Stressed, Sick, and Sad: A Transdiagnostic Investigation of the Translation of Stress into Depression through Neuroendoimmune Disruptions

by

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Abstract

Depression is a prevalent and disabling form of psychopathology that is frequently precipitated by experiences of stress. Disruptions in stress-sensitive biological systems, notably the immune system and hypothalamic-pituitary-adrenal axis, are strongly implicated in depression, and disturbances in these systems could reflect potential pathways through which experiences of stress are translated into depression. These systems, broadly subsumed within the neuroendoimmune system, may be highly responsive to stress and cognitive abilities related to stress management. To characterize the links between stress and depression and the potential influence of immune activity, the present study investigated the following: (1) the associations among perceived stress (across different time periods of life), proinflammatory immune markers, and depressive symptoms; (2) whether neuroendoimmune activity mediates the relationship between perceived stress and depressive symptoms; and (3) whether perceived stress and immune activity mediate the relationship between cognitive control and depressive symptoms. Fifty-nine medically healthy adult females with varying levels of depression participated in the study. Participants provided dimensional ratings of their depression symptoms and perceived life stress, and they completed a neuropsychological test of cognitive control. Plasma biomarkers of

stress, including pro-inflammatory cytokines, C-reactive protein, and free cortisol, were assayed following a fasted morning blood draw. Consistent with hypotheses, both greater perceived stress and higher concentrations of the proinflammatory immune marker, interleukin-6 (IL-6), were associated with greater depressive symptoms. Although levels of IL-6 alone did not significantly mediate the relationship between perceived stress and depressive symptoms, when considered together, elevated concentrations of IL-6 and lower free cortisol mediated the relationship between severity of childhood stress and current depressive symptoms. Contrary to expectations, cognitive control was not significantly associated with stress, immune markers, or depression. The findings are interpreted in the context of a potentially long-term reduction in glucocorticoid tone triggered by early life stress that curbs cortisol output, produces chronic low-grade immune activation, and leads to depression vulnerability later in life. Overall, the study provides new insights into potential pathways among stress, the neuroendoimmune system, and depression, shedding light on how early life stress may be translated into depression in adulthood.

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Chapter 1

1 Introduction

Depression is a pressing public health concern that results in immense personal suffering and functional limitations, affecting 300 million people worldwide (World Health Organization, 2018). Depression is a pervasive form of psychopathology with diverse manifestations and a multifactorial etiology that is not yet completely understood (Ruscio & Ruscio, 2000; Fried & Nesse, 2015a; Watson, 2003; Slavich & Cole, 2013). In authoritative psychiatric nosologies (e.g., Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition [DSM-5]; American Psychiatric Association, 2013), depression is classified as a discrete psychiatric disorder referred to as major depressive disorder (MDD). Within categorical frameworks, there are many different symptoms that comprise a diagnosis of MDD, and the number of symptom combinations are many, suggesting the possibility of unique symptom constellations resulting from relatively distinct etiologies (Goldberg, 2011; Watson, 2005; Kotov et al., 2017). However, the clinical syndrome and its constituent symptoms can also be conceptualized dimensionally (Patrick & Hajcak, 2016; Kotov et al, 2017). Despite these nuances, depression typically presents recurrently and is marked by episodes of low mood, higher risk of suicide, functional disability, and frequent comorbid physical and mental health conditions (American Psychiatric Association, 2013; Reddy, 2010).

The heterogeneity of MDD symptoms and corresponding range of potential etiological factors may help to explain why—despite decades of research on psychological and pharmacological interventions for depression—treatments are only effective for some (Carvalho, Berk, Hyphantis, & McIntyre, 2014; Biesheuvel-Leliefeld et al., 2015) and no progress has been made to reduce prevalence rates (Hidaka, 2012). To date, the vast majority of interventions that exist were born out of the conceptualization of depression as a disease of the mind (e.g., cognitions) or brain (e.g., neurotransmitters). Theories underlying the development of pharmacological treatments for depression have been based on notions that depression arises from disruptions in brain chemistry. For instance, the “monoamine theory of depression” has influenced the development of the majority of pharmacological interventions for depression since the early 1950s (Hirschfeld, 2000; López-Muñoz, Álamo, Juckel, & Assion, 2007). According to this theory, depression is

the result of deficient monoaminergic neurotransmission (e.g., serotonin, dopamine, and norepinephrine). As such, pharmacological agents are presumed to normalize this deficit and thereby improve depression symptoms (Hirschfeld, 2000). On the other hand, cognitive models of depression center on psychological processes, positing that dysfunctional ways of thinking contribute to the etiology, maintenance, and recurrence of depressive episodes (Beck, 1967). According to these models, biased negative thinking processes (e.g., automatic and pervasive negative thoughts about the self, world, and future) cause individuals to be more prone to experience depression. As these ways of thinking spiral out of control, this leads an individual to experience disruptions in affect, perception, memory, and so-called "neurovegetative" functions, such as sleep, appetite and weight. This implies that depressed individuals think in systematically different ways than non-depressed individuals, that these changes in thinking precede the depressed mood, and that relief will be found by targeting these ways of thinking in psychotherapy (Beck, 1967; Beck, Rush, Shaw, & Emery, 1979).

Despite these well-established brain- or mind-centric theories of depression, 30% of individuals with depression do not show a beneficial response to antidepressant medications, even after multiple medication trials (Souery & Pitchot, 2013; Luther et al., 2006). Of those that do respond, an additional 30% relapse while on continuous medication use, and up to 75% relapse after medication is withdrawn (Vittengl, Clark, Dunn, & Jarrett, 2007; Hollon et al., 2005). Similar to the effects of antidepressant medications, about two-thirds of patients respond to initial cognitive psychotherapy (DeRubeis, Siegle, & Hollon, 2008), but approximately 30% relapse after one year, and 54% within two years (Hollon et al., 2005; Vittengl, Clark, Dunn, & Jarrett, 2007). The beneficial effects of these interventions are relatively low considering that up to 53% of individuals will remit from depression without any intervention within a 12-month period (Whiteford et al., 2013), only to experience later depressive episodes (Boland & Keller, 2009). The high relapse rates suggest that available interventions do not fully address the range of factors that cause and maintain depression.

Therefore, a more complete understanding of these factors may help to advance treatment research on depression and curb its soaring prevalence rate (Hidaka, 2012; Raison, 2016). Rather than being considered solely a disorder of the brain or mind, depression is increasingly considered a system-wide disturbance that affects multiple interconnected biological,

psychological, and behavioural systems (Slavich & Cole, 2013; Miller & Raison, 2016). In line with this notion, an accumulating body of research has investigated immune activity as a biological factor in depression. Immune activity refers to the activation of inflammatory molecules by the immune system. Importantly, the immune system interacts with other central and peripheral biological systems implicated in depression, including the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system (ANS), and the brain's neurochemistry (see Appendix A for a list of abbreviations used throughout this review) (Thayer & Lane, 2007; Pavlov & Tracey, 2015; Harrison, 2017; Brunoni, Lopes, & Fregni, 2008; Yirmiya & Goshen 2011; Dantzer, 2018; Ménard, Pfau, Hodes, & Russo, 2017). Furthermore, a combination of immune related and other biological factors might influence psychological and behavioural systems that increase risk for depression.

The notion that immune activity might play a role in depression came from initial observations that behaviours related to infection and immune activity resemble symptoms of depression (Hart, 1988; Dantzer & Kelley, 1989; Smith 1991). In what has become known as the macrophage theory, inflammatory proteins are believed to interact with the brain through peripheral and central signaling pathways (e.g., sympathetic nervous system, vagus nerve, microglial cells) to inhibit neurogenesis, increase monoamine transporter activity, and alter glutamate and monoamine metabolism and synthesis, all of which may ultimately contribute to depressive states (Smith, 1991; Maes et al., 1992; Raison, Felger, & Miller, 2013). The theory is well supported by both human and animal research (Capuron et al, 2003; Shah, Kadia, Bawa, & Lippmann, 2013; Felger et al., 2016) and by the high levels of co-occurrence of inflammatory states or conditions with depression, including the post-partum period, cardiovascular disease, cancer, HIV, diabetes, multiple sclerosis, neurodegenerative disease, and irritable bowel disease, among other conditions (Graff, Walker, & Bernstein, 2009; Leonard, 2007; Evans et al., 2005; Slavich, 2016; Scarpioni, Ricardi, & Albertazzi, 2016). Individuals with these conditions experience depression at rates five times higher than the general population (Evans et al., 2005). Furthermore, otherwise medically healthy individuals with MDD demonstrate higher levels of inflammatory biomarkers in both cross-sectional (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020) and prospective studies (Valkanova, Ebmeier, & Allan, 2013; Lamers et al., 2019). Accordingly, anti-inflammatory interventions (e.g., nonsteroidal anti-inflammatory drugs

(NSAIDs), omega-3 fatty acids, and cytokine antagonists) are effective at reducing depression in individuals who carry a high inflammatory load, but not in those without high levels of inflammation (Raison, 2016). Interestingly, one of the proposed mechanisms of the success of some antidepressant medications is that they have an immunomodulatory effect—that is, impacting levels of inflammation (Strawbridge et al., 2015). Based on these findings, there is reason to believe that an increased understanding of immune factors in depression could lead to more targeted treatments for patients with specific underlying vulnerabilities (e.g., higher inflammatory load). However, more research is needed to better understand how immune activity influences and interacts with psychological and behavioral systems involved in depression.

The concept of stress can be used to bridge immune responses with psychological and behavioural systems involved in depression. Across multiple theories, stress is acknowledged as a common risk factor for the development, maintenance, and recurrence of depression (Beck, 1967; Maes et al., 1998; Capuron et al., 2003). It is well-documented that both internal stressors (due to infection, injury, or medical illness) and external stressors (psychosocial stressors, such as work strain, divorce, or childhood adversity) are important predictors of depression onset (Kessler, 2002; Monroe, Slavich, Georgiades, 2014), and thus hold a central role in prevailing theories of depression (Beck, 1967; Maes et al., 1998). For instance, in accordance with contemporary cognitive theories of depression, stress is considered a strong etiological factor that significantly impacts cognitive processes commonly associated with depression (Beck, 1967; Hollon, 2010; Colodro-Conde et al., 2018). Within cognitive models, individuals are presumed to develop negative self-schemas as a result of early life stress or trauma that can be re-activated by stressors experienced later in life—predisposing an individual to negative thinking and depression (Disner, Beevers, Haigh, & Beck, 2011; Colodro-Conde et al., 2018).

This link between stress and depression appears to be central to biological models (such as the macrophage theory) as well, although the connection has not always been apparent. As will be developed in more detail throughout this chapter, the links among internal physical stressors, (e.g., disease or infection), higher inflammatory loads, and depression have been more discernible in the research literature. However, the source of inflammation in otherwise medically healthy individuals with depression was less clear (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Berk, 2013). That is, it was not immediately apparent until researchers

discovered that psychosocial stressors, too, activate the immune system (Maes et al., 1998; Steptoe, Hamer, & Chida, 2007). The observation that exposure to psychosocial stressors can activate immune responses provides a new pathway through which to understand the relationship between inflammation and depression. As will be reviewed in the sections that follow, the impact of immune activity on states of mood and affect may be heavily influenced by both internal and external stressors, biological systems that respond to stress, and other cognitive capacities such as stress appraisal and executive functioning (Cohen et al., 2012; Moons & Shields, 2015; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016).

Recent conceptualizations of immune activity as a stress response radically changed the focus of immune activity in mental health research (Slavich & Irwin, 2014). Extensive research in the field of neuroendoimmunology has produced growing evidence for an interconnection between stress and biological responses from the ANS, immune system, and HPA axis (Ménard, Hodes, & Russo, 2016). Furthermore, dysregulation (i.e., both increases and decreases in activity) of these systems in isolation is reported extensively in relation to depression (Thayer & Lane, 2007; Harrison, 2017). However, capturing the interplay of these systems in relation to both stress and depression has not been thoroughly addressed. These are critical lines of investigation because they may help to uncover specific stress-related biomarkers that confer vulnerability in some individuals to develop symptoms of depression that add to the global burden of disease (World Health Organization, 2018).

To advance our understanding of these dynamics, evolutionary and integrated (i.e., biopsychosocial) theories of depression have been articulated (Raison, Capuron, & Miller, 2006; Berk, 2013; Miller & Raison, 2016; Slavich & Irwin, 2014; Slavich & Cole, 2013). In these theories, molecular and cellular pathways that were historically advantageous for healing injuries, eliminating infections, and responding to predators, are theorized to also be sensitive to psychosocial threats that, in modern day, can be far more pervasive (American Institute of Stress, 2013; Miller & Raison, 2016; Slavich & Irwin, 2014). Importantly, these integrative theories of depression posit much of an integration and synthesis of both cognitive and macrophage theories in relation to stress (Slavich & Irwin, 2014). In these models, stressors are hypothesized to interact with both perceptual and neurobiological systems to alter physiology and neuroendoimmune systems that although advantageous in some contexts, in other circumstances

can ultimately manifest as symptoms of depression. Overall, these perspectives suggest that a shift in neuroendoimmune activity caused by experiences of stress can contribute to the risk of depression (and symptoms of other forms of psychopathology). Several biological mechanisms are proposed to account for the effect of immune activity on depressive states (e.g., activation of the tryptophan degrading enzyme indoleamine 2,3-dioxygenase, increased expression of serotonin transporter, neuroinflammation, glucocorticoid resistance). In the present dissertation, the focus is on the relationships among stress, immune activity, and cortisol production and signaling, which is one proposed pathway through which immune activity may exert depressogenic effects (Raison & Miller, 2003; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019).

Importantly, it is unclear how these systems respond to stress experienced across different time periods in one's life (e.g., childhood, recent months or weeks). Whether these systems are responsive to more recently experienced stress that translates into current depression, or whether shifts in biological signaling occur as a result of more distal stressors (e.g., during childhood), remains a critical question for further investigation. It is well documented that prolonged periods of stress are associated with immune and HPA axis dysregulation (von Känel, Bellingrath, & Kudielka, 2008; Cohen et al., 2012; Miller et al., 2008). Whether more acute or chronically extended disruptions in neurobiological systems associated with the stress response contribute to the translation of stress and adversity into depression are central theoretically questions (Häfner et al., 2011; Jacob, Haro, & Koyanagi, 2019).

As will be detailed throughout this chapter, testing immune and neuroendoimmune hypotheses presents many challenges due to, for example, the limitations associated with conceptualizing depression as a discrete condition (as in prevailing psychiatric classification systems, such as the DSM-5), the limited number of scales available to dimensionally assess specific symptoms of depression that may be more relevant to immune activity, and the many potential factors that can influence stress biomarkers (e.g., sleep quality, meal timing, body mass, time of measurement, etc.; O'Connor et al., 2009). This dissertation proposes that research on the relationship between the immune system, stress, and depression may benefit from analyses of depression symptoms across the spectrum of symptom severity—regardless of clinical diagnosis—to include

subclinical levels of depression and individuals with multiple diagnoses in addition to depression (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Furthermore, findings that psychosocial stressors modulate immune activity have important implications for not only depression, but any stress-sensitive psychopathology that may also demonstrate elevated immune activity (Michopoulos et al., 2017; Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011; Jokinen & Nordströ, 2009; van der Werf-Eldering et al., 2012; Kahl et al., 2006; 2009; Masi, Glozier, Dale, & Guastella, 2017).

There remain many unanswered questions regarding the integration of biological processes and stress that may contribute to symptoms of depression. More specifically, the question of which biological systems are impacted by experiences of stress, how they are impacted by experiences of stress, and what time periods of stress (e.g., early life versus current day experiences) are most detrimental to the biological processes that may add to depressive symptoms will be critical questions to address. As will be presented throughout Chapter 1, a substantial body of research demonstrates a link between stress and immune activity (Irwin & Cole, 2011; Steptoe, Hamer, & Chida, 2007), as well as immune activity and depression (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020); however, less empirical work has directly assessed how stress may affect immune system activity to contribute to depression. Although many theoretical models exist to describe these potential pathways and their mechanisms (Raison, Capuron, & Miller, 2006; Berk, 2013; Iwata et al., 2016; Miller & Raison, 2016; Slavich & Irwin, 2014; Slavich & Cole, 2013), there is a dearth of empirical studies available to tease apart these relationships.

