alpha (TNF- α) were associated with heightened activation in frontolimbic regions (e.g., amygdala, medial prefrontal cortex [MPFC]) on an fMRI stressor task, which was associated with greater stress-related anxiety and negative appraisals. Among adolescents who exhibited high sleep variability, greater levels of interferon gamma (IFN γ) were associated with lower activation in lateral PFC regions during stress, which was associated with poorer cognitive performance on the stress task. These immune-brain associations were attenuated among those who reported low negative affect, long sleep duration, and low sleep variability, suggesting that negative affect and poor sleep habits may sensitize the brain to peripheral immune signaling. When homeostasis is imbalanced (e.g., insufficient sleep), higher levels of TNF- α and IFN γ may reflect a homeostatic drive to induce sickness-type behaviors/states (e.g., negative affect, increased anxiety, poorer cognition) to restore homeostasis (e.g., promote sleep) via modulation of respective neurocircuitry in adolescents.

The dissertation of Jessica Phuong Uy is approved.

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2020

To my mom, who always encouraged me to follow my heart.

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Chapter 1. Introduction

Adolescence is a unique developmental period of transition from childhood to adulthood during which significant physical maturation occurs. Alongside hormonally-driven physical transformations arise notable changes across psychosocial and cognitive domains (Crone & Dahl, 2012; Steinberg, 2008), brain structures and functions (Casey, Getz, & Galván, 2008; Galván et al., 2006; Giedd et al., 1999; Mills et al., 2016; Sowell et al., 2004), and neuroendocrine systems (Dahl & Gunnar, 2009; Romeo, 2017; Romeo et al., 2014). There is growing evidence that the immune system also undergoes important development during adolescence (Brenhouse & Schwarz, 2016). Yet, the relation between the immune system and brain development and behavior is not clearly understood. Emerging evidence from human adult and animal work implicate the immune system in psychological functioning and well-being. However, it is unclear whether psychoneuroimmunological models in animals and adults translate to adolescent samples. The robust plasticity across these systems during adolescence presents a period of both opportunity for enrichment as well as increased vulnerability. Indeed, a number of psychiatric disorders manifest during adolescence and early adulthood disproportionately more often compared to other developmental stage in the lifespan (Mojtabai, Olfson, & Han, 2016; Substance Abuse and Mental Health Services Administration, 2017). Understanding the interplay between physical and psychological well-being will provide novel perspectives on elucidating the mechanisms of adolescent depression for interventions.

Stress and depressive symptoms

There is a strong association between stress and depressive symptoms across the lifespan (Dean & Keshavan, 2017; Hammen, 2015; Pizzagalli, 2014). Proposed mechanisms that explain this association include dysfunctions in HPA axis function and cortico-limbic circuitry involved

in emotion regulation – particularly characterized by heightened responses to negative events and blunted reactivity to positive events. Indeed, stressful experiences in early life may sensitize cortico-amygdala circuitry to threat, which could potentiate the neuroimmune systems and perpetuate the cycle of vulnerability (Nusslock & Miller, 2016). Regional variation in developmental timing of the cortico-limbic circuitry – that is, greater engagement of limbic systems relative to prefrontal systems and the increasing engagement of prefrontal regulation of limbic systems during adolescence – is a proposed mechanism by which stress in early life increases risk for poorer physical and mental health in adolescence and adulthood. However, depressive symptoms still emerge in adolescents without a history of early adversity, suggesting that stress need not be traumatic or occur early in life to alter the links between cortico-limbic circuitry and depressive symptoms in otherwise healthy adolescents.

Inflammation and depressive symptoms in adolescents

There is an extensive body of literature demonstrating a link between psychological well-being and immunity in adult human and animal work. The comorbidity of major depressive disorder and physical illness and the similarities between depressive symptoms and sickness behavior (e.g., fever, decreased appetite, cognitive dysfunction) have enhanced our understanding and recognition of the bidirectional effects of inflammation and depression (Dantzer, 2001; Hart, 1988; Kelley et al., 2003; Quan & Banks, 2007).

Studies that have examined the associations between immune markers and depression in adolescents have typically focused on group differences in immune markers between individuals with a diagnosis of major depressive disorder (MDD) and/or those who experienced early life stress or adversity (Miller & Cole, 2012). The few studies that have investigated these associations in typically developing youth either only reported data from females who were at

high-risk for depression (Miller & Cole, 2012) or youth under 13 years of age (Caserta, Wyman, Wang, Moynihan, & O'Connor, 2011; Keller, El-Sheikh, Vaughn, & Granger, 2010), which does not reflect the span and variability of adolescence. Moreover, differences in how immune markers were acquired and assayed – through saliva (Keller et al., 2010) and dried blood spots (DBS) (Guan et al., 2016) – make it challenging to compare findings across studies. Recent studies examining depressive symptoms and immune processes during adolescence have found that greater levels of depressive symptoms were associated with greater stress-related increases in circulating inflammatory markers among adolescents with greater adiposity (Chiang, Bower, Irwin, Taylor, & Fuligni, 2017), higher levels of CRP among those with low parental support (Guan et al., 2016), and upregulated expression of inflammation-related genes and downregulated expression of antiviral-related genes (Chiang et al., 2019b). These studies demonstrate that associations between depressive symptoms and immunity can be detected during adolescence and in otherwise healthy adolescents. What is not well elucidated are the neural mechanisms that may mediate the link between inflammation and affective symptoms during adolescence.

Stress and the immune system

Overview of the immune system

The purpose of the immune system is to recognize and defend the organism against invasion from viruses, bacteria, and other antigens. As part of the immune response, immune cells secrete elevated levels of immune molecules, including cytokines and chemokines, which promote an inflammatory response that coordinates a cellular attack against pathogens (Medzhitov, 2008). The immune system is comprised of two interconnected branches: innate immunity and adaptive or acquired immunity. The innate immune response allows a rapid,

robust immune response to a pathogen through highly conserved mechanisms without requiring that the organism has previous exposure to the pathogen. Upon detection of a pathogen, regulatory transcription factors (e.g., nuclear factor- κ B [NF- κ B] and interferon [IFN]) are activated and drive the expression of pro-inflammatory genes (e.g., interleukin-1 [IL-1] and tumor necrosis factor- α [TNF- α]) that produce cytokines, the main contributors of the inflammatory response. Inflammation is a response by which the innate immune cells induce cytokines and chemokines to eradicate pathogens and promote tissue healing. These cytokines are classified as either pro-inflammatory (stimulate immune response; e.g., IL-1 β , IL-6, or TNF- α) or anti-inflammatory (attenuate immune response; e.g., IL-10). If a pathogen survives or evades the action of the innate immune response, the adaptive/acquired immune response becomes activated. In contrast to the non-specific response of innate immunity, adaptive/acquired immunity involves proliferation of memory-based (i.e., previous exposure to specific pathogen) microbial-specific white blood cells (lymphocytes, such as helper T cells, cytotoxic T cells, and B cells) to eliminate microbes. In response to a cellular pathogen (e.g., virus), a subset of T-helper lymphocytes (Th1 cells) produce cytokines, including IFN γ , to promote inflammation and activate macrophages and antigen-specific cytotoxic T cells to lyse the infected cells. Both pro- and anti-inflammatory responses are necessary for proper immune function. Dysregulation of the inflammatory response could lead to low-grade, chronic inflammation, which has implications for the pathogenesis of certain psychiatric and physical illnesses.

The immune system matures and changes throughout the lifespan, thereby differentially impacting the brain and behavior (Ellis, Mouihate, & Pittman, 2005; Levy, 2007; Ortega, Jadeja, & Zhou, 2011). For example, rats that have been challenged neonatally with lipopolysaccharide

(LPS) toxin show suppressed febrile responses, amplified HPA response as adults compared to those who received saline neonatally (Ellis et al., 2005), and increased mRNA expression levels of cytokines in the brain in adulthood (Ortega et al., 2011). Additionally, cytokine secretion of newborn cells is characterized by decreased IFN, decreased production of TNF- α and IL-1 β , and decreased IL-10 (Kollmann, Levy, Montgomery, & Goriely, 2012; Lee et al., 2008). In contrast, older adults evince elevated levels of proinflammatory cytokines and increased immunosenescence (Kollmann et al., 2012). Compared to the known changes in the immune response at the extreme ends of development, less is known about normative changes in the immune system from childhood to young adulthood, and how these changes may modulate stress sensitivity in the neuroimmune system.

Immune response to stress

When confronted with a physical or psychological stressor, a cascade of events occurs to prepare the organism to respond to the stressor, including release of neurotransmitters (e.g., epinephrine and norepinephrine) via the sympathetic nervous system (SNS) and release of hormones along the hypothalamic-pituitary-adrenal (HPA) axis. The rapid SNS response increases gene expression of pro-inflammatory cytokines, presumably to prepare for pathogen removal and wound healing as a result of fight-or-flight (Irwin & Cole, 2011). The slower glucocorticoid response, on the other hand, initially reinforces SNS-mediated pro-inflammatory response, and then releases anti-inflammatory cytokines to attenuate the stress response. These molecules bind to stress-sensitive receptors throughout the brain (or activate immune cells in the brain in the case of cytokines) to direct adaptive physiological and behavioral responses to overcome challenge. Impaired HPA axis function, as is common in situations of chronic stress and stress-related disorders, has consequences for the regulation and effectiveness of the immune

system. For example, a blunted HPA axis response may promote inflammation while excess circulating glucocorticoids may suppress immune function and increase susceptibility to infections (Sternberg, 2006). Therefore, a delicate balance of glucocorticoids is necessary to maintain homeostasis of the immune system. That these systems are undergoing considerable changes during adolescence makes it imperative to take a systems approach and utilize multiple methods across levels of analyses to better characterize adolescent development.

Immune system and the brain

In addition to neural regulation of the immune system, evidence suggests that the immune system also shapes the brain and behavior. Research shows that peripheral immune mediators (including IL-6 and TNF- α) can be transported across the blood brain barrier (BBB) to activate astrocytes and microglia (the macrophages of the brain), which make more cytokines in the brain. Cytokines could also signal the brain indirectly through the vagus nerve (Watkins, Maier, & Goehler, 1995). These signals act on relevant brain regions to modify feeding and sleeping behaviors, cognition, and social interactions, inducing generalized “sickness behaviors” (Banks, 2015; Vitkovic et al., 2000). These behavioral responses to immune activation are conserved across many species and are the mechanism by which our bodies coordinate the brain and behavior during sickness to promote rest and recovery from infection. Hence, neurons are sensitive to the inflammatory signals produced in the periphery and in the brain (Brenhouse & Schwarz, 2016). In rodents, repeated stress increased circulating cytokines and brain macrophages in the parenchyma of the PFC, amygdala, and hippocampus, which explained increases in anxiety behaviors (Wohleb, Powell, Godbout, & Sheridan, 2013). Chronic exposure to glucocorticoids in rats also primed hippocampal microglia to pro-inflammatory stimuli and potentiated the microglial pro-inflammatory response (Frank, Hershman, Weber, Watkins, &

Maier, 2014), suggesting heightened sensitivity of the brain to threat. In humans, peripheral IL-6 was inversely associated with gray matter volume of the hippocampus and medial PFC in middle-aged adults (Marsland, Gianaros, Abramowitch, Manuck, & Hariri, 2008), demonstrating that the hippocampus and PFC are targets of inflammation. The increased neuroplasticity during adolescence might present an opportunity for intervention to reverse the negative effects of stress. For example, rats that were exposed to early life stress via maternal separation as pups and then exposed to enriched environments during adolescence showed reduced cognitive deficits that was mediated by reductions in pro-inflammatory cytokine TNF- α relative to control (do Prado et al., 2016), demonstrating that the neural and cognitive effects of stress is amenable to interventions during adolescence.

Inflammation and the adolescent brain

There are very few studies that have examined the associations between peripheral immune markers and brain structure or function in adolescents. Research in adults has found that endotoxin administration, which elicits an inflammatory response, was associated with heightened neural reactivity to negative social experiences in socioemotional and pain regions compared to placebo, particular in the dorsal anterior cingulate cortex (dACC), anterior insula, and amygdala (Eisenberger, Inagaki, Rameson, Mashal, & Irwin, 2009; Eisenberger, Moieni, Inagaki, Muscatell, & Irwin, 2017; Inagaki, Muscatell, Irwin, Cole, & Eisenberger, 2012; Muscatell et al., 2016). Moreover, those who showed greater increases in pro-inflammatory cytokines in response to the inflammatory challenge showed greater activity in the dACC and anterior insula in response to social exclusion (Eisenberger et al., 2009). These studies suggest that inflammation might sensitize the brain to negative social experiences, including the social evaluative aspect of stress, which has implications for stress-related psychiatric disorders (e.g.,

depression). Therefore, it is possible that adolescents who exhibit higher levels of peripheral pro-inflammatory markers may show greater neural sensitivity to stress. One study that examined the associations between peripheral inflammatory markers and resting-state functional connectivity in African American adults (25 years) and adolescents (13-14 years) found that higher levels of inflammation were associated with lower resting-state functional connectivity in the emotion regulation network in adults and adolescents, with adolescents additionally showing a negative association with the central executive network (Nusslock et al., 2019). These findings suggest that the prefrontal cortex and regulatory processes may also be targets of inflammation during adolescence.

Adolescents' daily affective experiences, sleep habits, and inflammation

Adolescents report experiencing more stress and show heightened and protracted HPA activity in response to stress (Dahl & Gunnar, 2009; Romeo, 2010; Romeo et al., 2014; Stroud et al., 2009), which has implications for prolonging the effects of stress on brain and immune function. Indeed, adolescents who reported having more negative social interactions with friends and family members showed higher levels of CRP (Fuligni, Telzer, Bower, Cole, & Irwin, 2009) and upregulation of inflammation-related genes (Chiang et al., 2019a). Whether daily stress and negative affect alter how the adolescent brain responds to stress, and whether inflammatory markers play a role in sensitizing regions implicated in stress responding, remains unexplored.

Sleep also suffers during adolescence (Carskadon, Vieira, & Acebo, 1993; CDC, 2011; Kann et al., 2014). Using a wide array of sleep manipulations or observations (e.g., experimental partial or total sleep deprivation, naturalistic sleep disturbance, poor sleep efficiency), research shows that poor sleep is associated with increases in inflammatory markers such as CRP, IL-6, and TNF- α (Irwin, 2015; Irwin, Olmstead, & Carroll, 2016). Research examining how

adolescents' sleep habits relate to inflammation have found that shorter sleep duration was associated with higher levels of CRP among young adolescents and that greater variability in sleep duration was associated with higher levels of CRP (Park et al., 2016). Shorter sleep duration was also associated with upregulation of inflammation-related genes and downregulation of antiviral-related genes (Chiang et al., 2019a). Research in adults has shown that poor sleep and acute stress act on similar neural and physiological systems (C. Anderson & Platten, 2011; Balbo, Leproult, & Van Cauter, 2010; Minkel et al., 2014; Spiegel, Leproult, & Van Cauter, 1999; Vgontzas et al., 2004; Yoo, Gujar, Hu, Jolesz, & Walker, 2007). Surprisingly, very few studies have examined these processes together in adolescents. In addition to demonstrating independent associations between daily stress and sleep on inflammation-related gene expression, Chiang et al. (2019a) also found that the association between daily stress and inflammation-related gene expression was exacerbated in the context of shorter sleep duration, suggesting that daily stress and poor sleep, both separately and together, shape inflammatory processes during adolescence. These findings suggest that one way by which insufficient sleep might contribute to increased inflammation may be through sensitizing the brain to stress, which would amplify the body's inflammatory state and compound the immune system's effects on the brain.

What has yet to be investigated are the neural correlates that link daily emotional experiences and sleep habits to inflammatory processes. There is a need to fill this gap in the literature considering growing evidence of heightened response sensitivity to stress and that the brain regions most sensitive to stress (e.g., hippocampus, PFC, amygdala) continue to develop during adolescence (Giedd et al., 1999; Giedd & Rapoport, 2010; Lupien, McEwen, Gunnar, & Heim, 2009; Sowell et al., 2004) and are also targets of poor sleep (Dutil et al., 2018). Animal

research suggests that experience of daily stressors during adolescence (even in the absence of early life stress exposure) has the potential to affect behavior and brain development in limbic and cortical regions (Eiland, Ramroop, Hill, Manley, & McEwen, 2012; Isgor, Kabbaj, Akil, & Watson, 2004; McCormick & Green, 2013; McCormick, Nixon, Thomas, Lowie, & Dyck, 2010), which may increase psychological and biological sensitivity to subsequent stress and contribute to low-grade chronic inflammation, which in turn would continue this positive feedback loop and increase risk for poor health outcomes.

Overview of studies

The overarching goal of my dissertation was to investigate whether and how inflammation (as a proxy for exposure to stress) modulated neural function of stress circuitry during adolescence, with implications for understanding how these processes relate to mental health and well-being. Specifically, I investigated whether and how individual differences in peripheral immune markers related to neural response to stress in adolescents (Study 1), and whether these immune-brain associations could be predicted by or moderated by adolescents' daily experiences of stressors (Study 2) and sleep habits (Study 3).

The proposed studies employed self-reported measures of adolescents' depressive symptoms and daily experiences, a multiplex assay of immune cytokines obtained via venipuncture, and a functional magnetic resonance imaging (fMRI)-adapted laboratory stressor to investigate how the adolescent brain experiences acute stress.

Study 1 examined the associations between peripheral immune markers, neural response to stress, and depressive symptoms in healthy adolescents (14-15 years). Most studies of stress during childhood and adolescence have been limited to examining the SNS and HPA stress systems. My dissertation focused on immune markers because of the emergent significance of

the immune system in neurodevelopment and mental health in addition to the general scarcity of neuroscientific studies related to immune markers in humans. The current study used venipuncture draws to assess plasma levels of immune markers, which has been the method by which a majority of studies collect immune information in humans. The current study also used a multiplex immunoassay, which provided information on the concentrations of multiple cytokines – pro- and anti-inflammatory – to inform better speculation of the mechanisms involved. The use of the multiplex immunoassay extends from current studies that primarily report on pro-inflammatory cytokines IL-6 and C-reactive protein (CRP). To assess depressive symptoms, I utilized adolescents' self-report of depressive symptoms on the Center for Epidemiological Studies Depression (CES-D) Scale. To assess neural response to stress, I used a modified version of the Montreal Imaging Stress Task (MIST) paradigm (Dedovic et al., 2005), an fMRI-adapted lab stressor. On the MIST, participants perform arithmetic problems under a non-evaluative “practice” condition and under a challenging and evaluative “test” condition, which contains both performance and social evaluative aspects of a robust stressor (Dickerson & Kemeny, 2004). Previous research on the MIST has found an increase in salivary cortisol levels in the stress relative to control condition (Dedovic et al., 2005) as well as greater activation in stress-related circuitry (e.g., cingulate, thalamus, insula, lateral and medial aspects of prefrontal cortex) in adults (Dedovic et al., 2005; Ming et al., 2017) and adolescents (Strang, Pruessner, & Pollak, 2011). Moreover, greater activation in ventromedial and dorsolateral PFC regions in response to challenge was associated with smaller increases in salivary cortisol and lower depression scores in adults (Ming et al., 2017), implicating the role of the PFC in stress regulation. The current study extends from previous research to assess the role of peripheral immune markers in stress-related circuitry during adolescence.

Study 2 investigated how adolescents' experiences of daily stressors, daily negative affect, and stressor reactivity (i.e., affective responses to daily stressors) related to peripheral immune markers and neural response to stress. Participants were asked, each night for 7 days, to indicate on a checklist whether they experienced stressful demands from various sources and arguments with various people in their lives. Participants also rated how stressful they perceived the stressors to be, if any were indicated, and reported on their current experience of positive and negative affect. I investigated whether adolescents who 1) reported more stressors, 2) experienced greater negative affect, and/or 3) showed greater stressor reactivity (i.e., stronger correlation between number of daily stressors and negative affect) exhibited greater levels of peripheral inflammation and/or heightened neural response to stress, and explored mediating and moderating effects of these variables. The use of daily diary in this study captures variability in adolescents' daily experiences and allows investigation of how the type of experiences and variability in those experiences in daily life relate to immune and neural outcomes.

Study 3 investigated how adolescents' average sleep duration and variability in sleep duration across the week related to peripheral immune markers and neural response to stress. Adolescents reported daily sleep duration for 7 days prior to coming into the lab for a blood draw and performing the fMRI stressor task in the scanner. I examined how adolescents' sleep habits related to functional activation when performing the fMRI stressor task and explored whether inflammation was a mediating factor of sleep-related differences in stress response (if any) in adolescents and/or whether adolescents sleep habits moderated the association between inflammation and neural response to stress.

Chapter 2. Corticolimbic circuitry activation during stress relates to stress-related performance and anxiety in adolescents

There is a significant increase in the onset and prevalence of depression during adolescence (Mojtabai et al., 2016; Substance Abuse and Mental Health Services Administration, 2017). Notable changes in brain structure and function and neuroendocrine systems during adolescence have been posited to underlie the onset of depression. Recent evidence from human adult and animal work also implicate the immune system in psychological functioning and well-being. However, the associations between the immune system, brain function, and behavior during development are not clearly understood. Understanding the interplay between physical and psychological well-being will provide novel perspectives on elucidating the mechanisms of adolescent depression for interventions.

There is a strong association between stress and depressive symptoms (Elovainio et al., 2012; McLaughlin et al., 2012; Miller & Cole, 2012). Stress is also highly correlated with physical illnesses such as metabolic syndrome, coronary heart disease, and certain cancers (Braveman, Cubbin, Egerter, Williams, & Pamuk, 2010; Danese & McEwen, 2012; Danese & Tan, 2014). Indeed, many people who have depression are likely to have concomitant physical illnesses (R. J. Anderson, Freedland, & Lustman, 2001; Carney et al., 1988; McDaniel, Musselman, Porter, Reed, & Nemeroff, 1995). Inflammatory processes have been hypothesized to be a key mechanism explaining the links between, stress, depressive symptoms, and physical illnesses (Hiles, Baker, de Malmanche, & Attia, 2012; Mitchell & Goldstein, 2014; Slavich & Irwin, 2014). Because immune processes interact with endocrine processes that are in flux during adolescence, understanding the role of the immune system during this sensitive period of

development has implications for reducing the risk of stress-related psychological and physical illnesses in adulthood.

Research demonstrates that inflammatory markers are elevated in adolescents with major depressive disorder (MDD) compared to healthy controls (Gabbay et al., 2009; Henje Blom et al., 2012), which was positively correlated with self-reported anxiety and depressive symptoms (Henje Blom et al., 2012). Post-mortem samples of adolescents who completed suicide showed greater mRNA and protein expression levels of IL-1 β and TNF- α in PFC compared to controls (Pandey et al., 2012). Moreover, within individuals, higher levels of inflammation (IL-6 and CRP) were present during major depressive episodes relative to euthymic periods, with elevated CRP persisting over 6 months and elevated IL-6 predicting future major depressive episode (Miller & Cole, 2012).

In the few studies that have examined inflammatory markers in healthy youth without clinical depression, greater levels of salivary IL-6 were associated with adjustments problems in children (mean age = 9.85 years) (Keller et al., 2010) whereas higher perceived self-efficacy was associated with lower plasma IL-6 concentrations in 7-13 year-olds (Caserta et al., 2011). Recent studies examining depressive symptoms and immune processes during adolescence have found that greater levels of depressive symptoms were associated with greater stress-related increases in circulating inflammatory markers among adolescents with greater adiposity (Chiang et al., 2017), higher levels of CRP among those with low parental support (Guan et al., 2016), and upregulated expression of inflammation-related genes and downregulated expression of antiviral-related genes (Chiang et al., 2019b). Taken together, these findings demonstrate associations between inflammatory markers and indicators of depressive symptoms or distress among otherwise healthy youth.

Extant research on immune-to-brain signaling in humans demonstrated that, compared to placebo, inflammatory challenge was associated with heightened neural reactivity to negative social experiences in regions implicated in socioemotional and pain processing, particular in the dorsal anterior cingulate cortex (dACC), anterior insula, amygdala, and MPFC (Eisenberger et al., 2009, 2017; Inagaki et al., 2012; Muscatell et al., 2016). Moreover, those who showed greater increases in pro-inflammatory cytokines in response to the inflammatory challenge showed greater activity in the dACC and anterior insula in response to social exclusion (Eisenberger et al., 2009) and increased depressed mood (Eisenberger, Inagaki, Mashal, & Irwin, 2010; Moieni et al., 2015; Reichenberg et al., 2001). These studies suggest that inflammation might sensitize the brain to negative social experiences, including the social evaluative aspect of stress, which has implications for stress-related psychiatric disorders. In addition to heightened threat sensitivity, peripheral immune markers have also been shown to influence PFC structure and associated functioning. For example, inflammation has been associated with smaller MPFC volume (Marsland et al., 2008) and reduced subgenual cingulate cortex connectivity to amygdala, MPFC, and nucleus accumbens in response to an affective processing task in adults (Harrison et al., 2009). Though not discussed further in the current study, inflammatory processes have also been shown to attenuate reward-related processes that underlie feelings of anhedonia (reviewed in Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Eisenberger et al., 2017; Nusslock & Miller, 2016). A meta-analysis on the associations between peripheral immune markers and brain function in adults revealed that inflammatory markers showed consistent effects in limbic and basal ganglia regions (amygdala, hippocampus, striatum, thalamus), brainstem regions, cortical regions (ACC, DMPFC, VMPFC, OFC, insula), and temporal regions (Kraynak, Marsland, Wager, & Gianaros, 2018). Given the protracted

development of the PFC into adulthood, the implications of inflammatory processes sensitizing an already sensitive limbic system in conjunction with diminishing function of a developing regulatory system for mental and physical health warrants further research in adolescents. However, there are virtually no studies, to my knowledge, that have investigated the associations between inflammatory processes and frontolimbic circuitry function in adolescents. One study that examined the associations between peripheral inflammatory markers and resting-state functional connectivity in African American adolescents (13-14 years) found that higher levels of inflammation were associated with lower resting-state functional connectivity in the emotional regulation and central executive networks (Nusslock et al., 2019), suggesting that the PFC and regulatory processes may be targets of inflammation during adolescence.

Although it is unknown if peripheral immune markers have differential associations with the brain during adolescence, peripheral changes in the modulators of the immune system (e.g., sex hormones, HPA axis) and substantial neural remodeling during adolescence suggest that differential effects are likely. That is, it is possible that inflammatory challenges during adolescence can have differential effects on brain development compared to those experienced earlier or later in development (Schwarz & Bilbo, 2013). Additional research is necessary to advance our understanding of the bidirectional effects of immune and brain development during adolescence.

The goal of the current study was to characterize the associations between peripheral immune markers, depressive symptoms, and neural reactivity to a stressor in adolescents (14-15 years). This age range represents a time when MDD prevalence and stress reactivity is increasing (Dahl & Gunnar, 2009; Romeo, 2010; Romeo et al., 2014; Stroud et al., 2009; Substance Abuse and Mental Health Services Administration, 2017). The current study utilized a) venipuncture

draws and multiplex immunoassays to assess plasma levels of multiple peripheral immune markers, b) a well-validated self-report measure of depression (CES-D) to assess depressive symptoms in adolescents, and c) a well-validated fMRI stressor task (Dedovic et al., 2005) to assess neural reactivity to stress. I hypothesized that higher levels of peripheral pro-inflammatory markers (e.g., IL-6, TNF- α) would be associated with heightened neural response to stress in regions previously shown to respond to stress (e.g., anterior insula, anterior cingulate cortex) and/or diminished response in prefrontal regions, which would be associated with higher levels of self-reported depressive symptoms.

Methods

Participants

Self-report questionnaires, daily diary (used in studies 2 and 3), and neuroimaging data were collected from 40 adolescents (14.03-15.99 years, $M = 15.076$, $SD = 0.646$, 17 females) who participated in a larger study conducted by Drs. Galván, Fuligni, and Eisenberger (NSF BSC 1551952). Participants were recruited using flyers posted on university campus, in local child and adolescent-friendly locations, on community websites (e.g., Craigslist), and flyer distributions at local high schools. Inclusion criteria required all participants be right-handed, free from metal objects in the body, speak fluent English, be in the appropriate age range, and have no previously diagnosed psychiatric, neurological, or developmental disorders. Parents of adolescent participants provided written consent and adolescents provided assent in accordance with the University of California, Los Angeles (UCLA) Institutional Review Board. Participants were also provided the opportunity to consent to an optional blood draw. All participants were compensated for their participation.

Of the 40 participants, one participant was excluded from neuroimaging analyses due to a neuroanatomical abnormality and two participants were excluded for excessive motion across both runs of the task. Of the remaining 37 (18 females) participants with usable neuroimaging data, 23 (62%; 9 females) participated in the blood draw. Four out of the 37 participants (which included two participants with blood data) did not complete measures of depressive symptoms. Analyses were conducted with the maximum number of subjects for each analysis.

Procedure

Participants completed two visits at UCLA (Figure 2.1). During the first visit, after providing consent, participants completed questionnaires about demographic information and depressive symptoms and were trained on how to complete the daily diary measures. For 7 days after the first visit, participants received a text message each evening with a URL to an online survey asking about their day that they completed. After 7 days (but within two weeks), participants returned to UCLA to complete their second visit. Participants who consented to the blood draw had their blood drawn by a certified phlebotomist at the clinical lab in the Peter Morton Medical Building at UCLA. After the blood draw, participants completed a brain scan while performing the fMRI stressor task at the Center for Cognitive Neuroscience (CCN) at UCLA. Participants who did not consent to the blood draw only completed the brain scan portion of the study. Participants' height and weight were measured to calculate body mass index (BMI). BMI ranged from 14.337 to 45.154 ($M = 23.298$, $SD = 6.299$). After the brain scan, participants completed additional questionnaires about their experiences regarding the stressor task, were debriefed about the goals of the study, and received compensation.

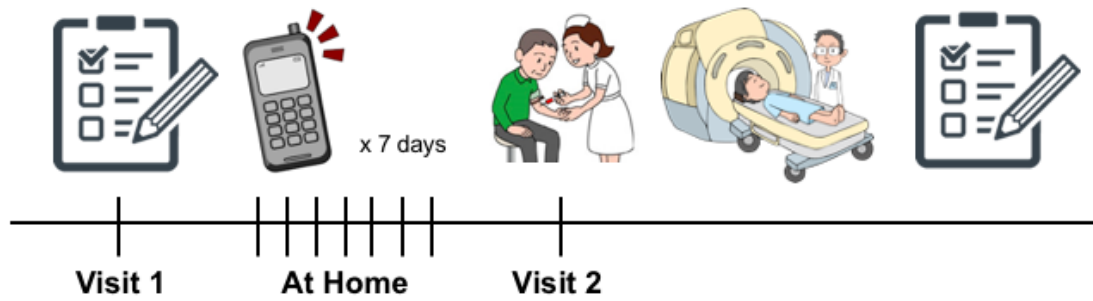


Figure 2.1. Schematic of experimental procedure.

Measures

Center for Epidemiological Studies – Depression (CES-D) Scale. Participants rated how often they felt or experienced 20 items that are indicative of depressive symptoms (e.g., “I felt that everything I did was an effort”, “I talked less than usual”, “I had crying spells”) on a 4-point scale (0 = rarely or none of the time, 3 = most or all of the time). Items on this scale were summed to create a composite score for each individual. CES-D scores of 16 or higher suggest clinical levels of depression. CES-D scores for our sample ranged from 2 to 51 ($M = 13.06$, $SD = 9.069$). Eight (24%) participants reported CES-D scores of 16 or higher. CES-D scores did not differ by gender, $t(30) = -1.565$, $p = .128$.

State Trait Anxiety Inventory (STAI). After the fMRI Stressor Task (described below), participants were asked to indicate to what extent (1 = not at all, 4 = very much so) they experienced 15 items relating to positive and negative affect and psychosomatic symptoms during the test trials of the task (e.g., “I felt calm”, “My heart was beating fast”, “I felt nervous”). After reverse-coding positive items, items on this scale were summed to create an index of task-related anxiety. Higher scores indicate greater anxiety symptoms. STAI scores ranged from 16 to 41 ($M = 26.54$, $SD = 5.615$).

Perceived Task Difficulty and Self-Rated Performance. After the fMRI Stressor Task, participants were asked to indicate how much control they felt they had during the test, how evaluated, effortful, challenging, threatening, and difficult they found the test trials of the task to be (1 = not at all, 7 = very much so) as well as how well they thought they did on the test overall (1 = not well at all, 7 = very well). Items were averaged to create an index of perceived task stressfulness. Higher scores indicate greater stress. Scores ranged from 2.833 to 5.667 (M = 4.338, SD = .8045) for task stressfulness. Participants self-rated performance on the test ranged from 1 to 7 (M = 3.32, SD = 1.415).

Immunological Measures

Blood samples were collected in EDTA tubes. After collection, samples were centrifuged at 4°C, plasma were harvested into multiple aliquots, and stored in a -80°C freezer until all blood samples for the study have been collected. All plasma samples from a single subject were assayed together on the same 96-well plate to minimize effects of inter-assay variation. All samples were assayed in duplicate and an internal quality control sample was included on every plate. IL-6, IL-8, IL-10, TNF- α , and IFN γ were measured in a multiplex assay utilizing a V-PLEX Custom Human Cytokine Proinflammatory Panel on the Meso Scale Discovery (MSD) electrochemiluminescence platform (MSD, Rockville, MD). Samples were assayed at a 2-fold dilution according to the manufacturer's protocol, with an eight-point standard curve with tripling dilutions. Analyte-specific lower limits were calculated for each assay plate (IL-6: 0.21 pg/mL, IL-8: 0.17 pg/mL, IL-10: 0.11 pg/mL, TNF- α : 0.11 pg/mL, IFN γ : .42 pg/mL). For all plasma biomarkers, inter-assay coefficients of variation were less than or equal to 10% and mean intra-assay coefficients of variation were less than 6.5%.

After excluding one subject with an acute viral infection and extreme value on IFN γ (40.49 pg/mL), values for immune markers were natural log-transformed to correct for non-normality. Descriptive statistics and bivariate correlations between immune markers and BMI are displayed in Table 2.1. Levels of immune markers did not differ by gender (p 's > .26).

| | M (SD) | TNF-α | IL-8 | IL-10 | IFNγ | BMI |
|--------------------------------|---------------|--------------------------------|-------------|--------------|-------------------------------|------------|
| IL-6 | 0.591 (.704) | .140 | -.037 | .131 | .247 | .719** |
| TNF-α | 2.031 (.329) | | .140 | .160 | .073 | -.123 |
| IL-8 | 3.959 (3.555) | | | -.272 | -.208 | .007 |
| IL-10 | 0.264 (.0873) | | | | .368 | -.074 |
| IFNγ | 5.066 (2.561) | | | | | .136 |
| BMI | 23.30 (6.39) | | | | | |

Table 2.1. Bivariate correlations between peripheral immune markers and BMI. Descriptive statistics are presented in raw values. Correlations were conducted using natural log-transformed values. ** $p < .01$

fMRI

fMRI Stressor Task. The current study used a modified version of the well-validated Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005) task. On the stressor task, participants were asked to perform a series of mental arithmetic challenges that have social evaluative components integrated into the task (Figure 2.2). To assess the effects of stress, the stressor task consisted of 2 experimental conditions (practice and test) that were presented in an alternating block design. In the practice condition, participants completed a series of easy mental arithmetic problems on the computer screen. Each series or block contained 6 trials. On practice trials, easy arithmetic problems with no answer choices were shown. Participants were given 5 seconds to solve each problem and were told to press 1 once they mentally solved each problem.

In the test condition, participants completed a series of challenging mental arithmetic problems on the computer screen. Each series or block contained 6 trials. On test trials, challenging arithmetic problems with 4 possible answer choices were presented to participants and they had 5 seconds to choose the correct answer before time ran out. The difficulty of the problems in the test condition were chosen to be just slightly beyond individuals' mental capacity to solve within the time limit, though it is possible to solve the problems within the time limit. After each arithmetic problem in the test condition, participants were shown feedback on their performance (i.e., "correct", "incorrect", or "out of time" if participants did not choose an answer in time). At the end of each test block, participants were shown a rating scale of their performance relative to that of their peers to increase the social evaluative threat of the task. This performance evaluation rating was manipulated by the experimenters such that the participants' performance evaluation rating was declining over time and at a faster rate than that of their peers. Participants were told that the performance rating takes into account their accuracy and speed on the test trials to circumvent suspicion of deception in those who may have greater accuracy. After each experimental block, participants were asked to rate their stress levels (1 = not at all stressful, 4 = very stressful). Participants performed 4 practice blocks and 4 test blocks that alternated in sequence.

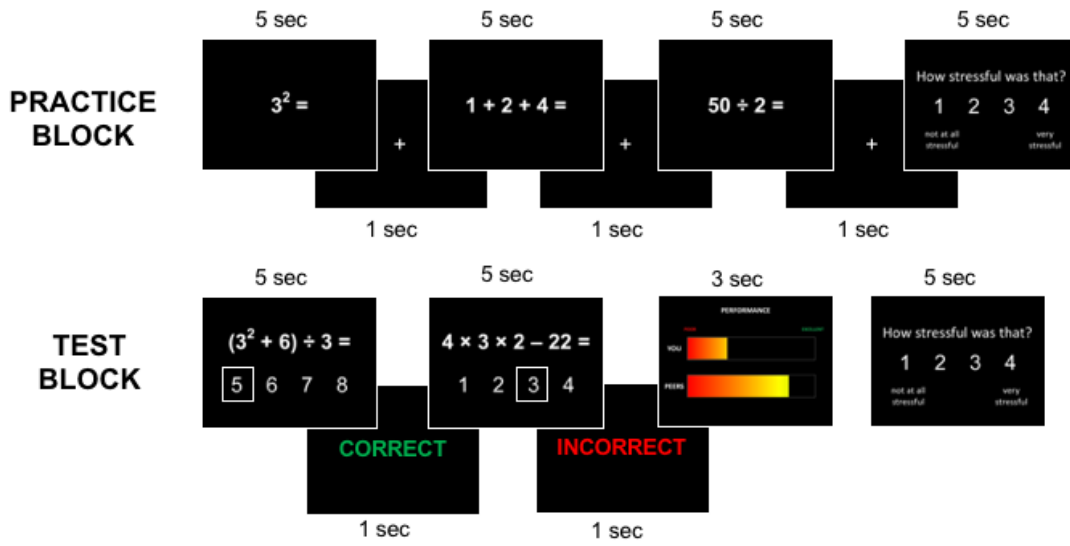


Figure 2.2. Diagram of the fMRI Stressor Task.

One of the goals of the larger study was to assess the effect of stress on giving behavior. As such, the stressor task was modified to include blocks of a Dictator-type game where participants were asked to make decisions to accept or reject certain monetary offers. The decision blocks occurred after each alternating practice and test block. A rest period of 10 seconds occurred between each decision block and the subsequent practice or test block where participants looked at a static computer screen on which no tasks were shown. Decision blocks were modeled but not analyzed in the current study. Participants completed two functional runs of approximately 8 minutes each.

fMRI Data Acquisition. Functional imaging data were collected on a 3 Tesla Siemens Magnetom Prisma MRI scanner with a 20-channel head coil using a gradient-echo, echo-planar image (EPI) sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90 degrees, FOV = 192 mm, 260 volumes, 34 slices, slice thickness = 4 mm). A T2-weighted, matched bandwidth (MBW), high-

resolution anatomical scan (TR = 5000ms, TE = 35ms, FOV = 192mm, flip angle = 90 degrees, 34 slices, slice thickness = 4.0 mm) and magnetization-prepared rapid-acquisition gradient echo (MPRAGE) scan were acquired for registration purposes (TR = 2000 ms, TE = 2.52 ms, FOV = 256 mm, matrix =, sagittal plane, slice thickness = 1 mm, 192 slices).

fMRI Preprocessing. Preprocessing and statistical analyses were performed using FMRIB's Software Library (FSL) 5.0.9. Preprocessing included motion correction, non-brain matter removal using FSL brain extraction tool (BET), spatial smoothing (5mm FWHM Gaussian kernel) to increase the signal-to-noise ratio and filtered in the temporal domain using a nonlinear high-pass filter (100s). Images with greater than 10% of TRs indicating framewise displacement > .9 mm were excluded from analyses. EPI images were registered to the MBW scan, then to the MPRAGE scan, and finally into standard Montreal Neurological Institute (MNI) space (MNI152, T1 2mm) using linear registration with FSL FMRIB's Linear Image Registration Tool (FLIRT).

Analytic Plan

All reported analyses covaried for gender. All analyses consisting of peripheral immune markers additionally covaried for body mass index (BMI).

Regression analyses were used to relate participants' levels of peripheral immune markers to depressive symptoms, controlling for gender and BMI.

Behavioral Analysis of Stressor Task. Repeated-measures analyses of covariance (ANCOVAs) were used to test the effect of stress condition (practice vs. test blocks) on stress ratings to confirm that participants indeed found the test blocks to be more stressful than the practice blocks. Response time to solve arithmetic problems between stress conditions were also compared to confirm that test trials were more challenging than practice trials. Additional

ANCOVAs and regression analyses were conducted to determine whether stress ratings, behavior on stressor task, participants' responses to the test-related STAI, and post-task questionnaire differed by depressive symptoms and peripheral immune markers.

fMRI Data Analysis. Imaging data were modeled using a block design. General linear models (GLM) with multiple explanatory variables (regressors) were used for fMRI analyses. For each run, 4 explanatory variables were modeled: 1) practice blocks; 2) test blocks; 3) decision blocks; 4) instruction and stress rating screens. Each explanatory variable was convolved with a canonical double-gamma hemodynamic response function (HRF). Onset time for practice and test blocks were defined as the onset of the first arithmetic problem in each block. Onset time for decision blocks was defined as the onset of the first decision trial. Offset time for practice blocks was defined as the offset of the last arithmetic problem in the practice block. Offset time for each test block was defined as the offset of the performance rating screen (Inagaki et al., 2016). Offset time for decision blocks was defined as the offset of the last decision trial. The duration of each block was the duration between each blocks' respective onset and offset times. "Rest" screens were not explicitly modeled and therefore served as an implicit baseline.

Analyses focused on the contrast between test blocks and practice blocks (Test > Practice, Practice > Test). A fixed effects voxel-wise analysis combined each of the two runs at the second level. Regression analyses were conducted at the group level using the FMRIB local analysis of mixed effects (FLAME1) module in FSL with mean-centered regressors of interest (e.g., peripheral immune markers, depressive symptoms) entered in each respective model in whole brain analyses. Z (Gaussianized T) statistic images were thresholded at $Z > 2.3$ (unless otherwise noted) by a corrected cluster significant threshold of $p < .05$ using Gaussian Random

Field theory and corrected for family-wise errors. Anatomical localization within each cluster were obtained by searching within maximum likelihood regions from the FSL Harvard-Oxford probabilistic atlas.

Region-of-interest (ROI) Analyses. In addition to whole-brain analyses, ROI analyses were also conducted in regions previously implicated in stress reactivity and regulation (e.g., dACC, left and right anterior insula, left and right amygdala, and left and right hippocampus). dACC and bilateral anterior insula ROIs were structurally defined using the Automated Anatomical Labeling (ALL) atlas. The dACC ROI combined Brodmann Areas 32 and 25 and used a rostral boundary of $y = 36$ and a caudal boundary of $y = 0$ (Dedovic, Slavich, Muscatell, Irwin, & Eisenberger, 2016). The anterior insula ROIs were constructed by dividing the AAL insula ROI at $y = 0$, approximately separating dysgranular and granular insula (Slavich, Way, Eisenberger, & Taylor, 2010). Amygdala and hippocampus ROIs were anatomically defined using the FSL Harvard-Oxford probabilistic atlas and thresholded at 50% (Figure 2.3).

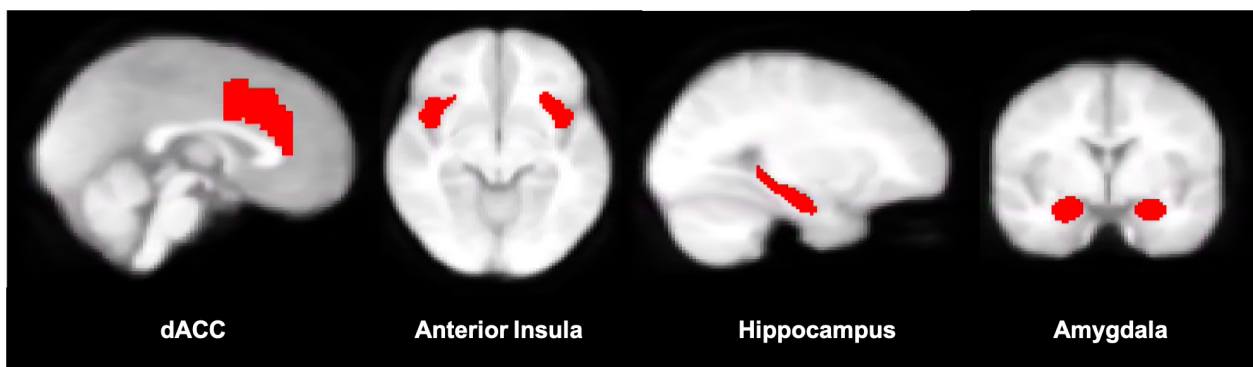


Figure 2.3. ROIs for ROI analyses

Results

Depressive symptoms and inflammation

Controlling for gender and BMI, self-reported depressive symptoms were not associated with levels of TNF- α ($B = -5.684$, $SE = 9.876$, $t(16) = -.575$, $p = .573$), IFN γ ($B = -2.005$, $SE = 3.977$, $t(16) = -.504$, $p = .621$), IL-10 ($B = -4.444$, $SE = 4.815$, $t(16) = -.923$, $p = .370$), IL-8 ($B = 4.505$, $SE = 2.320$, $t(16) = 1.941$, $p = .070$), or IL-6 ($B = -1.969$, $SE = 2.705$, $t(16) = -.728$, $p = .477$).

Behavior on fMRI Stressor Task

Stress ratings. Controlling for gender, repeated measures ANCOVA revealed that, on average, participants rated the test block ($M = 2.899$, $SD = .644$) as more stressful than the practice block ($M = 1.578$, $SD = .618$), $F(1, 35) = 171.809$, $p < .001$ (Figure 2.4A). Differences in stress ratings did not differ by depressive symptoms ($F(1, 30) < .001$, $p = .987$), though adolescents who reported greater depressive symptoms reported higher stress ratings overall ($F(1, 30) = 7.774$, $p = .009$). Controlling for gender and BMI, differences in stress ratings did not differ by levels of TNF- α ($F(1, 18) = .477$, $p = .499$), IFN γ ($F(1, 18) = 1.175$, $p = .293$), IL-8 ($F(1, 18) = .120$, $p = .733$), or IL-6 ($F(1, 18) = .070$, $p = .794$). Controlling for gender and BMI, adolescents with higher levels of IL-10 showed smaller differences in stress ratings between test and practice ($F(1, 18) = 4.667$, $p = .044$); however, simple slopes between IL-10 and stress ratings were not significant (practice: $B = .264$, $SE = .281$, $t(18) = .940$, $p = .360$; test: $B = -.632$, $SE = .434$, $t(18) = -1.456$, $p = .163$).

Response time. On average, controlling for gender, participants took longer to respond to test problems ($M = 3.535$ seconds, $SD = .399$) than practice problems ($M = 2.054$ seconds, $SD = .531$), $F(1, 33) = 376.046$, $p < .001$ (Figure 2.4B). Controlling for gender, response time

difference did not differ by depressive symptoms ($F(1, 28) = .850, p = .364$). Controlling for gender and BMI, response time difference did not differ by levels of TNF- α ($F(1, 18) = .142, p = .711$), IFN γ ($F(1, 18) = .045, p = .834$), IL-10 ($F(1, 18) = 1.455, p = .243$), IL-8 ($F(1, 18) = .490, p = .493$), or IL-6 ($F(1, 18) = 2.656, p = .121$).

Test Accuracy. Accuracy on test problems ranged from 0% to 87.5% ($M = 44.6\%$, $SD = 19.79\%$). Controlling for gender, adolescents who reported higher stress ratings on the test had lower test accuracy ($B = -.123, SE = .048, t(34) = -2.536, p = .016$). Adolescents who responded faster on test trials ($B = -.192, SE = .079, t(32) = -2.149, p = .021$) and practice trials ($B = -.215, SE = .051, t(32) = -4.197, p < .001$) had greater accuracy on the test. Controlling for gender, test accuracy did not differ by depressive symptoms ($B = -.002, SE = .004, t(30) = -.483, p = .632$). Controlling for gender and BMI, test accuracy did not differ by levels of TNF- α ($B = .162, SE = .268, t(18) = .604, p = .553$), IFN γ ($B = .068, SE = .100, t(18) = .679, p = .506$), IL-10 ($B = -.008, SE = .131, t(18) = -.057, p = .955$), IL-8 ($B = .074, SE = .068, t(18) = 1.082, p = .293$), IL-6 ($B = -.063, SE = .074, t(18) = -.857, p = .403$).

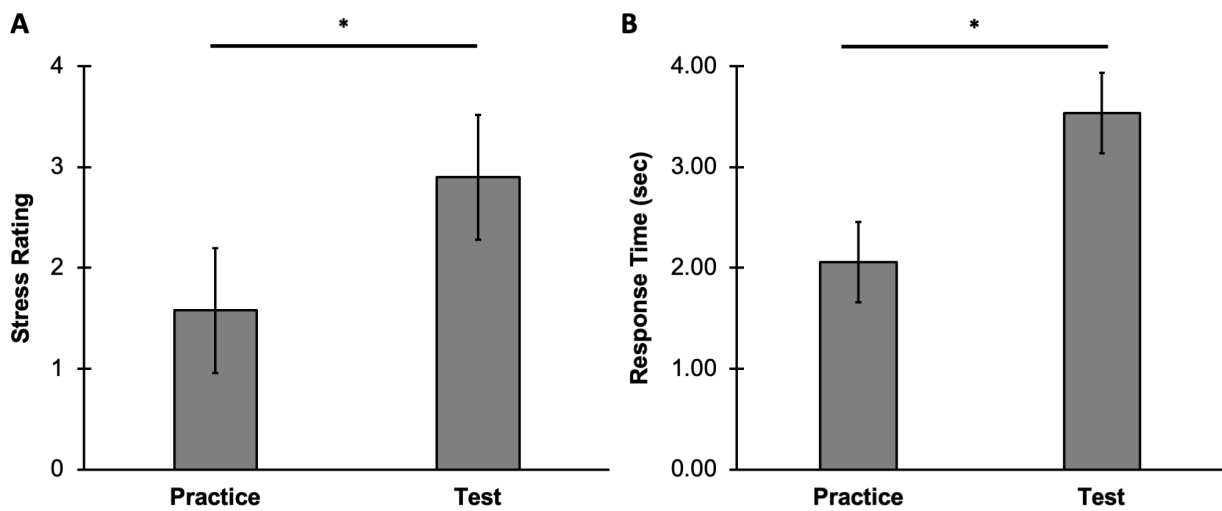


Figure 2.4. Stress ratings (A) and response times (B) as a function of task condition. * $p < .05$

Evaluation of fMRI Stressor Task

Anxiety. Anxiety symptoms were not related to test accuracy ($B = -.005$, $SE = .006$, $t(34) = -.782$, $p = .439$) or response times (practice RT: $B = .754$, $SE = 1.811$, $t(32) = .416$, $p = .680$; test RT: $B = 1.266$, $SE = 2.454$, $t(32) = .516$, $p = .610$). However, adolescents who endorsed higher stress ratings on the test also reported greater test-related anxiety ($B = 5.292$, $SE = 1.200$, $t(34) = 4.411$, $p < .001$). Self-reported feelings of anxiety were not associated with depressive symptoms ($B = .152$, $SE = .115$, $t(30) = 1.319$, $p = .197$). Controlling for gender and BMI, test-related anxiety symptoms were not associated with levels of TNF- α ($B = 5.918$, $SE = 6.202$, $t(18) = .954$, $p = .353$), IFN γ ($B = 1.756$, $SE = 2.336$, $t(18) = .752$, $p = .462$), IL-10 ($B = -3.847$, $SE = 2.944$, $t(18) = -1.307$, $p = .208$), IL-8 ($B = .036$, $SE = 1.656$, $t(18) = .021$, $p = .983$), IL-6 ($B = .498$, $SE = 1.759$, $t(18) = .283$, $p = .780$).

Perceived Task Difficulty. Adolescents who thought the test trials were more difficult/challenging were less accurate on the test ($B = -.081$, $SE = .039$, $t(34) = -2.098$, $p = .043$), endorsed higher stress ratings on the test ($B = .484$, $SE = .205$, $t(34) = 2.357$, $p = .024$), and reported greater task-related anxiety ($B = .083$, $SE = .021$, $t(34) = 3.974$, $p < .001$). Perceived task stressfulness was not associated with depressive symptoms ($B = .002$, $SE = .017$, $t(30) = .146$, $p = .885$). Controlling for gender and BMI, perceived task stressfulness was not associated with levels of TNF- α ($B = -1.027$, $SE = 1.052$, $t(18) = -.975$, $p = .342$), IFN γ ($B = -.207$, $SE = .400$, $t(18) = -.517$, $p = .612$), IL-10 ($B = -.223$, $SE = .521$, $t(18) = -.429$, $p = .673$), IL-8 ($B = -.099$, $SE = .280$, $t(18) = -.353$, $p = .728$), IL-6 ($B = .052$, $SE = .299$, $t(18) = .175$, $p = .863$).

Self-Rated Performance. Adolescents who reported greater test-related anxiety ($B = -.148$, $SE = .035$, $t(34) = -4.172$, $p < .001$) and greater perceived test difficulty ($B = -.818$, $SE = .260$, $t(34) = -3.143$, $p = .003$) thought that they performed worse on the test. However, self-rated performance was not related to test accuracy ($B = .012$, $SE = .024$, $t(34) = .515$, $p = .610$). Self-rated performance on the math test did not differ by depressive symptoms ($B = -.053$, $SE = .028$, $t(30) = -1.872$, $p = .071$). Controlling for gender and BMI, self-reported performance was not associated with levels of TNF- α ($B = -.720$, $SE = 1.706$, $t(18) = -.422$, $p = .678$), IFN γ ($B = -.696$, $SE = .619$, $t(18) = -1.124$, $p = .276$), IL-10 ($B = -.339$, $SE = .827$, $t(18) = -.410$, $p = .687$), IL-8 ($B = .308$, $SE = .441$, $t(18) = .699$, $p = .494$), IL-6 ($B = .157$, $SE = .474$, $t(17) = .331$, $p = .745$).

Main effects of fMRI Stressor Task

On average, participants engaged lateral prefrontal regions (dorsolateral prefrontal cortex, middle frontal gyrus, inferior frontal gyrus), anterior cingulate gyrus, anterior insula, orbitofrontal cortex, thalamus, and visual cortex more during test blocks compared to practice

blocks (Test > Practice) (Figure 2.5, Table 2.2). In contrast, participants engaged medial prefrontal regions (left frontal pole, right ventromedial prefrontal cortex), left amygdala, left hippocampus, right posterior insula, right posterior cingulate gyrus, left angular gyrus, and left temporal pole more during practice than test blocks (Practice > Test) (Figure 2.6, Table 2.2). One sample t-tests of activation in ROIs during Test > Practice revealed that participants engaged dACC ($M = .03533$, $SD = .0376$, $t(35) = 5.639$, $p < .001$) and bilateral anterior insula (left: $M = .0281$, $SD = .0385$, $t(35) = 4.375$, $p < .001$; right: $M = .0269$, $SD = .045$, $t(35) = 2.581$, $p = .001$) more during test relative to practice block. In contrast, participants engaged bilateral amygdala (left: $M = -.04055$, $SD = .0503$, $t(35) = -4.839$, $p < .001$; right: $M = -.0317$, $SD = .0518$, $t(35) = -3.672$, $p = .001$) and bilateral hippocampus (left: $M = -.0240$, $SD = .0348$, $t(35) = -4.137$, $p < .001$; right: $M = -.0195$, $SD = .0352$, $t(35) = -3.324$, $p = .002$) more during practice relative to test blocks (Figure 2.7).

Regression analyses were conducted to determine whether activation in ROIs (dACC, bilateral anterior insula, bilateral amygdala, bilateral hippocampus) were associated with task behavior and evaluation. Controlling for gender, analyses revealed that greater dACC activation during Test > Practice was marginally associated with greater test accuracy ($B = 1.658$, $SE = .850$, $t(33) = 2.1.951$, $p = .060$). Greater bilateral anterior insula activation during Test > Practice were associated with greater test accuracy (left: $B = 2.065$, $SE = .819$, $t(33) = 2.521$, $p = .017$; right: $B = 1.655$, $SE = .719$, $t(33) = 2.302$, $p = .028$) (Figure 2.8). Bilateral amygdala and bilateral hippocampus activation were not associated with test accuracy ($ps > .681$). In contrast, greater bilateral amygdala activation during Practice > Test were marginally associated with greater test-related anxiety (left: $B = -31.487$, $SE = 16.547$, $t(33) = -1.903$, $p = .066$; right: $B = -30.236$, $SE = 16.329$, $t(33) = -1.852$, $p = .073$). Similarly, right hippocampus activation during

Practice > Test was also marginally associated with greater test-related anxiety (right: $B = -41.804$, $SE = 23.555$, $t(33) = -1.775$, $p = .085$) (Figure 2.9).

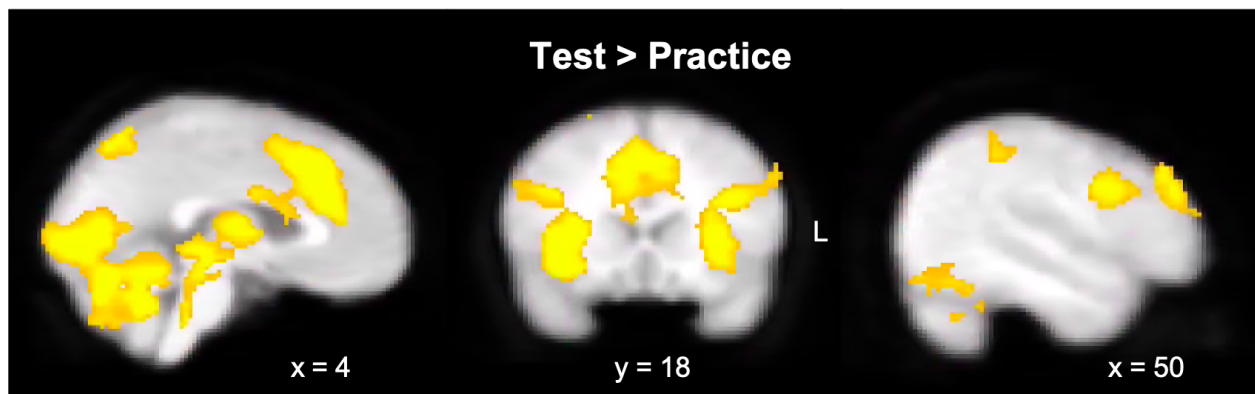


Figure 2.5. Whole-brain analyses revealed that, on average, adolescents engaged corticolimbic regions (DLPFC, MFG, IFG, dACC, anterior insula, and OFC) more during test relative to practice blocks, cluster-corrected at $Z > 3.1$, $p < .05$.

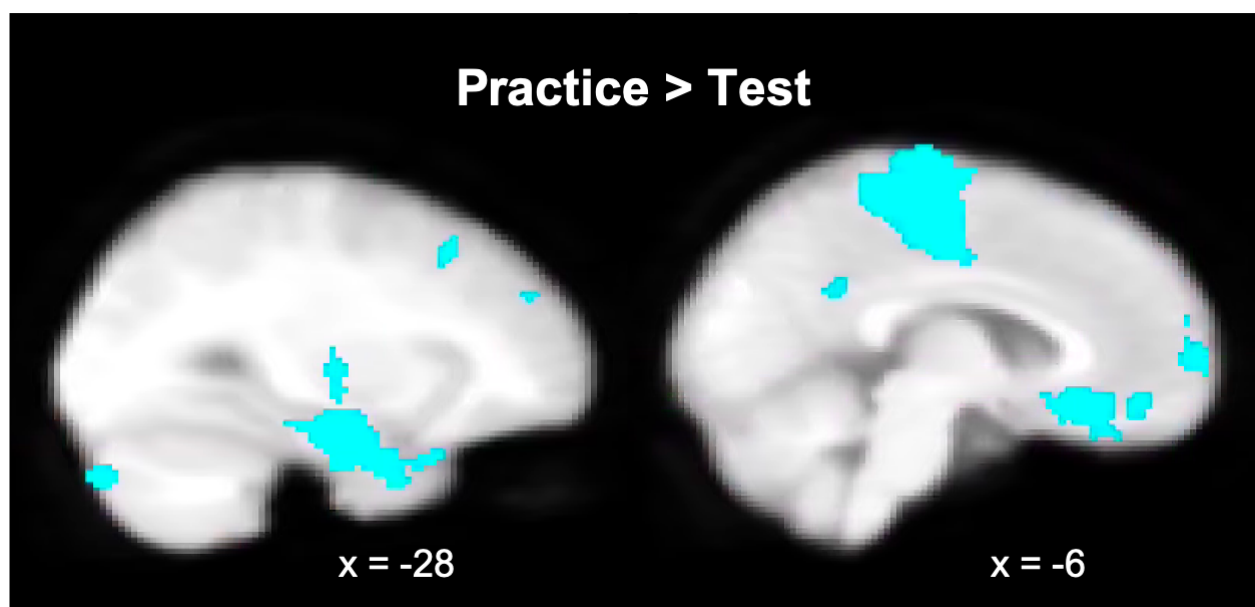


Figure 2.6. Whole-brain analyses revealed that, on average, adolescents engaged VMPFC, amygdala, and hippocampal regions more during practice relative to test blocks, cluster-corrected at $Z > 3.1$, $p < .05$.

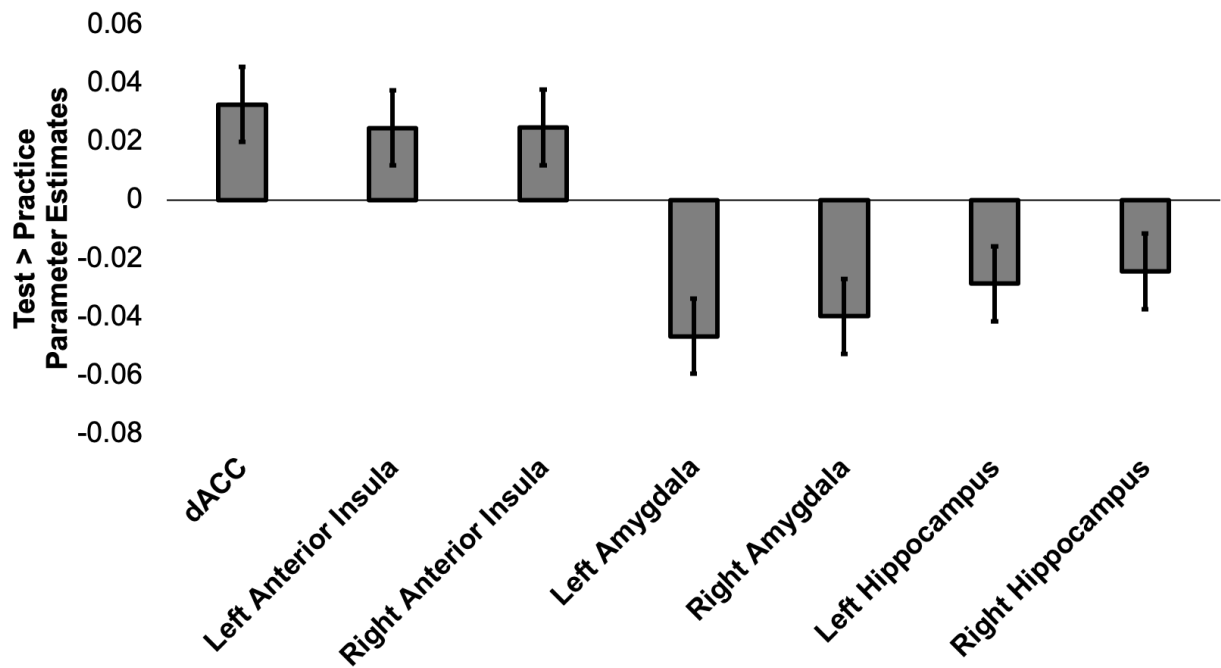


Figure 2.7. ROI analyses revealed significant activation in dACC and bilateral anterior insula during Test > Practice and significant activation in bilateral amygdala and hippocampus during Practice > Test.