The purpose of this dissertation is to present an integrative model of depression and test whether psychological experiences of stress, as well as a cognitive capacity related to stress management, may influence neuroendoimmune activity in relation to depression. To accomplish these goals, Chapter 1 presents a comprehensive review of the research literature, first by providing an overview of current conceptualizations of depression, including both categorical and dimensional approaches. Next, three components of the model presented in this chapter will be reviewed: stress, biological systems that respond to stress, and psychological or cognitive factors that may interact with biological stress systems to contribute to depression. The concept of stress will be

presented, along with research on associated biomarkers, including those of the immune system and HPA axis. Subsequently, the research literature on stress, neuroendoimmune activity, and depression will be summarized. This is followed by a review of the psychological and cognitive factors involved in stress appraisal and the influence these might have on biological systems that respond to stress and contribute to depression.

Chapter 2 presents the methods of an original empirical investigation that tests components of the model presented in Chapter 1. Blood plasma markers are analyzed to assess whether neuroendoimmune activity mediates the relationship between psychological experiences of stress and depression in a transdiagnostic sample of individuals with and without clinical levels of depression. Additionally, the severity of stress at different time periods (i.e. childhood, recent months or weeks) are evaluated to determine whether specific time periods drive the relationship between potential neuroendoimmune disruptions and depression. To examine psychological and cognitive aspects of the model, variables related to stress appraisal and cognitive performance are examined in relation to neuroendoimmune activity and depressive symptom severity, helping to determine whether cognitive ability alters biological responses to stress to influence depression outcomes. This approach, which adopts a dimensional assessment of depression, incorporates multiple measures of stress biomarkers, and uses a transdiagnostic sample of participants with varying levels of depression, could provide a more nuanced understanding of the relationship between stress and depression. These are important lines of inquiry because they could shed light on how the neuroendoimmune system potentially translates stress into depression, what periods of stress are most pertinent over the lifespan, and how stress and inflammation might mediate the relationship between cognitive ability and depression.

1.1 Depression

1.1.1 Conceptualizing depression

1.1.1.1 Categorical approaches to depression

Depression has traditionally been conceptualized as a discrete disorder in prevailing diagnostic systems; however, this categorical approach to the study of psychopathology is a comparatively new tradition, with only a brief history when contrasted against other areas of medicine

(Moriyama, Loy, Robb-Smith, 2011). It was not until the 1950s that the American Psychiatric Association (APA) and the World Health Organization (WHO) developed categorical systems to conceptualize mental health pathology, formalized within the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Diseases (ICD), respectively (Wilson, 1993). To date, there have been five editions of the DSM (American Psychiatric Association, 2013), and 11 editions of the ICD (World Health Organization, 2019). These manuals identify clinically meaningful disorders that are intended to aid in research, diagnosis, treatment, communication, public health statistics, policy making, education, training, and insurance coverage (American Psychiatric Association, 2013). Accordingly, the categorically-defined disorders included in these diagnostic manuals have shaped current conceptualizations of psychiatric illness and play a central role in both research and treatment.

To receive a diagnosis of MDD according to the DSM-5, five of nine criteria must be present for a two-week period, more days than not, that represent a change from previous functioning. These symptoms include the following: low mood; anhedonia (i.e., loss of pleasure in previously enjoyed experiences); changes in appetite, weight, and sleep; fatigue; diminished concentration and indecisiveness; psychomotor agitation (e.g., fidgeting, restlessness, pacing) or retardation (e.g., slowed speech, thinking, or movement); and feelings, thoughts, and behaviours related to guilt, worthlessness, or suicide.

Across the various iterations of the DSM, research has been conducted to understand the prevalence and developmental course of MDD. The 12-month prevalence rate of MDD in the United States is 7%, and females are 1.5 – 3 times more likely to experience MDD than males (American Psychiatric Association, 2013). This translates into approximately one in four females and one in six males experiencing depression in their lifetime (Kessler et al., 2010). Up to 85% of individuals experience more than one episode, and relapse risk increases by 16 – 18% after each subsequent episode (Mueller et al., 1999; Kruijshaar et al., 2005; Boland & Keller, 2009). Recurrent episodes typically begin within five years of the first episode (Belsher & Costello, 1988; Eaton et al., 2008; Mueller et al., 1999), with an average of five to nine episodes across one's lifetime (Burcusa & Iacono, 2007). When examined as a whole, MDD is considered a leading cause of disability worldwide (Marcus, Yasamy, Ommeren, Chisholm, & Saxena, 2012).

Importantly, as will be reviewed in more depth throughout this chapter, alterations in immune system and HPA axis activity are reported in MDD compared to healthy controls and during periods of remission from a major depressive episode (Fischer, Strawbridge, Vives, & Cleare, 2017; Dowlati et al., 2010; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015). However, these biological disruptions are only present in a subset of those suffering from MDD, leading to small albeit reliable effect sizes (Raison & Miller, 2011). Furthermore, these patterns of disruptions appear to overlap with many other medical and psychiatric disorders that are often excluded from such research (e.g., Grassi-Oliveira et al., 2009; Michopoulos et al., 2017). Given these nuances, categorical approaches to MDD research may limit progress into these lines of investigation. Determining why such alterations occur, and how they impact symptomatology, may be critical to advancing our understanding of experiences of depression, improving assessment, and targeting treatment. As such, the section to follow examines the pros and cons of categorical approaches to biobehavioural investigations of MDD, highlighting the potential value of dimensional approaches in depression-immune research.

1.1.1.2 Dimensional approaches to depression

Although categorical approaches to psychiatric illness are rooted in the well-established medical model of disease classification (Moriyama, Loy, Robb-Smith, 2011), the development of categories within mental health research did not adopt the same biological approach that is central to medical disease classification (Wilson, 1993). Whereas medical disease classification developed through empirical investigation related to biological underpinnings of disease (e.g., insulin resistance in type 2 diabetes; autoimmune origins of rheumatoid arthritis; the destruction of CD4 T lymphocytes by human immunodeficiency viruses in acquired immune deficiency syndrome; genetic inheritance of the mutation for hemochromatosis, etc.) (Dalal & Sivakumar, 2013), diagnostic criteria for mental disorders largely ignore biological factors in favour of clusters of behavioural symptoms when designing criteria related to diagnoses (North & Surís, 2017). This is largely because the behavioural symptoms were viewed as the target of treatment, and thus an assumption was made that establishing a clinical nosology based on behavioural commonalities would translate into optimal clinical communication and mental health treatment (Nelson, Strickland, Krueger, Arbisi, & Patrick, 2015).

Although the categorical approach represented in the DSM-5 and ICD-11 provides a common language for clinicians and researchers, and has significantly shaped the scientific literature, the approach does not reflect recent advances in our understanding of psychopathology (Lux & Kendler, 2010; Goldberg, 2011; Patrick & Hajcak, 2016; Kotov et al, 2017). While behavioural symptoms are a critical component of psychiatric nosology, categorizing disorders based primarily on clusters of behaviours has led to research challenges. The reliability and validity of traditional categorical systems is impacted by high levels of comorbidity, overlapping boundaries between different diagnoses, and arbitrary cut-offs between psychopathology and normalcy (Markon, Chmielewski, & Miller, 2011). By placing individuals into what on the surface may appear as homogeneous categories of mental disorders, biobehavioural commonalities across disorders are largely ignored in categorical systems. To complicate matters further, within-category heterogeneity, and masked individual differences, hinder attempts at understanding the biological factors that are involved in producing the specific symptoms associated with psychopathology (Goldberg, 2011; Patrick & Hajcak, 2016; Kotov et al, 2017).

Research on MDD—among the most highly studied of psychiatric disorders—has also been stymied by the aforementioned limitations of the categorical approach to diagnosing mental disorders in the prevailing psychiatric diagnostic systems (Monroe & Anderson, 2015; Fried & Nesse, 2015a; Fried, 2017). One reason that research on MDD may be hindered is because the DSM criteria capture a considerable range of symptoms. This can lead to two people receiving the same diagnosis, despite having very different symptom presentations. For example, within the context of the DSM-5 criteria for MDD, not only must a person have five of nine of the symptoms (leading to different possible symptom combinations between individuals), but as Goldberg (2011) described, many of the criteria can be coded as opposites. For example, one individual might present with hypersomnia, weight gain, and psychomotor retardation, while another individual can also receive the same diagnosis yet have insomnia, decreased appetite, weight loss, and psychomotor agitation. Within the categorical system, these individuals receive the same diagnosis and therefore are frequently considered as one group in research studies. This within-diagnosis variability led to the development of subtypes of depression (e.g., anxious, melancholic, atypical, etc.) (Lux & Kendler, 2010; Lamers et al., 2012; Rudolf, Greggersen, Kahl, Hüppe, & Schweiger, 2014; Hickman, Khambaty, & Stewart, 2014; Goldberg, 2011). However, most of these subtypes were not derived empirically, and do not reflect current

advancements in the field (Watson, 2005; Kotov et al., 2017). Although the subtypes appear qualitatively divergent on the surface, they do not demonstrate high reliability or external validity (Ruscio & Ruscio, 2000; Fried & Nesse, 2015b), and their mere existence signals that the broader MDD category is significantly heterogenous (Watson, 2003).

In addition to the in-group heterogeneity problems, the high levels of comorbidity between MDD and other disorders further muddles the research. There are estimates that only about 25% of those affected by MDD experience MDD in isolation (Kessler et al., 1996; Melartin et al., 2002). For example, 57% of individuals with MDD also experience an anxiety disorder (Clark, 1989; Kessler, Chiu, Demler, & Walters, 2005) and 38.6% have concurrent substance use disorders (Kessler et al., 1996). It is estimated that approximately 70% of MDD diagnoses occur within the context of a personality disorder, with about 30% of all MDD patients suffering from borderline personality disorder (BPD) (Rossi et al., 2001). In addition to overlapping diagnoses, MDD is highly comorbid with medical diseases such as autoimmune conditions, cardiovascular disease, cancer, and neurodegenerative disease (Graff, Walker, & Bernstein, 2009; Leonard, 2007; Evans et al., 2005; Slavich, 2016; Scarpioni, Ricardi, & Albertazzi, 2016). Until more recently, it was unclear why these rates of comorbidity are so pervasive. However, research over the past two decades shed some light as to common biological processes, such as immune activity and neuroendocrine responses that may link these categorically separate psychiatric and physical disorders, especially within the context of stress (Michopoulos et al., 2017; Slavich & Cole, 2013; Slavich & Irwin, 2014). At present, however, even within biological research, the majority of research studies screen out various comorbid conditions, potentially yielding an unrepresentative sample of patients that fit within specific diagnostic confines (e.g., Raison & Miller, 2011; Slavich, Graham-Engeland, Smyth, & Engeland, 2015).

To address some of these empirical challenges, there is now a push to study symptoms within and across disorders (i.e., “transdiagnostically”) to more fully elucidate the biological underpinnings of symptoms, and to facilitate targeted interventions (Nelson, Strickland, Krueger, Arbisi, & Patrick, 2015). Given that stress is linked to many psychiatric illnesses, including MDD, BPD, bipolar disorder, generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD), and schizophrenia (among others) (Colodro-Conde et al., 2018; Kapczinski et al., 2008; Howes & McCutcheon, 2017), it is important to clarify the nature of the relationship between

stress and different dimensions of psychopathology, especially depression, because many of these symptoms may result from common neurobiological causes. One proposed explanation for the strong relationship between stress and disorders such depression, anxiety, and trauma-related disorders, relates to the dynamics of the biological systems that respond to stressors including the immune system and HPA axis (Michopoulos et al., 2017). Importantly, all of the stress-linked psychiatric disorders listed above, are also characterized by disruptions in neuroendoimmune biomarkers, signaling potential common biological disruptions that may contribute to symptom presentations (Michopoulos et al., 2017; Jokinen & Nordströ, 2009; van der Werf-Eldering et al., 2012; Kahl et al., 2006; 2009).

While it is exciting to investigate new avenues for research that might significantly impact our conceptualization of psychopathology and approaches to treatment, the process is currently constrained by examining artificial categories and groups, when in reality, the biological response to stress cuts across a range of psychiatric and medical disorders (Michopoulos et al., 2017; Slavich, 2016; Scarpioni, Ricardi, & Albertazzi, 2016; Masi, Glozier, Dale, & Guastella, 2017). Instead, this empirical evidence may be more well suited to a dimensional and transdiagnostic approach to more precisely assess the relationship between biomarkers of stress and specific symptom outcomes.

One way to facilitate more precision in research is to use dimensional measures that capture the specific nature and degree of severity of symptoms. Dimensions—in the field of psychopathology research—are empirically-based characteristics that assess components of mental health on a continuum, in contrast to binary assessments of symptoms (i.e., present or absent) or cut-points with set numbers of symptom combinations (i.e., disorders) (Patrick & Hajcak, 2016; Kotov et al, 2017). Importantly, understanding common dimensional factors that occur within and across disorders, medical conditions, and individuals free of psychopathology, may substantially advance our understanding of psychopathology and health. For example, symptoms common to MDD (e.g., poor concentration, sleep disruptions, anhedonia) can be studied dimensionally and transdiagnostically as they often occur across disorders (e.g., PTSD, schizophrenia, anxiety, medical illness), and can be measured within ranges (i.e., mild, moderate, and severe) or using Likert scales (i.e., degrees of severity) to assist in providing more fine-grained detail regarding the relationship between such symptoms and their biological correlates.

As will be reviewed in more detail in later sections of this chapter, this kind of dimensional approach greatly informed pioneering immune research in depression because it revealed the relationship between specific symptoms of depression and severity ratings related to elevations in immune activity that could be tracked across time (Capuron, Ravaud, & Dantzer, 2000; Capuron, Ravaud, Miller, & Dantzer, 2004; Suarez, Lewis, Krishnan, & Young, 2004). Future research will benefit from more precisely designed dimensional measures, as the majority of measures available were not designed with this purpose in mind. That said, even commonly utilized measures, such as the Hamilton Depression Rating Scale (HAMD) and Beck Depression Inventory (BDI)-II, provide ratings for each symptom that can be adopted for dimensional research purposes (Suarez, Sundy, Erkanli, 2015).

At present, the field is straddling the divide of these two systems—categorical versus dimensional. The categorical system is still firmly implemented within clinical care settings, and the extant literature is largely formulated from this perspective, carrying the bulk of scientific evidence to date. As such, it is necessary to consider the categorical system and its relevant data before bridging to new dimensional approaches. More research is needed to start to untangle the linking threads between and across diagnostic categories. Like any good archeological dig, as the presence and implications of biological disruptions related to stress and depression were partially uncovered in categorical research, additional transdiagnostic and dimensional investigations are needed to sweep away the sand and carve out the specific dynamics of these systems. In line with this, the remainder of Chapter 1 will examine research on stress and depression with the bulk of data drawn from research on the categorical diagnosis of MDD. Then, Chapter 2 will adopt a multidimensional and transdiagnostic approach to the investigation of stress and neuroendoimmune activity in relation to depressive symptom severity, to begin the refining process and to advance biobehavioural research.

1.2 Stress

The devastating impacts of stress are estimated to cost the American economy $300 billion annually (American Institute of Stress, 2013), with comparable estimates reflected in Canada and other countries (Crompton, 2011; Hassard, Teoh, Cox, & Dewe, 2014). As our scientific understanding of the relationship between stress and physical and mental health continues to

expand, there is increasing appreciation for the biopsychosocial processes that integrate our environmental, biological, and psychological worlds. In line with this, this section will examine physical and psychological elements of stress. To begin, Section 1.2.1 focuses on conceptualizations of stress. Then, Section 1.2.2 examines stress measurement, recognizing both subjective and objective methodologies, and highlighting biobehavioural factors related to stress that should be considered in stress research. Section 1.2.3 provides a detailed exploration of the neuroendoimmune system associated with both acute and chronic states of stress. This will set the stage for an in-depth discussion of integrative models of stress and depression to follow in Section 1.3 of this chapter.