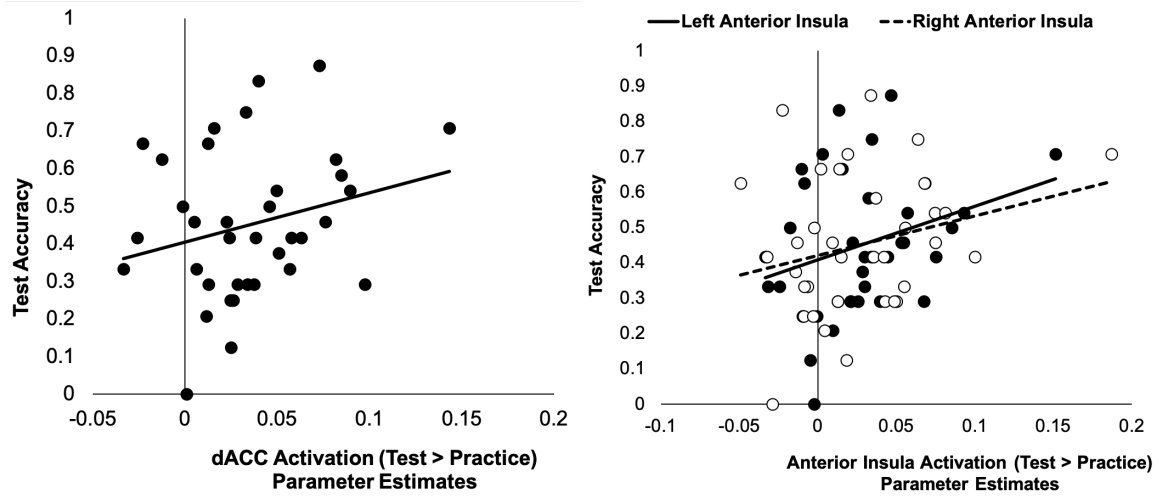


Figure 2.8. Greater activation in dACC and bilateral anterior insula during Test > Practice were associated with greater test accuracy, controlling for gender.

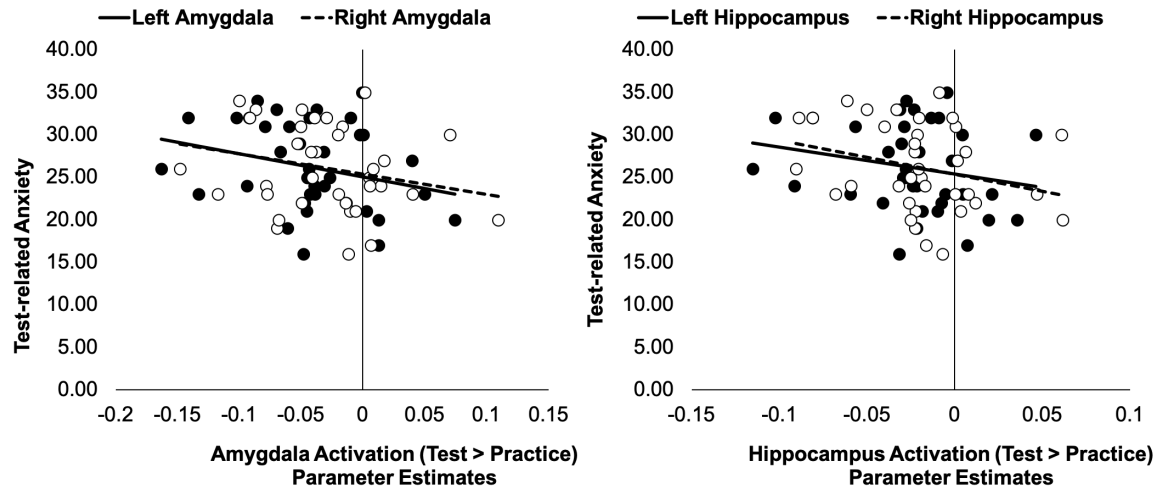


Figure 2.9. Greater activation in bilateral amygdala and hippocampus during Practice > Test were marginally associated with greater test-related anxiety, controlling for gender.

| | Hemisphere | Region | x | y | z | Z-max |
|---------------------------|------------|---------------------|-----|-----|-----|-------|
| Test > Practice | | | | | | |
| | L/R | Occipital pole | -18 | -84 | -10 | 7.99 |
| | | | 18 | -94 | 14 | 8.00 |
| Practice > Test | | | | | | |
| | L | Frontal pole | -10 | 66 | 18 | 5.73 |
| | R | VMPFC | 6 | 26 | -14 | 5.34 |
| | L | Amygdala | -20 | -6 | -22 | 5.90 |
| | L | Hippocampus | -28 | -12 | -24 | 5.87 |
| | R | Posterior insula | 34 | -20 | 14 | 5.67 |
| | R | Posterior cingulate | 6 | -16 | 46 | 5.98 |
| | R | Precentral gyrus | 18 | -26 | 68 | 5.89 |
| | L | Angular gyrus | -56 | -60 | 30 | 6.32 |
| | L | Temporal pole | -48 | 6 | -36 | 6.10 |

Table 2.2. Results from whole-brain analyses. Averaged across all participants, clusters of activation for Test > Practice and Practice > Test, cluster-corrected at $Z > 3.1$, $p < .05$. Note: x, y, and z refer to MNI coordinates, Z-max refers to peak level of activation intensity. L = left, R = right.

Depressive symptoms and neural response to fMRI Stressor Task

Whole-brain analyses revealed no significant associations between depressive symptoms and neural activation during Test > Practice. ROI analyses revealed that depressive symptoms were not associated with dACC ($B = -.001$, $SE = .001$, $t(30) = -1.604$, $p = .119$), bilateral anterior insula (left: $B = -.001$, $SE = .001$, $t(30) = -1.00$, $p = .325$; right: $B = -.001$, $SE = .001$, $t(30) = -.935$, $p = .357$), or bilateral amygdala (left: $B = -.002$, $SE = .001$, $t(30) = -1.587$, $p = .123$; right: $B = -.002$, $SE = .001$, $t(30) = -1.068$, $p = .294$), or bilateral hippocampus (left: $B = -.001$, $SE = .001$, $t(30) = -1.427$, $p = .164$; right: $B = -.001$, $SE = .001$, $t(30) = -1.059$, $p = .298$) activation during Test > Practice.

Inflammatory markers and neural response to fMRI Stressor Task

IL-6. Whole-brain analyses revealed that levels of IL-6 were not associated with activation for Test > Practice contrast. ROI analyses revealed that IL-6 was not significantly associated with dACC ($B = -0.021$, $SE = .015$, $t(18) = -1.462$, $p = .161$), bilateral anterior insula (left: $B = -0.018$, $SE = .017$, $t(18) = -1.047$, $p = .309$; right: $B = -0.026$, $SE = .020$, $t(18) = -1.308$, $p = .207$), bilateral amygdala (left: $B = -.028$, $SE = .018$, $t(18) = -1.1611$, $p = .125$; right: $B = -.030$, $SE = .019$, $t(18) = -1.534$, $p = .142$), or bilateral hippocampus (left: $B = -.016$, $SE = .010$, $t(18) = -1.580$, $p = .132$; right: $B = -.021$, $SE = .012$, $t(18) = -1.672$, $p = .112$) activation during Test > Practice.

TNF- α . Whole-brain analyses revealed that levels of TNF- α were negatively associated with activation in right occipital cortex for Test > Practice contrast (Table 2.3). ROI analyses revealed that levels of TNF- α were not associated with dACC ($B = -0.056$, $SE = .054$, $t(18) = -1.034$, $p = .315$), bilateral anterior insula (left: $B = -0.058$, $SE = .062$, $t(18) = -.940$, $p = .360$; right: $B = -0.027$, $SE = .073$, $t(18) = -.371$, $p = .715$), bilateral amygdala (left: $B = -.034$, $SE = .068$, $t(18) = -.507$, $p = .619$; right: $B = -0.012$, $SE = .075$, $t(18) = -.161$, $p = .874$) or bilateral hippocampus (left: $B = -.019$, $SE = .039$, $t(18) = -.494$, $p = .627$; right: $B = -.042$, $SE = .047$, $t(18) = -.890$, $p = .385$) activation during Test > Practice.

IL-10. Whole-brain analyses revealed that levels of IL-10 were not associated with activation for Test > Practice contrast. ROI analyses revealed that levels of IL-10 were not significantly associated with dACC ($B = -.004$, $SE = .027$, $t(18) = -0.144$, $p = .887$), bilateral anterior insula (left: $B = .014$, $SE = .030$, $t(18) = .446$, $p = .661$; right: $B = .003$, $SE = .036$, $t(18) = .083$, $p = .935$), or bilateral amygdala (left: $B = .003$, $SE = .033$, $t(18) = .080$, $p = .937$; right: $B = -0.020$, $SE = .036$, $t(18) = -0.558$, $p = .584$), bilateral hippocampus (left: $B = -.015$, $SE = .019$,

$t(18) = -.786, p = .442$; right: $B = -.026, SE = .023, t(18) = -1.132, p = .273$) activation during Test > Practice.

IL-8. Whole-brain analyses revealed that levels of IL-8 were positively associated with activation in left superior frontal gyrus, left precentral gyrus, left postcentral gyrus, left superior parietal cortex, right lingual gyrus, and left lateral occipital cortex for Test > Practice contrast (Table 2.3). ROI analyses revealed that levels of IL-8 were not significantly associated with dACC ($B = .001, SE = .015, t(18) = .079, p = .938$), bilateral anterior insula (left: $B = -.009, SE = .016, t(18) = -0.577, p = .571$; right: $B = -.004, SE = .019, t(18) = -.220, p = .828$), bilateral amygdala (left: $B = .008, SE = .018, t(18) = .455, p = .654$; right: $B = .026, SE = .018, t(18) = 1.385, p = .183$), bilateral hippocampus (left: $B = .009, SE = .010, t(18) = .896, p = .382$; right: $B = .014, SE = .012, t(18) = 1.174, p = .256$) activation during Test > Practice.

IFN γ . Whole-brain analyses revealed that levels of IFN γ were negatively associated with activation in right postcentral gyrus, bilateral precuneus, and occipital regions for Test > Practice contrast (Table 2.3). ROI analyses revealed that levels of IFN γ were marginally negatively associated with dACC activation during Test > Practice ($B = -0.035, SE = .019, t(18) = -1.847, p = .081$). IFN γ was not associated with bilateral anterior insula (left: $B = -0.015, SE = .023, t(18) = -0.644, p = .528$; right: $B = -0.013, SE = .027, t(18) = -0.464, p = .648$), bilateral amygdala (left: $B = .004, SE = .025, t(18) = .154, p = .880$; right: $B = -0.034, SE = .027, t(18) = -1.283, p = .216$), or bilateral hippocampus (left: $B = -.016, SE = .014, t(18) = -1.141, p = .269$; right: $B = -.017, SE = .018, t(18) = -.948, p = .356$) activation during Test > Practice.

| | Direction | Hemisphere | Region | x | y | z | Z-max |
|--------------------------------|-----------|------------|--------------------------|-----|-----|-----|-------|
| Test > Practice | | | | | | | |
| <i>TNF-α</i> | Negative | R | Lateral occipital cortex | 38 | -78 | -12 | 4.22 |
| | | R | Lingual gyrus | 14 | -82 | -12 | 3.94 |
| <i>IL-8</i> | Positive | L | Superior frontal gyrus | -20 | -8 | 72 | 3.64 |
| | | L | Superior parietal lobule | -42 | -44 | 62 | 3.88 |
| | | L | Precentral gyrus | -38 | -8 | 60 | 3.34 |
| | | L | Postcentral gyrus | -48 | -32 | 60 | 3.72 |
| | | L | Lateral occipital cortex | -42 | -88 | -6 | 3.68 |
| | | R | Lingual gyrus | 18 | -52 | 0 | 3.60 |
| <i>IFNγ</i> | Negative | L | Lateral occipital cortex | -36 | -86 | -18 | 4.20 |
| | | L/R | Precuneous | -4 | -66 | 30 | 3.62 |
| | | | | 2 | -66 | 54 | 4.02 |
| | | R | Postcentral gyrus | 40 | -36 | 62 | 3.12 |

Table 2.3. Results from whole-brain analyses. Associations between peripheral immune markers and neural activation during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$. Note: x, y, and z refer to MNI coordinates, Z-max refers to peak level of activation intensity. L = left, R = right.

Discussion

The goal of the current study was to characterize the associations between peripheral immune markers, depressive symptoms, and neural reactivity to a stressor in adolescents (14-15 years). Analyses revealed that self-reported depressive symptoms, as indexed by the CES-D scale, were not associated with peripheral immune markers (TNF- α , IFN γ , IL-8, IL-10, or IL-6). While contrary to hypothesis, these findings are consistent with previous research showing that, in otherwise healthy adolescents, depressive symptoms were not related to baseline levels of IL-6 or IL-6 reactivity to a laboratory stressor in late adolescents, but that greater depressive symptoms were only associated with greater IL-6 reactivity for adolescents with high adiposity

(Chiang et al., 2017), suggesting that associations between peripheral immune markers and depressive symptoms during adolescence are complex and may be conditional upon many factors.

On the fMRI stressor task, adolescents endorsed higher stress ratings during test blocks relative to practice blocks and took longer to complete test trials than practice trials, indicating that the test trials were indeed more challenging and stressful than the practice trials. Adolescents who endorsed higher stress ratings were less accurate on the test, reported experiencing greater test-related anxiety, and thought the test trials were more difficult and challenging. However, whereas perceived task difficulty was negatively associated with test accuracy, test-related anxiety was not associated with accuracy, suggesting perhaps that feelings of anxiety regarding a challenge may not necessarily compromise performance on a challenge. In relation to immune markers, peripheral immune markers were not associated with task performance (stress ratings, response time, accuracy), reported test-related anxiety, perceived task difficulty, or self-rated performance. The only exception was found for anti-inflammatory marker IL-10, whereby greater levels of IL-10 were associated with smaller difference in stress ratings between practice and test trials, suggesting smaller psychological stress reactivity to the task for those with greater levels of IL-10.

In the brain, participants engaged regions in corticolimbic circuitry (DLPFC, MFG, IFG, OFC, dACC, anterior insula) more during test blocks relative to practice blocks, consistent with previous research that utilized this task in adults and adolescents to elicit stress (Inagaki et al., 2016; Strang et al., 2011). Adolescents who exhibited greater dACC and bilateral anterior insula activation during stress (Test > Practice) were more accurate on the test. Activation in other regions during Test > Practice was not associated with task performance or task evaluation. The

anterior insula and dACC are part of the salience network (Seeley et al., 2007) and play a role in integrating cognitive, affective, and physiological processes to regulate autonomic responses to stress (Gianaros et al., 2005; Gianaros & Wager, 2015; Strang et al., 2011). Greater anterior insula activation might reflect better integration and control of the stress response, which was reflected in better performance on the test/stressor. However, dACC and anterior insula activation were not associated with psychological reactivity or affective responses to the stressor in the current study, in contrast to what previous studies in adults have found (e.g., Eisenberger et al., 2009). Future analyses that examine functional connectivity of the anterior insula with cortical/affective regions would provide insight into the mechanisms by which anterior insula activation relates to stressor performance.

In contrast, participants engaged regions implicated in emotion/stress regulation (VMPFC, amygdala, hippocampus) more during periods of relatively low stress (Practice > Test). Greater activation in bilateral amygdala and hippocampus during Practice > Test were marginally associated with greater test-related anxiety. While this pattern of results was unexpected, these findings suggest that activation of these emotion regulation regions during lower relative to higher periods of stress might reflect the neural processes underlying stress recovery or anticipation. Additionally, that greater amygdala and hippocampus activation during low stress were associated with greater test-related anxiety suggest that greater engagement of these regions under conditions of relatively lower stress may reflect protracted affective recovery from stress in adolescents. Previous research that utilized the MIST showed that, in a sample of adults, greater amygdala, VMPFC, DMPFC, and DLPFC activation during the stress condition relative to control (analogous to Test > Practice in the current study) were associated with greater changes in skin conductance response (an index of stress reactivity) and self-reported stress

(Orem et al., 2019), suggesting that variability in amygdala and PFC function may play a role in individual variability to psychological and physiological response to stress (LeDoux, Iwata, Cicchetti, & Reis, 1988; Ochsner et al., 2004). Future analyses that examine functional connectivity of the amygdala with PFC regions during stress reactivity (or recovery/anticipation) in comparison with an adult sample would provide insight into the functional networks that are relevant for stress processing for adolescents as well as whether these processes might differ developmentally from adults.

In relation to immune markers, whole-brain and ROI analyses revealed no significant associations between peripheral immune markers and activation in corticolimbic circuitry during Test > Practice. Failure to detect significant associations could be attributed to several factors: 1) insufficient statistical power due to the small sample size; 2) the possibility that peripheral immune markers may be associated with network-level functional connectivity rather than regional activation during adolescents (e.g., Nusslock et al., 2019); 3) the possibility that the current fMRI task may not be sensitive to variability in immune markers in adolescents (e.g., previous studies that found associations between inflammation and brain function utilized threatening social stimuli such as negative facial expressions (Inagaki et al., 2012; Muscatell et al., 2016; Slavich et al., 2010); and 4) the possibility that the associations between peripheral immune markers and activation in corticolimbic circuitry may depend on other factors (i.e., moderators), which is explored in subsequent studies of the current dissertation.

The current study also has additional limitations that should be considered. First, the cross-sectional and correlational study design precludes drawing any conclusions about causality or directionality of the relation between immune markers and brain function. Second, the age-range of our adolescents was restricted to 14-15 years of age, which precludes generalization of

our findings to younger or older adolescents. There also may have been self-selection bias of subjects, as immune data were only available from those who opted in for the blood draw. Additionally, while we have good variability in self-reported depressive symptoms, only a small proportion (24%) of our sample reported depressive symptoms that would meet and exceed the clinical threshold. Moreover, adolescents tend to have relatively intact immune systems that keep inflammatory activity from fostering a chronic inflammatory state, therefore having relatively low levels of inflammation (Miller & Chen, 2010). As a result, our sample may have had restricted range for discovery of substantial mind/brain-body associations. However, whereas Chiang et al., (2017) did not find associations between depressive symptoms and peripheral levels of IL-6, Chiang et al., (2019b) found that depressive symptoms was associated with transcriptional profiles of immune cells, specifically gene expression of the conserved transcriptional response to adversity (CTRA) pattern (i.e., upregulation of pro-inflammatory gene expression and downregulation of antiviral gene expression) in a sample of late adolescents. These findings suggest that the expression of inflammation-related genes may represent a multitude of pro-inflammatory signals beyond IL-6 and may be sensitive enough to detect in otherwise healthy youth. Moreover, whereas gene expression is probed specifically in immune cells, the cellular origins of circulating IL-6 cannot be precisely determined as multiple tissues release IL-6, which confounds circulating immune markers in the periphery with immune function. Future research with larger samples, that utilize a longitudinal design, and probe both circulating levels of cytokines as well as gene expression of cytokines immune cells in addition to utilizing rigorous neuroimaging techniques would be well-positioned to elucidate the mind-body connection across development in health and disease.

Despite these limitations, the current study was one of the first studies to investigate the associations between peripheral immune markers (using a multiplex immunoassay) and brain function in adolescents. While no significant associations between peripheral immune markers and corticolimbic activation during stress were detected, the current study contributes to the literature by demonstrating that frontolimbic activation during periods of low relative to high stress may play an informative role in understanding stress-related anxiety in otherwise healthy adolescents.

Chapter 3. Daily affective experiences moderate associations between immune markers and activation in corticolimbic circuitry during stress

One characteristic of adolescence is the pubertal-driven change in stress reactivity. Adolescents report perceiving and experiencing more stress and show heightened and protracted HPA activity in response to stress relative to individuals in other developmental stages (Dahl & Gunnar, 2009; Romeo, 2013; Romeo et al., 2014; Stroud et al., 2009). These changes have implications for prolonging the effects of stress on brain and immune function. Indeed, animal research has shown that adolescent animals exhibit greater neural activity in the paraventricular nucleus (PVN) of the hypothalamus in response to stress (Romeo et al., 2006; Viau, Bingham, Davis, Lee, & Wong, 2005) as well as less effective glucocorticoid-dependent negative feedback of the HPA axis compared to adult rats (Goldman, Winget, Hollinshead, & Levine, 1973).

The frequency and type of stressors can shape one's hormonal response to stress (Grissom & Bhatnagar, 2009). For example, repeated exposure to the same stressor (homotypic stress) can lead to a habituated hormonal response compared to novel exposure to that stressor (Romeo et al., 2006). However, after repeated exposure to the same stressor, the introduction of a novel stressor (heterotypic stress) induces a heightened HPA response compared to that elicited by the novel stressor alone. Interestingly, this pattern of response to stressors is different between adults and adolescents. Whereas homotypic stress leads to habituation in adults, pre-adolescent males do not show similar patterns of habituation (Lui et al., 2012). Moreover, while heterotypic stress induces similar peak response for both age groups, pre-adolescent animals show slower recovery compared to adults (Lui et al., 2012). It has been suggested that these age-related differences in HPA function might be mediated by greater PVN activation after both homotypic and heterotypic stress during adolescence compared to adulthood (Lui et al., 2012). These animal

models suggest that adolescents are likely to be exposed to more stress-related hormones than adults when confronted with similar acute or repeated stressors. Indeed, work in humans has shown that adolescents (13-17 years of age in Stroud et al., 2009 and 15 years of age in Dahl & Gunnar, 2009) exhibited greater cortisol reactivity to laboratory stress compared to children (7-12 years in Stroud et al., 2009; 9-13 years of age in Dahl & Gunnar, 2009). These ontogenetic changes in HPA function have implications for immune function and inflammation-related effects on brain and behavior during adolescence. Animal research has demonstrated that the effects of an inflammatory challenge on sickness behavior and increased mRNA expression of inflammatory proteins (e.g., IL-1 β , IL-6, TNF- α) in the hippocampus and PFC were augmented in animals that experienced psychosocial stress (Gibb, Hayley, Gandhi, Poulter, & Anisman, 2008; Gibb, Hayley, Poulter, & Anisman, 2011). That is, psychosocial stress enhanced the effects of inflammation on brain and behavior. It is yet unknown whether this interactive effect is also observed during adolescence.

Extant studies that examined the associations between stress and immune function during adolescence have found that greater frequency of daily interpersonal stress was associated with elevated levels of CRP in adolescents one year later (Fuligni et al., 2009). In contrast, other studies have found that daily interpersonal stress was not concurrently associated with circulating levels of CRP (Chiang et al., 2015) or IL-6 reactivity to a laboratory-based stressor (Chiang et al., 2017). However, greater daily interpersonal stress was associated with greater pro-inflammatory gene expression and inflammatory transcription factor (NF-kB) activity (Chiang et al., 2019a). These findings suggest that frequent daily stressors may affect upstream molecular inflammatory processes at the genomic level that may translate to changes in downstream circulating markers later. In addition to experiencing daily stressors, examining associations

between negative affect and affective reactivity (i.e., changes in negative affect in relation to those stressors) and immune processes would provide novel insight into the links between daily affective experiences and immunity during adolescence.

Very little is known about how stressors and immune processes affect brain structure and function during adolescence. There is a need to fill this gap considering growing evidence of a hyper-responsive stress system during adolescence and the strong evidence that the brain regions known to be most sensitive to stress in adulthood (e.g., hippocampus, PFC, amygdala) continue to develop during adolescence (Giedd & Rapoport, 2010; Lupien et al., 2009; McEwen & Morrison, 2013). Animal studies have begun to shed light on these questions and provide a springboard from which we can begin to assess these questions in humans. For example, male rats exposed to chronic variable stress (daily exposure to physical stressors) throughout adolescence (for 4 weeks) showed initial increases in hippocampal volume (only in CA1), but exhibited impairment in volumetric growth in CA1, CA3, and dentate gyrus three weeks after stress termination compared to controls (Isgor et al., 2004). These structural changes were related to spatial impairments (Isgor et al., 2004). In another study, rats exposed to chronic restraint stress for 6 hours per day for 21 days during adolescence exhibited elevated depressive and anxious behaviors in addition to reduced dendritic complexity of pyramidal neurons in the PFC while neurons in the basolateral amygdala showed increased complexity (Eiland et al., 2012). Finally, exposure to social stress (e.g., social instability, isolation) during adolescence (for 15 days) have also led to decreases in hippocampal neurogenesis and survival (McCormick et al., 2010). Taken together, these findings suggest that experience of daily stressors during adolescence (even without exposure to early life stress) has the potential to affect behavior and

brain development in limbic and cortical regions. What remains unknown is the role that immune processes might play in these stress-related changes in brain and behavior.

The purpose of the current study was to investigate whether adolescents' experiences of daily stressors, daily negative affect, and stressor reactivity (Lippold, Davis, McHale, Buxton, & Almeida, 2016) related to peripheral immune markers and neural response to stress. Adolescents' levels of perceived stress were also examined to assess global levels of feelings of stress. It was hypothesized that adolescents who reported greater levels of perceived stress, endorsed more stressors, reported higher negative affect, and/or showed greater stressor reactivity across the week would exhibit higher levels of peripheral pro-inflammatory markers (e.g., IL-6, TNF- α), which would be associated with heightened neural response to stress. Additionally, in line with the idea that psychosocial stress may enhance the effects of inflammation on brain function, it was also hypothesized that daily experiences would moderate the associations between peripheral immune markers and neural response to stress in regions previously shown to respond to stress (e.g., anterior insula, anterior cingulate cortex, and prefrontal regions).

Methods

Participants

Self-report questionnaires, daily diary, and neuroimaging data were collected from 40 adolescents (14.03-15.99 years, $M = 15.076$, $SD = 0.646$, 17 females) who participated in a larger study conducted by Drs. Galvan, Fuligni, and Eisenberger (NSF BSC 1551952).

Participants were recruited using flyers posted on university campus, in local child and adolescent-friendly locations, on community websites (e.g., Craigslist), and flyer distributions at local high schools. Inclusion criteria required all participants be right-handed, free from metal objects in the body, speak fluent English, be in the appropriate age range, and have no previously

diagnosed psychiatric, neurological, or developmental disorders. Parents of adolescent participants provided written consent and adolescents provided assent in accordance with the University of California, Los Angeles (UCLA) Institutional Review Board. Participants were also provided the opportunity to consent to an optional blood draw. All participants were compensated for their participation.

Of the 40 participants, one participant was excluded from neuroimaging analyses due to a neuroanatomical abnormality and two participants were excluded for excessive motion across both runs of the task. Of the remaining 37 (18 females) participants with usable neuroimaging data, 23 (62%; 9 females) participated in the blood draw. Four out of the 37 participants (which includes two participants with blood data) did not complete measures of perceived stress. Analyses were conducted with the maximum number of subjects for each analysis.

Procedure

Participants completed two visits at UCLA. During the first visit, after providing consent, participants completed questionnaires about demographic information and depressive symptoms and were trained on how to complete the daily diary measures. For 7 days after the first visit, participants received a text message each evening with a URL to an online survey asking about their day that they completed. After 7 days (but within two weeks), participants returned to UCLA to complete their second visit. Participants who consented to the blood draw had their blood drawn by a certified phlebotomist at the clinical lab in the Peter Morton Medical Building at UCLA. After the blood draw, participants completed a brain scan while performing the fMRI stressor task at the Center for Cognitive Neuroscience (CCN) at UCLA. Participants who did not consent to the blood draw only completed the brain scan portion of the study. Participants' height and weight were measured to calculate body mass index (BMI). BMI ranged from 14.337 to

45.154 ($M = 23.298$, $SD = 6.299$). After the brain scan, participants completed additional questionnaires about their experiences regarding the stressor task, were debriefed about the goals of the study, and received compensation.

Measures

Perceived Stress Scale (PSS). Participants rated how often in the last month they felt or experienced 10 items indicative of stress perception (e.g., “felt you were unable to control the important things in your life”, “felt nervous and stressed”) on a 5-point scale (0 = never, 4 = very often). Responses on each item were summed to create a composite score for each individual. PSS scores ranged from 4 to 32 ($M = 17.03$, $SD = 6.682$).

Daily Stressors. Each night, participants were asked to indicate on a checklist whether they experienced stressful demands from various sources (e.g., a lot of work at school, a lot of demands made by my family) and arguments with various people (e.g., family member, friend). These events were selected because they represent psychological stressors for adolescents across domains of family, peers, and school (Chiang et al., 2015; Chung, Flook, & Fuligni, 2009; Nishina & Juvonen, 2005). Participants endorsed 0 to 3 stressors per day (average number of stressors endorsed per day = 0.6535, $SD = 0.585$). To capture the recurrence or chronicity of daily stress, a summary score reflecting the proportion of days participants experienced some degree of stress were calculated (Chiang et al., 2019a). Endorsed items were summed and recoded as 0 or 1 for each day to indicate whether any one of the stressors occurred that day. Recoded scores were then averaged across days to index the proportion of days that at least one stressor occurred. Proportions of daily stressors across the week ranged from 0.00 (no stressors endorsed during the week) to 1.00 (at least one stressor endorsed each day of the week) ($M = .4342$, $SD = .2833$).

Daily Affect. Each night, participants were asked to rate the extent to which they experienced six negative (e.g., on edge, sad, unable to concentrate, uneasy, hopeless, nervous) and eight positive (e.g., joyful, happy, calm, interested, excited, enthusiastic, cheerful, attentive) affect (1 = not at all, 5 = extremely). Items were taken from the Profile of Mood States (McNair, Lorr, & Droppleman, 1971) and the Positive and Negative Affect Schedule (Watson, Clark, & Tellegen, 1988). Previous studies that have used these items for daily diary showed good internal consistency of both negative affect (alpha = .94) and positive affect (alpha = .94) (Chiang et al., 2015; Chiang, Kim, et al., 2017; Fuligni et al., 2009). Ratings for negative affect items were averaged across each day, which were then averaged across the week to obtain an index of average daily negative affect. Higher scores indicate greater average daily negative affect (range: 1.033 – 3.833, M = 1.761, SD = .5677).

Stressor Reactivity. Separate linear regression analyses ($\text{Negative Affect}_{ti} = b_{0i} + b_1 (\text{Number of Daily Stressors})_{ti} + e_{ti}$) were conducted for each participant to calculate each person's stressor reactivity (b_1 = changes in negative affect on days when he or she endorses more daily stressors). Coefficients could not be calculated for 8 individuals because they did not endorse at least one stressor during the week (thus having zero variability in daily stressors for analyses). Higher stressor reactivity scores indicate greater increases in negative affect on days when individuals endorsed more stressors. Stressor reactivity ranged from -.7083 to 1.089 (M = .1676, SD = .4638).

Immunological Measures

A detailed description of blood sample collection, processing, and immunological assays are reported in the *Methods: Immunological Measures* section of Chapter 2/Study 1.

fMRI

A detailed description of the fMRI Stressor Task, Data Acquisition, Data Preprocessing, and Level 1 analyses are previously reported in the *Methods: fMRI* section of Chapter 2/Study 1.

Analytic Plan

All reported analyses covaried for gender. All analyses consisting of peripheral immune markers additionally covaried for body mass index (BMI).

Regression analyses were used to relate participants' perceived stress, proportion of daily stressors, average daily negative affect, and stressor reactivity to levels of peripheral immune markers.