1.2.1 Conceptualizing stress

The term *stress* is used quite casually both colloquially and across the research literature (Kagan, 2016). There are many different terms utilized within stress research (e.g., stress, stressor, psychosocial stress, stress response, etc.). However, these terms can carry different meanings, and thus it is important to define each term explicitly. *Stress*, within the context of the present research, whether physiological (e.g., heart rate, sweat, pupil dilatation), biological (e.g., cortisol, adrenaline), or psychological (e.g., anxiety, pressure, strain), refers to a *response* to an external (e.g., argument, car accident, exercise) or internal (e.g., infection, injury, disease, thought, emotion) event that challenges an individual physically or psychologically (i.e., disrupts homeostasis) (Kagan, 2016). Within the present review, the terms “stress” and “stress response” are used interchangeably and are considered to be part of the individual’s physical and psychological reaction in response to an event.

Stressors, on the other hand, are events that disrupt a person’s psychological or physical equilibrium, causing stress or a stress response (Schneiderman, Ironson, & Siegel, 2004). Although prior research and specific models of stress (such as the diathesis-stress model) commonly utilize the terms stress and stressor interchangeably (for a review, see Segerstrom & Miller, 2004), within the present review, these are not assumed to be synonymous, and the term *stressor* is only utilized when referring to an event that either could, or has, caused stress. The term *psychosocial stress* implies that the stress response was caused by a psychological or social stressor (as opposed to a physical assault or physical challenge of any kind).

It can also be noted that some conceptualizations of stress focus largely on the subjective psychological experience of stress alone (Lazarus & Folkman, 1984), while others use the term stress to encompass the stressor, the individual's appraisal of the stressor, and the biobehavioural response (Segerstrom & Miller, 2004). However, these dimensions are empirically dissociable (Segerstrom & Miller, 2004). As such, although the term stress within the present review includes both psychological and biological responses to an event, what the conscious mind perceives as stressful (i.e., subjective appraisals of stress), and what information the body holds in relation to stress (i.e., objective, biological responses), are addressed separately, and will be evaluated empirically in the research presented in Chapter 2. As such, the terms "psychological experience of stress" and "stress appraisals" refer to one's psychological experience of stress, and "biological (or) physical stress response" to one's physiological response.

Stress and stressors come in many forms and can be characterized in many ways (e.g., distant, brief, acute, or chronic) (Segerstrom & Miller, 2004). For the purposes of the present review, the two primary distinctions of interest are acute and chronic. An acute stressor involves a time-limited, short-term challenge that does not demand a sustained stress response. Acute stressors are a common everyday occurrence. The acute stress response to acute stressors can be both healthy and adaptive (e.g., waking up, exercise, sauna heat, sprinting to catch a bus) (Lazarus & Folkman, 1984). Chronic stressors, on the other hand, extend the stress response to a point that could increase health risk (Oliveira et al., 2016). Chronic stress can be caused by a stressor that persists for an extended period of time (e.g., disease, ongoing abuse), or when the source of stress resolves but the appraisal of the stressor remains (e.g., cognitions that the world is unsafe following an acute trauma, ruminating about one's performance at work after the workday is over). In both cases, the stress response can be extended past adaptive levels, leaving one vulnerable to health risk (Segerstrom & Miller, 2004). These chronic stress states have implications for psychopathology and medicine, as chronic stress is known to precipitate, exacerbate, and maintain both physical and psychological conditions (Juster, McEwen, & Lupien, 2010).

1.2.2 Measuring stress

The psychological and physical stress response can be measured in various ways, whether queried subjectively (i.e., self-report), manipulated experimentally (e.g., using acute or chronic stress research paradigms), or measured using objective metrics (e.g., blood pressure, heart rate variability, galvanic skin response, cortisol, cytokines). As research advances, best practices continue to emerge surrounding the measurement of stress, as various aspects of both stressors and stress hold clinically relevant meaning. As such, indexing the type of stressor, the timing and duration of a stressor, as well as the subjective and objective components of stress, are all critically important in research on this topic (Monroe, 2008; Slavich & Shields, 2018).

1.2.2.1 Subjective stress reports

Self-report measures can be used to index types of stressors, the period of time during which the stressors had an impact, as well as one's subjective experience of stress (i.e., how intense and distressing it was). Although stressors are commonly measured and implemented as an indirect index of stress (for example, as is often the case in diathesis-stress models; Howes & McCutcheon, 2017; Colodro-Conde et al., 2018), the way people perceive stressors, psychologically, is assumed to play an important role on the impact those stressors have on psychological states, behaviour, and well-being (Yamakawa et al., 2009; Aschbacher et al., 2012; Shields & Slavich, 2017). While broadly useful, measuring stressors can be a poor metric of stress because what one person finds stressful may be unalarming to another (or at the very least, more manageable). Similarly, what may be stressful for an individual at one given time, may be unprovoking to them at a different time. Lastly, indexing stressors can be ineffective as a metric for stress, as we often experience stress even when we are unaware of the stressor or cannot make a connection between our current experience and a specific stressor (e.g., micronutrient deficiency, poor sleep, weather, automatic thoughts, emotions, infection, etc.). Given this, when using stressors from subjective reports as a metric for stress, it is important to try to establish the relationship between the stressor and the experience of stress, or instead to use the experience of stress in and of itself (Slavich & Sheilds, 2018).

Measuring the psychological experience of stress can be captured directly through self-report assessments to gauge the level of stress an individual may experience. One way to assess the impact of a stressor is to measure an individual's perception of a given stressor and their ability to cope. Humans perceive stress when they feel psychological strain, pressure, distress, or when

they evaluate something as threatening and doubt their ability to cope or respond to the threat or challenge (Lazarus & Folkman, 1984; Slavich & Shields, 2018). There are several measures designed to index perceived stress or stress severity (e.g., the Transactional Stress Questionnaire, Perceived Stress Scale [PSS], Stress and Adversity Inventory for Adults [STRAIN], Childhood Trauma Questionnaire, Life Events and Difficulties Schedule) (Lazarus & Folkman, 1984; Cohen, Kamarck, & Mermelstein, 2006; Slavich & Shields, 2018). Using measures of perceived stress in and of themselves, and also to clearly assess the relationship between external stressors and stress, is critical to establishing the role of psychological processes in mental health and disease. Measures of stressors and perceived stress severity are reviewed below to highlight relevant instruments that are incorporated into the empirical research presented in Chapter 2 of this dissertation.

1.2.2.1.1 Stressors and perceived stress severity

As mentioned, stress is known to increase the risk for a wide variety of physical and psychological conditions, including (but not limited to) cardiovascular disease, autoimmunity, cancer, Alzheimer's disease, accelerated aging, chronic pain, depression, and anxiety disorders (Graff, Walker, & Bernstein, 2009; Leonard, 2007; Slavich, 2016; Michopoulos et al., 2017). However, it remains unclear how one's perception about their ability to cope with life stressors influences health outcomes.

The PSS was developed by Cohen, Kamarck, and Mermelstein (2006) to measure the degree to which individuals appraise events in their life as stressful over the past month (without any specific information collected about the stressors) (Cohen, Kamarck, & Mermelstein, 2006). The PSS is a 10-item self-report inventory that presents questions to gauge how well an individual coped with, or responded to, stressors (e.g., "In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?"), rated from 0 (*Never*) to 4 (*Very Often*). Four items are coded in reverse directions and are inverted when calculating total scores, with total scores ranging from 0 to 40. The PSS demonstrates high construct validity and internal consistency (Cronbach's alpha = .89; Roberti, Harrington, & Storch, 2011). High PSS scores are associated with increased sickness, decreased health behaviours, and greater vulnerability to depressive symptoms (Roberti, Harrington, & Storch, 2011).

While the PSS captures proximal (past month) stress perception and coping, it is theorized that one's exposure to stressors across the lifespan may exert an effect on biological processes that contribute to widespread impacts on health (Slavich & Shields, 2018). If exposure to significant stressors across the lifespan shifts the balance of neural and peripheral systems that respond to threats (i.e., immune and HPA axis activity), individuals may become more vulnerable to disease states and psychological disturbances (Lupien, McEwen, Gunnar, & Heim, 2009). As such, indexing stressors and perceived stress severity that occur across the lifespan may provide important information as to the relationship between experiences of stress and health.

The term "cumulative life stress" was coined to capture the summation of one's experience of stress across the lifespan (Slavich & Shields, 2018). To date, few, if any measures exist to assess this central theoretical concept apart from the STRAIN. The STRAIN is an online questionnaire that assesses the frequency of stressors (i.e., total count), timing of stressors (i.e., age at which they occurred), and perceived stress associated with the stressors (i.e., stress severity; "At its worst, how stressful or threatening was this for you?"). Life stressors on the STRAIN include those related to work, finances, loss, bereavement, medical concerns, caregiving, substance use, interpersonal strain, and divorce among others (Slavich & Shields, 2018). The STRAIN provides scores on the frequency (to produce a total count score) and intensity of stressors (to produce a severity score) and categorizes stressors and perceived stress severity into clinically meaningful timelines (e.g., early life adversity, college years, adulthood exposure, recent past six months). The timing of stressful events is relevant, as, for example, research demonstrates that early life trauma is strongly associated with psychopathology and immune and endocrine disruptions in adulthood (Danese & Baldwin, 2017; Slopen, Kubzansky, McLaughlin, & Koenen, 2013). The measure takes approximately 18 minutes to complete and demonstrates good psychometric properties (Toussaint, Shields, Dorn, & Slavich, 2016; Dooley, Slavich, Moreno, & Bower, 2017). Scores on the STRAIN are associated with health complaints and diagnoses, disrupted sleep, and poor executive functioning (Slavich & Shields, 2018).

To provide both proximal and distal assessments of subjective experiences of stress, the research presented in Chapter 2 includes the PSS and the STRAIN. The PSS provides an assessment of current levels of perceived stress (defined as within the past month), while the STRAIN captures

the cumulative perceived severity of stressors across the lifespan (including within different times periods of life, such as childhood).

1.2.2.2 States of stress and experimental paradigms

The stress response can be studied in a variety of contexts and with various paradigms. Important information can be gathered from basal states, acute stress paradigms, and chronic states of stress. Although they are beyond the scope of the present review, studies employing stress reduction paradigms have also illuminated the overall profile of stress dynamics (Nater, Skoluda, & Strahler, 2013). In total, these paradigms can offer interesting insights into the underlying mechanisms that transform stress into various forms of pathology. This section reviews the basic paradigms utilized to investigate the effects of stress under basal, acute, and chronic states. These paradigms will be referred to throughout the remainder of this chapter when exploring research on the biological response to stress and depression.

1.2.2.2.1 Basal states

Basal (or base) assessments refer to measurements of stress biomarkers at rest. Basal state assessments can (a) provide a baseline assessment for statistical contrast against activated stress states, and (b) provide an indication of biomarkers levels compared to pre-established normal ranges in the scientific and medical literature. This latter comparison can signal a disrupted system that may result from ongoing chronic stress states (to be discussed in Section 1.2.2.2.3 to follow). Most biomarkers exist on a continuum from diminished to excessive levels. Like many biological systems, there appears to exist a “goldilocks” position, or inverted u-shape trajectory for biomarkers of stress, where mid-range levels are associated with positive health outcomes (Goshen & Yirmiya, 2009), and excessive or diminished concentrations typically reflect unfavourable health conditions (Goshen et al., 2007; Müschen, 2018).

1.2.2.2.2 Acute stress paradigms

As mentioned, acute stressors are a time-limited challenge that do not demand a sustained stress response. In general, acute stressors are not considered detrimental to health unless they become persistent and repetitive in a way that over-extends the stress response (as will be discussed within the context of chronic stress in the section to follow) (Hänsel, Hong, Cámara, von Känel,

2010). While not the focus of the empirical research presented in this dissertation, acute stress paradigms are briefly reviewed below to provide appropriate context for interpreting the research approaches commonly used to investigate biomarkers of the stress response.

Acute stressors stimulate various central and peripheral biological systems into action, including the ANS, HPA axis, and immune system (Foley & Kirschbaum, 2010). Acute stressors can also be perceived subjectively as both negative (eustress) or positive (distress), as for example, one might view the acute challenge of exercise in a positive or negative light. In either case, the biological stress response is called into action.

To study acute stress experimentally, it is critical to use stressors that evoke reliable psychological and biological stress responses in laboratory settings. Acute stress paradigms commonly implemented in human research include the Trier Social Stress Test (TSST) (Kudielka, Hellhammer, & Kirschbaum, 2007; Foley & Kirschbaum, 2010), the cold pressor task (Schwabe, Haddad, & Schachinger, 2008), and the CO_2 challenge test (Vickers, Jafarpour, Mofidi, Rafat, & Woznica, 2012). These paradigms reliably activate the biological stress response, including biomarkers of the HPA axis and immune system (Kudielka, Hellhammer, & Kirschbaum, 2007; Vickers, Jafarpour, Mofidi, Rafat, & Woznica, 2012).

Of all the above measures, the TSST is the most empirically validated as a reliable acute stressor paradigm (Kudielka, Hellhammer, & Kirschbaum, 2007; Foley & Kirschbaum, 2010). The TSST requires participants to prepare a three-minute speech about why they would be the perfect candidate for a job, and to present a mock job interview to a panel of judges. This speech is then followed by an oral arithmetic task (Kudielka, Hellhammer, & Kirschbaum, 2007). Within this paradigm, stress assessments can be administered at multiple time points before and after the stressor. It is hypothesized that the TSST is so effective as an acute stressor in humans because it presents a social evaluative threat that is uncertain and uncontrollable (Dickerson, Gruenewald, & Kemeny, 2009), which are aspects of psychosocial threat that are particularly evocative to humans (Slavich & Irwin, 2014). As will become more apparent in Section 1.2.2.3, acute stress paradigms not only allow opportunities to gain insights into individual differences in the biological stress response (Kudielka, Hellhammer, & Wüst, 2009), but they can also isolate

dysregulation of the stress response under chronic stress conditions and in group-based analyses of individuals with psychiatric and medical conditions (Foley & Kirschbaum, 2010).

1.2.2.2.3 Chronic stress paradigms

Unlike acute stressors, chronic stressors persist over extended periods of time and prolong the stress response to a point that increases health risk (Hänsel, Hong, Cámara, von Känel, 2010). Chronic stressors tend to cause behavioural (e.g., exhaustion, fatigue, burnout), social (e.g., isolation, avoidance, withdrawal), and biological (e.g., ANS, HPA axis, immune system) shifts as the individual fails to adapt to the circumstances that persist (Selye, 1963; Slavich, 2016). Chronic stress paradigms are not commonly implemented in experimental contexts with human participants for ethical reasons. Instead, chronic stress conditions are often characterized and then utilized to organize individuals into meaningful groups (Slavich & Shields, 2018). For example, experiences of trauma, low socioeconomic status (SES), caregiver stress (e.g., providing care to a family member with dementia, looking after disabled children), and loneliness (i.e., perceived social isolation) are indexed as chronic stress conditions, and all have been investigated as models of chronic stress in humans (Miller et al., 2014). While some studies use past events (e.g., early life stress) as indicators of chronic stress (whether resolved or ongoing), other research characterizes chronic stress by identifying current ongoing, but extended, difficulties, by rank-ordering severity and duration of current stressors and selecting the most salient ongoing and extended stressor as the chronic stress variable for analytic purposes (Segerstrom & Miller, 2004). Importantly, as will be discussed throughout this chapter, these chronic stress conditions are associated with immune and HPA axis dysregulation (von Känel, Bellingrath, & Kudielka, 2008; Cohen et al., 2012; Miller et al., 2008; Slavich, 2016).

Early life stress (e.g., foster care; sexual, physical, or emotional abuse; separation from parents; unpredictability) is considered a chronic stressor, as many individuals demonstrate alterations in biological processes, even into adulthood, long after the specific stressor has resolved (Danese & Baldwin, 2017; Slopen, Kubzansky, McLaughlin, & Koenen, 2013). Childhood trauma and early life stress are risk factors for many forms of psychopathology, and it could be the case that extended disruptions in neurobiological systems associated with the stress response may contribute to the translation of childhood adversity to adult mental health disturbances (Danese & Baldwin, 2017). This is one chronic stress condition that may carry clinically significant

meaning for health across the lifespan (Slavich & Shields, 2018). Theorized mechanisms related to this category of chronic stress will be reviewed in later sections of this dissertation (see Section 1.2.3.2).