Behavioral Analysis of Stressor Task. Repeated-measures ANCOVAs and regression analyses were conducted to determine whether stress ratings, behavior on stressor task, participants' responses to the test-related STAI, and post-task questionnaire differed by perceived stress, proportion of daily stressors, average daily negative affect, and stressor reactivity.

fMRI Data Analysis. A detailed description of level 1 analyses is previously reported in *Methods: Analytic Plan* section of Chapter 2/Study 1. Analyses focused on the contrast between test blocks and practice blocks (Test > Practice, Practice > Test). A fixed effects voxel-wise analysis combined each of the two runs at the second level. Regression analyses were conducted at the group level using the FMRI local analysis of mixed effects (FLAME1) module in FSL with mean-centered regressors of interest (e.g., daily stressors, daily negative affect, stressor reactivity) entered in each respective model in whole brain analyses. Z (Gaussianized T) statistic images were thresholded at $Z > 2.3$ (unless otherwise noted) by a corrected cluster significant threshold of $p < .05$ using Gaussian Random Field theory and corrected for family-wise errors.

Anatomical localization within each cluster were obtained by searching within maximum likelihood regions from the FSL Harvard-Oxford probabilistic atlas.

Average number of daily stressors, average daily negative affect, and participants' stressor reactivity were entered as mean-centered regressors of interest in separate GLMs for whole-brain fMRI analyses to assess their associations with neural response to stress.

Moderation analyses for each daily experience measure and each immune marker were conducted at the whole-brain level to assess whether daily affective experiences moderated associations between immune markers and neural response to stress.

ROI Analyses. In addition to whole-brain analyses, ROI analyses were also conducted in regions previously implicated in stress reactivity and regulation (e.g., dACC, left and right anterior insula, left and right amygdala, and left and right hippocampus). A detailed description of the ROIs is previously reported in *Methods: Analytic Plan* section of Chapter 2/Study 1.

Results

Bivariate correlations between perceived stress and daily measures are presented in Table 3.1. Analyses revealed that greater levels of perceived stress were associated with greater average daily negative affect. There were no significant associations between daily stressors, negative affect, and stressor reactivity.

| | M (SD) | Daily stressors | Negative affect | Stressor reactivity |
|----------------------------|---------------|------------------------|------------------------|----------------------------|
| Perceived stress | 17.03 (6.682) | .035 | .631** | -.215 |
| Daily stressors | .4342 (.2833) | | .133 | .216 |
| Negative affect | 1.751 (.5677) | | | .160 |
| Stressor reactivity | .1676 (.4638) | | | |

Table 3.1. Bivariate correlations between perceived stress, proportion of daily stressors, average daily negative affect, and stressor reactivity. ** $p < .001$

Perceived stress and inflammation

Controlling for gender and BMI, greater perceived stress was associated with lower levels of IFN γ (B = -8.366, SE = 3.475, t(16) = -2.408, p = .028). Perceived stress was not significantly associated with levels of TNF- α (B = -14.561, SE = 9.417, t(16) = -1.546, p = .142), IL-10 (B = -7.889, SE = 4.594, t(16) = -1.717, p = .105), IL-8 (B = 3.487, SE = 2.460, t(16) = 1.418, p = .175), or IL-6 (B = -4.265, SE = 2.570, t(16) = -1.660, p = .116).

Daily stressors and inflammation

Controlling for gender and BMI, daily stressors were not associated with IFN γ (B = .590, SE = .391, t(18) = 1.507, p = .149), IL-10 (B = -.169, SE = .318, t(18) = -.534, p = .600), IL-6 (B = -.029, SE = .559, t(18) = -0.053, p = .959), IL-8 (B = -.633, SE = .576, t(18) = -1.099, p = .286), or TNF- α (B = .109, SE = .153, t(18) = .710, p = .487).

Negative affect and inflammation

Controlling for gender and BMI, greater negative affect was marginally associated with lower levels of IL-10 ((B = -0.272, SE = .155, t(18) = -1.757, p = .096). Negative affect was not associated with IFN γ (B = .032, SE = .218, t(18) = .148, p = .884), IL-6 (B = -.085, SE = .293, t(18) = -.292, p = .774), IL-8 (B = .223, SE = .308, t(18) = .725, p = .478), or TNF- α (B = .015, SE = .081, t(18) = .180, p = .859).

Stressor reactivity and inflammation

Controlling for gender and BMI, stressor reactivity was positively associated with IL-6 (B = .662, SE = .253, t(15) = 2.620, p = .019). That is, adolescents who reported greater negative affect on days that they endorsed experiencing more stressors evinced greater levels of IL-6.

Stressor reactivity was not associated with IFN γ (B = -0.038, SE = .243, t(15) = -.156, p = .878),

IL-10 ($B = -0.145$, $SE = .176$, $t(15) = -0.823$, $p = .423$), IL-8 ($B = -.340$, $SE = .299$, $t(15) = -1.137$, $p = .273$), TNF- α ($B = .038$, $SE = .081$, $t(15) = .475$, $p = .642$).

Behavior on fMRI Stressor Task

Stress ratings. Main effects of stress ratings were reported in Chapter 2/Study 1. Differences in stress ratings did not differ by perceived stress ($F(1,30) = 1.755$, $p = .195$), but adolescents who reported greater perceived stress reported higher stress ratings overall ($F(1, 30) = 5.783$, $p = .023$). Differences in stress rating did not differ by daily stressors ($F(1, 33) = .329$, $p = .570$), negative mood ($F(1, 33) = .026$, $p = .874$), or stressor reactivity ($F(1, 33) = .040$, $p = .843$). However, adolescents who reported greater negative affect reported higher stress ratings overall ($F(1, 33) = 8.641$, $p = .006$).

Response time. Main effects of response time were reported in Chapter 2/Study 1. Controlling for gender, response time difference did not differ by perceived stress ($F(1, 28) = .001$, $p = .979$), daily stressors ($F(1, 33) = 3.510$, $p = .070$), or stressor reactivity ($F(1, 25) = .002$, $p = .961$). Adolescents who reported greater negative affect showed greater difference in response time between test and practice trials ($F(1, 33) = 6.266$, $p = .017$) and were faster overall ($F(1, 33) = 4.362$, $p = .045$).

Test Accuracy. Controlling for gender, test accuracy did not differ by perceived stress ($B = -.005$, $SE = .006$, $t(30) = -.943$, $p = .353$), daily stressors ($B = .129$, $SE = .124$, $t(33) = 1.042$, $p = .305$), negative affect ($B = .012$, $SE = .061$, $t(33) = .194$, $p = .847$), or stressor reactivity ($B = -0.056$, $SE = .068$, $t(25) = -0.819$, $p = .420$).

Evaluation of fMRI Stressor Task

Anxiety. Controlling for gender, adolescents who reported greater perceived stress reported experiencing greater test-related anxiety symptoms ($B = .400$, $SE = .145$, $t(30) = 2.767$,

$p = .010$). Adolescents who reported greater daily negative affect reported experiencing greater test-related anxiety symptoms ($B = 4.311$, $SE = 1.558$, $t(33) = 2.766$, $p = .009$). Test-related anxiety symptoms were not associated with daily stressors ($B = 1.732$, $SE = 3.545$, $t(33) = .489$, $p = .628$) or stressor reactivity ($B = .550$, $SE = 2.217$, $t(25) = .248$, $p = .806$).

Perceived Task Difficulty. Controlling for gender, adolescents who reported greater perceived stress reported that they thought the test trials were more difficult/challenging ($B = .052$, $SE = .021$, $t(30) = 2.500$, $p = .018$). Perceived task difficulty was not associated with daily stressors ($B = -0.036$, $SE = .511$, $t(33) = -0.071$, $p = .944$), negative affect ($B = .138$, $SE = .247$, $t(33) = .558$, $p = .581$), or stressor reactivity ($B = -0.182$, $p = .319$, $t(25) = -0.570$, $p = .574$).

Self-Rated Performance. Adolescents who reported greater perceived stress were more likely to report that they thought they performed worse on the test ($B = -.088$, $SE = .038$, $t(30) = -2.336$, $p = .026$). Self-rated performance was not associated with daily stressors ($B = -1.545$, $SE = .868$, $t(33) = -1.779$, $p = .084$), negative mood ($B = -.626$, $SE = .428$, $t(33) = -1.463$, $p = .153$), stressor reactivity ($B = .453$, $SE = .485$, $t(25) = .934$, $p = .359$).

Main effects of fMRI Stressor Task

Detailed analyses of the main effects of fMRI Stressor Task were previously reported in Chapter 2/Study 1. Briefly, on average, participants engaged DLPFC, ACC, anterior insula, and OFC regions more during test blocks compared to practice blocks. Greater bilateral anterior insula (ROIs) activation during Test > Practice were associated with greater test accuracy. In contrast, participants engaged VMPFC, amygdala, hippocampus, and posterior cingulate regions more during practice blocks compared to test blocks. Greater bilateral amygdala and hippocampal activation (ROIs) during Practice > Test were marginally associated with greater test-related anxiety.

Perceived stress and neural response to fMRI Stressor Task

Whole-brain analyses revealed that perceived stress was not associated with activation during Test > Practice. ROI analyses revealed that perceived stress was not associated with dACC (B = -.0003, SE = .001, t(30) = -.248, p = .806), bilateral anterior insula (left: B = -.0002, SE = .001, t(30) = -.146, p = .885; right: B < .001, SE = .001, t(30) = -.063, p = .950), bilateral amygdala (left: B = -.003, SE = .002, t(30) = -1.510, p = .142; right: B = -.002, SE = .002, t(30) = -.802, p = .429), or bilateral hippocampus (left: B = -.001, SE = .001, t(30) = -.863, p = .395; right: B = -.001, SE = .001, t(30) = -.499, p = .621) activation during Test > Practice.

Daily stressors and neural response to fMRI Stressor Task

Adolescents who reported greater proportion of daily stressors during the week showed greater frontostriatal activation (left frontal pole, OFC, and putamen) during Test > Practice (Table 3.2; Figure 3.1). Parameter estimates (5mm spheres around peak activation) from left frontal pole, OFC, and putamen were extracted to determine whether activation related to task behavior and evaluation. Controlling for gender and daily stressors, there were no significant associations between frontostriatal activation and task behavior or evaluation.

ROI analyses revealed that daily stressors were positively associated with bilateral anterior insula activation during Test > Practice (left: B = .048, SE = .023, t(33) = 2.078, p = .046; right: B = .057, SE = .026, t(33) = 2.162, p = .038) (Figure 3.2). Controlling for gender and daily stressors, bilateral anterior insula activation was positively associated with test accuracy (left: B = 1.955, SE = .883, t(32) = 2.215, p = .034; right: B = 1.545, SE = .778, t(32) = 1.985, p = .056). Daily stressors were not significantly associated with dACC (B = .031, SE = .026, t(34) = 1.185, p = .244), bilateral amygdala (left: B = -.025, SE = .040, t(34) = -.627, p = .535; right: B = -.037, SE = .046, t(34) = -.801, p = .428), or bilateral hippocampus (left: B = -.010, SE = .029,

$t(34) = -.354, p = .725$; right: $B = -.005, SE = .030, t(34) = -.167, p = .868$) activation during Test > Practice.

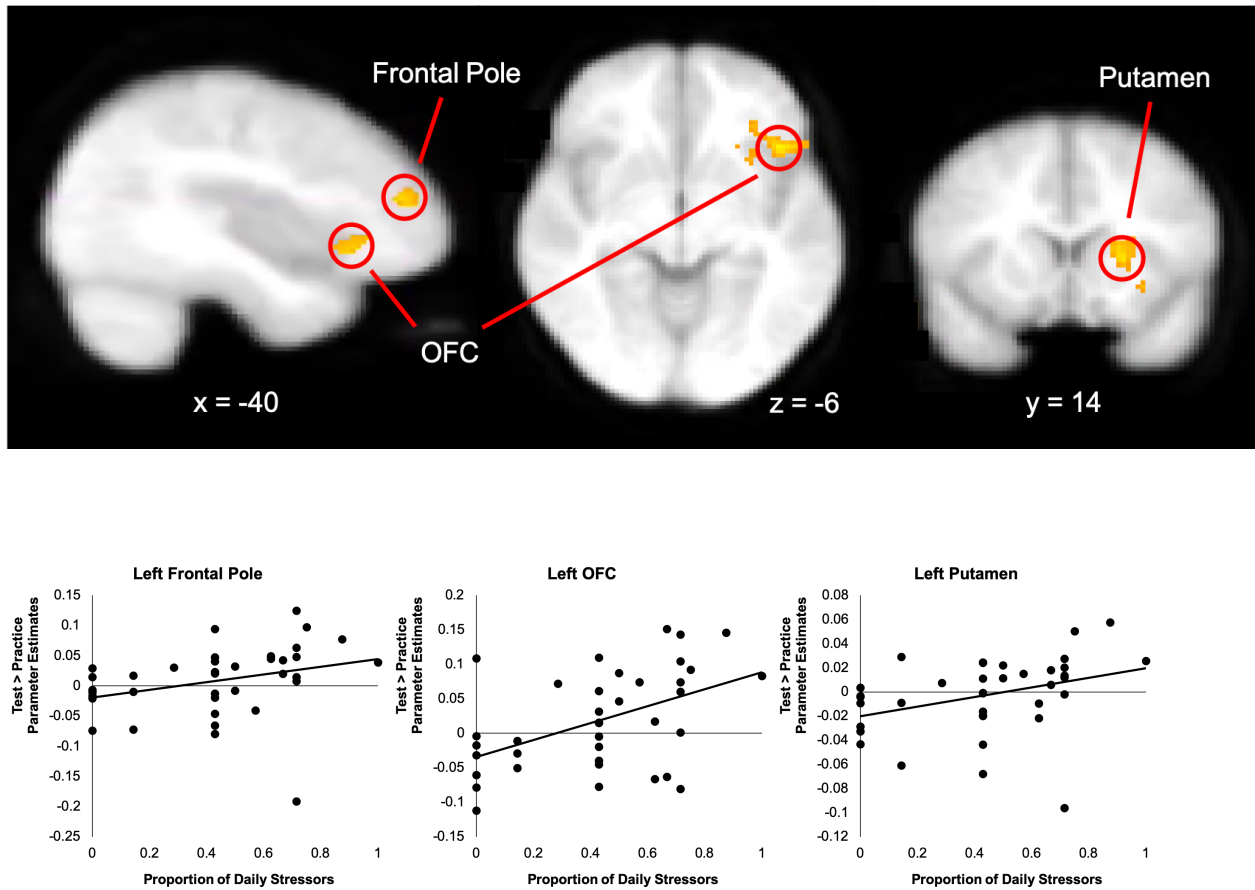


Figure 3.1. Greater proportion of daily stressors was associated with greater activation in left frontal pole, OFC, and putamen during Test > Practice, cluster-corrected at $Z > 2.3, p < .05$. Scatterplots displayed for visual purposes.

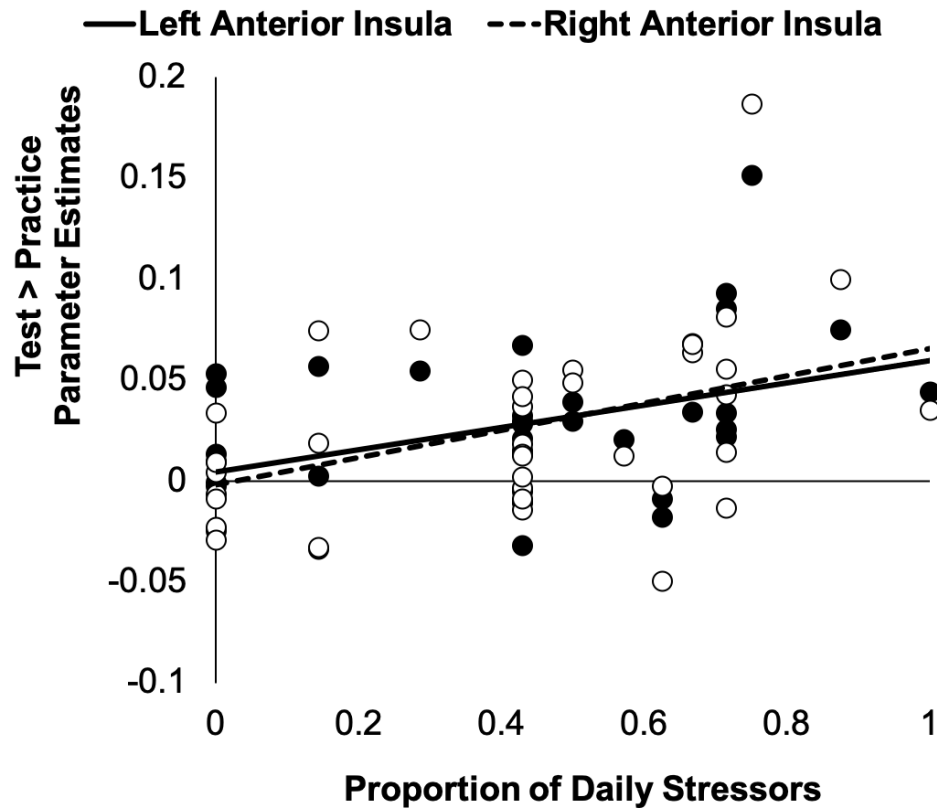


Figure 3.2. ROI analyses revealed that daily stressors were positively associated with bilateral anterior insula during Test > Practice.

Negative affect and neural response to fMRI Stressor Task

Lower negative affect was associated with greater activation in right prefrontal regions (DLPFC, MPFC, middle frontal gyrus, OFC), right superior frontal gyrus, right temporal pole, posterior cingulate, and left parahippocampal gyrus during Test > Practice (Table 3.2, Figure 3.3). Parameter estimates (5mm spheres around peak activation) from right DLPFC, MPFC, and OFC were extracted to determine whether activation related to task behavior and evaluation. Controlling for gender and daily negative affect, greater levels of MPFC activation during Test > Practice were associated with lower perceived test difficulty ($B = -7.117$, $SE = 3.036$, $t(33) = -$

2.344, $p = .025$) (Figure 3.4). Activation in DLPFC and OFC were not significantly associated with behavior or self-reported evaluation.

ROI analyses revealed that negative affect was negatively associated with dACC ($B = -.023$, $SE = .011$, $t(33) = -2.087$, $p = .045$) and right anterior insula ($B = -.026$, $SE = .013$, $t(33) = -1.984$, $p = .056$) activation during Test > Practice, and positively associated with bilateral amygdala (left: $B = -.046$, $SE = .014$, $t(33) = -3.352$, $p = .002$; right: $B = -.037$, $SE = .015$, $t(33) = -2.519$, $p = .017$) and bilateral hippocampus (left: $B = -.032$, $SE = .010$, $t(33) = -3.265$, $p = .003$; right: $B = -.027$, $SE = .010$, $t(33) = -2.650$, $p = .012$) activation during Practice > Test (Figure 3.5). Negative affect was not significantly associated with left anterior insula ($B = -.017$, $SE = .011$, $t(33) = -1.465$, $p = .152$). Controlling for gender and negative affect, dACC ($B = 1.967$, $SE = .904$, $t(32) = 2.175$, $p = .037$) and right anterior insula ($B = 1.926$, $SE = .759$, $t(32) = 2.538$, $p = .016$) activation during Test > Practice were positively associated with test accuracy. Bilateral amygdala and hippocampus activation were not significantly associated with test-related anxiety after controlling for negative affect (left amygdala: $B = -14.641$, $SE = 18.536$, $t(32) = -.790$, $p = .435$; right amygdala: $B = -16.819$, $SE = 17.117$, $t(32) = -.983$, $p = .333$; left hippocampus: $B = -5.439$, $SE = 26.591$, $t(32) = -.205$, $p = .839$; right hippocampus: $B = -21.383$, $SE = 24.892$, $t(32) = -.859$, $p = .397$). However, negative affect remained positively associated with test-related anxiety over and above bilateral amygdala and bilateral hippocampus activation ($B = 3.324$, $SE = 1.745$, $t(29) = 1.905$, $p = .067$).

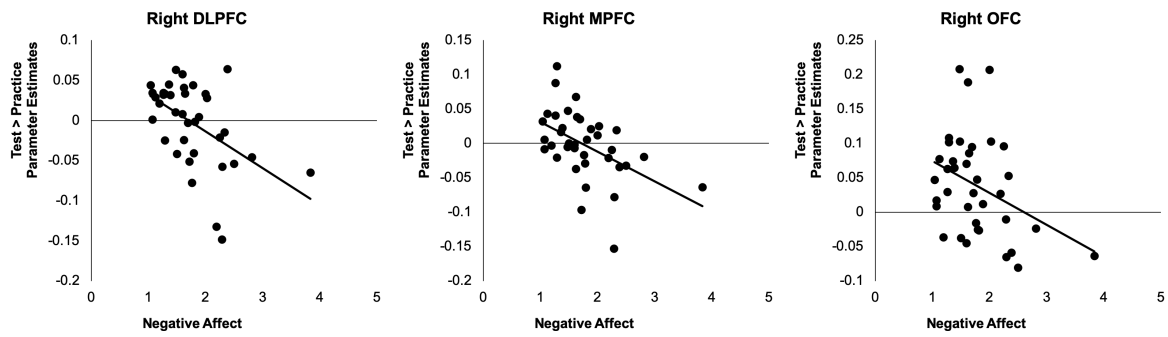
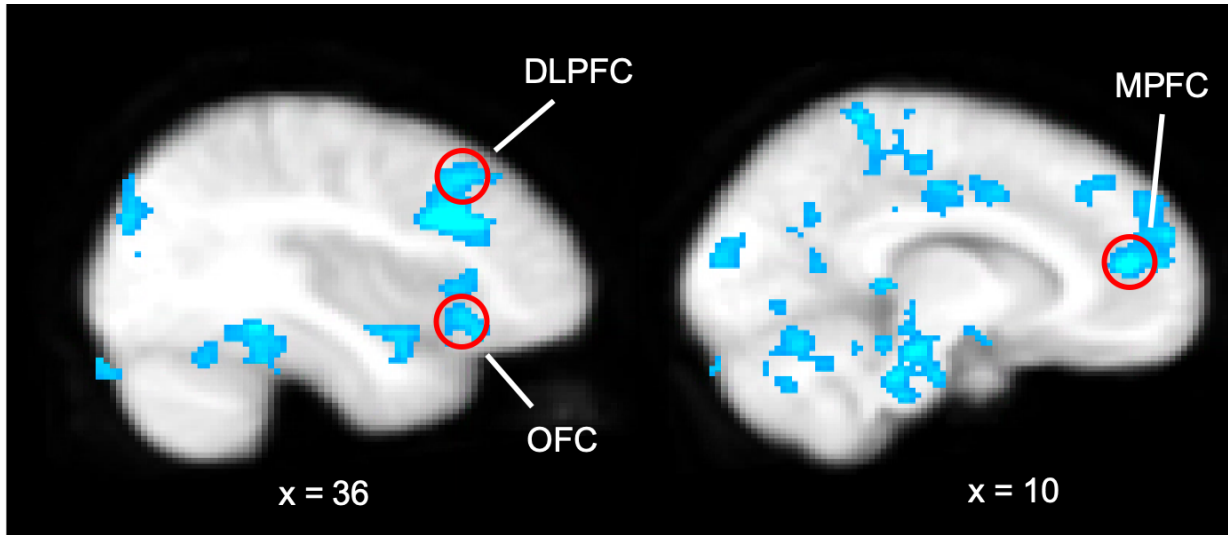


Figure 3.3. Whole-brain analyses revealed that daily negative affect was negatively associated with activation in right DLPFC, MPFC and OFC during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$. Scatterplots displayed for visual purposes.

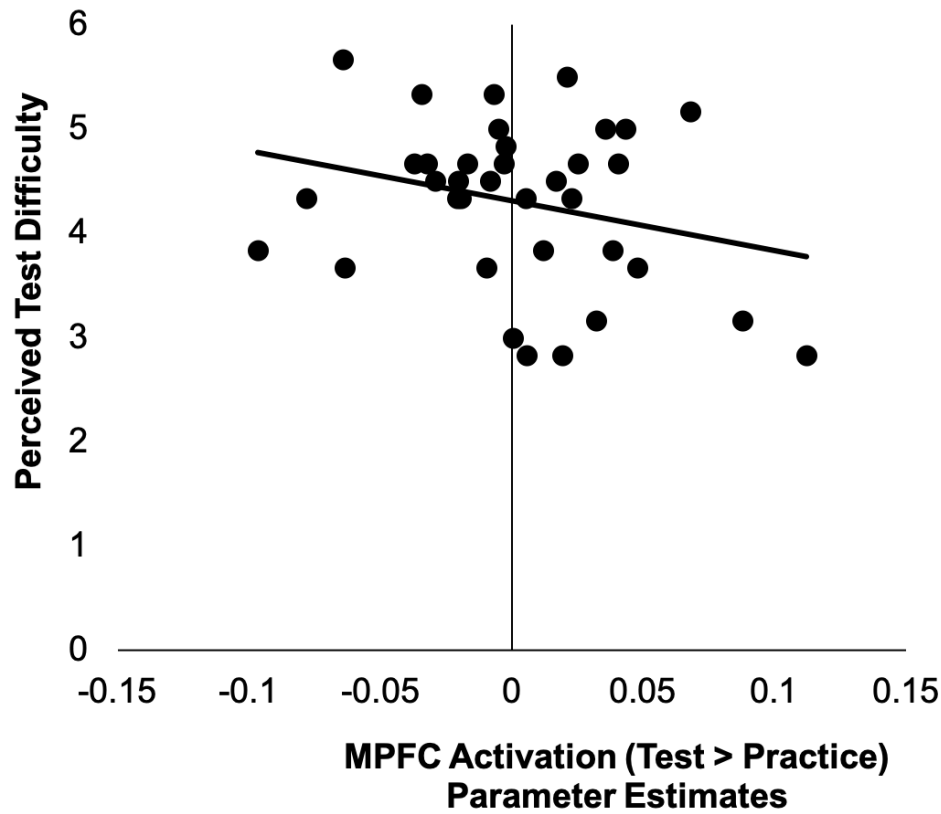
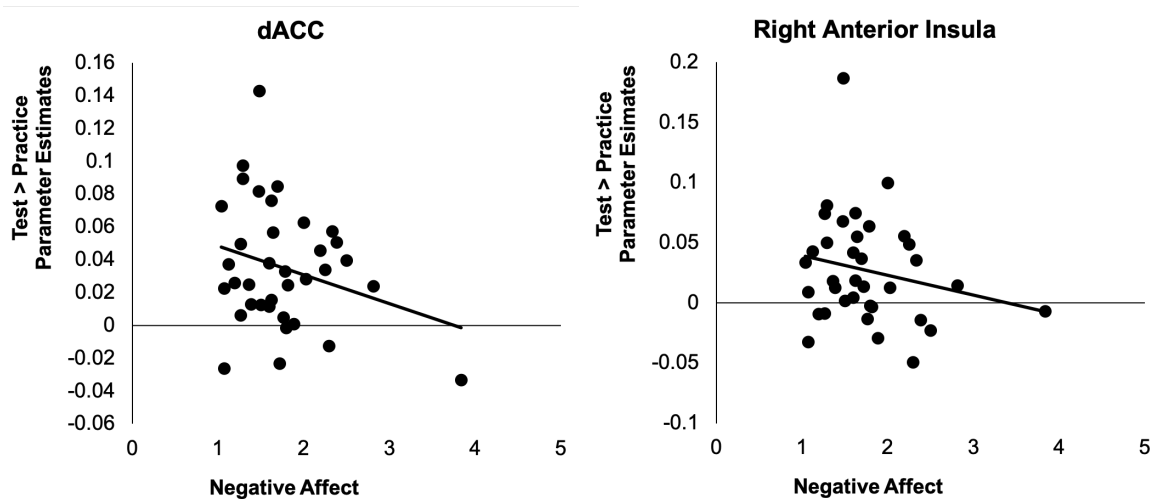


Figure 3.4. Controlling for gender and negative affect, greater levels of MPFC activation during Test > Practice were associated with lower perceived test difficulty.



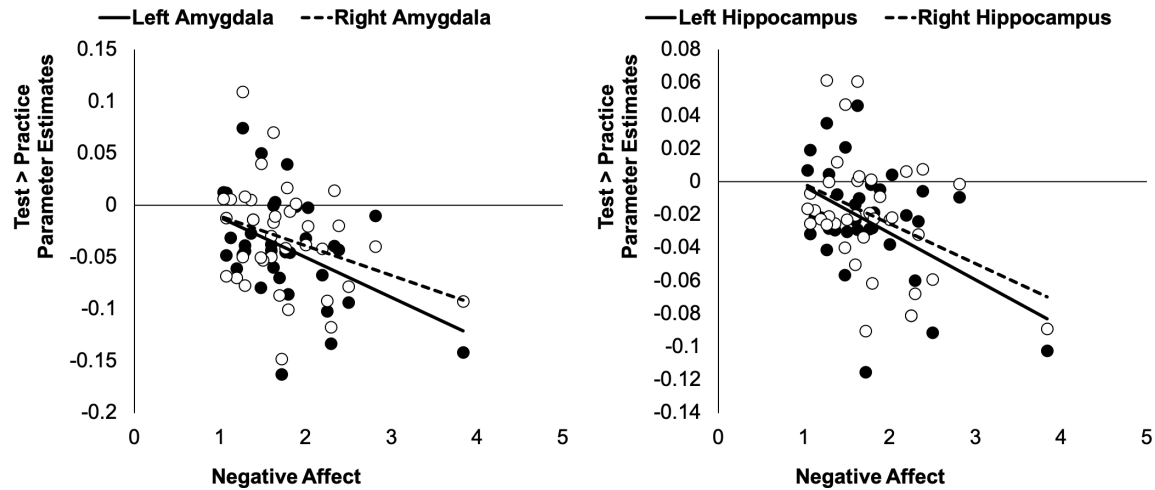


Figure 3.5. ROI analyses revealed that negative affect was associated with dACC, right anterior insula, bilateral amygdala, and bilateral hippocampus activation during Test > Practice.

Stressor reactivity and neural response to fMRI Stressor Task

Greater stressor reactivity was associated with lower activation in right middle temporal gyrus during Test > Practice (Table 3.2).

ROI analyses revealed that greater stressor reactivity was associated with lower (greater) bilateral amygdala (left: $B = -.061$, $SE = .017$, $t(25) = -3.484$, $p = .002$; right: $B = -.058$, $SE = .020$, $t(25) = -2.843$, $p = .009$) and bilateral hippocampus (left: $B = -.026$, $SE = .013$, $t(25) = -1.992$, $p = .057$; right: $B = -.039$, $SE = .014$, $t(25) = -2.812$, $p = .009$) activation during Test > Practice (Practice > Test) (Figure 3.6). Stressor reactivity was not significantly associated with dACC ($B = -.019$, $SE = .016$, $t(25) = -1.197$, $p = .243$), bilateral anterior insula (left: $B = -.011$, $SE = .016$, $t(265) = -.648$, $p = .523$; right: $B = -.028$, $SE = .019$, $t(25) = -1.527$, $p = .139$) activation during Test > Practice.

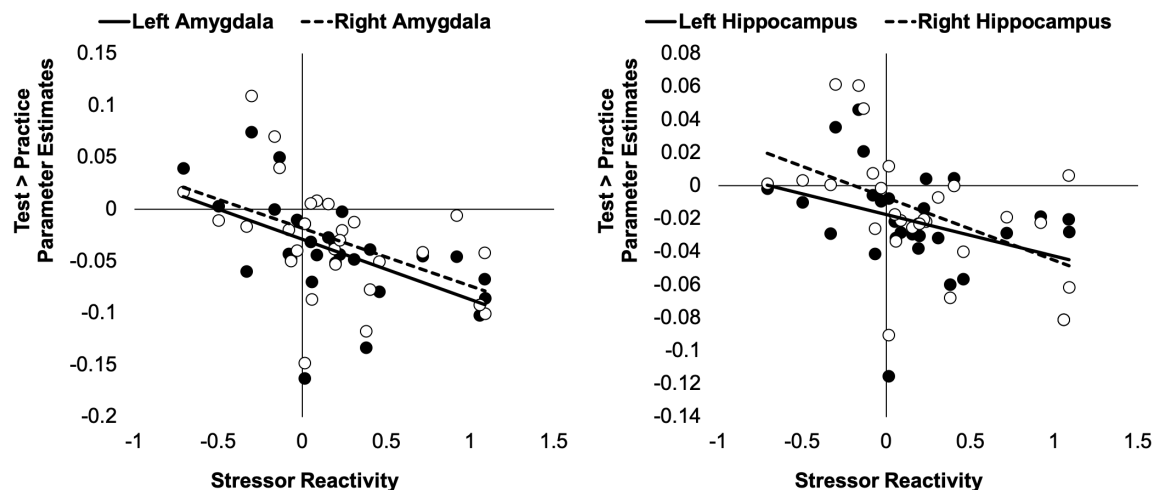


Figure 3.6. ROI analyses revealed that stressor reactivity was negatively (positively) associated with bilateral amygdala and bilateral hippocampus activation during Test > Practice (Practice > Test).

| | Direction | Hemisphere | Region | x | y | z | Z-max |
|---------------------------|-----------|------------|-------------------------|-----|-----|-----|-------|
| Test > Practice | | | | | | | |
| Daily Stressors | Positive | L | Frontal pole | -40 | 44 | 14 | 3.13 |
| | | L | OFC | -42 | 22 | -6 | 3.46 |
| | | L | Putamen | -22 | 14 | 4 | 3.19 |
| Negative Affect | Negative | R | MPFC | 10 | 48 | 14 | 4.26 |
| | | R | DLPFC | 36 | 22 | 40 | 3.43 |
| | | R | Middle frontal gyrus | 38 | 26 | 26 | 3.93 |
| | | R | Superior frontal gyrus | 4 | 30 | 48 | 3.72 |
| | | R | OFC | 40 | 24 | -10 | 3.24 |
| | | R | Temporal pole | 46 | 20 | -16 | 3.50 |
| | | R | Superior temporal gyrus | 54 | -30 | 6 | 3.11 |
| | | R | Posterior cingulate | 6 | -20 | 44 | 3.73 |
| | | L | Parahippocampal gyrus | -16 | -36 | -18 | 4.04 |
| Reactivity | Negative | R | Middle temporal gyrus | 50 | -30 | -6 | 3.78 |

Table 3.2. Associations between daily stressors, negative affect, and stressor reactivity on neural response to stress (Test > Practice), cluster-corrected at $Z > 2.3$, $p < .05$. Note: x, y, and z refer to MNI coordinates, Z-max refers to peak level of activation intensity. L = left, R = right.

Immune markers and neural response to fMRI Stressor Task

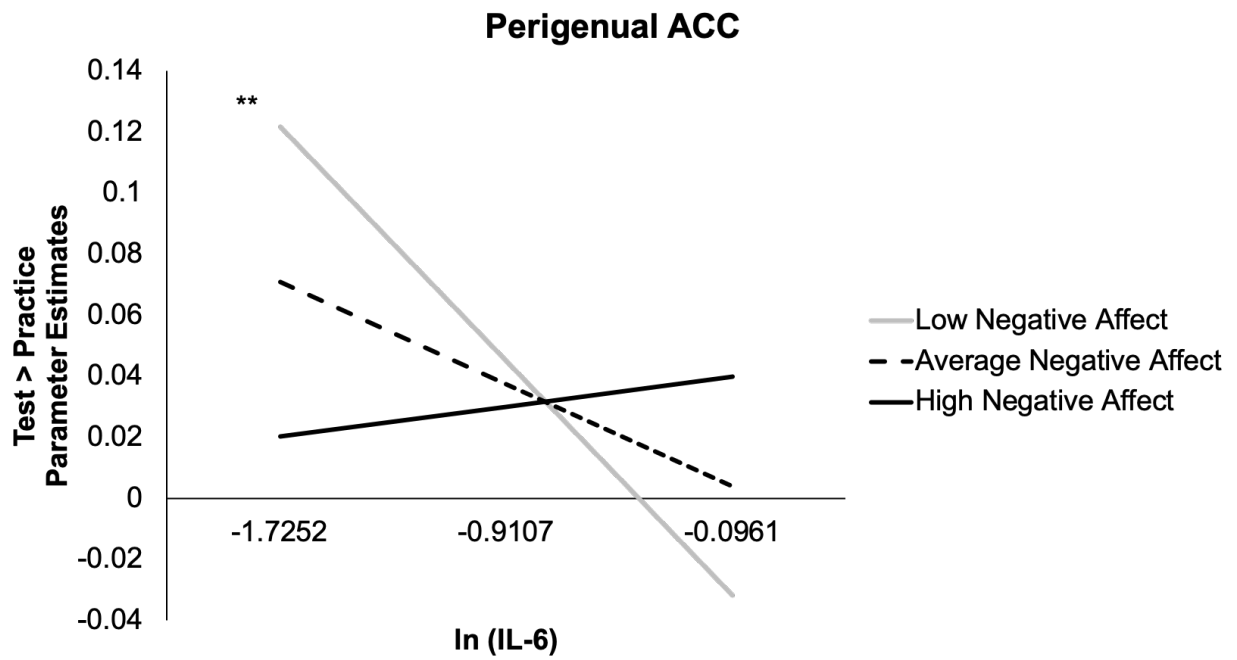
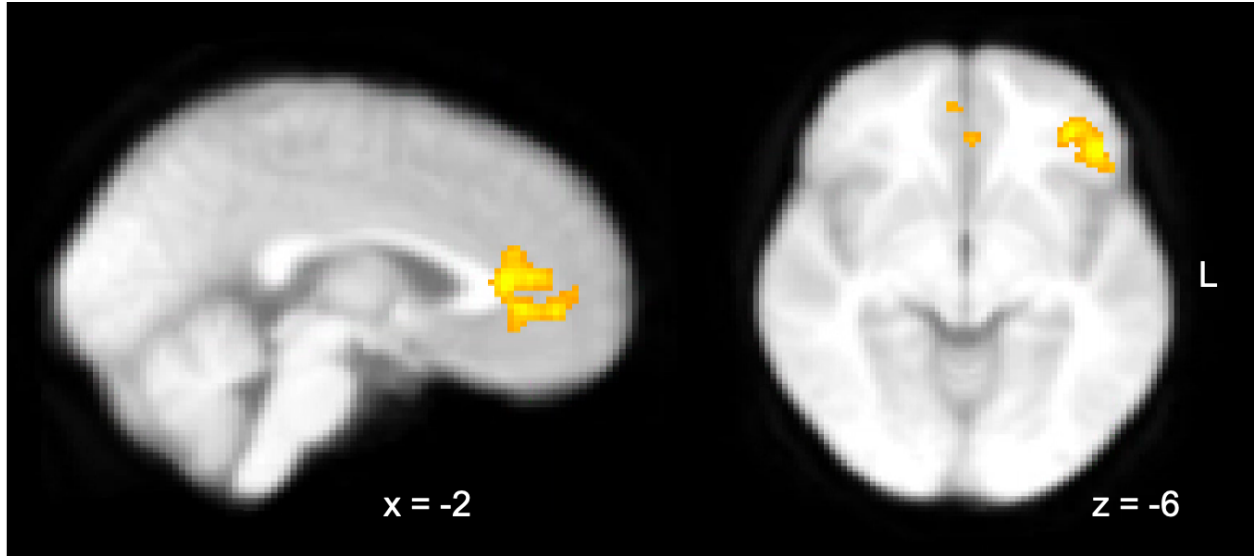
Detailed analyses of the associations between immune markers and neural response to stress were previously reported in Chapter 2/Study 1. Briefly, levels of immune markers were not significantly associated with regions in corticolimbic circuitry during Test > Practice, precluding testing of mediation between daily affective experiences and neural response to stress by immune markers. Therefore, moderation analyses were conducted to determine whether the relation between peripheral immune markers and brain function depended on daily affective experiences.

Interactions with peripheral immune markers

Daily stressors. Whole-brain analyses testing for interactions between daily stressors and immune markers on neural response to stress (Test > Practice) revealed significant interactions between daily stressors and TNF- α in bilateral temporal poles; between daily stressors and IL-8 in right superior frontal gyrus, precentral gyrus and bilateral occipital regions; and between daily stressors and IFN γ in left parietal and occipital regions (Table 3.3).

Negative affect. Whole-brain analyses testing for interactions between negative affect and immune markers on neural response to stress (Test > Practice) revealed significant interactions between IL-6 and negative affect in perigenual ACC, MPFC, and left lateral OFC during Test > Practice (Table 3.3, Figure 3.7). Parameter estimates (5mm spheres around peak activation) from perigenual ACC, MPFC, and left OFC were extracted to probe the nature of the interaction.

Follow up analyses revealed that among those who reported low negative affect (1 SD below mean = 1.1264), lower levels of IL-6 were associated with greater activation in perigenual ACC and MPFC during Test > Practice (ACC: $B = -.0942$, $SE = .0256$, $t(16) = -3.6808$, $p = .002$; MPFC: $B = -.0650$, $SE = .0315$, $t(16) = -2.0626$, $p = .0558$). Levels of IL-6 were not associated with perigenual ACC and MPFC activation among those who reported average levels of negative affect (mean negative affect = 1.611; ACC: $B = -.0411$, $SE = .0217$, $t(16) = -1.8972$, $p = .0760$; MPFC: $B = -.0064$, $SE = .0267$, $t(16) = -.2416$, $p = .8122$) or high levels of negative affect (1 SD above mean = 2.0958; ACC: $B = .0119$, $SE = .0313$, $t(16) = .3800$, $p = .7090$; MPFC: $B = .0521$, $SE = .0386$, $t(16) = 1.3497$, $p = .1959$) (Figure 3.7). In contrast, among those who reported high negative affect (1 SD above mean = 2.0958), greater levels of IL-6 was associated with greater activation in left lateral OFC during Test > Practice. IL-6 was not associated with left lateral OFC activation among those who reported average ($B = .02224$, $SE = .0144$, $t(16) = 1.5580$, $p = .1388$) or low levels of negative affect ($B = -.0273$, $SE = .0170$, $t(16) = -1.6101$, $p = .1269$) (Figure 8). Additionally, controlling for gender, BMI, daily negative affect, and levels of IL-6, greater OFC activation was associated with lower self-rated performance on the test ($B = -12.621$, $SE = 5.431$, $t(16) = -2.324$, $p = .034$).



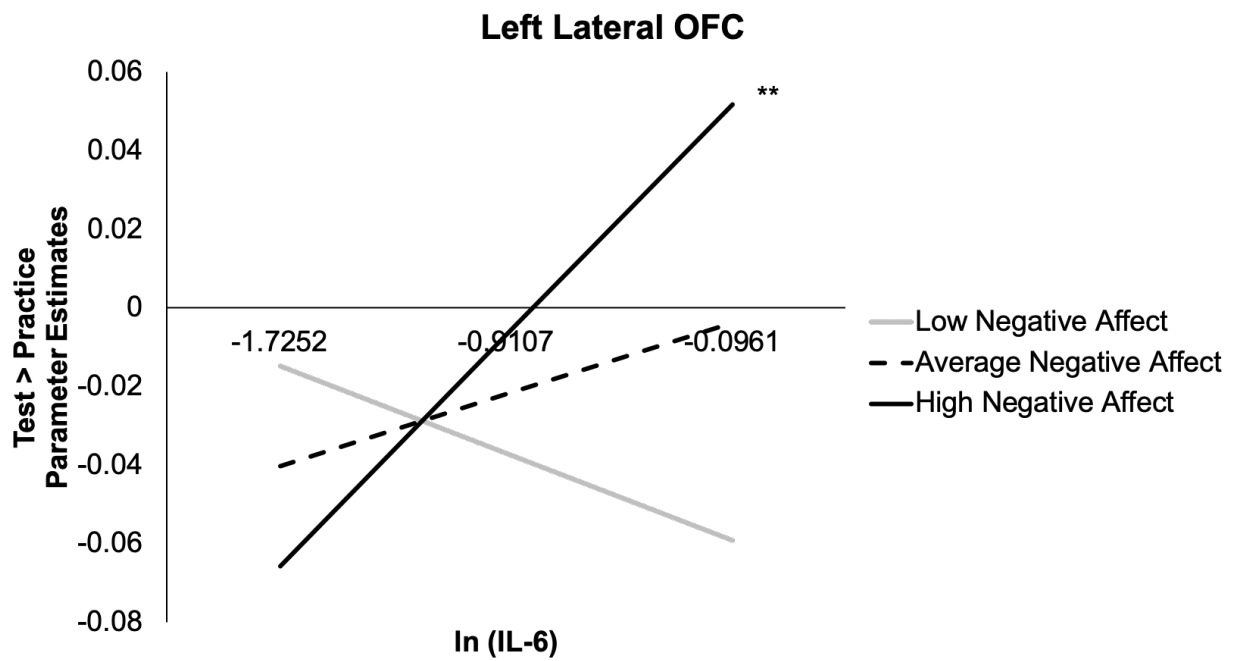
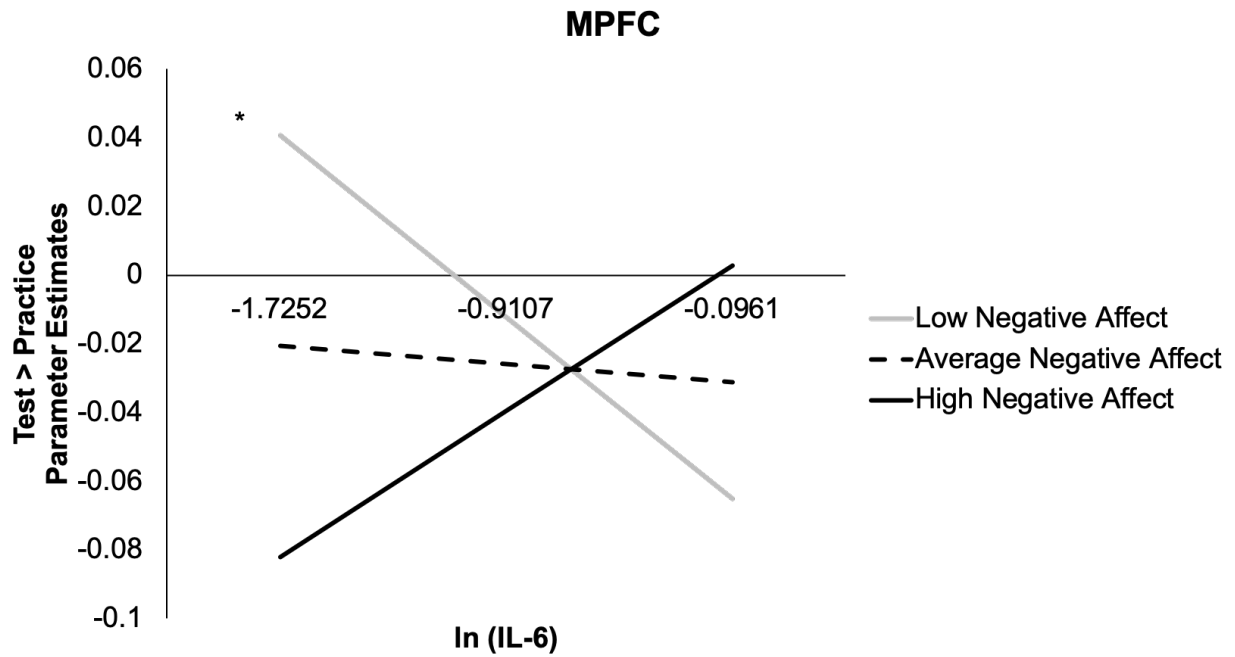
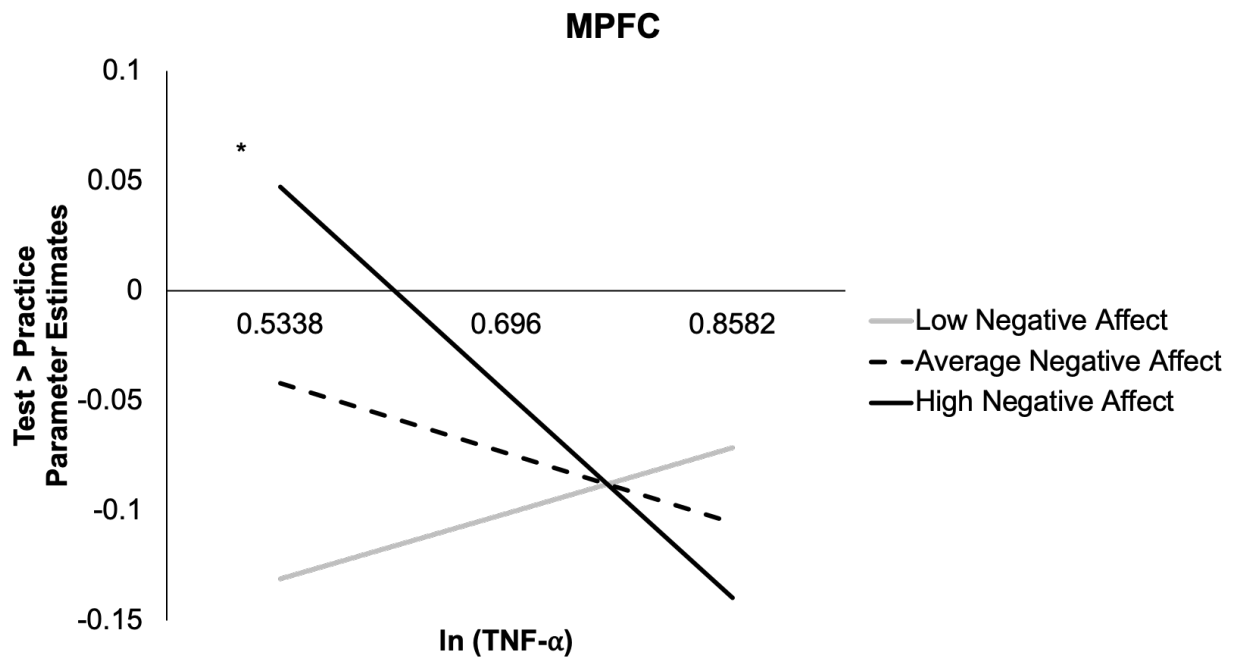
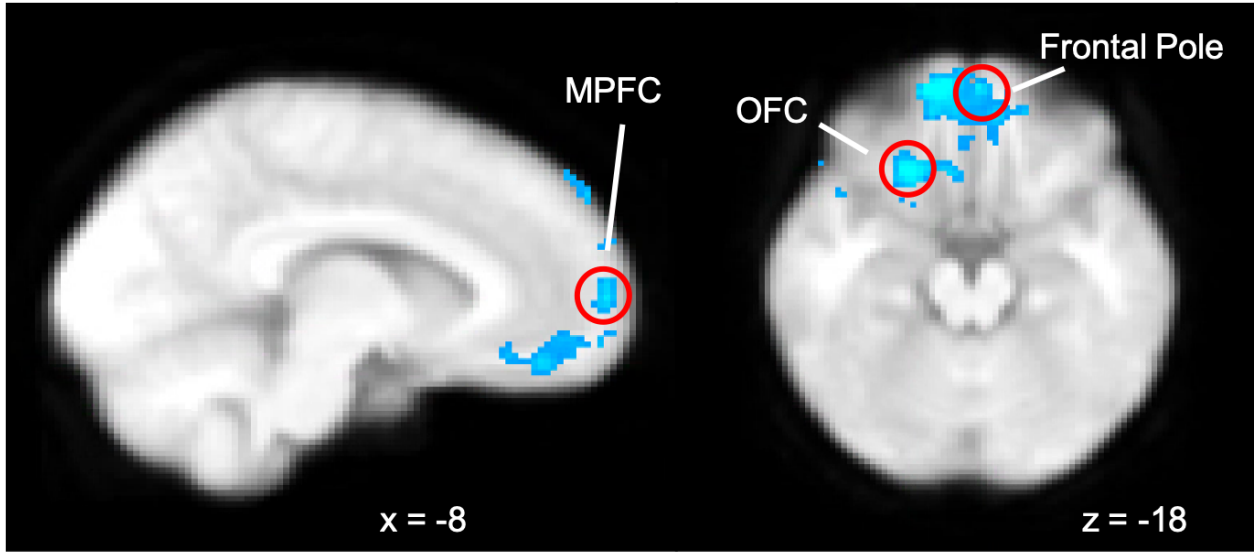


Figure 3.7. Daily negative affect moderated associations between IL-6 and activation in perigenual ACC, MPFC, and OFC during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

Analyses also revealed a significant interaction between TNF- α and negative affect in medial prefrontal regions (MPFC, left frontal pole, right medial OFC), left superior frontal gyrus, right temporal pole, and right occipital cortex during Test > Practice (Table 3.3, Figure 3.8). Parameter estimates (5mm spheres around peak activation) from MPFC, left frontal pole, and right medial OFC were extracted to probe the nature of the interaction. Follow up analyses revealed that among those who reported high negative affect, higher levels of TNF- α were associated with lower (greater) activation in MPFC, left frontal pole, and right OFC during Test > Practice (Practice > Test) (MPFC: $B = -.5757$, $SE = .2015$, $t(16) = -2.8575$, $p = .011$; frontal pole: $B = -.2862$, $SE = .1292$, $t(16) = -2.2157$, $p = .0416$; OFC: $B = -.6002$, $SE = .1389$, $t(16) = -4.3212$, $p = .0005$). Levels of TNF- α were not significantly associated with MPFC activation during Test > Practice among those who reported low ($B = .1838$, $SE = .2059$, $t(16) = .8926$, $p = .3853$) or average levels of negative affect ($B = -.1959$, $SE = .1337$, $t(16) = -1.4658$, $p = .1621$). In contrast, among those who reported low negative affect, higher levels of TNF- α were associated with higher (lower) activation in left frontal pole and right OFC during Test > Practice (Practice > Test) (frontal pole: $B = .2990$, $SE = .1320$, $t(16) = 2.2650$, $p = .0377$; OFC: $B = .3400$, $SE = .1420$, $t(16) = 2.3948$, $p = .0292$) (Figure 3.8). Activation in these regions were not associated with task behavior or evaluation.

There were no significant interactions between negative affect and IL-8, IL-10, or IFN γ on neural response to stress (Test > Practice).



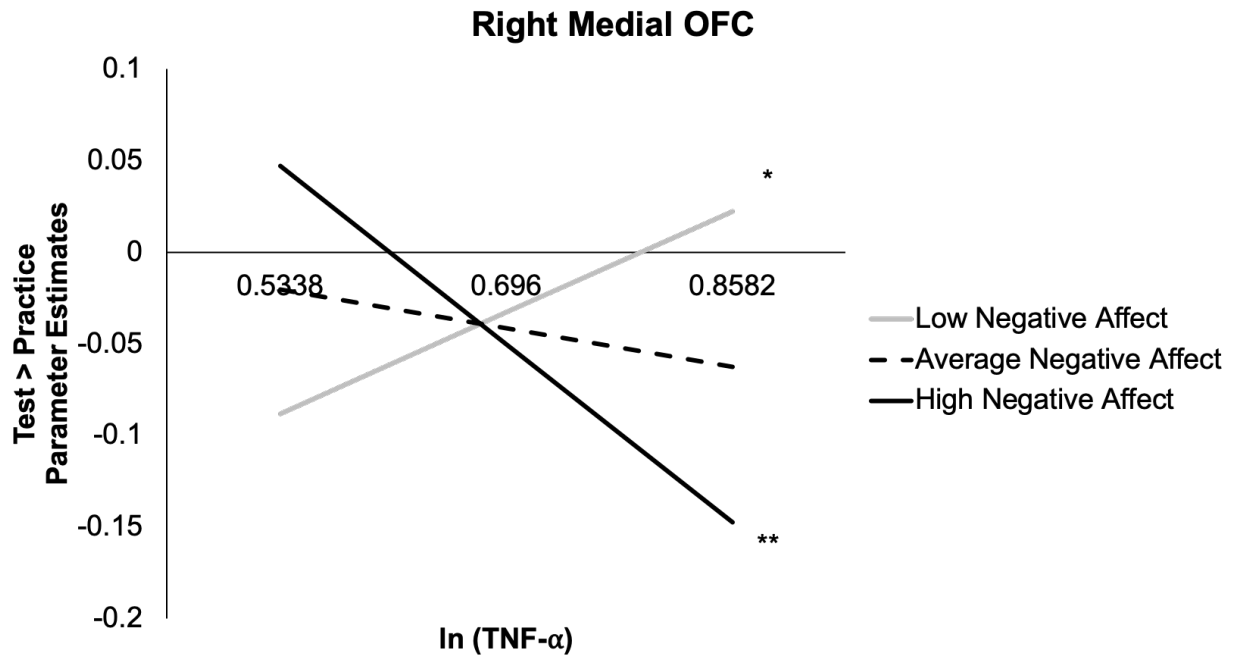
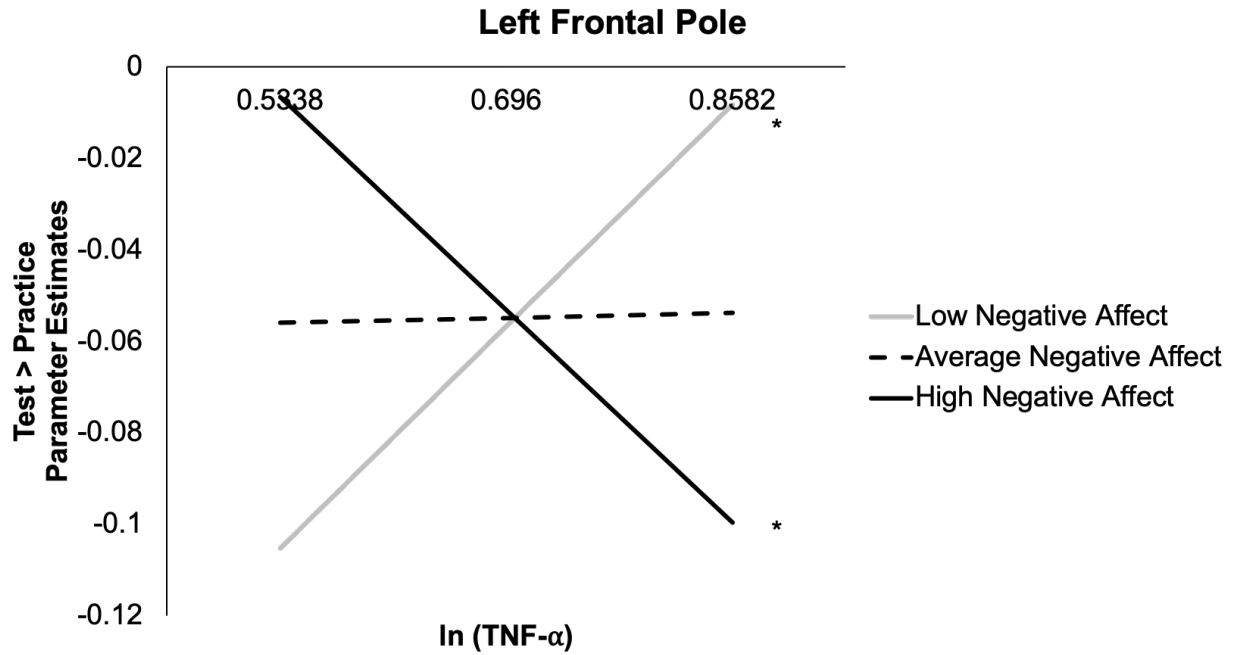


Figure 3.8. Daily negative affect moderated associations between TNF- α and activation in MPFC, left frontal pole, and right OFC during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

Stressor reactivity. Whole-brain analyses testing for interactions between negative affect and immune markers revealed a significant interaction between IL-6 and stressor reactivity in left MPFC during Test > Practice (Table 3.3; Figure 3.9). Parameter estimates (5mm spheres around peak activation) from MPFC were extracted to probe the nature of the interaction. Follow up analyses revealed that among those who evinced average levels of stressor reactivity (mean = .1931), greater levels of IL-6 were associated with higher (lower) activation in MPFC during Test > Practice (Practice > Test) ($B = .1064$, $SE = .0488$, $t(13) = 2.1818$, $p = .0481$). This effect was stronger for those who evinced high stressor reactivity (1 SD above mean = .6647; $B = .2988$, $SE = .0860$, $t(13) = 3.4743$, $p = .0041$). Levels of IL-6 were not associated with MPFC activation for those who showed low stressor reactivity (1 SD below mean = -.2785; $B = -.0860$, $SE = .0533$, $t(13) = -1.6117$, $p = .1310$) (Figure 3.9). MPFC activation was not associated with task behavior or evaluation.