Beyond early life experiences, chronic stressors throughout adulthood are also problematic for health. For example, loneliness due to perceived social isolation is considered a form of chronic stress (Jacob, Haro, & Koyanagi, 2019). It is estimated that having quality social connections decreases mortality by up to 50%, which is similar to the benefit incurred by quitting smoking, and has a stronger relationship with mortality than many other known risk factors, such as physical inactivity, obesity, or excessive alcohol consumption (Holt-Lunstad, Smith, & Layton, 2010). Similarly, low SES and caregiver strain are factors in adulthood that are correlated with negative health outcomes (Kiecolt-Glaser, Gouin, et al., 2011; Miller et al., 2014). Importantly, these chronic forms of stress in adulthood are all associated with dysregulation of stress biomarkers of both the immune system and HPA axis (Häfner et al., 2011), as well as physical illness and psychopathology (Jacob, Haro, & Koyanagi, 2019).

Importantly, the dysregulation of systems caused by chronic stressors often results in altered assessments of stress biomarkers under both basal and acute states of stress (Miller et al., 2011; Slavish, 2016). As such, researchers can examine the impacts of chronic stress under both basal and acute conditions to observe the effects of chronic stressors on stress-sensitive biological systems and with symptoms and disorders associated with chronic stress.

1.2.2.3 Biomarkers of stress

In addition to subjective metrics of stress, the stress response can also be assessed by measuring reliable biomarkers that generate neuroendoimmunological profiles of stress (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Slavich & Cole, 2013; Leonard, 2018). This is an important additional metric for two reasons: first, biological markers may provide additional information about states of stress that are not captured by self-report. Although subjective (i.e., self-report) measures provide essential information about psychological experiences of stress, it is also possible that the body may be in a state of stress that is not subjectively perceived at a psychological level. These additional metrics are important to capture the full range of the stress response because the total experience of stress may have implications for depression and other

mental and physical health disturbances (Couzin-Frankel, 2010; Slavich, 2016; Slavich & Shields, 2018). Secondly, biomarkers may help to uncover mechanistic pathways that translate psychological experiences of stress into depression, as will be explored in more detail in Section 1.3 of this chapter.

Biomarkers of stress include molecules and substrates that transform psychological or physical stressors into activity within the body and brain (Foley & Kirschbaum, 2010). In terms of human biology, the stress response consists of a complex series of central and peripheral nervous system exchanges including the activation of the ANS, immune system, and HPA axis (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Slavich & Cole, 2013).

It is worth noting that biomarkers of stress exist in a range: basal, hypo-active (diminished), or hyper-active (excessive) levels. As such, terminology regarding these biomarkers often uses words such as “increased” or “elevations” to refer to changes from known basal levels and normal ranges in healthy humans. This does not necessarily imply that the researchers measured pre-and post-levels of immune or HPA axis activity, but rather that these biomarkers are compared to “normal” lab range values, groups of healthy controls, or relative to the given sample (Raison & Miller, 2003; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). The term “dysregulation” within the present review refers to situations of either elevated (hyper-active) or deficient (hypo-active) biomarker concentrations, or a slow return to baseline (Hiles, Baker, de Malmanche, & Attia, 2012).

It is beyond the scope of the present review to discuss all biomarkers of stress that have been studied. This dissertation focuses on the biomarkers of the immune system and HPA axis that are most commonly studied in relation to stress and depression. These biomarkers, which are reviewed in detail below, are also incorporated in the empirical research presented in this dissertation.

1.2.2.3.1 Immune System

It is well-recognized that disruptions in immune activity are risk factors for a number of illnesses and disorders, including autoimmune disorders, cardiac disease, diabetes, asthma, and Alzheimer’s disease, among many others (Bower et al., 2007; Heinz et al., 2003; Jehn et al.,

2010; Papadopoulos, & Cleare, 2012; Scarpioni, Ricardi, & Albertazzi, 2016). However, research has now shown that the immune system interacts with more than physical health—it also interacts with mental health (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020; Michopoulos et al., 2017). Traditionally, immune activity was associated with response to injury or infection. However, in addition to these physical threats, the immune system is also sensitive to psychosocial stressors, which may play a partial role in the observed relationship between depression and immune activity that will be outlined in sections to follow (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Slavich & Cole, 2013; Slavich & Irwin, 2014; Leonard, 2018). Here, a brief description of the immune system is provided before outlining key immune biomarkers that appear to respond to both physical and psychological stress.

1.2.2.3.1.1 *Immune system terminology*

The immune system is a complex set of structures and processes that function to support cellular operations and to protect an individual against infection, injury, and disease. To do this, the immune system recruits inflammatory biomarkers to carry out the task of surveillance, maintenance, and protection of bodily tissues and cellular functions throughout the body and brain (Zmora, Bashiardes, Levy, & Elinav, 2017). In the case of immune biomarkers, the majority of research measures immune markers peripherally in serum or plasma of humans, or centrally in the brain in rodents (Hodes et al., 2014; Zmora, Bashiardes, Levy, & Elinav, 2017).

When used colloquially, the word “inflammation” is often used to refer to the swelling, pain, and redness that is commonly associated with response to injury. However, the onset of these symptoms is orchestrated by a vast number of immune cells, including macrophages, lymphocytes, leukocytes, neurophils, platelets, endothelial, and T helper cells, as well as protein molecules involved in cell signalling, including histamines, chemokines, and cytokines (Hodes, Kana, Menard, Merad, & Russo, 2015). As such, the term inflammation is used, scientifically speaking, to refer to the activation of inflammatory molecules, rather than to characterize the symptoms (e.g., swelling, redness, brain fog, fatigue, or fever) they may cause.

In keeping with the research literature on stress, it is important to differentiate between acute and chronic forms of inflammation when discussing clinical research (Miller et al., 2008; Yamakawa

et al., 2009). Acute and chronic forms of inflammation present different profiles of inflammatory activity (Slavish, Graham-Engeland, Smyth, & Engeland, 2015; Slavich & Irwin, 2014; Ménard, Pfau, Hodes, & Russo, 2017). Acute inflammation occurs in response to stressors lasting a few days. This is characterized by rapid immune activity needed to repair tissue damage, eliminate infection, or respond to an external stressor, followed by the downregulation of the immune response in an effort to maintain the body's homeostasis (Slavich & Irwin, 2014). In contrast, chronic inflammation is characterized by prolonged or altered immune activity, at which point immune molecules and protein cells can disrupt cellular processes (Couzin-Frankel, 2010; Zmora, Bashiardes, Levy, & Elinav, 2017). The balance of immune activity is critical to health, as hyper- or hypo-activation of immune proteins can, paradoxically, be damaging to the very systems they have evolved to protect (i.e., the "goldilocks principle" in effect) (Hänsel, Hong, Cámara, & von Känel, 2010). Although immune activity is critical to overall health, dysregulation of this system is associated with many negative health consequences (Couzin-Frankel, 2010). One factor that can tilt the balance from adaptive to chronic inflammation is stress (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Slavich & Cole, 2013).

Although there is a myriad of immune signalling proteins, the sections to follow provide detail on the inflammatory biomarkers commonly related to stress and depression. To start, the relationship between these common immune messengers and stress is discussed, setting the stage for a review of the relationship between these immune biomarkers and depression.

1.2.2.3.1.2 *Biomarkers of inflammation*

Although there are many signalling proteins involved in the immune system response (e.g., cytokines, eicosanoids, leukotrienes, histamines, and growth factors), cytokines are the immune system's central messengers and appear to have particular relevance to stress (Hodes, Kana, Menard, Merad, & Russo, 2015). Cytokines include a broad category of protein molecules. These molecules are often equated to hormones or neurotransmitters, as they mediate immune system communication. They are suitable biomarkers of inflammation because they are produced by activated immune cells and interact with surrounding cells to coordinate the inflammatory process (Zmora, Bashiardes, Levy, & Elinav, 2017). Throughout this chapter, the terms "immune activity," "immune activation," and "inflammation" imply the production of cytokines.

The various cytokines and their receptors are extensive, but there are roughly five broad categories of cytokine families: interleukins, lymphokines, tumor necrosis factors, interferons, and chemokines. Cytokines can generally be classified as either pro- or anti-inflammatory. The word "generally" is appropriate because many of the cytokines can have both pro- and anti-inflammatory properties depending on the context to which they are deployed (Scheller, Chalaris, Schmidt-Arras, Rose-John, 2011). Both pro- and anti-inflammatory cytokines are critical to health, and respond to infection, trauma, disease, reproduction, maintenance, and decay (Berk et al., 2013). Proinflammatory cytokines include interleukin 1-beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), interferon-alpha (INF-α); whereas anti-inflammatory cytokines include IL-1 receptor antagonist (IL-1Ra), interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor (TGF- β) (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). While both pro- and anti-inflammatory molecules are paramount to a healthy immune system, proinflammatory cytokines are most consistently linked to negative health outcomes as these protein messengers can become overactive, preventing the downregulation of the immune system response, causing cellular damage and communication disruptions (Scarpioni, Ricardi, & Albertazzi, 2016; Couzin-Frankel, 2010; Zmora, Bashiardes, Levy, & Elinav, 2017).

Stress activates the immune system, mobilizing inflammatory cytokines (Slavish, Graham-Engeland, Smyth, & Engeland, 2015; Slavich & Irwin, 2014; Ménard, Pfau, Hodes, & Russo, 2017). It is well established in animal research that stress can be used to evoke both peripheral and central immune activity (Felger et al., 2016; Goshen et al., 2007). The cytokines IL-1β, IL-6, and TNF-α, are the most commonly studied cytokines in the stress research literature, in addition to the acute phase reactant, C-reactive protein (CRP), which is stimulated into production by proinflammatory cytokines (i.e., IL-6) (Valkanova, Ebmeier, & Allan, 2013). IL-1β, IL-6, and TNF-α are mainly classified as proinflammatory cytokines as they stimulate the immune system to up-regulate systemic inflammation (Zmora, Bashiardes, Levy, & Elinav, 2017) (see Table 1 for a summary of relevant biomarkers).

In acute states of stress, these biomarkers contribute to healing, prevent infection, and monitor the body for potential threats. Under chronic stress, these immune biomarkers are associated with disease and psychopathology (Couzin-Frankel, 2010; Zmora, Bashiardes, Levy, & Elinav, 2017).

Chronic stress-induced cytokine production may thus represent a neurobiological process that transforms psychosocial stressors into psychopathology. The sections to follow review TNF-α, IL-1β, IL-6, and CRP in relation to stress. In later sections of this chapter, research studies on these immune biomarkers and stress-associated psychopathology are summarized, with special attention paid to depression in relation to immune activity.

TNF-α. To begin the inflammatory cascade, TNF-α is a cytokine protein that upregulates inflammatory activity by signaling and promoting the further release of both IL-1β and IL-6 (Sekiyama, Yoshiba, & Thomson, 2008). TNF-α can cause fever, muscle aches, loss of appetite, apoptotic cell death, swelling and redness associated with injury, and can inhibit tumour growth, viral replication, and sepsis (Huizinga, Nigrovic, Ruderman, & Schulze-Koops, 2011). In serum and plasma, TNF-α responds to acute stressors such as the TSST (Bower et al., 2007; Dickerson, Gruenewald, & Kemeny, 2009). TNF-α is also positively associated with chronic stress conditions such as early life stress (Kiecolt-Glaser et al., 2011), interpersonal conflict (Chiang, Eisenberger, Seeman, & Taylor, 2012; Kiecolt-Glaser et al., 2005), loneliness (Jaremka et al., 2013) and burnout at work (von Känel, Bellingrath, & Kudielka, 2008).

IL-1β. IL-1β is a proinflammatory cytokine that carries out a number of functions to aid in the defense against pathogens and injury to promote healing (Dinarello, 2009). To do so, it is involved in cell differentiation, proliferation, and apoptosis (Dinarello, 2009). In plasma and serum, IL-1β increases under acute stressors, such as during an examination period in school (Mahmood & Ibrahim, 2016), an oral examination (Heinz et al., 2003), and the TSST (Bower et al., 2007). In terms of chronic stress, increased IL-1β levels have been found in the hippocampus following social isolation in rodents (Ben Menachem-Zidon et al., 2008; Goshen & Yirmiya, 2009), and are similarly associated with loneliness in humans (Jaremka et al., 2013).

IL-6. IL-6 is a cytokine protein produced in response to infection or injury, and also contributes to the fever response (Valkanova, Ebmeier, & Allan, 2013). It serves as the primary activator of CRP release from the liver, and like IL-1β, is involved in cell differentiation, proliferation, and apoptosis (Valkanova, Ebmeier, & Allan, 2013). Associations between IL-6 and psychosocial stress appear to be relatively consistent across stress studies. Increased levels of IL-6 are associated with acute stressors in laboratory settings (e.g., TSST, cold pressor task) in

healthy adults (Bower et al., 2007; Carpenter et al., 2010). In terms of more chronic stress conditions, levels of IL-6 were found to be higher in adults who experienced early life stress or low SES in childhood (Kiecolt-Glaser, Gouin, et al., 2011; Carpenter et al., 2010; Slopen, Kubzansky, McLaughlin, & Koenen, 2013). Furthermore, adults who experienced early life stress also showed higher IL-6 production to acute stressors in daily life (Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012), as well as laboratory measures of acute stress (i.e., TSST) (Carpenter et al., 2010; Danese & Baldwin, 2017; Leonard, 2018). In fact, one study found that childhood abuse was associated with a 2.35 times larger IL-6 response to stress experienced in daily life in a sample of older adults with a history of childhood abuse compared to controls (Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012). It may be that states of chronic stress can promote sustained immune activity and contribute to the sensitivity of some individuals to experience heightened biological responses to acute stressors across the lifespan (Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012; Cohen et al., 2012).

IL-6 is also associated with chronic stress conditions in adulthood such as low SES (Slopen, Graham-Engeland, Smyth, & Engeland, 2015), acute and chronic interpersonal conflict (Chiang, Eisenberger, Seeman, & Taylor, 2012; Kiecolt-Glaser et al., 2005), social isolation (Häfner et al., 2011; Jaremka et al., 2013; Slavich & Irwin, 2014), and caregiver strain (Kiecolt-Glaser et al., 2003; Kiecolt-Glaser et al., 2011). More specifically, socially isolated depressed men were found to express 3.8 times higher levels of IL-6 compared to socially integrated non-depressed men (Häfner et al., 2011). Caregivers looking after dementia patients were also found to demonstrate a fourfold increase in IL-6 compared to controls over a 6-year longitudinal study (Kiecolt-Glaser et al., 2003).

CRP. Acute phase reactants, such as CRP, are produced in response to proinflammatory cytokines. CRP is secreted by the liver in response to IL-6 and further promotes the production of proinflammatory cytokines (Valkanova, Ebmeier, & Allan, 2013). As such, CRP is considered a broad measure of systemic inflammation (Valkanova, Ebmeier, & Allan, 2013). Like the proinflammatory cytokines above, CRP increases under acutely stressful situations such as a mock job interview (Miller, Rohleder, Stetler, & Kirschbaum, 2005) and cognitive challenge (Steptoe, Hamer, & Chida, 2007). Elevations in CRP are also associated with chronic stress states, such as childhood adversity (Danese & Baldwin, 2017; Slopen, Kubzansky, McLaughlin,

& Koenen, 2013), low SES (Deverts, Cohen, Kalrab, & Matthews, 2012), sustained interpersonal conflict (e.g., negative relationships at home or work; Chiang, Eisenberger, Seeman, & Taylor, 2012), and loneliness (Häfner et al., 2011).

1.2.2.3.2 HPA axis

The HPA axis is the body's central stress response system and also contributes to a vast array of homeostatic functions, including digestion, sexual behaviour, mood, metabolism, and immunity (Kudielka & Wüst, 2010). The HPA axis is a major neuroendocrine system that includes communication between the hypothalamus, pituitary, and adrenal glands. This system also interacts with both the ANS and immune system to regulate reactions to stressors (Kudielka & Wüst, 2010). Below, biomarkers of the HPA axis will be reviewed within the context of stress.

1.2.2.3.2.1 *Biomarkers of the HPA axis*

Although there are many hormones associated with HPA axis activity (e.g., corticotrophin-releasing hormone, vasopressin, glucocorticoids), the two hormones of central relevance to the present review are adrenocorticotropic hormone (ACTH) and the glucocorticoid, cortisol, due to their effects on the immune system and their relevance to stress and depression (see Table 1 for descriptions of these biomarkers) (Irwin & Cole, 2011; Foley & Kirschbaum, 2010; McEwen, Gray, & Nasca, 2015).