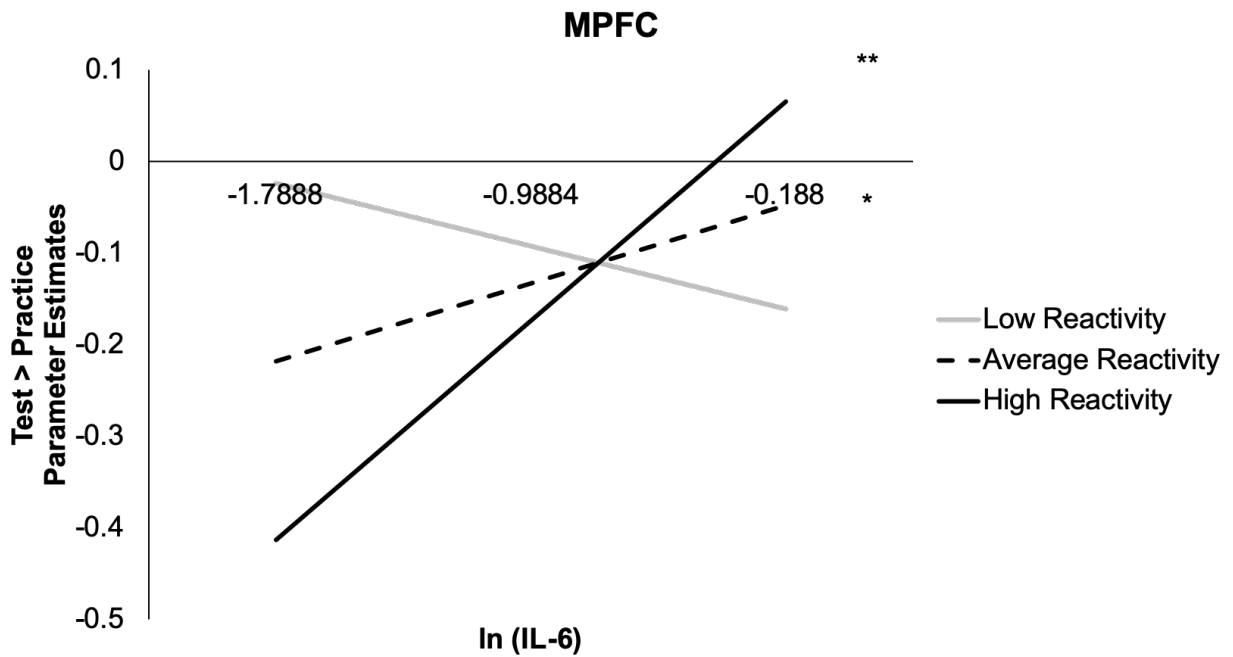
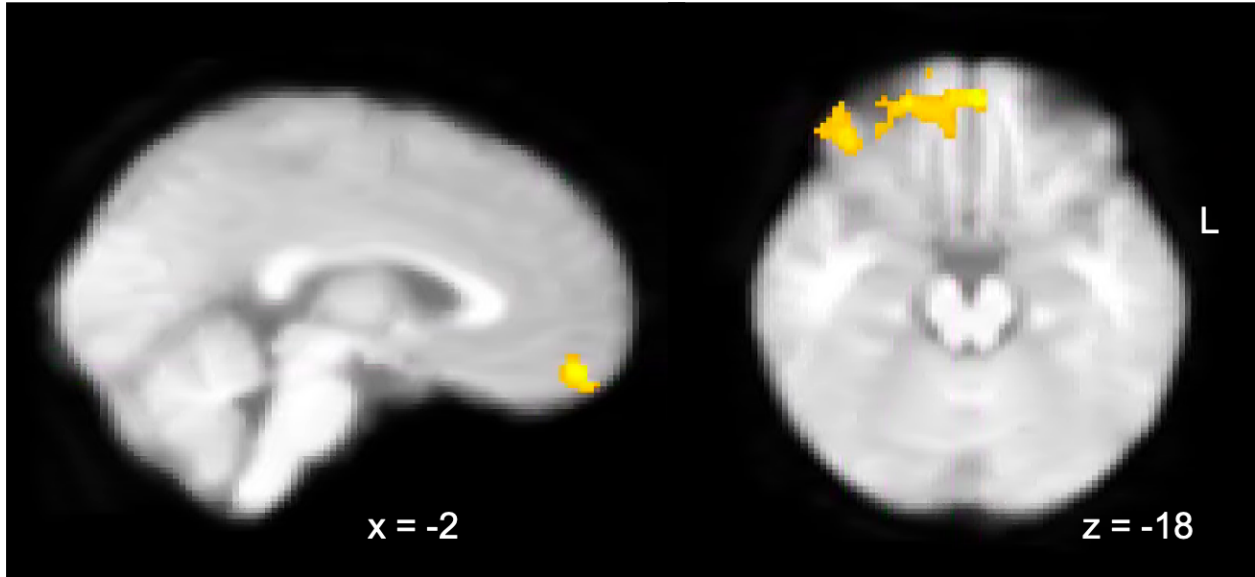
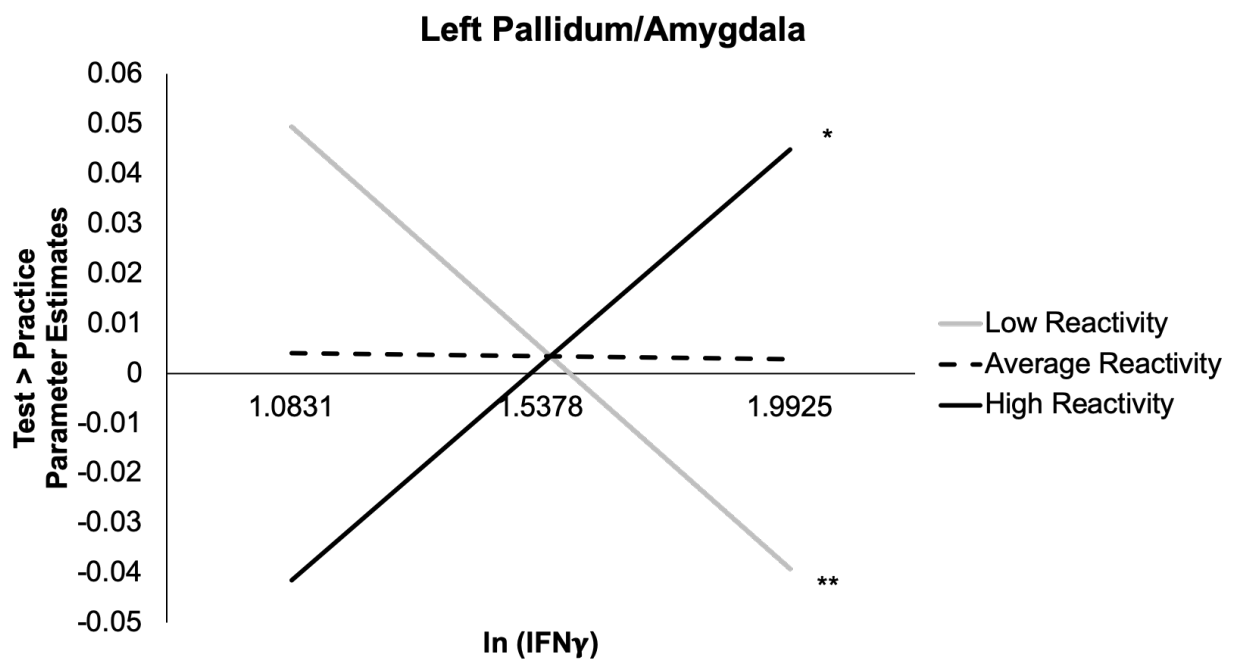
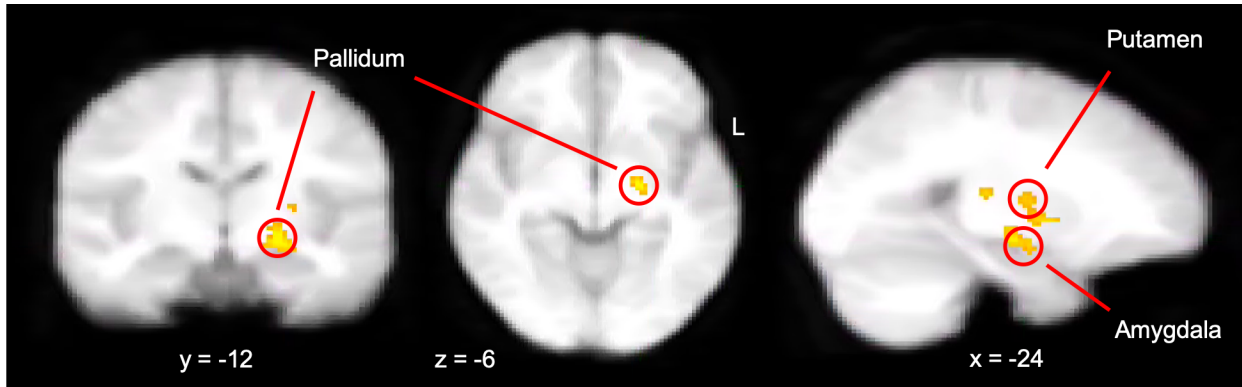


Figure 3.9. Stressor reactivity moderated associations between IL-6 and activation in MPFC during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

Analyses also revealed a significant interaction between IFN γ and stressor reactivity in left pallidum, which extended out to left amygdala, and left putamen (Table 3.3; Figure 3.10). Parameter estimates (5mm spheres around peak activation) from left pallidum/amygdala and left putamen were extracted to probe the nature of the interaction. Follow up analyses revealed that among those with low stressor reactivity, greater levels of IFN γ were associated with greater activation in left pallidum/amygdala ($B = -.0975$, $SE = .0244$, $t(13) = -3.9953$, $p = .0015$) and left putamen during Practice > Test ($B = -.0433$, $SE = .0176$, $t(13) = -2.4612$, $p = .0286$). In contrast, among those with high stressor reactivity, greater levels of IFN γ were associated greater activation in left pallidum/amygdala ($B = .0949$, $SE = .0320$, $t(13) = 2.9617$, $p = .0110$) and left putamen ($B = .0709$, $SE = .0231$, $t(13) = 3.0741$, $p = .0089$) activation during Test > Practice (Figure 3.10). Levels of IFN γ were not significantly associated with left pallidum or putamen activation during Test > Practice for those with average levels of stressor reactivity (pallidum: $B = -.0013$, $SE = .0161$, $t(13) = -.0852$, $p = .9355$; putamen: $B = .0138$, $SE = .0116$, $t(13) = 1.1905$, $p = .255$). Controlling for gender, BMI, stressor reactivity, and IFN γ , greater activation in left pallidum/amygdala was associated with greater difference in stress ratings between test and practice blocks ($F(1, 13) = 5.885$, $p = .031$); this effect was driven by greater stress ratings of test blocks among those with greater left pallidum/amygdala activation during Test > Practice ($B = 9.253$, $SE = 2.614$, $t(13) = 3.540$, $p = .004$ (Figure 3.11).

There were no significant interactions between stressor reactivity and IL-8, IL-10, or TNF- α on neural response to stress (Test > Practice).



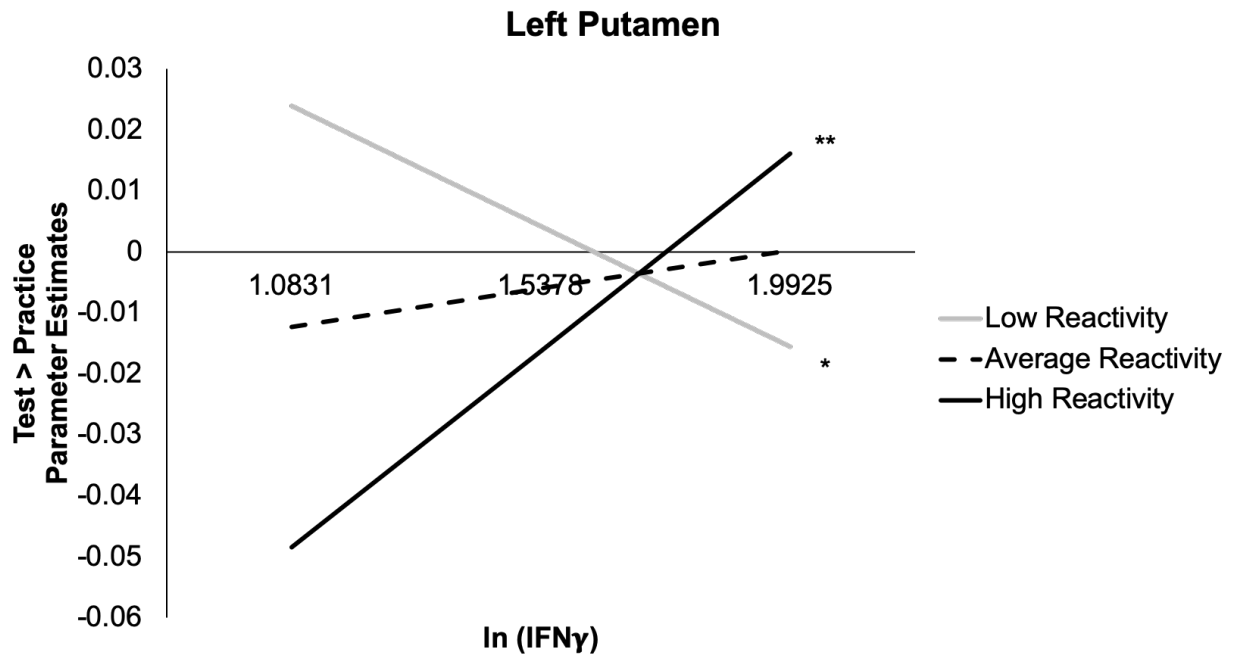


Figure 3.10. Stressor reactivity moderated associations between IFN γ and activation in left pallidum/amygdala and left putamen during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

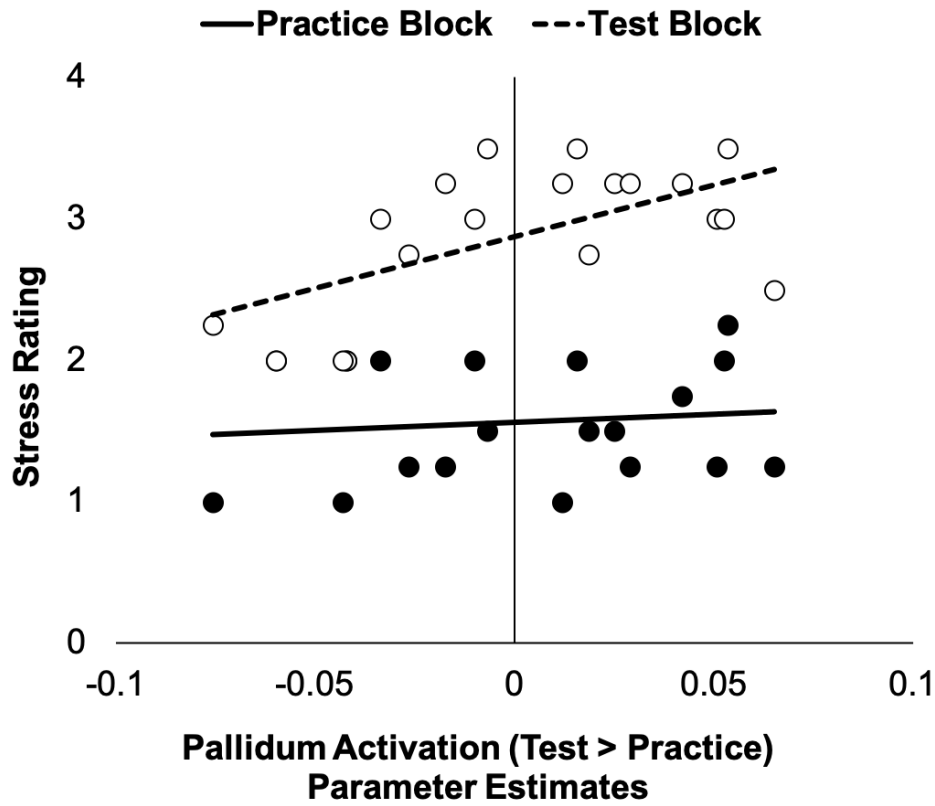


Figure 3.11. Controlling for gender, BMI, negative affect, and levels of $IFN\gamma$, greater activation in left pallidum/amygdala during Test > Practice was associated with greater stress reactivity (difference in stress ratings between test and practice blocks).

| | Immune Marker | Hemisphere | Region | x | y | z | Z-max | |
|-------------------------------|--------------------------------|--------------------------------|--------------------------|--------------------------|-----|-----|-------|------|
| Test > Practice | | | | | | | | |
| Daily Stressors | TNF-α | L/R | Temporal pole | -42 | 4 | -22 | 3.36 | |
| | | | | 48 | 16 | -30 | 3.15 | |
| | IL-8 | R | | Precentral gyrus | 28 | -16 | 52 | 3.95 |
| | | | | Superior frontal gyrus | 26 | -6 | 60 | 3.25 |
| | | | | Lateral occipital cortex | 56 | -68 | 8 | 3.56 |
| | | | | Occipital pole | -28 | -92 | 28 | 4.56 |
| | IFNγ | L | | Lateral occipital cortex | -38 | -64 | 48 | 4.17 |
| | | | | Angular gyrus | -44 | -54 | 20 | 3.82 |
| Negative Affect | IL-6 | L | Anterior cingulate | -2 | 40 | 10 | 3.41 | |
| | | | MPFC | -2 | 48 | -2 | 3.33 | |
| | | | OFC | -44 | 32 | -6 | 3.60 | |
| | TNF-α | R | | OFC | 22 | 24 | -18 | 4.16 |
| | | | | Frontal pole | -2 | 56 | -18 | 3.45 |
| | | | | MPFC | -8 | 64 | 4 | 3.34 |
| | | | | Superior frontal gyrus | -2 | 40 | 52 | 3.53 |
| | | | | Temporal pole | 40 | 18 | -28 | 3.70 |
| | IFNγ | R | | Occipital pole | 24 | -98 | 6 | 3.49 |
| | | | | Lateral occipital cortex | 46 | -78 | 16 | 3.41 |
| | Reactivity | IL-6 | L | MPFC | -2 | 54 | -18 | 4.10 |
| | | TNF-α | L | Lateral occipital cortex | -48 | -80 | 20 | 4.57 |
| IL-8 | | L | Lateral occipital cortex | -42 | -84 | 8 | 3.90 | |
| IL-10 | | L/R | | Occipital pole | -10 | -90 | 22 | 4.10 |
| | | | | | 22 | -90 | 26 | 4.23 |
| IFNγ | | L | | Pallidum | -22 | -12 | -6 | 3.59 |
| | | | | Putamen | -24 | -8 | 10 | 2.79 |

Table 3.3. Significant interactions between daily measures and peripheral immune markers on neural activation during Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$. Note: x, y, and z refer to MNI coordinates, Z-max refers to peak level of activation intensity. L = left, R = right.

Discussion

The goal of the current study was to investigate whether adolescents' perceived stress, experiences of daily stressors, daily negative affect, and stressor reactivity (Lippold et al., 2016) related to peripheral immune markers, neural response to stress, and whether these daily experiences moderated the associations between peripheral immune markers and neural response to stress. It was hypothesized that adolescents who reported greater levels of perceived stress, endorsed more stressors, reported higher negative affect, and/or showed greater stressor reactivity would exhibit higher levels of pro-inflammatory markers. Results revealed that greater perceived stress was associated with lower levels of IFN γ . Results also revealed that stressor reactivity was positively associated with levels of IL-6. That is, adolescents who, on average, reported greater negative affect on days that they endorsed experiencing more stressors had higher levels of circulating IL-6. Stressor reactivity was not associated with any other immune markers. Additionally, greater negative affect was marginally associated with lower levels of anti-inflammatory markers, IL-10. Negative affect was not associated with other immune markers. Daily stressors were not associated with any of the immune markers. These findings suggest that experiences of normative stressors in daily life, such as demands from school or arguments with others, themselves may not necessarily have effects on immunity; rather, it is the negative appraisals and reactivity to the stressors that appear to have consequences for inflammation. These findings extend from previous research that reported elevated levels of inflammation and pro-inflammatory gene expression in relation to increased stress in adolescents (Chiang et al., 2019a; Fuligni et al., 2009) by demonstrating that stressor reactivity also relates to elevated levels of inflammation. The current study further extends previous research by showing

that greater daily negative affect and greater levels of perceived stress were negatively associated with anti-inflammatory and antiviral processes, respectively.

On the fMRI stressor task, adolescents who reported greater levels of perceived stress reported higher stress ratings overall, greater test-related anxiety symptoms, greater perceived task difficulty, and worse self-rated performance. Despite these elevated levels in negative evaluation and affect, levels of perceived stress were not related to test accuracy. Additionally, perceived stress was not associated with neural activation on the task.

While proportion of daily stressors was not associated with behavior or participants' self-reported evaluation of the task, adolescents who reported more daily stressors showed greater frontostriatal (left lateral prefrontal and orbitofrontal cortex, left putamen) activation during Test > Practice. Activation in these regions were not associated with behavior or self-reported evaluation of the task. ROI analyses revealed that daily stressors were positively associated with bilateral anterior insula activation during Test > Practice, which were positively associated with greater test accuracy. These findings suggest that adolescents who experience more daily stressors recruit frontostriatal circuitry to a greater extent than those with fewer daily stressors when engaging with stress, which bolsters their performance in overcoming the stressor. Daily stressors did not moderate the associations between immune markers and neural activation in corticolimbic circuitry.

Adolescents who reported greater negative affect reported higher stress ratings for both practice and test blocks, showed greater differences in response times between practice and test trials, and were faster on trials overall. They also reported experiencing greater test-related anxiety symptoms. However, negative affect was not associated with test accuracy. In the brain, greater negative affect was associated with lower activation in regions implicated in stress

reactivity (dACC, right anterior insula) and prefrontal regions implicated in regulation (DLPFC, MPFC, OFC/VLPFC) during stress. Controlling for gender and negative affect, lower activation in dACC and right anterior insula were associated with lower test accuracy. Additionally, lower MPFC activation during stress (or greater MPFC activation during Practice > Test) was associated with greater perceived test difficulty, over and above negative affect. Moreover, greater negative affect was associated with greater activation in bilateral amygdala and bilateral hippocampus during periods of low relative to high stress (i.e., Practice > Test). Results from Chapter 2/Study 1 indicated that greater activation in regions implicated in emotion/stress regulation (e.g., amygdala, hippocampus, VMPFC) during low stress (Practice > Test) were associated with greater test-related anxiety, suggesting perhaps that engaging these emotion regulation regions during periods of relatively lower stress might reflect poorer stress regulation or recovery from stress, which might explain the heightened anxiety symptoms. In the current study, that greater daily negative affect was associated with greater activation in these regions during Practice > Test as well as greater test-related anxiety might suggest a positive feedback loop whereby individuals with greater negative affect might have exaggerated negative appraisals of stress, which are paralleled by under-engagement of brain regions that help regulate and overcome stress, leading to exacerbated negative appraisals associated with the stressor (indicated by higher stress ratings) and increased anxiety symptoms and negative affect.

Negative affect moderated the associations between IL-6 and perigenual ACC, MPFC, and lateral OFC activation during Test > Practice. Among those who reported low negative affect, lower levels of IL-6 were associated with greater perigenual ACC and MPFC activation during Test > Practice. IL-6 was not associated with perigenual ACC or MPFC activation among those who reported average or high negative affect. Controlling for gender, BMI, daily negative

affect, and levels of IL-6, perigenual ACC and MPFC activation were not associated with behavior or self-report. In contrast, among those who reported high negative affect, higher levels of IL-6 were associated with greater lateral OFC activation during Test > Practice. Controlling for gender, BMI, daily negative affect, and levels of IL-6, greater OFC activation was associated with lower self-rated performance on the test. Research suggests that the perigenual ACC is a region that is responsive to social-environmental factors and has implications for stress and health depending on whether those factors are risk or resilience factors (Holz, Tost, & Meyer-Lindenberg, 2020). These findings suggest that greater perigenual ACC and MPFC activation during Test > Practice (as opposed to Practice > Test) might be a marker of resiliency in stress, which could explain why these individuals evince lower levels of negative affect and IL-6. Alternatively, it could be possible that these differences in activation could be explained by individuals with low negative affect and IL-6 experiencing fewer daily stressors relative to those with high negative affect and IL-6.

Negative affect also moderated the associations between TNF- α and activation in medial prefrontal and orbitofrontal regions during Test > Practice. Among those who reported high negative affect, greater levels of TNF- α were associated with greater activation in these medial frontal regions during low stress (Practice > Test). This effect was attenuated or in the opposite direction among those who reported low negative affect. These findings extend from the negative affect findings above to specify that individuals with high daily negative affect *and* high levels of inflammation might evince poorer stress regulation via greater medial frontal activation during periods of low stress. These findings also suggest that immune-brain associations may be more readily detected during conditions of high negative affect, supporting the notion that negative

affect may sensitize the signaling between stress/emotion regulation regions and inflammatory markers.

Stressor reactivity was not associated with behavior or self-report or prefrontal activation during Test > Practice. Greater stressor reactivity was associated with greater bilateral amygdala and hippocampus activation during Practice > Test, suggesting that adolescents who endorse greater negative affect on days that they experience stress may be less likely to regulate stress/negative emotions under periods of relatively lower stress. Additionally, stressor reactivity moderated associations between IL-6 and MPFC activation during Test > Practice such that among those with high reactivity, greater levels of IL-6 was associated with greater MPFC activation during Test > Practice (or lower MPFC activation during Practice > Test). Stressor reactivity also moderated associations between IFN γ and striatal activation (pallidum/amygdala, putamen) during Test > Practice. Among those with high stressor reactivity, greater levels of IFN γ was associated with greater pallidum/amygdala and putamen activation during Test > Practice. In contrast, among those with low reactivity, greater levels of IFN γ was associated with lower pallidum/amygdala and putamen activation during Test > Practice. Controlling for gender, BMI, stressor reactivity, and IFN γ , greater activation in left pallidum/amygdala was associated with greater difference in stress ratings between test and practice blocks, driven by greater stress ratings of test blocks among those with greater left pallidum/amygdala activation. These findings suggest that the associations between inflammatory markers and frontolimbic response to stress depends on individual differences in reactivity to stressors in daily life. That is, those who reported greater negative affect in response to stressors in daily life *and* have higher levels of inflammatory markers are more likely to engage frontolimbic circuitry while undergoing a

stressor, which was associated with greater stress reactivity (i.e., difference in stress ratings between test and practice blocks) to stress, but no differences in performance.

The current study has several limitations to note. First, the correlational design of the study precludes drawing any conclusions regarding the directionality of the relations among the daily diary measures, immune markers, and brain function. Based on our hypotheses, the current study tested whether daily measures moderated the associations between immune markers and brain function and reported findings accordingly, but analyses that test the interaction of daily measures and brain function to predict immune markers could have also been conducted and different interpretations/conclusions could have possibly been drawn. Second, while we found significant effects for the interactions between daily measures and immune markers on brain function, the sample size for those analyses were very small, so it remains to be tested whether these effects would replicate with larger samples. Moreover, the small sample size could also provide insufficient power for us to detect associations between daily stressors and immune markers, which previous studies have found. Third, the current sample of adolescents reported relatively low levels of daily negative affect (average = 1.751 out of possible 5) and showed relatively low levels of circulating immune markers, which limit the generalizability of the findings to adolescents who have more adverse experiences. Additionally, the current study's assessment of stressors was limited to demands and arguments; it could be possible that adolescents experienced additional stressors that were not captured by our measure (e.g., finding out that a loved one is sick, parents losing jobs, etc.). Future studies conducted in larger samples that utilize longitudinal or experimental designs with more extensive measures of daily experiences, rigorous neuroimaging techniques, and that probe circulating levels of immune

markers would be better positioned to delineate the associations between daily experiences and the brain-body connection during adolescence.

Despite these limitations, the current study was the first to investigate the associations between daily affective experiences (daily stressors, negative affect, and stressor reactivity), peripheral immune markers, and brain function in adolescents. It provided empirical evidence that even in a relatively healthy sample of adolescents, variability in daily affective experiences and immune markers relate to variability in neural response to stress in corticolimbic circuitry in a sample of older adolescents.

Chapter 4. Sleep duration and variability moderate the associations between immune markers and corticolimbic function during stress

During adolescence, there is a shift in chronotype such that adolescents prefer a later bedtime and waketime (Carskadon et al., 1993) as well as accumulate sleep pressure at a slower rate (Jenni, Achermann, & Carskadon, 2005). Coupled with early school start times (Carskadon, Wolfson, Acebo, Tzischinsky, & Seifer, 1998), adolescents represent one of the most sleep-deprived populations – over 60% of U.S. high school students receive less than the recommended 7-9 hours of sleep (CDC, 2011; Kann et al., 2014). In addition to negatively affecting learning and memory (Walker & Stickgold, 2006), cognition (C. Anderson & Platten, 2011; Telzer, Fuligni, Lieberman, & Galván, 2013), and decision making (Killgore, Balkin, & Wesensten, 2006; Killgore, Kamimori, & Balkin, 2011), individuals who receive insufficient sleep are also more likely to experience physical and psychological health problems (Vgontzas et al., 2004). For example, across healthy and clinical populations, various forms of poor sleep (e.g., experimental partial or total sleep deprivation, naturalistic sleep disturbance, poor sleep efficiency) have typically been associated with elevated levels of inflammatory markers such as CRP, IL-6, and TNF- α (Irwin, 2015; Irwin et al., 2016). There is also a strong association between poor sleep and depressive symptoms (Baglioni et al., 2011). Given the links between stress, inflammation, and depressive symptoms reviewed in Chapter 2/Study 1, it is likely that stress-related processes are candidate mechanisms by which poor sleep relates to depressive symptoms. This area of research has been relatively under-explored, especially in adolescents.

Emerging evidence suggests that sleep influences the systems that respond to stress (e.g., SNS and HPA axis) (Irwin, 2015), which has been shown to regulate immune responses (as reviewed in Chapter 2/Study 1). During sleep, blood levels of cortisol, epinephrine, and

norepinephrine lower while hormones that subserve cell growth show a steep increase (Besedovsky, Lange, & Born, 2012). These processes are disrupted in the context of poor sleep, which results in increased SNS and HPA axis activity and have implications for stress responding during wake. Compared to well-rested adults, sleep-deprived adults exhibit higher baseline cortisol levels and heightened cortisol response to psychosocial stress (Minkel et al., 2014). Furthermore, the effects of sleep and stress are bidirectional: stressors experienced throughout the day and levels of inflammatory cytokines may also influence sleep quality (Gordon, Mendes, & Prather, 2017; Raison et al., 2010). Taken together, evidence suggests that poor sleep influences immune function and health not only through disruption of processes that subserve cell growth and recovery, but also by sensitizing the systems that respond to stress. Surprisingly, sleep and stress processes on brain development and immune activity are rarely studied together in adolescents. The confluence of changes in HPA function, sleep habits, and corticolimbic circuitry that occur during adolescence confers a period of vulnerability to negative health outcomes. Burgeoning research examining the links between sleep habits and immune markers during adolescence have found that shorter sleep duration was associated with higher levels of CRP (Park et al., 2016) and greater likelihood of high risk CRP levels ($> 3\text{mg/L}$) (Hall, Lee, & Matthews, 2015). Greater variability in sleep duration was also associated with higher levels of CRP (Park et al., 2016). In relation to the upstream molecular immune processes, shorter sleep duration was associated with greater gene expression of pro-inflammatory proteins, increased signaling of pro-inflammatory transcription factor NF- κ B, downregulation of antiviral gene expression, and decreased signaling of interferon response factors in adolescents (Chiang et al., 2019a). Moreover, shorter sleep duration strengthened the associations between daily stress and NF- κ B activity. That is, greater daily stress was more strongly associated with greater

inflammatory NF- κ B activity among adolescents with shorter sleep duration (Chiang et al., 2019a). These findings suggest that poor sleep may sensitize the brain to the psychological and physiological effects of stress, including the effects of inflammation, which may further potentiate stress sensitivity and responses. However, the effects of sleep and inflammation on the developing brain's response to stress are unknown.

The current study examined whether sleep duration and variability relate to levels of peripheral immune markers and the brain's response to stress in adolescents. I hypothesized that adolescents who reported shorter sleep duration and greater variability in sleep duration would exhibit higher levels of pro-inflammatory markers and heightened neural response to stress. The current study also explored whether sleep habits moderate the association between inflammation and neural responses to stress.

Methods

Participants

Self-report questionnaires, daily diary, and neuroimaging data were collected from 40 adolescents (14.03-15.99 years, $M = 15.076$, $SD = 0.646$, 17 females) who participated in a larger study conducted by Drs. Galvan, Fuligni, and Eisenberger (NSF BSC 1551952). Participants were recruited using flyers posted on university campus, in local child and adolescent-friendly locations, on community websites (e.g., Craigslist), and flyer distributions at local high schools. Inclusion criteria required all participants be right-handed, free from metal objects in the body, speak fluent English, be in the appropriate age range, and have no previously diagnosed psychiatric, neurological, or developmental disorders. Parents of adolescent participants provided written consent and adolescents provided assent in accordance with the University of California, Los Angeles (UCLA) Institutional Review Board. Participants were

also provided the opportunity to consent to an optional blood draw. All participants were compensated for their participation.

Of the 40 participants, one participant was excluded from neuroimaging analyses due to a neuroanatomical abnormality and two participants were excluded for excessive motion across both runs of the task. Of the remaining 37 (18 females) participants with usable neuroimaging data, 23 (62%; 9 females) participated in the blood draw. Analyses were conducted with the maximum number of subjects for each analysis.

Procedure

Participants completed two visits at UCLA. During the first visit, after providing consent, participants completed questionnaires about demographic information and depressive symptoms and were trained on how to complete the daily diary measures. For 7 days after the first visit, participants received a text message each evening with a URL to an online survey asking about their day that they completed. After 7 days (but within two weeks), participants returned to UCLA to complete their second visit. Participants who consented to the blood draw had their blood drawn by a certified phlebotomist at the clinical lab in the Peter Morton Medical Building at UCLA. After the blood draw, participants completed a brain scan while performing the fMRI stressor task at the Center for Cognitive Neuroscience (CCN) at UCLA. Participants who did not consent to the blood draw only completed the brain scan portion of the study. Participants' height and weight were measured to calculate body mass index (BMI). BMI ranged from 14.337 to 45.154 ($M = 23.298$, $SD = 6.299$). After the brain scan, participants completed additional questionnaires about their experiences regarding the stressor task, were debriefed about the goals of the study, and received compensation.

Measures

Sleep Duration. Each night, participants were asked to report how much sleep they received the night before. Daily sleep duration was averaged across the 7 days to assess participants' average nightly sleep duration. Average weekly sleep duration ranged from 259.285 minutes to 585.857 minutes (M = 467.452 minutes, SD = 60.479).

Sleep Duration Variability. For each participant, standard deviation in self-reported sleep duration across the week was calculated to assess variability in sleep duration. Variability ranged from 23.604 minutes to 292.391 minutes (M = 85.139 minutes, SD = 53.593).

Immunological Measures

A detailed description of blood sample collection, processing, and immunological assays are reported in the *Methods: Immunological Measures* section of Chapter 2/Study 1.

fMRI

A detailed description of the fMRI Stressor Task, Data Acquisition, Data Preprocessing, and Level 1 analyses are previously reported in the *Methods: fMRI* section of Chapter 2/Study 1.

Analytic Plan

All reported analyses covaried for gender. All analyses consisting of sleep variability additionally covaried for average sleep duration. All analyses consisting of peripheral immune markers covaried for gender and body mass index (BMI).

Regression analyses were used to relate participants' average sleep duration and variability in sleep duration to levels of peripheral immune markers.

Behavioral Analysis of Stressor Task. Repeated-measures ANCOVAs and regression analyses were conducted to determine whether stress ratings, behavior on stressor task, participants' responses to the test-related STAI, and post-task questionnaire differed by average sleep duration and sleep variability.

fMRI Data Analysis. A detailed description of level 1 analyses is previously reported in *Methods: Analytic Plan* section of Chapter 2/Study 1. Analyses focused on the contrast between test blocks and practice blocks (Test > Practice, Practice > Test). A fixed effects voxel-wise analysis combined each of the two runs at the second level. Regression analyses were conducted at the group level using the FMRIB local analysis of mixed effects (FLAME1) module in FSL with mean-centered regressors of interest (e.g., sleep duration, sleep variability) entered in each respective model in whole brain analyses. Z (Gaussianized T) statistic images were thresholded at $Z > 2.3$ by a corrected cluster significant threshold of $p < .05$ using Gaussian Random Field theory and corrected for family-wise errors. Anatomical localization within each cluster were obtained by searching within maximum likelihood regions from the FSL Harvard-Oxford probabilistic atlas.

Average sleep duration and sleep duration variability were entered as mean-centered regressors of interest in separate GLMs for whole-brain fMRI analyses to assess their associations with neural response to stress. Moderation analyses for each sleep measure and each immune marker were conducted at the whole-brain level to assess whether average sleep duration and/or variability moderated associations between immune markers and neural response to stress.

ROI Analyses. In addition to whole-brain analyses, ROI analyses were also conducted in regions previously implicated in stress reactivity and regulation (e.g., dACC, left and right anterior insula, left and right amygdala, and left and right hippocampus). A detailed description of the ROIs is previously reported in *Methods: Analytic Plan* section of Chapter 2/Study 1.

Results

Sleep duration and inflammation

Controlling for gender and BMI, average sleep duration across the week was not associated with levels of IFN γ (B = -0.00015, SE = .002, t(18) = -0.097, p = .924), IL-10 (B = .001, SE = .001, t(18) = 1.00, p = .331), IL-6 (B = -.001, SE = .002, t(18) = -.310, p = .760), IL-8 (B = -.004, SE = .002, t(18) = -1.720, p = .103), or TNF- α (B = -.000194, SE = .00057, t(18) = -.338, p = .739).

Sleep duration variability and inflammation

Controlling for gender, BMI, and average sleep duration, sleep duration variability was marginally negatively associated with IL-10 (B = -.002, SE = .001, t(17) = -2.023, p = .059). Sleep duration variability was not associated with levels of IFN γ (B = -.001, SE = .002, t(17) = -.783, p = .444), IL-6 (B = -.001, SE = .002, t(17) = -.330, p = .746), IL-8 (B = -.001, SE = .002, t(17) = -.301, p = .767), or TNF- α (B = -.00029, SE = .001, t(17) = -.440, p = .666).

Behavior on fMRI Stressor Task

Stress ratings. Main effects of stress ratings were reported in Chapter 2/Study 1. Controlling for gender, differences in stress ratings did not differ by sleep duration (F(1, 32) = .287, p = .596) or sleep variability (F(1, 31) = 3.299, p = .079).

Response time. Main effects of response time were reported in Chapter 2/Study 1. Controlling for gender, differences in response time did not differ by sleep duration (F(1, 32) = .607, p = .441) or sleep duration variability (F(1, 31) = 1.218, p = .278).

Test Accuracy. Controlling for gender, test accuracy did not significantly differ by sleep duration (B = .000273, SE = .001, t(32) = .471, p = .641) or sleep duration variability (B = -.0001, SE = .001, t(31) = -0.829, p = .414).

Evaluation of fMRI Stressor Task

Anxiety. Controlling for gender, test-related anxiety did not differ by sleep duration ($B = .001$, $SE = .015$, $t(32) = .036$, $p = .972$) or sleep variability ($B = -.030$, $SE = .016$, $t(31) = -1.825$, $p = .078$).

Perceived Task Difficulty. Controlling for gender, perceived task difficulty did not differ by sleep duration ($B = -0.002$, $SE = .002$, $t(32) = -1.047$, $p = .303$) or sleep variability ($B = -.0001$, $SE = .003$, $t(31) = -.070$, $p = .994$).

Self-Rated Performance. Greater sleep variability was associated with better self-rated performance, controlling for gender and average sleep duration ($B = .010$, $SE = .004$, $t(31) = 2.469$, $p = .019$). Self-rated performance did not differ by sleep duration ($B = .004$, $SE = .004$, $t(32) = 1.052$, $p = .301$), controlling for gender.

Main effects of fMRI Stressor Task

Detailed analyses of the main effects of fMRI Stressor Task were previously reported in Chapter 2/Study 1. Briefly, on average, participants engaged DLPFC, ACC, anterior insula, and OFC regions more during test blocks compared to practice blocks. Greater bilateral anterior insula (ROIs) activation during Test > Practice were associated with greater test accuracy. In contrast, participants engaged VMPFC, amygdala, hippocampus, and posterior cingulate regions more during practice blocks compared to test blocks. Greater bilateral amygdala and hippocampal activation (ROIs) during Practice > Test were marginally associated with greater test-related anxiety.

Sleep duration and neural response to stress

Whole-brain analyses revealed that, controlling for gender, average sleep duration was not associated with activation during Test > Practice. ROI analyses revealed that, controlling for gender, average sleep duration was not significantly associated with dACC ($B < .001$, $SE < .001$,

$t(32) = -.119, p = .906$), bilateral anterior insula (left: $B < .001, SE < .001, t(32) = -.386, p = .702$; right: $B < .001, SE < .001, t(32) = -.369, p = .715$), bilateral amygdala (left: $B < .001, SE < .001, t(32) = -.016, p = .987$; right: $B < .001, SE < .001, t(32) = .172, p = .865$), or bilateral hippocampus (left: $B < .001, SE < .001, t(32) = -.238, p = .813$; right: $B < .001, SE < .001, t(32) = -.332, p = .742$) activation during Test > Practice.

Sleep variability and neural response to stress

Whole-brain analyses revealed that, controlling for gender and average sleep duration, sleep variability was not associated with activation during Test > Practice. Controlling for gender and average sleep duration, ROI analyses revealed that sleep variability was not significantly associated with dACC ($B < .001, SE < .001, t(31) = -0.501, p = .620$), bilateral anterior insula (left: $B < .001, SE < .001, t(31) = -1.550, p = .131$; right: $B < .001, SE < .001, t(31) = -1.484, p = .148$), bilateral amygdala (left: $B < .001, SE < .001, t(31) = .441, p = .662$; right: $B < .001, SE < .001, t(31) = .743, p = .463$), or bilateral hippocampus (left: $B < .001, SE < .001, t(31) = .600, p = .553$; right: $B < .001, SE < .001, t(31) = .371, p = .714$) activation during Test > Practice.

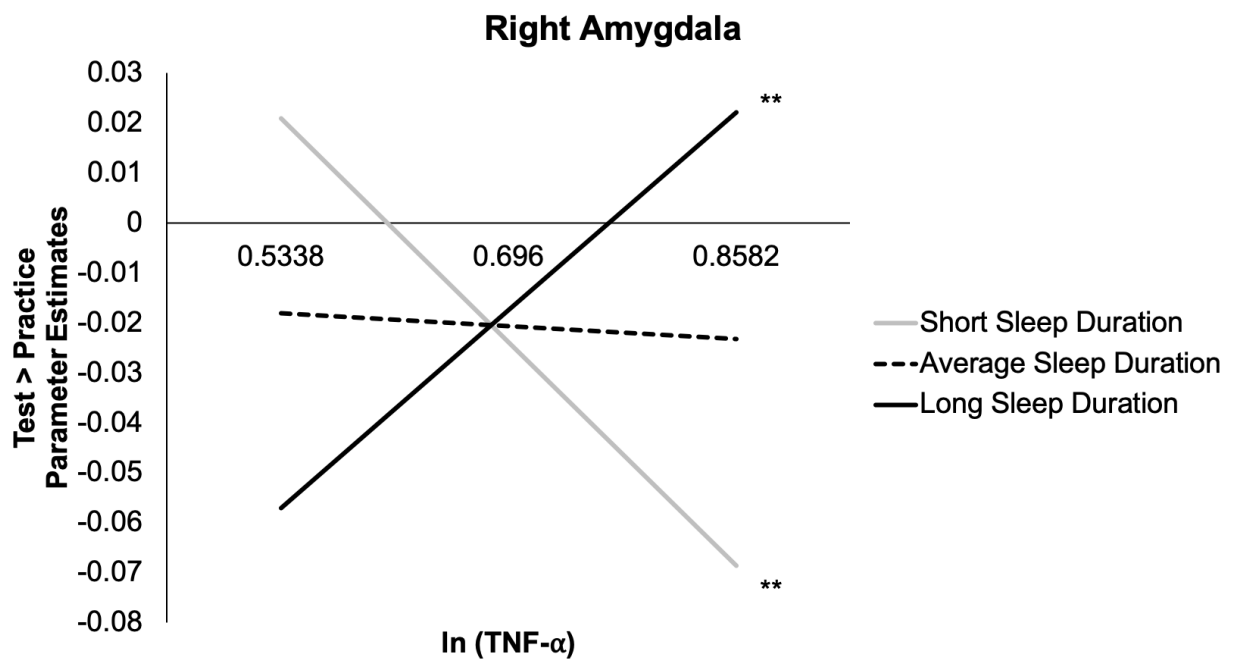
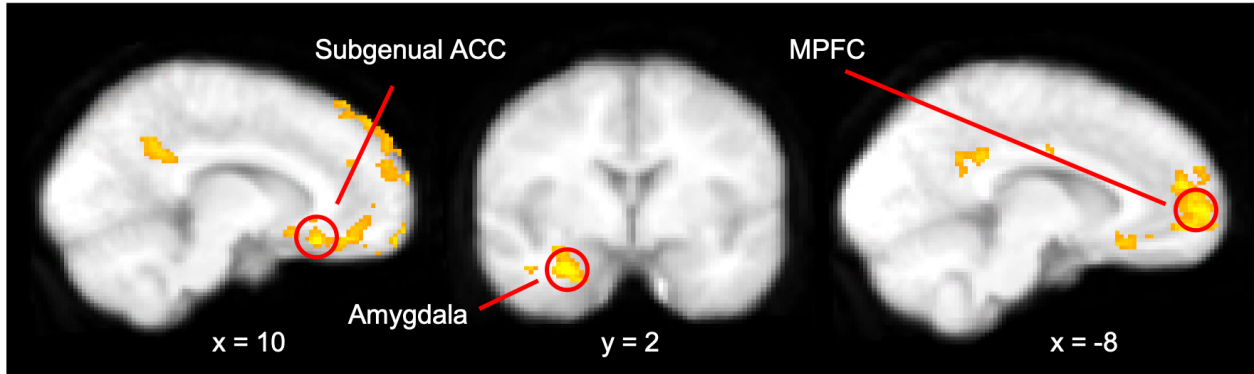
Immune markers and neural response to stress

Detailed analyses of the associations between immune markers and neural response to stress were previously reported in Chapter 2/Study 1. Briefly, levels of immune markers were not significantly associated with regions in corticolimbic circuitry during Test > Practice.

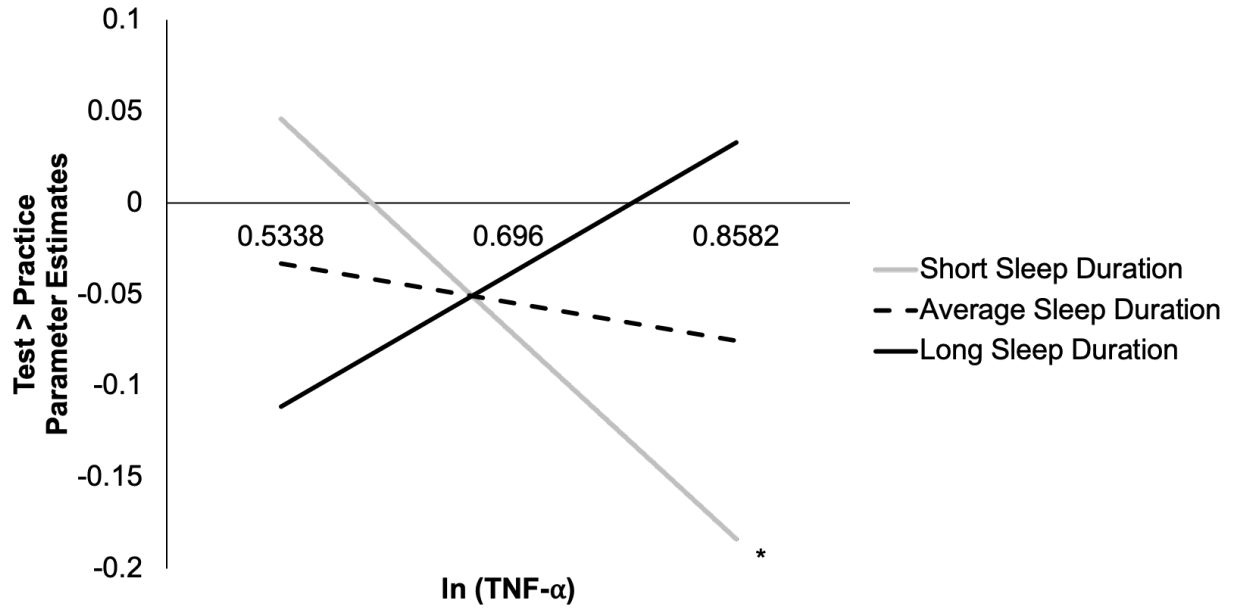
Interactions between sleep variables and peripheral immune markers on neural response to stress

Sleep duration. Whole-brain analyses testing for interactions between average sleep duration and immune markers on neural response to stress (Test > Practice), controlling for gender and BMI, revealed significant interactions between sleep duration and TNF- α in right

amygdala, right subgenual ACC, left MPFC and frontal pole, right temporal pole, left posterior cingulate, left precuneus and occipital cortex, right angular gyrus, and bilateral supramarginal gyrus during Test > Practice (Table 4.1; Figure 4.1). Parameter estimates (5mm spheres around peak activation) from right amygdala, right subgenual ACC, and left MPFC were extracted to probe the nature of the interaction. Follow up analyses revealed that among individuals who reported short sleep duration (1 SD below mean = 386.879 minutes), greater levels of TNF- α were associated with lower activation in right amygdala, right subgenual ACC, and left MPFC during Test > Practice (or greater activation during Practice > Test) (amygdala: $B = -.2757$, $SE = .0724$, $t(16) = -3.8058$, $p = .0016$; subgenual ACC: $B = -.7072$, $SE = .2443$, $t(16) = -2.8952$, $p = .0105$; MPFC: $B = -.2954$, $SE = .1215$, $t(16) = -2.4316$, $p = .0272$). Additionally, among those who reported long sleep duration (1 SD above mean = 530.660), greater levels of TNF- α were associated with greater amygdala and MPFC activation during Test > Practice (or lower activation during Practice > Test) (amygdala: $B = .2441$, $SE = .0772$, $t(16) = 3.1624$, $p = .006$; MPFC: $B = .4134$, $SE = .1294$, $t(16) = 3.1939$, $p = .0056$). In contrast, levels of TNF- α were not associated with subgenual ACC activation during Test > Practice among those who reported long sleep duration ($B = .4465$, $SE = .2603$, $t(16) = 1.7151$, $p = .1056$). Among those who reported average sleep duration, levels of TNF- α were not associated with amygdala, subgenual ACC, or MPFC activation during Test > Practice (amygdala: $B = -.0158$, $SE = .0459$, $t(16) = -.3437$, $p = .7356$; subgenual ACC: $B = -.1304$, $SE = .1549$, $t(16) = -.8417$, $p = .4124$; MPFC: $B = .0590$, $SE = .0770$, $t(16) = .7663$, $p = .4546$) (Figure 4.1). Controlling for gender, BMI, sleep duration, and levels of TNF- α , activation in these regions were not associated with behavior or task evaluation.



Right Subgenual ACC



Left MPFC

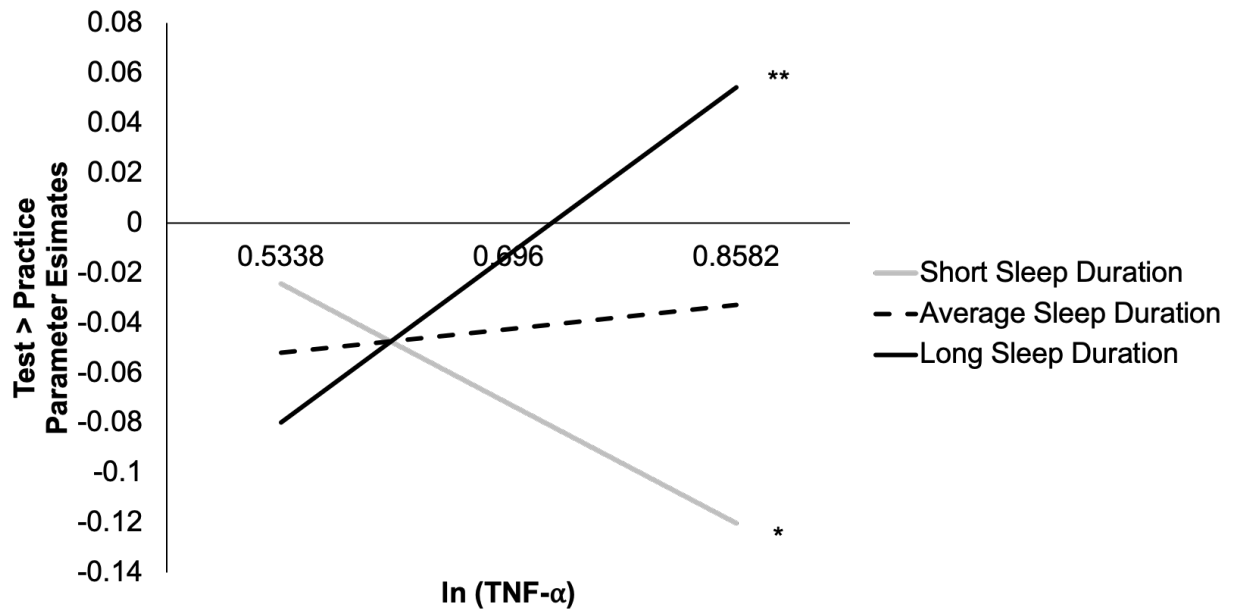
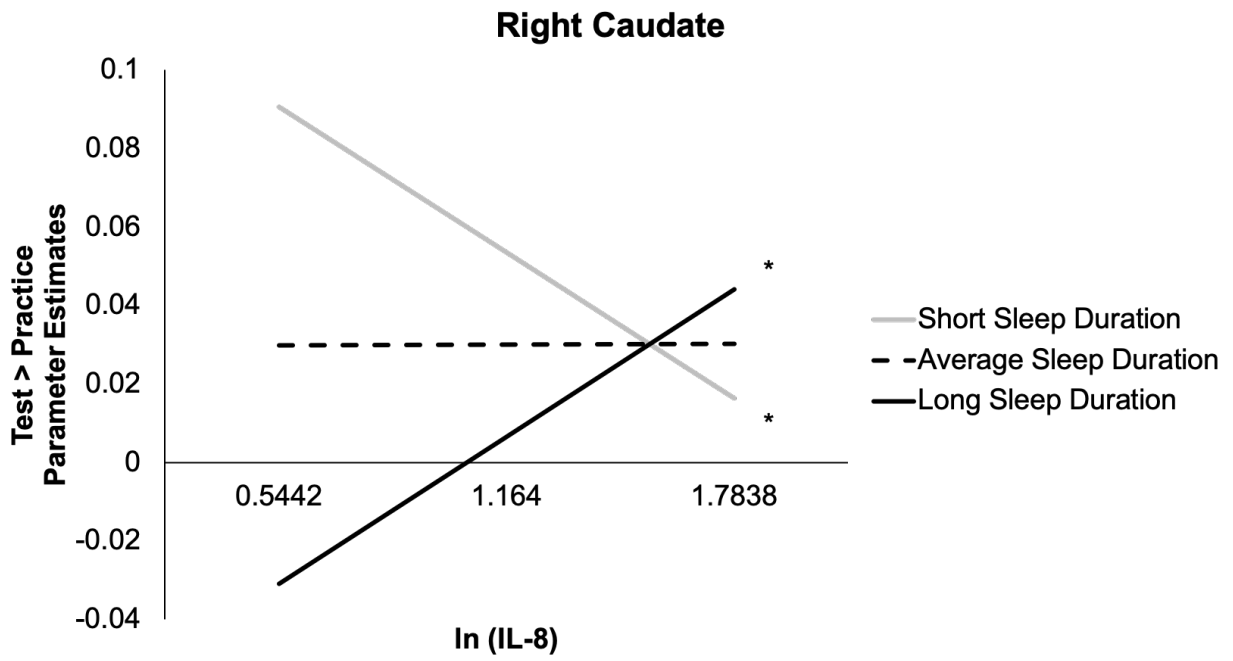
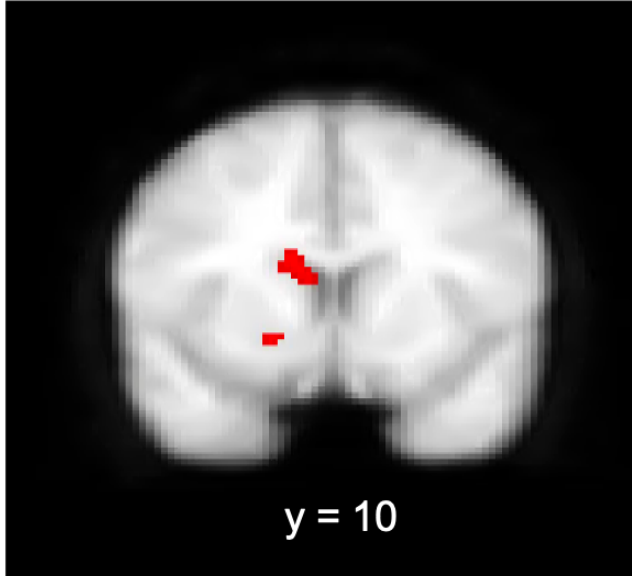


Figure 4.1. Sleep duration moderated the associations between TNF- α and activation in amygdala, subgenual ACC, and MPFC for Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

Analyses also revealed significant interactions between sleep duration and IL-8 in right caudate and putamen, left precuneus, and right occipital pole during Test > Practice (Table 4.1; Figure 4.2). Parameter estimates (5mm spheres around peak activation) from right caudate and putamen were extracted to probe the nature of the interaction. Follow up analyses revealed that among individuals who reported short sleep duration, greater levels of IL-8 were associated with lower caudate and putamen activation during Test > Practice (caudate: $B = -.0600$, $SE = .0180$, $t(16) = -3.3285$, $p = .0043$; putamen: $B = -.0673$, $SE = .0287$, $t(16) = -2.3435$, $p = .0323$). Additionally, among individuals who reported long sleep duration, greater levels of IL-8 were associated with greater caudate activation during Test > Practice ($B = .0606$, $SE = .0156$, $t(16) = 3.8753$, $p = .0013$). In contrast, levels of IL-8 were not significantly associated with putamen activation during Test > Practice among those who reported long sleep duration ($B = .0416$, $SE = .0249$, $t(16) = 1.6707$, $p = .1142$). Among individuals who reported average sleep duration, levels of IL-8 were not associated with caudate or putamen activation during Test > Practice (caudate: $B = .0003$, $SE = .0095$, $t(16) = .0309$, $p = .9758$; putamen: $B = -.0128$, $SE = .0152$, $t(16) = -.8474$, $p = .4092$) (Figure 4.2). Controlling for gender, BMI, sleep duration, and levels of IL-8, activation in these regions were not associated with behavior or task evaluation.



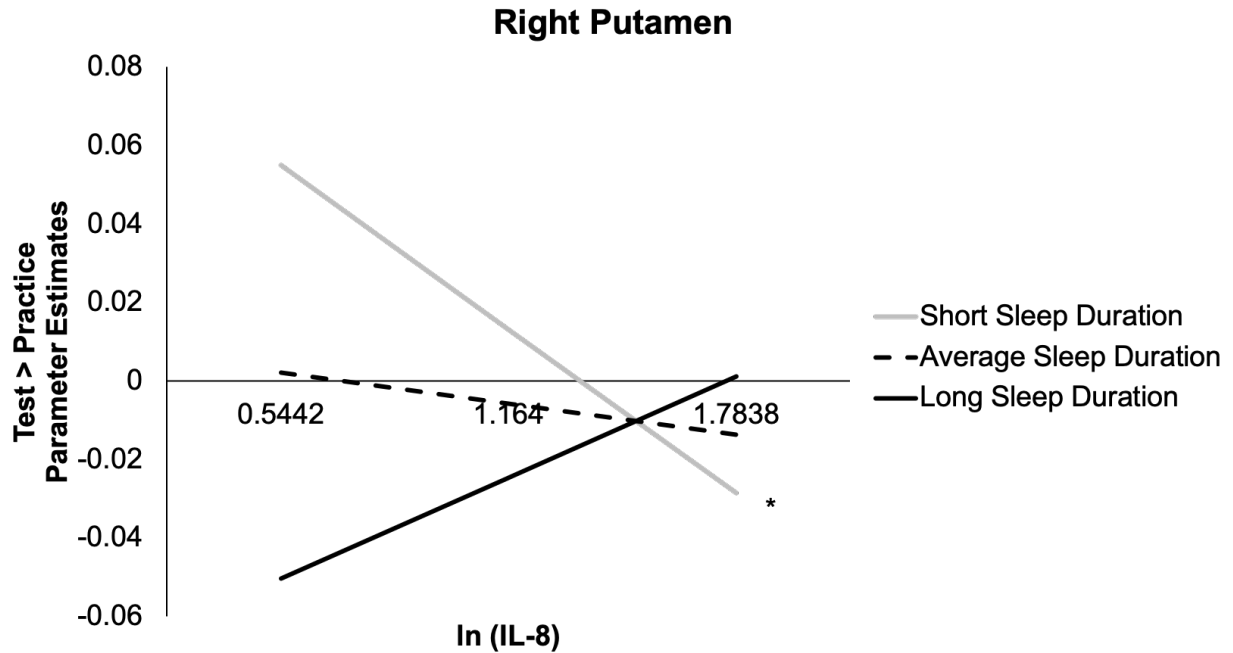


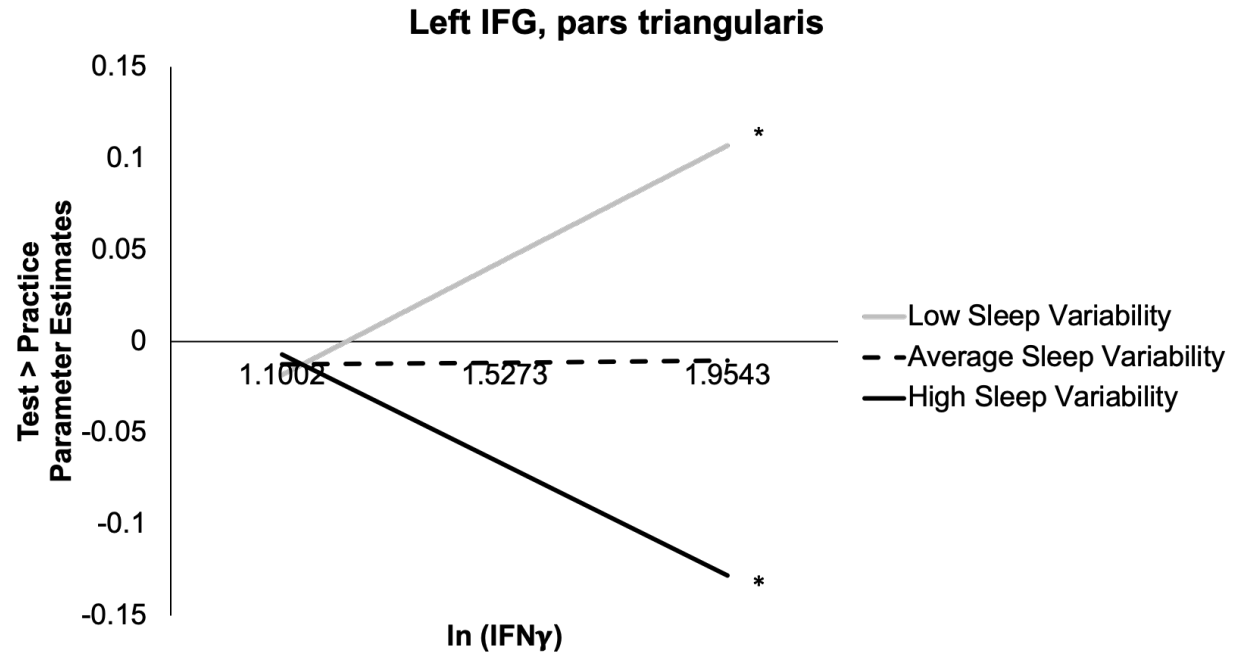
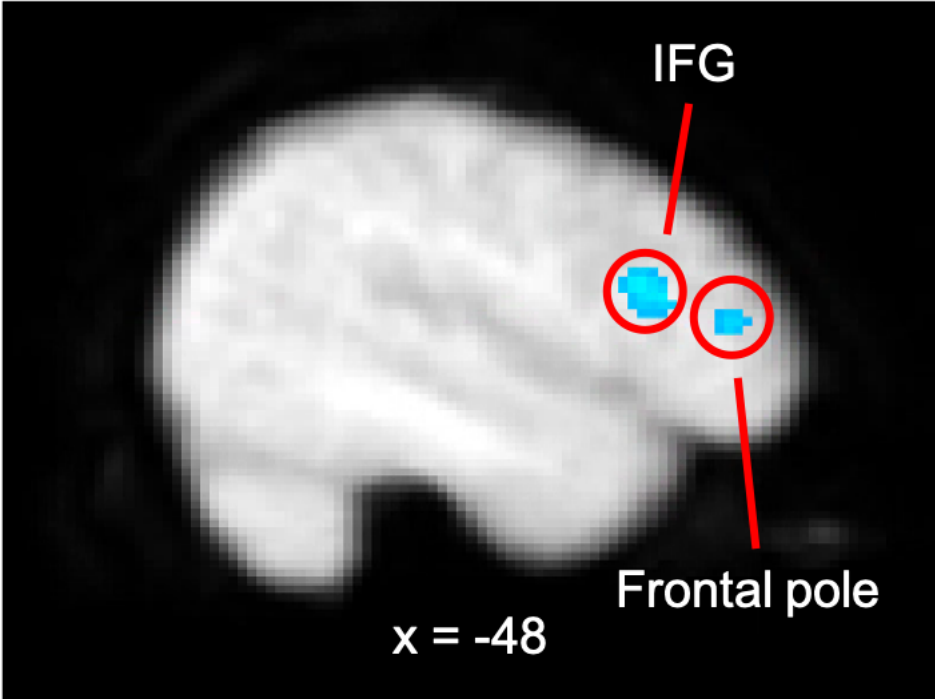
Figure 4.2. Sleep duration moderated the associations between IL-8 and activation in right caudate and putamen for Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

Sleep duration also moderated the associations between IL-6 and left occipital activation; and between IL-10 and bilateral precentral gyrus activation during Test > Practice (Table 4.1).

Sleep variability. Whole-brain analyses testing for interactions between sleep variability and immune markers on neural response to stress (Test > Practice), controlling for gender, BMI, and average sleep duration, revealed significant interactions between IFN γ and left inferior frontal gyrus (pars triangularis and opercularis) and left frontal pole activation during Test > Practice (Table 4.1, Figure 4.3). Parameter estimates (5mm spheres around peak activation) from left IFG (pars triangularis and opercularis) and frontal pole were extracted to probe the nature of the interaction. Follow up analyses revealed that among individuals who exhibit low sleep variability (1 SD below mean = 34.4094), greater levels of IFN γ were associated with greater

activation in IFG (triangularis) during Test > Practice ($B = .1468$, $SE = .0516$, $t(15) = 2.8430$, $p = .0123$). In contrast, levels of IFN γ were not associated with activation in left IFG (opercularis) and frontal pole among those with low sleep variability (IFG opercularis: $B = .0498$, $SE = .0296$, $t(15) = 1.6794$, $p = .1138$; frontal pole: $B = .0626$, $SE = .0383$, $t(15) = 1.6340$, $p = .1231$).