As the HPA axis responds to stressors, it increases the production of corticotrophin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus, which stimulates the production of ACTH from the anterior lobe of the pituitary gland into the bloodstream. ACTH then leads to the secretion of glucocorticoids, such as cortisol, from the adrenal glands. To complete this cycle, cortisol modulates physiological, metabolic, and immunological processes as part of the inhibitory feedback loop of the HPA axis, downregulating secretion of CRH and ACTH to bring the system back to homeostasis after a stressful encounter (Irwin & Cole, 2011; Foley & Kirschbaum, 2010; McEwen, Gray, & Nasca, 2015). In the sections to follow, ACTH and cortisol will be described as HPA axis hormones related to the biological stress response.

ACTH. ACTH is a hormone secreted by the anterior pituitary gland as part of the response to stress by the HPA axis (Kumsta, Entringer, Hellhammer, & Wüst, 2007). As its name

suggests, the primary function of *adreno-cortico*tropic hormone is to stimulate the release of *corti*sol by binding to ACTH receptors of the *adren*al glands. Release of ACTH stimulates steroid (e.g., cortisol) hormone secretion through both rapid short-term mechanisms within minutes of a stressor, as well as through long-term actions over several hours of the stress response (Kumsta, Entringer, Hellhammer, & Wüst, 2007). In addition to this stress response, ACTH has a normal diurnal pattern across the day, peaking in the early morning, and then steadily decreasing across the day (see Table 1). Basal morning samples procured at 8a.m. are typically between 10 – 50 picograms per millilitre (pg/ml), less than 20pg/ml at 4p.m., and less than 5 – 10 pg/ml at midnight in healthy participants. ACTH reacts readily to acute stressors and is highly associated with cortisol levels as these two molecules regulate one another (Kumsta, Entringer, Hellhammer, & Wüst, 2007; Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008). In the following section, a greater emphasis is placed on the role of cortisol because of its direct effect on the immune system and its more consistent relationship with chronic stress. However, both ACTH and cortisol play a primary role in the biological and adaptive stress response.

Cortisol. Cortisol is one of the most commonly measured biomarkers of stress. It is consistently activated under stressful conditions and can be reliably measured in saliva, blood, urine, or hair (Nater, Skoluda, & Strahler, 2013). Cortisol is a steroid hormone produced by the adrenal glands in response to stress and is classified as a glucocorticoid. Glucocorticoids are a specific class of hormones that bind to glucocorticoid receptors. Cortisol serves many functions peripherally and centrally to increase blood sugar, regulate sleep/wake cycles, aid in digestion, and suppress immune system activity (Raison & Miller, 2003). Once released into the bloodstream, 90 – 95% of cortisol is bound to corticosteroid binding globulin (CBG) and other molecules with an affinity for cortisol (Foley & Kirschbaum, 2010). As a result, less than 10% of cortisol circulates in the body in the free fraction form. This is important because only free form cortisol is readily available to activate glucocorticoid receptors to stimulate and downregulate the stress response. As such, measuring free fraction cortisol in the saliva or blood is a more direct metric to gauge individual differences in biological adaptations to stressors (Foley & Kirschbaum, 2010).

In healthy individuals, and in basal conditions, cortisol follows a natural diurnal rhythm in tandem with ACTH, rising rapidly in the morning and steadily decreasing over the day. Normal ranges of cortisol assessed one hour after waking are typically between 193 – 690 nanomoles per litre (nmol/L), and 83 – 303 nmol in the evening (see Table 1 for biomarker ranges). Diurnal cortisol patterns can be tested across the entire day to gauge an individual's current cortisol rhythm (the slope) or diurnal output (average) (Woda, Picard, & Dutheil, 2016). However, the cortisol awakening response test is often used because it can be administered in a much shorter window (Nater, Skoluda, & Strahler, 2013). The cortisol awakening response is a test that is used to evaluate the natural course of cortisol increase across the first 30 minutes after waking (Steptoe & Serwinski, 2016). In healthy individuals, cortisol tends to increase about 50 – 75% immediately after waking, and peaks within approximately 30 minutes of waking (Pruessner et al., 1997). Although measuring waking and diurnal profiles can provide a reliable spectrum of cortisol activity over the morning or day, for practical reasons, the majority of studies sample cortisol at one time point in the morning (e.g., between 8 and 9a.m.) (Miller, Chen, & Zhou, 2007; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019).

Beyond basal diurnal patterns, cortisol is also responsive to acute, short-term stressors (Dickerson, Mycek, & Zaldivar, 2008). Within the context of acute stressors, cortisol concentrations tend to peak 10 – 30 minutes after the cessation of the stress exposure (Miller, Chen, & Zhou, 2007; Miller, Rohleder, Stetler, & Kirschbaum, 2005; Wielaard, Schaakxs, Comijs, Stek, & Rhebergen, 2018). Importantly, increased response to acute psychological stressors studied within laboratory settings predicts hypertension three years later (Hamer & Steptoe, 2012) suggesting that exaggerated responses to acute stressors have negative implications for health.

Variations in the diurnal cortisol profile are also associated with chronic stress (Miller, Chen, & Zhou, 2007; Vachon-Presseau et al., 2013; Miller et al., 2014). Early research into cortisol patterns proposed that elevated levels of the hormone are associated with stress (i.e., the glucocorticoid cascade hypothesis; Raison & Miller, 2003). Many chronic stress conditions, such as chronic pain (Woda, Picard, & Dutheil, 2016), loneliness (Steptoe, Hamer & Chida, 2007), and early life stress (Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008) are associated with hypercortisolism (e.g., increased hormone bioavailability). However, throughout the late

1990s and early 2000s, many reports of deficient cortisol production also began to surface in populations with high stress (Raison & Miller, 2003). For example, individuals with PTSD, as well as non-psychiatric individuals exposed to chronic stress (e.g., caregiver strain of relatives with dementia), demonstrate blunted cortisol activity, also known as hypocortisolism (i.e., diminished hormone bioavailability) (Seedat, Stein, Kennedy, & Hauger, 2003; Tops, Riese, Oldehinkel, Rijsdijk, & Ormel, 2008). This hypoactive response was also found in medical patients, such as those with irritable bowel syndrome, chronic fatigue, and fibromyalgia (Papadopoulos & Cleare, 2012; Suárez-Hitz et al., 2012).

To address these varying results in relation to stress, a meta-analysis by Miller, Chen, and Zhou (2007) separated key components of cortisol measurement (such as measurement timing and approach), patient population, time since onset of stress/trauma, type of stressor, and perceived stress. They found that exposure to chronic stress is associated with hypocortisolism in morning samples, hypercortisolism in afternoon and evening samples, a flatter diurnal rhythm, and higher daily output. This translates into a shifted diurnal pattern with lower than normal levels in the morning, but elevated levels across the day. Furthermore, they found an inverse relationship between onset of chronic stress and daily cortisol output, such that recent traumas were associated with higher cortisol measurements across the day, and more distant traumas were associated with hypocortisolism in the morning and flattened diurnal slopes (Miller, Chen, & Zhou, 2007). Miller and colleagues suggest that this implies a spike in HPA axis activity immediately following a trauma that then crashes to below normal morning ranges as time passes. Importantly, these studies provide evidence that chronic stressors may disrupt cortisol patterns even after the removal of the stressful stimulus.

1.2.2.3.3 Biobehavioural factors and methodological considerations related to biomarkers of stress

As described above, the biological stress response and the biomarkers of the immune system and HPA axis, all reliably respond to psychosocial stressors, and are associated with negative health outcomes (Thayer & Lane, 2007; Dooley, Slavich, Moreno, & Bower, 2017; Flynn, Moran, Rash, & Campbell, 2019; Vachon-Presseau et al., 2013; Woda, Picard, & Dutheil, 2016). Although changes in these biomarkers can be detected in relation to stressors in healthy controls

and patients, there exists considerable individual variability in terms of the response of biomarkers under both basal and stressful conditions (O'Connor et al., 2009). Some of this variability is accounted for by demographic, behavioural, and biological factors (e.g., ethnicity, age, sleep, exercise, sex, medication). A brief review of such factors is provided below, with particular attention paid to those factors most relevant to the empirical study described in Chapter 2.

1.2.2.3.3.1 *Biological sex and sex hormones*

The endocrine system exerts a significant effect on stress biomarkers between the sexes. In terms of HPA axis hormones, while some studies report equal cortisol responses to acute stressors across the sexes (Foley & Kirschbaum, 2010), others demonstrate higher ACTH and cortisol response in males (Veldhuis et al., 2009). The sex differences in HPA axis markers may reflect differences in pituitary output and adrenal sensitivity (Veldhuis et al., 2009; Foley & Kirschbaum, 2010).

Within females, menstrual cycle phase, pregnancy, and hormonal contraceptives are known to influence immune and HPA axis biomarkers (Bouman, Jan Heineman, Faas, 2005). Salivary cortisol response in females exposed to acute psychosocial stressors is influenced by menstrual cycle phase, such that females in the follicular phase of their menstrual cycle demonstrate lower responses (Foley & Kirschbaum, 2010). When females taking oral contraceptives are compared to females in the luteal phase of their menstrual cycle, those taking oral contraceptives demonstrate blunted free cortisol response but a significant increase in sensitivity to cortisol following an acute stressor (Rohleder, Wolf, Piel, & Kirschbaum, 2003). Lastly, pregnant females tend to have higher-than-normal levels of cortisol (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009).

The sex differences identified in HPA axis biomarkers are echoed in proinflammatory cytokine responses. For example, females demonstrate more reliable elevations in proinflammatory cytokines under chronic stress conditions compared to males (Birur, Amrock, Shelton, & Li, 2017). Proinflammatory molecules also fluctuate with menstrual cycling, as IL-1β, IL-6, and TNF-α can be 1.5 – 3 times higher during menses compared to the follicular phase (Whitcomb et al., 2014). Although lower levels of proinflammatory cytokines appear in healthy pregnancy

(Graham et al., 2017), pregnant females under chronic psychosocial stress were found to demonstrate associations between stress severity and IL-1β, IL-6, and CRP output across pregnancy (Coussons-Read, Okun, & Nettles, 2007).

Given the moderating effect of biological sex on biomarkers of stress, many studies opt to study only male or female samples in isolation, as results from one sex may not be generalizable to the other, and mixing sexes may significantly influence the findings (Otte et al., 2005; Foley & Kirschbaum, 2010). If studies do include both sexes, it is worth running separate analyses for each sex (Otte et al., 2005). Similarly, given the moderating effects of menstrual cycle, it is ideal to test females at the same point of their menstrual cycle. However, in complex research paradigms this may not always be feasible, and thus best practices instead monitor the phase of menstrual cycle at the time of data collection to include as a covariate (Kahl et al., 2006; O'Connor et al., 2009; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016).

1.2.2.3.3.2 *Age*

Chronological age interacts with both HPA axis and immune biomarkers. Healthy biological aging is associated with increases of proinflammatory cytokine activity and HPA axis biomarkers (Gouin, Hantsoo, Kiecolt-Glaser, 2008; Nater, Hoppmann, & Scott, 2013). Furthermore, a meta-analysis found that cortisol response to acute stressors amplifies with age, and this effect is three times higher in females than males (Otte et al., 2005). These studies demonstrate a natural relationship between age and biomarkers of stress, and thus most research studies target specific age ranges (e.g., children, adolescents, adults, older adults) to avoid age-related confounds in biomarker measurements.

1.2.2.3.3.3 *Exercise*

Exercise has a metabolic and immunological effect, upregulating biomarkers of stress in response to acute exercise and altering basal levels (Woods, Vieira, & Keylock, 2009). For instance, highly trained athletes often exhibit higher-than-normal levels of cortisol, and lower levels of CRP (Woods, Vieira, & Keylock, 2009). Due to these relationships, research studies often control for, or limit, exercise output when examining levels of cytokine activity in relation to health outcomes (Rudolf, Greggersen, Kahl, Hüppe, & Schweiger, 2014).

1.2.2.3.3.4 *Food and meal timing, alcoholic beverages, and recreational drugs*

It is beyond the scope of this review to expand all potential confounds related to foods, alcoholic beverages, and recreational drugs; however, the stress biomarkers listed above are also impacted by food quality and timing, alcohol consumption, and recreational drug use (Kirschbaum et al., 1997; González-Reimers, Santolaria-Fernández, Martín-González, Fernández-Rodríguez, & Quintero-Platt, 2014). Although it is not always feasible to control for food consumption, fasted morning blood draws are often conducted to avoid food-induced variability (Grassi-Oliveira et al., 2009). Similarly, research studies often preclude participants with problematic alcohol or drug use (Grassi-Oliveira et al., 2009), and ask participants to refrain from drug or alcohol use in advance of participation (Grassi-Oliveira et al., 2009).

1.2.2.3.3.5 *Medications and medical conditions*

Beyond hormonal contraceptives, many other medications impact biomarkers of stress. For example, any steroid medications will alter cortisol and immune activity (Granger, Hibel, Fortunato, & Kapelewski, 2009). Similarly, some antidepressants appear to have a dampening effect on immune activity (Strawbridge et al., 2015). To address this, some studies test participants free of medication, or limit recent medication changes or types of medications that interfere with biomarker activity (Granger, Hibel, Fortunato, & Kapelewski, 2009; Haroon et al., 2016). Pre-existing medical conditions are also likely to increase immune activity (Evans et al., 2005; Jehn et al., 2010). As such, the majority of studies control, or exclude, medical conditions including chronic illnesses and inflammatory diseases (Grassi-Oliveira et al., 2009).

1.2.2.3.3.6 *Body mass index*

Higher body fat is associated with higher proinflammatory cytokine levels (Rexrode, Pradhan, Manson, Buring, & Ridker, 2003; Mac Giollabhui et al., 2019), and predicts IL-6 production across time (Mac Giollabhui et al., 2019). This may be partially because weight gain and adipose tissue produce inflammatory cytokines such as TNFα and IL-6, and partially because of other side effects associated with excess weight (e.g., low food quality, lack of activity, body mass wear and tear on joints) (Rexrode, Pradhan, Manson, Buring, & Ridker, 2003). In general, the relationship between IL-6 and body mass index (BMI), or abdominal adiposity tissue, is known to be linear, with each percentage increase in body fat increasing inflammatory levels (Rexrode,

Pradhan, Manson, Buring, & Ridker, 2003; Mac Giollabhui et al., 2019). BMI is an approximate measure of body fat that is calculated based on bodyweight-to-height ratios. BMI is calculated by dividing body mass by the square root of height and is reported in kilograms per meter squared (kg/m^2). From a BMI calculation, an individual can be classified as either underweight (<18.5), normal weight (18.5 – 25), overweight (25 – 30), or obese (>30). Due to the relationship between measures of BMI and immune biomarkers, the vast majority of studies include BMI as a covariate in analyses (Kahl et al., 2006; O'Connor et al., 2009; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016). In line with this, studies that adjust for confounds such as BMI are typically less robust (Howren, Lamkin, & Suls, 2009), although research suggests adipose tissue is only a partial confound in the relationship between immune activity and MDD (Rudolf et al., 2014; Hickman et al., 2014; Osimo et al., 2020).

1.2.2.3.3.7 *Sleep*

Biomarkers of stress directly influence sleep cycles (Kudielka, Federenko, Hellhammer, & Wüst, 2006) and are also responsive to the quantity and quality of sleep (Clinton, Davis, Zielinski, Jewett, & Krueger, 2011). For instance, shift workers tend to have shifted cortisol levels compared to individuals who work a more typical 9a.m. – 5p.m. schedule (Manenschijn, Van Kruysbergen, De Jong, Koper, & Van Rossum, 2011). Furthermore, an individual's chronotype (i.e., their preferred waking hours) may correlate with individual variability in diurnal cortisol rhythms (Kudielka, Federenko, Hellhammer, & Wüst, 2006). Due to these variations, monitoring, prescribing, or controlling sleep (Shield, Moons, & Slavich, 2017), and measuring biomarkers at a set time of day is ideal within the context of research to control for common diurnal patterns associated with biomarkers of stress (Kudielka, Federenko, Hellhammer, & Wüst, 2006).

1.2.2.3.3.8 *Summary of biobehavioural factors related to stress biomarkers*

In summary, biobehavioural factors worth consideration in research include biological sex, hormonal patterns, age, exercise, food intake, medication use, drug and alcohol use, BMI, and sleep (O'Connor et al., 2009). This list is by no means comprehensive, as there are many biobehavioural factors related to immune and HPA markers; however, this selection highlights some of the most commonly controlled and monitored confounds in immune-depression research

(see Table 2 for a summary of biobehavioural factors and research approaches). However, as Slavich, Graham-Engeland, Smyth, and Engeland (2015) point out, none of these factors are simple to control, and the more control variables that are put in place, the less generalizable the results. It will be important for future research to examine how these factors interact with biomarkers of stress to promote a broad base of findings to further understand the biological basis of mental health.

1.2.3 The impact of stress on the immune system and HPA axis: The neuroendoimmune system

The biological response to stressors is integrated within the neuroendoimmune system. In this network, neurotransmitters, cytokines, and hormones facilitate the "cross-talk" between the brain, endocrine, and immune systems. Ultimately, these systems communicate to facilitate homeostasis—to repair damage, fight infection, and respond to threats (Dantzer, 2018). Critically, the activity of the immune system and HPA axis are highly intertwined, and the communication of these stress-sensitive networks may provide underlying information (over and above the information gleaned from the activity of each system in isolation) in regard to how and why stress makes some individuals more vulnerable to depression than other people.

The aim of this section is to characterize the relationships between stress-related biomarkers of the immune system and HPA axis. To do this, first, immune-HPA axis responses to acute stressors are reviewed, followed by a discussion of chronic stress conditions that may contribute to the imbalance of these systems.

1.2.3.1 The neuroendoimmune system under acute stress

Acute stressors—whether physical or psychosocial—reliably evoke neuroendoimmune activity (Goshen et al., 2007; Slavish, Graham-Engeland, Smyth, & Engeland, 2015; Slavich & Irwin, 2014; Ménard, Pfau, Hodes, & Russo, 2017). As highlighted above, this is observed via increased biomarker activity from the ANS, immune system, and HPA axis (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Irwin & Cole, 2011; Pavlov & Tracey, 2004; Slavich & Cole, 2013). Here, a brief description of the communication between these systems is provided, with

an emphasis on the relationship between the immune system and HPA axis, which are the central focus of the empirical research presented in Chapter 2.

The first system that responds to acute stressors and upregulates immune activity is the ANS. The ANS modulates heart rate and blood pressure through sympathetic and parasympathetic signaling. When an individual is thrown into a fight or flight response, this increases heart rate, muscle tone, immune activity, and glucose breakdown to increase energy availability through the production of catecholamines, including epinephrine and norepinephrine that bind to adrenergic receptors to stimulate immune system and HPA axis activity. Adrenergic receptor activation upregulates immune activity to prime the body to respond to threats (Irwin & Cole, 2011).

Following ANS signaling, the HPA axis is stimulated into action. As mentioned, the HPA axis plays a number of roles, regulating metabolic and homeostatic functions, and also serves as a major immune regulator, which may facilitate the relationship between psychosocial stress and immune activity (Kudielka & Wüst, 2010). Under acute and adaptive stress responses, the HPA axis acts to downregulate immune activity previously stimulated by the ANS. As an immune regulator, the HPA axis has a profound impact on immune activity in the presence of stressors because, as the HPA axis responds to stressors, it increases the production of ACTH, CRH, and glucocorticoids, such as cortisol.

Cortisol is the body's most potent anti-inflammatory molecule (Irwin & Cole, 2011; Ménard, Pfau, Hodes, & Russo, 2017). To unleash these anti-inflammatory effects, glucocorticoids, like cortisol, bind to glucocorticoid receptors in immune cells throughout the body and brain to suppress the ongoing release of proinflammatory cytokines and activate the synthesis of anti-inflammatory cytokines (Irwin & Cole, 2011; Foley & Kirschbaum, 2010; McEwen, Gray, & Nasca, 2015). The downregulation of immune activity by the HPA axis is essential to resolve inflammatory activity which—although critical to initial healing processes—if left unabated, can be deleterious to the body (Ménard, Pfau, Hodes, & Russo, 2017). This acute and adaptive upregulation and then downregulation of the neuroendoimmune system facilitates time-limited energy mobilization to respond to stressors before returning to homeostatic resting states. [In this way, when a person realizes they forgot to read a student's dissertation the day before the

defense, they can stay up late cramming, and still arrive calm, collected, and prepared to drill the student on their shortcomings the following day (coffee in hand).]

1.2.3.2 The neuroendoimmune system under chronic stress

When the components of the neuroendoimmune system are in balance, they facilitate adaptive stress responses by supporting the rise and fall of systems needed to cope with a stressor, and then return the systems to basal states. However, the dynamics of these systems may become altered in chronic states of stress, tilting the balance from helpful to harmful, leading to poor health outcomes and psychopathology (Slavich & Irwin, 2014). This section examines the mechanisms of the neuroendoimmune system that may contribute to these biological disruptions under chronic stress conditions.

It is well known that stress, and more specifically, chronic stress, has negative implications for human health (Dooley, Slavich, Moreno, & Bower, 2017; Flynn, Moran, Rash, & Campbell, 2019; Vachon-Presseau et al., 2013; Woda, Picard, & Dutheil, 2016; Oliveira et al., 2016). One reason that external stressors may translate into poor health is because the systems that respond to stressors become dysregulated (Bower et al., 2007; Slavich & Irwin, 2014). The balance of the neuroendoimmune system is considered to be critical to overall health, as dysregulation of these systems is common to numerous medical and psychological conditions (Pavlov & Tracey, 2015; Jehn et al., 2010; Papadopoulos, & Cleare, 2012). As such, stress and dysregulation of the neuroendoimmune system are implicated in the etiology of many mental and physical conditions (Michopoulos et al., 2017; van der Werf-Eldering et al., 2012; Scarpioni, Ricardi, & Albertazzi, 2016).

Heightened inflammatory activity under chronic stress conditions may be due, in part, to the communication between the immune system and HPA axis. As mentioned above, the distinction between acute and chronic stress is important for biological research: in acute stress, activity from the HPA axis is essentially anti-inflammatory, while under chronic stress, the prolonged activity of the HPA axis can lead to deficient glucocorticoid production or signaling, ultimately increasing the overall burden of proinflammatory cytokines (Raison & Miller, 2003; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). Although cortisol is considered the body's main anti-inflammatory hormone (Irwin & Cole, 2011; Foley & Kirschbaum, 2010; McEwen, Gray, &

Nasca, 2015), chronic stress can disrupt hormone production and signaling in two ways. On the one hand, excessive cortisol production under extended periods of stress can desensitize glucocorticoid receptors (i.e., glucocorticoid resistance—similar to the impact of excess sugar and insulin resistance in diabetes) (Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). On the other hand, extended periods of stress can deplete adrenal output at the hormone level, decreasing overall glucocorticoid tone or capacity (Raison & Miller, 2003). In both cases, cortisol signaling can become deficient, preventing the inhibitory feedback of cortisol on immune system responses (Cohen et al., 2012; Miller et al., 2008; Leonard, 2018). Metaphorically speaking, this takes the foot off of the break and puts it on the gas. As such, the system meant to downregulate immune activity (i.e., HPA axis and cortisol) ends up doing the opposite (Hänsel, Hong, Cámara, & von Känel, 2010). Disruptions to cortisol signaling (via glucocorticoid resistance) or production (via reduced glucocorticoid tone) may be a mechanism that explains the higher levels of immune activity found in otherwise healthy samples of individuals with MDD (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015).

Due to the complex dynamics of the neuroendoimmune system and its implications, future research can benefit from multidimensional measurement approaches to the stress-sensitive activity of the neuroendoimmune system. Measuring concurrent markers of activity from the ANS, HPA axis, and immune system will further identify patterns of dysregulation that may contribute to depression and potentially other forms of psychopathology.

1.3 Stress and depression

Exposure to a major life stressors (e.g., separation, divorce, childbirth, medical disease, financial or professional loss, trauma, or the death of a loved one) is considered to be a primary risk factor for the development of a major depressive episode (Kessler, 2002; Monroe, Slavich, & Georgiades, 2014). Depressed individuals are 2.5 times more likely to experience a stressful life event prior to the onset of depression compared to individuals who do not develop depression within the same time frame (for a review, see Monroe, Slavich, & Georgiades, 2014). This statistic is as high as 9.38 times more likely when precipitating the first episode of depression (Kendler, Thornton, & Gardner, 2000). It is estimated that a staggering 80% of depressive

episodes are preceded by significant life stressors (Mazure, 1998), and this association is especially strong for females (Harkness et al., 2010). Additionally, it is notable that early life stress predicts 20 – 25% of depressive disorders in adulthood (Green et al., 2010). In total, these statistics provide strong evidence for an etiological role of stress in depression across the lifespan.

As reviewed thus far, an especially exciting feature of this line of research is that peripheral biological systems are responsive to stressors in the physical and social environment, indicating potential mechanistic pathways that lead to the development and maintenance of depression. Considering the association between stressors and neuroendoimmune activity reviewed in the preceding sections, as well as the commonly studied relationship between stress and depression, an advanced understanding of the biological systems that may respond to stressors to increase depression vulnerability are of critical importance. The interrelationship between experiences of stress and peripheral biological systems may be a central component in the pathogenesis and recurrence of depression, and can help to explain the high levels of comorbidity between depression and other mental and physical health conditions that show similar immunological and endocrine disruptions (Michopoulos et al., 2017; van der Werf-Eldering et al., 2012; Scarpioni, Ricardi, & Albertazzi, 2016). Research into these dynamics may shed light on the nature of depression, including its etiology, stress vulnerability, recurrence, and high levels of comorbidity. Ultimately, a clear understanding of the mediators of the stress-depression relationship could also influence treatment and prevention of the disorder (Raison, 2016).

This section presents an in-depth examination of the relationship between depression, stress, and neuroendoimmune activity, to ultimately advance understanding of why and how shifts in biological systems that respond to stress may make some individuals more likely to experience depression than other people. It is not yet clear whether stress-related biological disruptions are a strong etiological factor in depression, and what kinds of stressors are most detrimental to these pathways. To address these questions, this section begins by reviewing research that links immune and HPA axis activity to depression. Then, emerging data that point to the dynamics of these neuroendoimmune systems in relation to stress are discussed as a potential biological pathway to depression. Integrative theories of depression that frame the immune system as part of the stress response are presented. Finally, a model is introduced to test the associations

between psychological factors, experiences of stress, and the neuroendoimmune system that may contribute to depression.

1.3.1 Immune activity and depression

1.3.1.1 Associations between proinflammatory cytokines and depression

Immune activity associated with psychiatric patients is often described as chronic "low-grade" inflammation or immune activity (Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016). Low-grade immune activity in such individuals is characterized by chronic production of inflammatory activity at concentrations that are elevated compared to basal levels or non-psychiatric controls but are at levels that may still be within the normal range (i.e. low-grade) (Berk et al., 2013). Low-grade immune activity typically occurs in individuals who do not have a known medical condition, infection, or injury causing the inflammatory response. While it is difficult to evaluate these differences at an individual-person level, group-based analyses demonstrate reliable elevations in low-grade immune levels associated with depression and other psychiatric disorders (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Michopoulos et al., 2017; Osimo et al., 2020).

Studies have commonly found associations between low-grade chronic systemic inflammation and depressive episodes as reflected in the elevation of cytokine proteins including TNF-α, INF-α and gamma, IL-1β, IL-6, the IL-1 receptor antagonist (IL-1ra), the soluble IL-2 receptor, and CRP (for a review, see Dowlati et al., 2010; Osimo et al., 2020). More specifically, alterations in immune activity are associated with depressive episodes compared to periods of remission or in contrast to healthy controls (Valkanova, Ebmeier, & Allan, 2013; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). Additionally, inflammatory activity is linked to the severity of depressive symptoms, relapse recurrence, and treatment non-response, such that patients with elevated inflammatory markers often exhibit more severe symptoms and lower responsiveness to antidepressant treatment (Raison, 2016; Strawbridge et al., 2015; Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016; Felger et al., 2016; Raison, Felger, & Miller, 2013). A meta-analysis of longitudinal studies found that immune activity was associated with an increased risk of subsequently developing depressive symptoms even after adjusting for age and socio-demographic variables (Valkanova, Ebmeier, & Allan, 2013), and this may be

especially true for females (Lamers et al., 2019). In line with these findings, gene polymorphisms associated with the inflammatory response have been associated with the development of depression, as well as a poor response to antidepressant medication (Raison, 2016). Similarly, lifestyle factors and comorbid conditions associated with inflammatory burden, such as childhood trauma, obesity, and medical illness, are associated with treatment-resistant depressive episodes (Raison, Felger, & Miller, 2013; Strawbridge et al., 2015). The term "treatment-resistant depression" typically refers to a continued episode even after at least one course of adequate treatment (usually in reference to antidepressant medication) (Souery & Pitchot, 2013; Raison, Felger, & Miller, 2013). Taken together, this research suggests that inflammation may be associated with a vulnerability to depressive episodes, and a more severe course of the disorder.

To consolidate the findings that accumulated after several decades of research, at least five separate meta-analyses have explored the relationship between proinflammatory cytokines related to MDD as compared to healthy controls (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). Four of the meta-analyses found that levels of IL-6 and TNF-α were significantly higher in otherwise medically healthy individuals with MDD (Dowlati et al., 2010; Liu, Ho, and Mak, 2012; Haapakoski et al., 2015; Osimo et al., 2020). Howren, Lamkin, and Suls (2009) found that CRP, IL-1β, and IL-6 levels were associated with depressive episodes, which was also confirmed by Liu, Ho, and Mak (2012), but only in participants with European ancestry compared to non-European participants. From the sum of these meta-analyses, the most consistent relationship between depression and inflammation is tied to the proinflammatory cytokines IL-1β, IL-6, TNF-α, as well as CRP, which are also the biomarkers most consistently studied in the stress literature reviewed above.

Excess levels of IL-6, IL-1β, and TNF-α are associated with depressive episodes (Raison, Capuron, & Miller, 2006; Dowlati et al., 2010; Liu, Ho, and Mak, 2012; Haapakoski et al., 2015; Howren, Lamkin, & Suls, 2009; Osimo et al., 2020; Lamers et al., 2012; Rudolf, Greggersen, Kahl, Hüppe, & Schweiger, 2014; Hickman, Khambaty, & Stewart, 2014), treatment resistance (Raison, Felger, & Miller, 2013; Osimo et al., 2020), as well as specific symptoms of depression, including psychomotor retardation (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Goldsmith et al., 2016), amotivation (Nunes et al., 2014), and learning and memory deficits

(Lieb et al., 2006; Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011; Felger & Treadway, 2016). In MDD research, IL-6 has specifically been associated with fatigue (Cavadini et al., 2007), sleep disturbances (Clinton, Davis, Zielinski, Jewett, & Krueger, 2011), lower immediate and delayed verbal recall on the logical memory subtests of the Wechsler Memory Scale-Revised (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011), and deficits in selective attention (Mac Giollabhui et al., 2019).

Although most studies investigating inflammatory cytokines in MDD focus on blood serum markers, immune activity has also been identified directly within the CNS (D'Mello & Swain, 2017; Dantzer, 2018; Setiawan et al., 2015). More specifically, evidence of neuroinflammation (i.e., increased presence of proinflammatory cytokines) has been reported in the post-mortem brain tissue of depressed individuals compared to deceased age-matched, non-psychiatric controls (Pandey, Rizavi, Ren, Bhaumik, & Dwivedi, 2014; Torres-Platas, Cruceanu, Chen, Turecki, & Mechawar, 2014). Additionally, studies using positron emission tomography (PET) found markers of neuroinflammation in living adults with MDD compared to non-psychiatric participants (Setiawan et al., 2015; Holmes et al., 2018). Of note, levels of neuroinflammation have been correlated with depressive symptom severity (Setiawan et al., 2015) and suicidal thinking (Holmes et al., 2018). The activation of immune cells in the brain, and the relation between immune activity and depressive symptoms, suggests that CNS inflammation could interact with cognitive and affective symptoms of the disorder (Dantzer, 2018; Setiawan et al., 2015; Byrne, Whittle, & Allen, 2016; Holmes et al., 2018).