Among those who exhibit high sleep variability (1 SD above mean = 158.2830), greater levels of IFN γ were associated with lower activation in IFG (triangularis and opercularis) and left frontal pole (IFG triangularis: $B = -.1415$, $SE = .0591$, $t(15) = -2.3925$, $p = .0303$; IFG opercularis: $B = -.1759$, $SE = .0339$, $t(15) = -5.1809$, $p = .001$; frontal pole: $B = -.1342$, $SE = .0439$, $t(15) = -3.0574$, $p = .008$). Additionally, among those who exhibit average sleep variability, greater levels of IFN γ were associated with lower levels of IFG opercularis activation during Test > Practice ($B = -.0630$, $SE = .0169$, $t(15) = -3.7386$, $p = .002$). In contrast, levels of IFN γ were not associated with left IFG triangularis or frontal pole activation for those who exhibit average sleep variability ($B = .0026$, $SE = .0294$, $t(15) = .0898$, $p = .9296$; frontal pole: $B = -.0358$, $SE = .0218$, $t(15) = -1.6414$, $p = .1215$) (Figure 4.3). Controlling for gender, BMI, average sleep duration, sleep variability, and levels of IFN γ , greater left frontal pole activation during Test > Practice was associated with better accuracy ($B = 1.891$, $SE = .880$, $t(15) = 2.149$, $p = .048$). Greater left IFG triangularis and frontal pole activation during Test > Practice were associated with lower perceived test difficulty (IFG triangularis: $B = -6.516$, $SE = 2.401$, $t(15) = -2.713$, $p = .016$; frontal pole: $B = -9.093$, $SE = 3.329$, $t(15) = -2.731$, $p = .015$) (Figure 4.4).



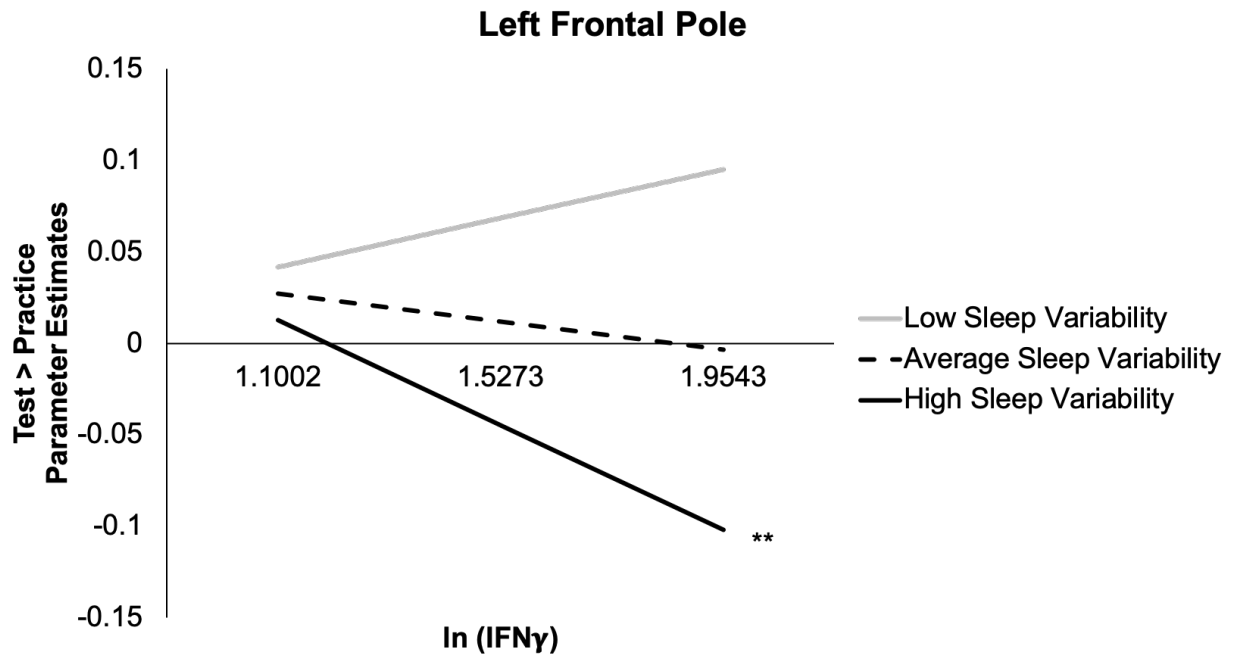
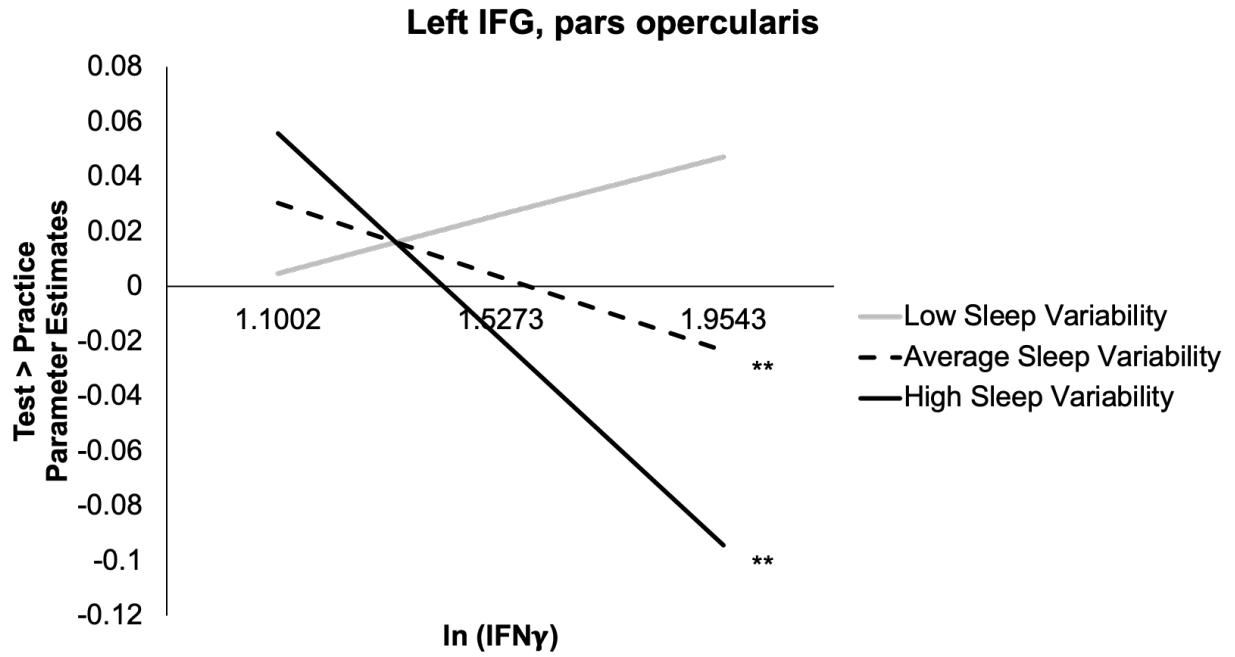


Figure 4.3. Sleep variability moderated the associations between IFN γ and activation in left IFG and frontal pole for Test > Practice, cluster-corrected at $Z > 2.3$, $p < .05$.

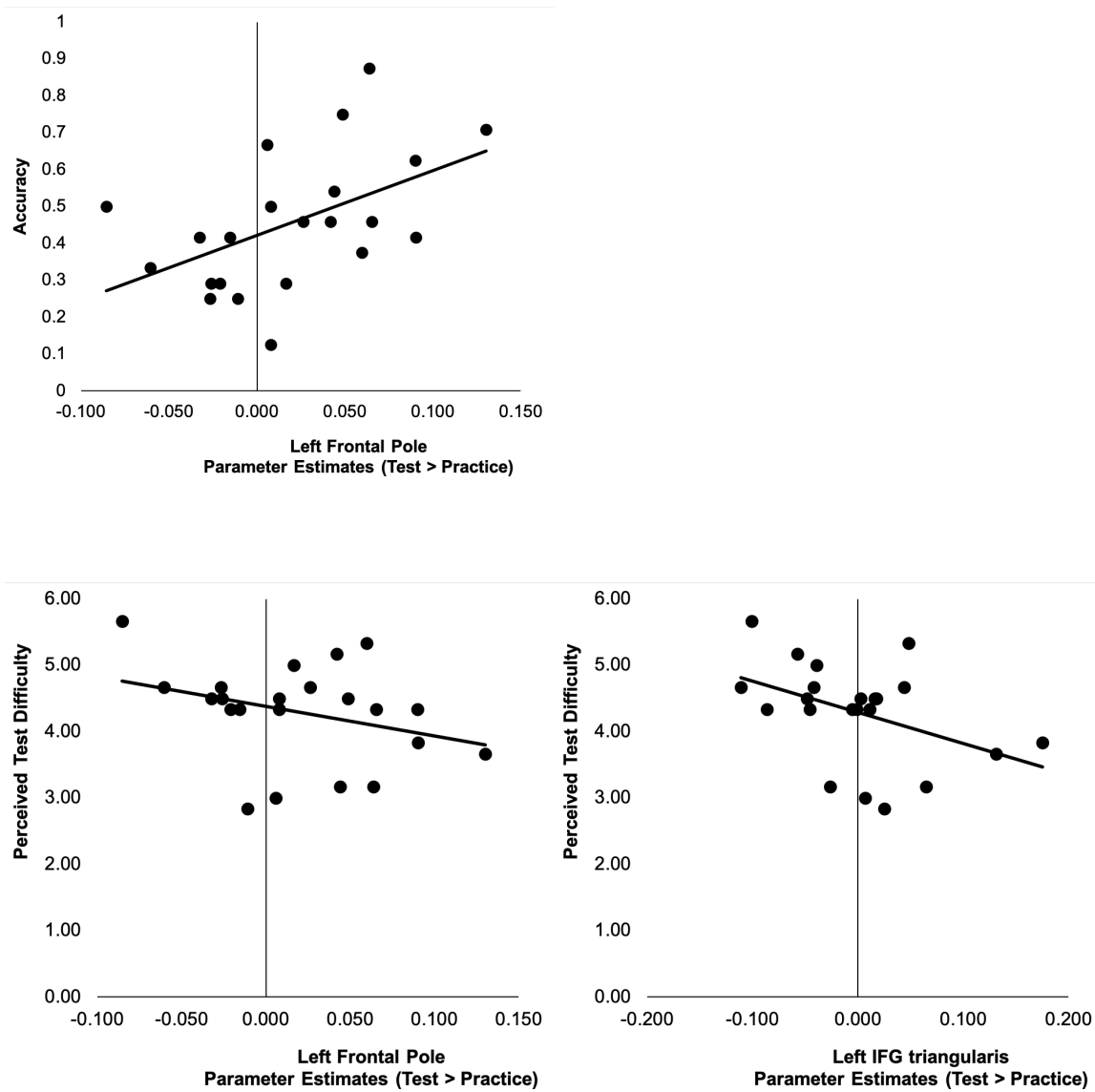


Figure 4.4. Greater activation in left frontal pole during Test > Practice was associated with greater accuracy and lower perceived test difficulty. Greater activation in left IFG triangularis during Test > Practice was associated with lower perceived test difficulty.

Sleep variability also moderated the associations between IL-6 and supramarginal gyrus, precentral gyrus, and postcentral gyrus activation during Test > Practice (Table 4.1).

| | Immune Marker | Hemisphere | Region | x | y | z | Z-max |
|---------------------------|--------------------------------|--------------------------|------------------------|-----|------|------|-------|
| Test > Practice | | | | | | | |
| Sleep Duration | IL-6 | L | Occipital pole | -16 | -104 | -10 | 4.60 |
| | | | | | | | |
| | TNF-α | R | Amygdala | 28 | 2 | -26 | 3.95 |
| | | L | Frontal Pole | -2 | 60 | 22 | 3.83 |
| | | L | MPFC | -8 | 60 | 2 | 3.65 |
| | | R | Subgenual ACC | 10 | 26 | -18 | 3.69 |
| | | R | Temporal pole | 56 | 12 | -28 | 3.77 |
| | | L | Posterior cingulate | -2 | -42 | 30 | 3.64 |
| | | L | Precuneus | -2 | -60 | 32 | 3.35 |
| | | R | Angular gyrus | 56 | -52 | 48 | 3.51 |
| | | L/R | Supramarginal gyrus | -64 | -44 | 30 | 4.24 |
| | | | | | 60 | -40 | 48 |
| | L | Lateral occipital cortex | -44 | -64 | 44 | 3.46 | |
| | IL-8 | R | Caudate | 10 | 6 | 18 | 3.07 |
| | | R | Putamen | 18 | 12 | -6 | 2.91 |
| L | | Precuneus | -24 | -50 | 6 | 3.47 | |
| R | | Occipital pole | 28 | -98 | 12 | 4.16 | |
| IL-10 | L/R | Precentral gyrus | -58 | -2 | 28 | 3.79 | |
| | | | 62 | 6 | 24 | 3.01 | |
| Sleep Variability | IL-6 | L | Postcentral gyrus | -54 | -22 | 42 | 3.53 |
| | | L | Precentral gyrus | -54 | -6 | 36 | 2.96 |
| | | L | Supramarginal gyrus | -36 | -38 | 34 | 3.29 |
| | IFNγ | L | IFG, pars triangularis | -52 | 36 | 10 | 3.23 |
| | | L | IFG, pars opercularis | -48 | 18 | 18 | 3.05 |
| | | L | Frontal pole | -44 | 38 | 10 | 2.97 |

Table 4.1. Significant interactions between sleep measures (average duration, duration variability) and peripheral immune markers on neural activation during Test > Practice, cluster corrected at $Z > 2.3$, $p < .05$. Note: x, y, and z refer to MNI coordinates, Z-max refers to peak level of activation intensity. L = left, R = right.

Discussion

The current study investigated whether adolescents' sleep patterns (average sleep duration and variability in sleep duration) related to peripheral immune markers and neural response to stress. Using a daily diary approach, adolescents self-reported their sleep time for the previous night each night for seven days. Average sleep duration was calculated by averaging sleep time reported across the week. Sleep duration variability was determined by calculating the standard deviation of sleep duration from each subject's average sleep duration. Results revealed that average sleep duration was not significantly associated with immune markers. However, greater sleep duration variability was marginally associated with lower levels of IL-10, controlling for gender, BMI, and average sleep duration. While these findings did not replicate previous research in regard to greater sleep variability being associated with greater inflammation in adolescents (e.g., Park et al., 2016), these findings complemented previous research by demonstrating that sleep variability may also have effects on anti-inflammatory processes.

Sleep duration and sleep variability were not significantly associated with behavior on the stressor task or evaluation of the stressor. Sleep duration and sleep variability were also not significantly associated with neural response to stress (Test > Practice).

Interestingly, sleep duration moderated the associations between inflammatory markers (TNF- α and IL-8) and neural response to stress in corticolimbic circuitry. Specifically, among adolescents who reported short sleep duration (about 386 minutes on average), greater levels of TNF- α were associated with greater activation in stress/emotion regulation regions (amygdala, subgenual ACC, MPFC) during periods of low stress (Practice > Test). In contrast, among adolescents who reported long sleep duration (about 530 minutes), greater levels of TNF- α were associated with lower activation in amygdala and MPFC during low stress (Practice > Test). In relation to IL-8, greater levels of IL-8 were associated with lower caudate and putamen activation during Test > Practice among adolescents who reported short sleep. Based on findings from Chapter 2/Study 1 and Chapter 3/Study 2, greater activation in these emotion regulation regions (e.g., amygdala, MPFC) during periods of lower stress (i.e., Practice > Test) were associated with greater self-reported anxiety and negative affect, suggesting possible protracted recovery from stress or amplified stress anticipation during periods of low stress. The current findings suggest that short/insufficient sleep might exacerbate the associations between inflammation and heightened activation in emotion reactivity/regulation regions during periods of relatively lower stress, which has implications for increased negative affect and potentiated stress reactivity. On the other hand, the current findings also suggest that long/sufficient sleep might buffer the effects that inflammation has on stress-related circuitry.

Sleep variability moderated associations between IFN γ and neural response to stress in regions related to cognitive control (left IFG/DLPFC), controlling for gender, BMI, and sleep duration. Specifically, among adolescents who exhibited high sleep variability (deviating about 158 minutes from average sleep duration across the week), greater levels of IFN γ were associated with lower activation in left IFG/DLPFC regions during Test > Practice. In contrast,

among adolescents who exhibited low sleep variability (deviating about 34 minutes from average sleep duration across the week), greater levels of IFN γ were associated with greater activation in left IFG/DLPFC during Test > Practice. Additionally, over and above levels of IFN γ and sleep variability, greater activation in left IFG/DLPFC regions during stress was associated with better accuracy and lower perceived test difficulty. These findings suggest that 1) engagement of lateral prefrontal regions during stress may be conducive to better performance/resilience under stress, consistent with previous work demonstrating that engagement of lateral prefrontal regions under stress facilitates better cognitive control and risky decision-making (Rahdar & Galván, 2014; Uy & Galván, 2017); and 2) that adolescents with lower sleep variability and higher levels of IFN γ may be better able to engage lateral prefrontal regions during stress that helps them overcome the stressor.

The immune system is influenced by both sleep and circadian processes. Research that characterized profiles of systemic and cellular inflammation over the course of a regular sleep-wake cycle relative to 24-hour of continuous wakefulness found that sleep increases levels of IL-6 and production of TNF- α , suggesting the involvement of these cytokines in the regulation of sleep-wake behavior. Sleep deprivation delays the nocturnal increase in IL-6 levels, attenuates nocturnal production of TNF- α , and shifts the pattern of IL-6 secretion from night-time to daytime, leading to an over-secretion of IL-6 during the day and excessive inflammation (Dimitrov, Besedovsky, Born, & Lange, 2015; Vgontzas et al., 1999).

In regard to adaptive immunity, circulating T and B cells peak early in the evening and migrate from circulation to lymphoid organs where they may come into contact with antigens, such as viruses. Sleep promotes the activation of T cells through their increased production of IL-2 and IFN γ , which induces Th1 cell-type adaptive immune response and increase immune

defense (Lange, Dimitrov, & Born, 2010). Sleep loss not only impacts the ability of these cells to be at the right place at the right time, but may also impair T cell functioning, which includes diminished antigen-specific response by helper T cells and declines in production of cytokines essential to T-cell maturation (Bollinger et al., 2009). Sleep disturbance also induces a shift away from Th1 cell-type adaptive immune response towards Th2 cell-type cytokine activity, leading to increased susceptibility to viral infections (Dimitrov, Lange, Tieken, Fehm, & Born, 2004; Lange, Dimitrov, Fehm, & Born, 2006). Indeed, short habitual sleep was associated with increased risk for the development of pneumonia (Patel et al., 2012) and higher incidences of reported respiratory infections compared to sufficient sleep duration (Prather & Leung, 2016).

Sleep stages also regulate inflammatory activity. Sleep disturbances have been found to decrease the duration of slow-wave sleep (SWS), a component of non-REM (NREM) sleep, and increase the duration of REM sleep (Irwin, 2015). During SWS, cortisol is at its lowest level, which promotes antiviral immune responses, as indicated by greater Th1/Th2 ratio (Dimitrov et al., 2004). Sympathetic nervous system (SNS) activity also decreases during NREM sleep. Sleep deprivation prevents this NREM/SWS-related decrease in SNS activity, leading to overall increased SNS activity during the night (Irwin, Thonpson, Miller, Gillin, & Ziegler, 1999) and disrupted antiviral immune response (Lange et al., 2010). Additionally, longer time spent in REM sleep has been correlated with greater morning levels of IL-6 as well as greater activation of the SNS. Thus, sleep disturbance results in persistent activation of the HPA axis, which can induce glucocorticoid resistance of immune cells (Abell, Shipley, Ferrie, Kivimäki, & Kumari, 2016; Castro-Diehl et al., 2015), decrease antiviral immune response, and increase SNS activity.

In addition to the effects of sleep disturbance on immune function, inflammatory and anti-viral signals could also signal the brain to induce sickness behaviors to aid recovery from

infection, including fatigue, sleepiness, negative mood, and hypersensitivity to pain. In relation to sleep, researchers have discovered that IL-1 and TNF cytokines play a role in homeostatic regulation of NREM sleep in animals. For example, blocking the actions of IL-1 and TNF led to a reduction in physiological NREM sleep duration and NREM sleep rebound after sleep deprivation (Krueger & Majde, 1995; Opp, 2005). Moreover, the production of IL-1 and TNF is enhanced after sleep deprivation, which correlates with greater amounts of recovery sleep in animals (Lue et al., 1988). In humans, low doses of endotoxin induce inflammatory activity and result in enhanced NREM and SWS sleep (Krueger, 2008; Mullington et al., 2000). While not as well-studied as IL-1 or TNF, research suggests that other pro-inflammatory cytokines (e.g., IFN γ and IL-6) may also have NREM sleep-promoting actions (Hogan, Morrow, Smith, & Opp, 2003; Kubota, Majde, Brown, & Krueger, 2001). Together, these findings suggest that inflammatory cytokines drive the propensity to sleep.

The current findings – that higher levels of TNF- α were associated with a pattern of brain activity during stress that was related to increased negative affect and anxiety among adolescents who report shorter sleep duration – are consistent with the role of TNF- α in regulating sleep and other sickness behaviors. That is, in the context of insufficient sleep, greater levels of TNF- α may modulate brain function in attempts to increase behaviors/states (e.g., negative affect) that would promote sleep. Unfortunately, the current study did not assess levels of sleepiness or attentiveness during the laboratory visit. The effects of TNF- α on brain function may not have been observed for adolescents with high sleep variability because of the likely increase in rebound sleep after acute sleep restriction. Indeed, studies found that plasma levels of TNF were mostly unchanged during or on the day after acute sleep deprivation/restriction in humans (Haack, Schuld, Kraus, & Pollmächer, 2001; Irwin, Olmstead, Valladares, Breen, & Ehlers,

2009; Ruiz et al., 2010; Shearer et al., 2001), but were increased after one week of sleep restriction (Vgontzas et al., 2004), suggesting that prolonged sleep deprivation or restriction may be required to detect sleep-related changes in TNF- α .

In regard to the IFN γ and sleep variability findings, it could be possible that higher levels of IFN γ may serve different functions among individuals with higher relative to lower sleep variability, reflected through divergent effects on the brain's cognitive system and associated outcomes. Higher levels of IFN γ in the context of high sleep variability may reflect a homeostatic drive by IFN γ to induce sleep and other energy-conserving practices, such as lowered cognitive function, whereas higher levels of IFN γ in the context of low sleep variability may reflect immune defense functioning, which engenders the organism to actively engage in stress/challenge. Although average sleep duration and sleep variability may be driven by similar physiological processes, sleep variability may additionally capture day-to-day situational changes in affect, work schedules, stress, or illness symptomatology that may be obscured when only examining mean sleep duration (Slavish, Taylor, & Lichstein, 2019).

The current study has several limitations to note. First, the correlational design of the study precludes drawing any conclusions about the directionality of the relations among the sleep measures, immune markers, and brain function. Second, while we found significant effects for the interactions between sleep habits and immune markers on brain function, the sample size for those analyses were very small, so it remains to be tested whether these effects would replicate in larger samples. Relatedly, the small sample size could have provided insufficient power to detect significant associations between sleep habits and immune markers, which previous studies have found. Third, sleep duration and sleep variability were determined via self-report from the adolescents and also at the end of the day rather than when they wake up that day, which may not

be as accurate as more objective measures of daily sleep such as actigraphy. Additionally, sleep duration was measured for only one week and it was assumed that this one week captured an average week in the adolescents' lives. However, it could be possible that sleep behavior during the measured week may not represent some adolescents' average week (e.g., adolescents could be on break from school, traveling, having a particularly challenging week, etc.). Unfortunately, typicality of the week was not assessed.

Despite limitations, the current study makes novel contributions to the associations between self-reported sleep habits, peripheral immune markers, and brain function in adolescents. It provided empirical evidence that even in a relatively healthy sample of adolescents, variability in sleep patterns and immune markers relate to variability in neural response to stress in corticolimbic circuitry in a sample of older adolescents.

Chapter 5. General Discussion

The goal of the current dissertation was to investigate whether and how peripheral immune markers modulate neural function of stress circuitry during adolescence, and whether these immune-brain associations are predicted or moderated by daily affective experiences and sleep habits. The ultimate goal is to uncover implications for understanding how these processes relate to mental health and well-being. During adolescence, there is a significant increase in the onset and prevalence of depression (Mojtabai et al., 2016; Substance Abuse and Mental Health Services Administration, 2017). Substantial changes in neurobiological and neuroendocrine systems, in addition to changes in adolescents' social environment and lifestyles (e.g., sleep habits), have been posited to underlie the onset and potentiation of depression. Research in animal and human adult work has implicated the immune system in psychological functioning and well-being through modulation of affective neural circuitry and stress systems (Kraynak et al., 2018). These systems are more sensitive and therefore more vulnerable to environmental influences during adolescence than in adulthood (e.g., Brenhouse & Schwarz, 2016; Crone & Dahl, 2012; Dahl & Gunnar, 2009; Romeo, 2017). Research in adolescents suggests that the link between immunity and depressive symptoms is not only present in youth who have depression (Gabbay et al., 2009; Henje Blom et al., 2012; Miller & Cole, 2012; Pandey et al., 2012), but also in those who are otherwise healthy (Chiang et al., 2017; Chiang et al., 2019b; Guan et al., 2016). Indeed, among healthy adolescents, variability in psychosocial factors (e.g., interpersonal stress, poor sleep) are related to immune functioning (Chiang et al., 2019a; Fuligni et al., 2009; Park et al., 2016). Health status during adolescence tends to predict health status later in adulthood. Therefore, understanding how experiences during adolescence relate to immune functioning is important to provide novel perspectives for interventions. Elucidating the

neurobiological correlates of immune functioning during adolescence would advance the field forward in determining whether there might be distinct neuroimmune effects during this formative developmental period.

The research presented provides novel evidence that peripheral immune markers may play a role in corticolimbic circuitry function during stress in an otherwise healthy sample of adolescents; however, the findings reveal a fairly nuanced understanding of the relations between peripheral immune markers and brain function during adolescence.

By combining daily diary methods with functional neuroimaging and multiplex cytokine assays of venipuncture samples, findings suggest that daily affective experiences and sleep habits are relevant moderators of the associations between certain peripheral immune markers and neural response to stress. Specifically, among adolescents who reported experiencing greater negative affect in their daily lives, higher levels of pro-inflammatory TNF- α were associated with greater medial prefrontal (MPFC) activation during periods of low relative to high stress (i.e., Practice > Test), which was found to be associated with greater negative affect, perceived stressor difficulty, higher stress ratings, and greater test-related anxiety symptoms. This effect was reduced among those who reported lower negative affect in their daily lives. A similar pattern of results was also observed for sleep duration and levels of TNF- α in regions implicated in emotion/stress regulation (MPFC, subgenual ACC, amygdala) such that higher levels of TNF- α were associated with greater activation in these regions during periods of low relative to high stress among adolescents who reported short/insufficient sleep duration whereas these associations were attenuated among those who reported sufficient sleep duration. These findings suggest that perhaps engaging these emotion regulation regions during periods of relatively lower stress might reflect protracted regulation or recovery from stress, which could potentiate a

positive feedback loop whereby high negative affect (and inflammation) could lead to exaggerated negative appraisals of stress, which could lead to a neural response pattern that may increase stress appraisal, negative affect, and anxiety symptoms. Levels of TNF- α are regulated by both stress and sleep processes whereby greater stress and sleep disturbance lead to greater levels of circulating levels of TNF- α . In turn, one of the roles of TNF- α is to induce sickness behaviors/states, including negative affect and sleepiness, to promote recovery from physical or psychological stress by acting on relevant systems in the central nervous system (Besedovsky, Lange, & Haack, 2019; Irwin, 2019; Prather, 2019). While speculative, the current findings suggest that higher levels of TNF- α in the context of short/insufficient sleep may reflect a homeostatic drive to induce sickness behaviors that would promote sleep via modulation of the stress/emotion regulation circuitry.

Another notable finding from the current research is that variability in sleep duration interacted with IFN γ to predict activation of lateral prefrontal regions during periods of high relative to low stress (i.e., Test > Practice). In particular, among adolescents who exhibited low sleep duration variability, higher levels of IFN γ were associated with greater lateral prefrontal activation during periods of high relative to low stress, which was associated with better performance on the stressor and lower perceived stressor difficulty. In contrast, the opposite pattern was observed among adolescents who exhibited high sleep duration variability – that is, higher levels of IFN γ were associated with greater lateral prefrontal activation during low relative to high stress, which was associated with poorer performance on the stressor and greater perceived stressor difficulty. Similar to TNF- α , stress and sleep disturbances also alter levels of IFN γ and its corresponding role in antiviral defense (Besedovsky et al., 2019; Irwin, 2019). The current findings suggest that whereas TNF- α appears to play a role in modulating stress/emotion

regulation circuitry, IFN γ might play a role in modulating cognitive/executive function circuitry. It is speculated that higher levels of IFN γ may serve different functions among individuals with higher relative to lower sleep variability, as reflected through divergent effects on the brain's cognitive system and associated outcomes.

Together, these findings support the notion that peripheral immune markers may promote sickness-type behaviors/states (e.g., negative affect, increased anxiety, poorer cognition) through modifying the respective neurocircuitry in response to stress/challenge. The interactive effects of these immune-brain associations by negative affect and poor sleep suggest that immune-brain signaling tend to occur (or may be more readily detected) under contexts that necessitate homeostasis, highlighting the intricate and sophisticated relationship between the co-evolved brain and immune systems. If homeostasis is not achieved due to chronic stress or prolonged sleep restriction, increased allostatic load on these biological systems could lead to dysregulation and increased risk for psychological and physical health problems. Indeed, research suggests a robust relationship between immune dysregulation and depressive symptoms, which share many features with the repertoire of sickness behaviors (Brymer, Romay-Tallon, Allen, Caruncho, & Kalynchuk, 2019; Cho, Eisenberger, Olmstead, Breen, & Irwin, 2016; Dantzer, 2009; Medina-Rodriguez, Lowell, Worthen, Syed, & Beurel, 2018; Slavich & Irwin, 2014).

The findings from the current dissertation need to be interpreted in the context of the studies' limitations. First, there may be limited generalizability of the current findings to younger and older adolescents, as the current sample only spanned 14-15 years of age. Additionally, self-selection bias may have influenced the results given that immune data were only available from adolescents who opted in for the blood draw, who could differ from those who chose not to provide a blood sample for reasons unknown. Second, the correlational nature of the design

precludes drawing any conclusions regarding directionality of the findings. Third, immune markers measured in the periphery may not represent/reflect the inflammatory environment in the brain. Fourth, because of the relatively small sample size, other relevant biological and health factors (e.g., ethnicity, socioeconomic status, physical activity, diet, smoking/drinking behaviors, medical history) that could potentially confound the findings could not all be taken into account in analyses. Nevertheless, despite the small sample size, the strengths of the current study lie in the multi-method and multi-system data collected, including fMRI data, extensive questionnaire and daily diary measures, and venipuncture draws with multiplex immunoassay. The daily diary measures allow one to link real-world experiences to laboratory findings while the multiplex immunoassay extends the literature by examining other inflammatory markers beyond IL-6 and CRP. While these data collection methods may have limited the sample size due to a relatively high demand placed on participants' time and energy, the richness of the collected data allowed novel research questions to be answered.

Future studies that manipulate cytokine levels and relate them to affective and cognitive neurocircuitry longitudinally would provide further insight into the complex relationship between the immune system and neurodevelopment during adolescence. Another avenue for future research would be to determine whether certain features/patterns of immune-brain associations are unique to adolescence relative to other developmental periods. Answers to these questions would elucidate novel pathways for intervention to reduce the risk of psychiatric and physical problems during adolescence and across the lifespan.

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