1.3.1.2 The Macrophage Theory: Causal links between inflammation and depression

For inflammation to play a causal role in the pathogenesis and behavioural profile of depression, inflammation must both precede the onset of depression and also induce symptoms of depression. Beyond the observational studies cited above, there is now substantial evidence that demonstrates a causal relationship between immune activity and depression from both the rodent and human literature that ultimately shaped the macrophage theory of depression (Smith 1991; Maes et al. 1992). In rodents, administration of inflammatory cytokines or pathogens activate a suite of "sickness behaviours" which, when extended over a prolonged period of time, mimic

depressive symptoms, including anhedonia, loss of appetite, weight loss, cognitive deficits, fatigue, sleep disruptions, anxiety behaviours, as well as reduced motivation, social behaviour, and motor activity (Raison et al, 2010; D'Mello & Swain, 2017; Dantzer, 2018). In contrast, when the genes encoding for these proinflammatory cytokines are deleted, or receptors are blocked, antidepressant-like behaviours are stimulated (e.g., increased hedonic behaviour and decreased helplessness response) (D'Mello & Swain, 2017). Sickness behaviours and neurovegetative symptoms (i.e., fatigue, lack of energy, motor retardation, and anhedonia) are assumed to serve an adaptive function by conserving energy intake and output (to reduce metabolic demands), and by reducing social contact during the acute phase of illness (Hart, 1988; Dantzer, 2018). However, when these behaviours become prolonged and extend beyond the acute sickness window after infection has cleared (Moreau et al., 2008), they resemble a depressed state and provide some indication that cytokines may induce behaviours that closely resemble depression in humans.

In keeping with the rodent literature, administration of inflammatory cytokines in humans also activates sickness behaviours and increases the risk of depression (Pasco et al., 2010; Maes, Mihaylova, Kubera, & Ringel, 2012). The bulk of evidence in support of the macrophage theory in humans comes from literature on hepatitis C and cancer treatment. As part of the treatment, these patient groups can be injected with the cytokine INF-α to bolster the immune system to fight disease (Shah, Kadia, Bawa, & Lippmann, 2013). While INF-α is itself an inflammatory cytokine, it also induces the activation of additional proinflammatory cytokines such as TNF-α, IL-6, and IL-1β (Capuron et al, 2003; Raison et al, 2009). Interestingly, when hepatitis C patients are injected with INF-α to combat the virus, up to 50% of patients develop a major depressive episode (Raison & Miller, 2011; Shah, Kadia, Bawa, & Lippimann, 2013; Bonaccorso et al., 2001), while up to 80% experience significant neurovegetative symptoms (Raison et al, 2009; Raison et al, 2010). In fact, these depressive symptoms and episodes are considered among the most serious side-effects of hepatitis C treatment (Shah, Kadia, Bawa, & Lippimann, 2013) and are treated prophylactically with antidepressants (Halaris, 2017). Along with depressive episodes, depressive symptom severity is also correlated with increases in peripheral blood cytokine concentrations following INF-α treatment in these patient groups (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008). This pattern has been replicated in healthy participants who developed depressive symptoms following injection of inflammatory endotoxins (i.e., gram

negative bacteria) or following typhoid vaccination (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Eisenberger et al., 2010). This line of research represents key early pieces of evidence linking inflammation to depression (Hart, 1988; Dantzer & Kelley, 1989; Smith 1991; Maes et al. 1992).

The relationship between inflammation and depression could help to explain the high levels of MDD comorbidity with other inflammatory medical diseases, such as chronic liver disease, obesity, cardiovascular disease, chronic pain, and autoimmune conditions (Leonard, 2007; Evans et al., 2005; Slavich, 2016; Scarpioni, Ricardi, & Albertazzi, 2016). These patient populations carry high levels of inflammatory burden and are at an increased risk of developing MDD, showing rates of clinical depression at 50% (Irwin & Miller, 2007). It is possible that the high levels of comorbidity could be due to common underlying biological disruptions.

Of course, comorbid medical conditions represent only a portion of the larger MDD population, and many MDD sufferers do not have known primary medical conditions (Osimo et al., 2020), suggesting that medically-derived inflammation may not be the only source of inflammation. Additionally, research suggests that approximately one third of otherwise medically healthy depressed patients demonstrate reliable elevations in inflammatory markers (Raison & Miller, 2011). Thus, inflammation is present in a substantial subset of otherwise medically healthy patients with MDD (~30%), and MDD is present in a significant proportion of medically ill patients with inflammation (~50%). Although meta-analyses do confirm associations between depression and immune activity, effect sizes vary greatly among individual studies (Dowlati et al., 2010). This variability could be due, in part, to methodological and sample characteristics (e.g., medication use, biological sex, in-patient/out-patient samples, age, life stress, etc.), as well as the heterogenous nature of the categorical diagnosis. As inflammation is not a necessary or sufficient component of a diagnosis of MDD, individuals that do carry higher levels of inflammation may represent a biological subtype of depression (Raison, 2016).

In line with the macrophage theory of depression, the aforementioned research demonstrates that inflammation can both precede depression and is associated with depressive symptoms. Although it is beyond the scope of the present review, it is worth noting that there is evidence that the reverse is also true, indicating the need for more advanced models of depression

(Dantzer, 2018). Although the majority of longitudinal studies have found a directional pattern of inflammation-to-depression, others demonstrate the opposite direction. For example, in a sample of otherwise healthy older adults, depression scores measured by the BDI-II predicted levels of IL-6 over a six-year follow-up period, implying that depression may lead to inflammation in some individuals (Stewart, Rand, Muldoon, & Kamarck, 2009). Although the research cited above demonstrates a causal relationship via injection of inflammatory molecules, it is considerably more difficult to study the reverse relationship because individuals cannot be randomly assigned to experience depression; however, these relationships are all likely to be bidirectional, as even in the case where inflammation develops after depression, it is still possible that the inflammatory response may further contribute to symptoms of depression, possibly by increasing the burden of neurovegetative symptoms, and ultimately delaying recovery. Many reviews describe the bidirectional relationship of these factors (Berk et al., 2013; Stewart, Rand, Muldoon, & Kamarck, 2009; D'Mello, & Swain, 2017; Dantzer, 2018).

In summary, several lines of investigation point to a link between inflammation and depressive symptoms. Various cytokine and immune markers are identified in depressed patients compared to controls, and these elevations appear to precede the development of depression in some prospective studies. Additionally, depression is common in patients that carry high inflammatory burden, and symptoms of depression appear to be sensitive to pharmaceutical interventions that target inflammation. In the experimental literature, casual relationships between immune challenge and depression are observed in rodents, and in both healthy samples and patient groups in humans, suggesting a direct link between elevations in immune system activity and depressive phenotypes. Taken together, this line of research provides substantial evidence linking immune activity to depression.

1.3.2 HPA axis activity and depression

Given the relationship between stress and HPA axis activity, as well as the strong association between stressful events and depression onset, it follows that HPA axis activity likely bears some relationship to depression. While cortisol peaks after waking and steadily decreases over the day in healthy individuals, depressive symptoms (Pruessner, Hellhammer, Pruessner, & Lupien, 2003) and MDD (Vreeburg et al., 2013) are typically associated with a higher cortisol awakening

response that does not decrease as steeply in the afternoon or evening compared to healthy controls (Raison & Miller, 2003; Pruessner, Hellhammer, Pruessner, & Lupien, 2003; Dienes, Hazel, & Hammen, 2013). This results in a flattened daily curve and higher diurnal cortisol output across the day (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013). Although there are some reports of hypocortisolism in morning samples with MDD (Tops, Riese, Oldehinkel, Rijsdijk, & Ormel, 2008; Burke, Davis, Otte, & Mohr, 2005; Vreeburg et al., 2013; Izakova et al., 2020; Wielaard, Schaakxs, Comijs, Stek, & Rhebergen, 2018), the bulk of evidence suggests hypercortisolism in this population from morning to night (Burke, Davis, Otte, & Mohr, 2005; Dienes, Hazel, & Hammen, 2013).

Elevated cortisol levels in MDD are significantly correlated with depressive symptoms, such as trait rumination and negative affect (Stewart, Mazurka, Bond, Wynne-Edwards, & Harkness, 2013; Dienes, Hazel, & Hammen, 2013). Furthermore, depressed individuals with elevated cortisol were found to be less responsive to psychotherapy treatment, suggesting a potentially more resistant form of depression related to HPA axis disruptions (Fischer, Strawbridge, Vives, & Cleare, 2017). In total, this research demonstrates higher levels and longer duration of cortisol activity in depressed individuals across the day. Considering the dynamics of the neuroendoimmune system described in previous sections of this dissertation, these deviations may be critically important to the relationship between immune activity and depression.

1.3.3 Integrative models of stress and depression

As outlined thus far, there is considerable evidence that proinflammatory cytokines are present in a subset of individuals with MDD and can stimulate depressive-like behaviours in animals and humans. This led to the macrophage theory of depression—a theory that posits a causal link between immune activity and depression. While the macrophage theory provided initial insights into an immunological variant of depression, it could not explain why depression also demonstrates such a heavy cognitive vulnerability and is so often precipitated by stressful life events. However, research demonstrating that all forms of stress—both physical and psychosocial—trigger the immune system, permits new lines of investigation into the immunological basis of depression. From here, it seems logical to consider whether and how stress might influence immune system activity to contribute to depression. It is well known that

depression is highly related to experiences of stress, but whether and how this is mediated by disruptions in biological pathways has yet to be determined.

Integrative, biologically-based, and evolutionary theories of depression have started to explore these dynamics (Irwin & Cole, 2011; Slavich & Irwin, 2014). Theories such as the inflammasome hypothesis (Iwata et al., 2016), the social signal transduction theory (Slavich & Irwin, 2014), and the pathogen host defense hypothesis of depression (Miller & Raison, 2016) describe biological processes that interact with cognitive and neural components to translate experiences of stress into depression within evolutionary frameworks. The various integrative and evolutionary models all provide very detailed mechanistic pathways linking genomic, immunological, neurological, and psychological components of a model that bias one toward depression, each with their own unique focus. For instance, while the pathogen host defense hypothesis focuses on genomic and cellular surveillance pathways that upregulate immune activity in the presence of stressors (Miller & Raison, 2016), the social signal transduction theory focuses on psychosocial factors that would be of particular relevance to these pathways and their transcription into depression (Slavich & Irwin, 2014). Broadly speaking, however, within these comprehensive and integrative theories of depression, psychological experiences of stress, and stress-related changes in immune activity, are considered a central pathway through which neurobiological changes may occur to influence depressogenic behaviour.

Within these models, one commonly proposed mechanism for the translation of stress into depression is via the disruption of neuroendoimmune activity (Slavich & Irwin, 2014; Miller & Raison, 2016). According to these theories, although the neuroendoimmune response to stressors was likely adaptive and critical to survival throughout our evolutionary history, when stress is chronic, or the biological response to a stressor is unresolved, it may contribute to the development of symptoms of depression (Irwin & Cole, 2011; Iwata et al., 2016; Slavich & Irwin, 2014; Miller & Raison, 2016). In the case of depression, this may begin with some of the common neurovegetative and sickness behaviours that promote withdrawal and healing, including psychomotor retardation, fatigue, anhedonia, loss of appetite, agitation, and sleep disruptions, which when overextended under chronic stress, lead to a maladaptive state that is no longer functional to survival (Harrison, 2017; Capuron & Castanon, 2017; Slavich & Irwin, 2014; Miller & Raison, 2016). Although these comprehensive accounts provide theoretical

models that highlight neuroendoimmune pathways that may contribute to depression, the authors emphasize the need for more clinical research that investigates the link between psychological experiences of stress, biological processes, and depression directly within the same study, as a dearth of such research exists.

In response to these calls for integrative clinical research, a simplified variant of these rather complex integrative models is developed in this dissertation and presented in Figure 1. This model will be used as the basis for the questions and variables of interest relevant to the empirical study described in Chapter 2. The model presented here places a particular focus on neuroendoimmune activity related to both physical and psychological experiences of stress and depression. Although the constituent parts of the model are all assumed to be bidirectional, the bold arrows indicate the directions of primary focus in this dissertation. The sections to follow, and the research presented in Chapter 2, examines two main components of the model in relation to stress and depression: (a) biological systems responsive to stress, and (b) psychological and cognitive factors relevant to stress-sensitive biological processes and depression.

A growing body of research suggests that neuroendoimmune activity may play a role in the translation of stress into depression; however, much more research is needed to understand the interplay of the systems that contribute to these disruptions in psychological and physical health. The central question of this dissertation is whether and how neuroendoimmune responses mediate the influence of stress on depression. Due to the vast complexity of the ways in which the functioning of the neuroendoimmune system can be conceptualized and correspondingly measured, in addition to inflammation being a relatively more recent player in the field of depression, the exact dynamics of neuroendoimmune activity are far from clearly understood. To this aim, the present research attempts to delineate aspects of neuroendoimmune activity to provide more information regarding mechanisms that may translate life stress into depression.

1.3.3.1 Biological components of the model

This section briefly summarizes research that links stress, immune activity, and depression, before moving on to the specific dynamics of the neuroendoimmune system that may mediate this relationship.

1.3.3.1.1 Stress, immune activity, and depression

To elucidate more advanced models related to the pathogenesis and maintenance of depression, this section reviews research on immune activity under acute and chronic stress conditions (see Section 1.2.1 for definitions) in depression research. To begin, under acutely stressful conditions in laboratory settings (e.g., public speech and mental arithmetic), individuals with depression exhibit more pronounced proinflammatory cytokine responses, including IL-6, TNF-α, and CRP, compared to healthy controls (Weinstein et al., 2010). In another study, IL-6 response to an acute stressor was positively correlated with depressive symptoms in a community sample of 138 participants (Fagundes, Glaser, Hwang, Malarkey, & Kiecolt-Glaser, 2013).

With regard to chronic stress, depressed individuals with early life stress are 1.48 times more likely to have clinically elevated levels of CRP (>3 mg/L) compared to depressed individuals without a history of early life stress (Danese & Baldwin, 2017; Slavich & Irwin, 2014). These associations were also detected in adolescents (Miller & Cole, 2012) and children (Danese & Baldwin, 2017) with depression and early life stress. To investigate the combined influences of chronic and acute stress responses, Pace et al. (2006) used the TSST to examine immune activity in depressed males with or without a history of early life stress. They found that individuals with early life stress exhibited higher levels of IL-6 in response to the TSST, which remained elevated throughout the stress recovery period. Considering the fact that elevated immune profiles are associated with a more treatment-resistant form of depression (Raison, Felger, & Miller, 2013; Strawbridge et al., 2015), and that immune activity may directly influence behavioural symptoms of depression (Raison, Capuron, & Miller, 2006; Shah, Kadia, Bawa, & Lippimann, 2013; Bonaccorso et al., 2001; Eisenberger et al., 2010), this research sheds light on a potential pathway through which early life stress may alter biological systems that produce and maintain depressive symptoms, even into adulthood when the source of stress has presumably resolved.

1.3.3.1.2 Neuroendoimmune activity and depression vulnerability

The preceding section presented evidence that exaggerated immunological responses to stressful situations leave individuals more vulnerable to depressive symptoms. Importantly, this stress-susceptibility and depression vulnerability may be tightly linked to, and impacted by, HPA axis activity and its regulatory control on the immune system. In addition to examining individual

components of the neuroendoimmune system in isolation (i.e., ANS, immune system, and HPA axis), it is possible that examining the relationships among these systems may illuminate factors leading to stress susceptibility and depression. Importantly, the dynamics of the neuroendoimmune system may mediate the effects of stress on depression and could partially explain why some individuals are more susceptible to experiencing depressive symptoms than other people.

Similarly, in the human experimental and treatment literature, patients that develop a full episode of depression following immunotherapy for hepatitis C or cancer produce three times as much ACTH and cortisol compared to those that do not meet for clinical depression (Capuron et al., 2003). This suggests that the HPA axis response to immune intervention is more pronounced in those susceptible to a treatment-induced depressive episode, which may relate to glucocorticoid resistance and lead to sustained immune activity (Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). In line with this thinking, a meta-analysis of acute stress paradigms in laboratory settings found that individuals with MDD demonstrated elevated cortisol over the recovery period compared to healthy controls (Burke, Davis, Otte, & Mohr, 2005). Furthermore, higher IL-6 concentrations are correlated with higher cortisol in MDD participants (Jehn et al., 2010), as well as patients with both MDD and BPD (Kahl et al., 2006; 2009), and glucocorticoid resistance has been identified repeatedly in patients with depression (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). Taken together, these studies highlight the role of hyperactive neuroendoimmune responses that may relate to depressogenic behaviours in rodents and episodes of depression in humans.

These findings suggest that cortisol-cytokine upregulation may play a role in the vulnerability of some individuals to develop significant depressive symptoms. This neuroendoimmune predisposition may explain why only approximately 50% of medically-ill patients with inflammatory burden are also affected by MDD (Raison, Capuron, & Miller, 2006; Shah, Kadia, Bawa, & Lippimann, 2013) and why only 50% of patients receiving immunotherapy become clinically depressed (Capuron & Miller, 2004). While some individuals may incur a protective benefit from a more subtle neuroendoimmune response to stressors, others may have intense biological sensitivities that leave them more vulnerable to depression.

While excessive cortisol levels may increase glucocorticoid resistance to contribute to elevated immune activity, and ultimately depression, hypocortisolism is also observed in relation to stress and depression. For instance, a blunted cortisol response in depressed patients has been observed in relation to acute stressors (Miller, Rohleder, Stetler, & Kirschbaum, 2005), and this may be particularly true for females (Ménard, Pfau, Hodes, & Russo, 2017). To examine this in more detail within the context of stress, when depressed females were asked to complete a mock job interview, levels of proinflammatory cytokines increased, while cortisol concentrations and sensitivity to cortisol decreased compared to controls (Miller, Rohleder, Stetler, & Kirschbaum, 2005). This research suggests that under acutely stressful conditions, depressed females demonstrate a blunted HPA axis response, which may ultimately extend immune system activity. In line with this, when a cortisol to CRP ratio (CORT/CRP) was calculated to provide a snapshot of neuroendoimmune activity in a cross-sectional study, females demonstrated lower ratios, suggesting dampened release of cortisol in response to inflammation (Suarez, Sundy, Erkanli, 2015). Furthermore, lower CORT/CRP ratios in females, but not males, were negatively correlated with depressive symptom severity as measured by the HAMD, such that lower CORT/CRP ratios were associated with higher levels of depression in females (Suarez, Sundy, Erkanli, 2015). The authors suggest that a CORT/CRP ratio may be a viable biomarker that captures the individual contributions of each system and their relationship, as well as variations related to biological sex. Although this ratio does appear to capture excessive neuroendoimmune biomarker production with more extreme ratio values (high or low values), and normal range neuroendoimmune production with moderate ratio values, a ratio variable would be difficult to interrupt in linear models and also runs the risk of misrepresenting the data. There is a high probability that different ratio combinations will yield similar values and confound the results. These disclaimers lower a ratio marker's reliability and validity.

Although the hypocortisolism findings are somewhat contrary to the hepatitis C literature discussed above, they signal the possibility of a range of neuroendoimmune profiles that may further delineate variability observed between the sexes. Variations in neuroendoimmune activity may also help to explain why, in part, females are approximately 1.5 – 3 times more likely to be diagnosed with MDD (American Psychiatric Association, 2013). Interestingly, females are more likely than men to develop depressive symptoms following an inflammatory challenge (Moieni et al., 2015). This was a surprising finding because even though the circulating levels of

inflammation were equal across the sexes, females experienced more severe depressive symptoms. This may signal some kind of sex-linked endocrine-immune interaction (for a review, see Ménard, Pfau, Hodes, & Russo, 2017), although much more research is needed.

Here, we are left with two possible neuroendoimmune profiles that may both uniquely contribute to runaway immune activity. The first may be of hypercortisolism leading to glucocorticoid resistance under stressful conditions, and the second may represent a depleted HPA axis response where hypocortisolism develops and, likewise, fails to sufficiently downregulate immune activity. Hypocortisolism may result from decreased production of upstream precursors and signaling molecules (e.g., CRH, ACTH), a deficit in hormonal production from the adrenal gland, or some other signaling disruption along the HPA axis (Raison & Miller, 2003). This may be especially true in cases of long-standing chronic stress or trauma (Raison & Miller, 2003; Miller, Chen, & Zhou, 2007). In either case, immune activity may be left unabated, and contribute to the depressogenic effects of immune activity (Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). As these are relatively new lines of inquiry, more research is needed to evaluate neuroendoimmune dynamics during basal, acute, and chronic states of stress to pinpoint deviations in pathways that may leave some individuals more vulnerable to the impacts of stress on depression.

This set of research studies highlights the need for more advanced methods for evaluating neuroendoimmune activity, and the relevance of sex-specific research in depression. These variations—whether hyper- or hypo-activity—and their relationship to one another, could provide critical information to aid in the understanding of biobehavioural components of depression. A maladaptive (either hyperactive, hypoactive, or some combination of both) neuroendoimmune response to stress could increase the vulnerability to affective symptoms and may contribute to sex-related variance (Moieni et al, 2015).

The question remains whether it will be possible to establish a resilient versus vulnerable neuroendoimmune profile. Research indicates that both a hyperactive and hypoactive HPA axis response (Irwin & Cole, 2011; Foley & Kirschbaum, 2010; McEwen, Gray, & Nasca, 2015) may interact with the immune system to leave individuals vulnerable to stress and symptoms of depression, although to date the research is very novel and unrefined. Assessing specific aspects

of HPA axis and immune biomarkers in relation to experiences of stress and depressive symptoms, both within and across diagnoses and between biological sexes, will be critical to advancing our understanding of individual vulnerability to stress and depression. These immune and endocrine signatures might predispose individuals to depression and the frequent comorbid health conditions associated with depression. As the dynamics of these systems are uncovered and mapped across multiple contexts (e.g., basal, acute, and chronic states of stress), groups with psychiatric diagnoses (e.g., MDD, BPD, PTSD, and schizophrenia), and severity levels of psychopathology (e.g., mild, moderate, and severe), researchers may gain valuable insights into how stress is embodied and manifested into psychopathology and disease. Research that adopts novel sampling methods and multidimensional measurements to investigate the translation of stress into depression across diagnostic populations will further our understanding of common neurobiological pathways that support health and can prevent disease.

1.3.3.2 Psychological components of the model

Stress Appraisals. One psychological variable examined in relation to stress-vulnerability and neuroendoimmune activity is stress appraisal, or one's emotional and cognitive reactivity under stress. As described in earlier sections of this chapter, stress appraisals appear to play a significant role in the impact that stress has on our physical and psychological health, and immune activity may be one pathway through which this occurs (Yamakawa et al., 2009; Aschbacher et al., 2012; Shields & Slavich, 2017). For example, participants who reported higher levels of perceived stress to the TSST demonstrated significantly increased IL-1β reactivity following stress induction (Yamakawa et al., 2009). Conversely, positive affect (e.g., confidence, excitement, hope) and positive attitudes (e.g., "I will complete the tasks successfully") toward the TSST were negatively associated with IL-1β reactivity in a sample of females, and predicted resiliency to depression over a one-year follow-up period (Aschbacher et al., 2012). As such, individuals who maintained positive cognitive-affective states during the TSST demonstrated less immune response to the acute stressor and more resiliency across the year compared to those who did not maintain positive cognitive-affective perspectives. These studies provide preliminary evidence that stress appraisals and perceptions may influence immune reactivity to lessen depression outcomes. In this way, how one perceives stress may decrease neuroendoimmune reactivity, dampening immune system responses and the potential

depressogenic effects of immune activity (Slavich & Irwin, 2014). However, more research is needed to investigate specific aspects of affect and cognition that may influence these biological systems to affect depression outcomes.

Cognitive Control. One cognitive capacity related to depression that may contribute to stress appraisals and neuroendoimmune activity is cognitive control. The terms "cognitive control" and "executive functioning" are often used interchangeably in a large body of research studies, although the latter tends to refer to a wider range of cognitive functions (Snyder, 2013; Grahek, Everaert, Krebs, & Koster, 2018). *Cognitive control* refers broadly to high-level mental processes that permit the flexible organization and manipulation of information (Grahek, Everaert, Krebs, & Koster, 2018; Friedman & Miyake, 2017). These processes are considered to be goal-directed and controlled, as opposed to more automatic or habitual processes that have been overlearned through repetition (for a review, see Grahek, Everaert, Krebs, & Koster, 2018). At a broad level, these capacities allow individuals to make decisions, plan, break habits, and to effectively organize and execute actions. Cognitive control is not considered a unitary construct, but rather consists of several components. The three-component model of cognitive control includes inhibition (over-riding automatic and prepotent responses), shifting (switching between tasks), and updating (adding relevant information and ignoring obsolete information) (Friedman et al., 2008; Friedman & Miyake, 2017). It is hypothesized that the ability to ignore (inhibit) and disengage from (update and shift) outdated and irrelevant information influences stress appraisals and depression outcomes by allowing one to flexibly adapt to stressful situations (Joorman & Vanderlind, 2014).

Cognitive control has consistently been associated with depressive symptoms in both cross-sectional and prospective studies (for a review, see Joormann & D'Avanzato, 2010). Furthermore, deficits in cognitive control have been identified in both currently depressed and remitted depressed samples (Hammar et al., 2011; Owens, Koster, & Derakshan, 2012; Vanderhasselt & De Raedt, 2009). In experimental contexts, cognitive control training has been shown to reduce negative thoughts and rumination following both a laboratory and naturalistic stressor (e.g., examination period) (Hoorelbeke, Koster, Vanderhasselt, Callewaert, & Demeyer, 2015), and led to a greater reduction in rumination and depressive symptomatology, and less need for outpatient services over a one-year follow-up (Siegle et al., 2014; Koster, Hoorelbeke,

Onraedt, Owens, & Derakshan, 2017). These findings suggest a causal role of cognitive control in depressive symptoms and signal the impact of cognitive control on resiliency to stressful situations (Siegle et al., 2014; Hoorelbeke, Faelens, Behiels, & Koster, 2017; Hoorelbeke, Koster, Vanderhasselt, Callewaert, & Demeyer, 2015).

Theoretical models developed to describe the relationship between cognitive control and depressive symptoms posit that deficits in cognitive control decrease the likelihood that someone will enact effective emotion regulation strategies and thereby increase depressive symptoms (Joormann & Vanderlind, 2014; Joorman & D'Avanzato, 2010). As such, deficits in cognitive control may prevent adaptive information processing and emotion regulation strategies to increase depressive symptomatology, and this may be particularly heightened under states of stress. If cognitive control is highly related to depression, and this relationship is most apparent under stressful conditions (Shield, Moons, & Slavich, 2017; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016), it begs the question whether stress appraisals and biological responses may mediate this relationship. If stress management and perceptions are dependent upon cognitive control, and stress reactivity and appraisals influence immune related responses that predict depressive outcomes (Yamakawa et al., 2009; Aschbacher et al., 2012), it is possible that stress reactivity and immune activity may mediate the relationship between cognitive control and depression outcomes, although these lines of inquiry have not been investigated.

Cognitive control can be tested using paradigms such as the Go/No-go task, n-back task, Colour-Word Interference Test (CWIT; i.e., Stroop), Tower Test, Trail Making Test (Part B), Digit Symbol Substitution Test, the forward and backward digit span task (a test of verbal working memory), and Wisconsin Card Sorting Test (WCST), among many others (see Snyder, 2013). These tasks require continuous updating, response selection, inhibition, and performance monitoring to complete the tasks efficiently and accurately (Snyder, 2013). Of relevance to the present research is the CWIT.

The CWIT is a test that is part of the Delis-Kaplan Executive Function System (DKEFS) and is designed to measure aspects of executive function and cognitive control, including goal maintenance, inhibitory control, cognitive flexibility, and performance monitoring (Delis, Kaplan, & Kramer, 2006). Also commonly referred to as the “Stroop task”, it challenges

individuals to inhibit learned behaviours (e.g., word reading) and instead exert controlled goal-oriented behaviours (e.g., saying the colour of the ink a word is printed in). The test requires participants to maintain a rule, override an automatic response (i.e., word reading), and instead execute a directed behaviour (i.e., naming the colour of the ink). Incongruent stimuli (e.g., the word "blue" printed in red ink) that require the suppression of word reading reliably increase response latencies compared to congruent stimuli (e.g., the word "blue" printed in blue ink). This phenomenon is referred to as the Stroop effect (MacLeod, 1991). Meta-analyses have detected medium effect size differences between MDD and non-psychiatric controls on the Stroop task, providing evidence for depression-related deficits in cognitive control on this task that consists of emotionally neutral stimuli (Hammar et al., 2011; Snyder, 2013; Rock, Roiser, Riedel, & Blackwell, 2014). More specifically, MDD participants produce larger interference scores (i.e., the difference score between incongruent and neutral condition response times) and are significantly less accurate on incongruent trials compared to controls (Snyder, 2013).

Cognitive control, stress, and inflammation. Executive functioning (and cognitive control, in particular) is hypothesized to be one domain of cognition that may influence stress appraisals and biological processes (Compton, Hofheimer, & Kazinka, 2013; Shield, Moons, & Slavich, 2017; Harrison, 2017). In a healthy sample, Shield, Moons, and Slavich (2017) found that individuals with better executive functioning under acute TSST laboratory stress reported less health complaints (i.e., lower scores on the Physical Health Questionnaire [PHQ]; Schat, Kelloway, & Desmarais, 2005) and rated recent life stressors (as measured by the STRAIN) as less severe than those with lower executive functioning. In this study, aspects of executive functioning were measured using a version of the WCST that most closely captures cognitive flexibility (Friedman & Miyake, 2017). The authors interpret these findings to suggest that better executive functioning may decrease stress appraisals and dampen the impacts of stress on negative health outcomes. If such high-level executive functions contribute to stress management capacities to lead to better health outcomes, this may due in part to the activation of stress-sensitive biological systems in response to stress appraisals (Aschbacher et al., 2012; Miller & Raison, 2016; Slavich & Irwin, 2014).

Although very limited research exists that directly assesses the impact of psychological variables on stress-related biological disruptions, Shields, Kuchenbecker, Pressman, Sumida, and Slavich

(2016) pioneered work investigating the relationship between cognitive control, stress, and inflammation directly. They found that higher levels of cognitive control predicted lower levels of proinflammatory cytokine activity (IL-1β, IL-6, IL-8) following an emotional stressor (i.e., an emotionally evocative video) compared to a non-stress condition (i.e., neutral video) in healthy females. In this study, cognitive control was assessed using an emotional Stroop task (i.e., a variant of the traditional Stroop that incorporates emotionally valanced test materials). From these findings, there is some indication that cognitive control may dampen the impact of stressors on immune activation. Whether stress management capacities may participate in this relationship to contribute to depressive states remains to be seen.

Deficits in cognitive control are associated with higher emotional reactivity to stress and difficulties downregulating negative emotions (Joormann & Vanderlind, 2014), which are features of some stress-linked disorders, such as MDD, BPD, and PTSD (Schulze, Bürkner, Bohländer, & Zetsche, 2018). The question remains as to whether cognitive control is associated with stress appraisals and neuroendoimmune activity to indirectly influence depression outcomes as these questions have not yet been applied to clinical samples. To address this question, Chapter 2 presents primary research investigating the relationship between cognitive control, stress appraisals, immune activity, and depressive symptom severity in a transdiagnostic sample of participants with varying levels of depressive symptoms. Individual variability in terms of how individuals process information may relate to psychological and biological responses to help uncover why some individuals are more vulnerable to the unfavourable impacts of stress on depression.

1.4 Limitations of Prior Research

There remain many unanswered questions regarding the factors that produce and maintain depression; however, it is clear that the symptoms of the disorder are impairing from both an individual and economic standpoint. The limited effectiveness of treatments and the soaring rates of depression may be due, in part, to an incomplete understanding of the biobehavioural factors that contribute to the disorder. This chapter comprehensively reviewed the research literature on the relationships among stress, stress-sensitive biological systems (immune system and HPA axis), psychological factors (cognitive control and stress appraisals), and depression (Slavich &

Irwin, 2014; Slavich, 2016; Ménard, Hodes, & Russo, 2016). The research presented thus far provides initial evidence that disruptions to neuroendoimmune activity may mediate the relationship between stress and depression. However, there are several key gaps in our knowledge that require further investigation.

First, while there is substantial evidence linking immune activity separately to stress and depression (Leonard, 2018; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020), there is very little research examining experiences of stress, immune activity, and depression conjointly within the same study. Investigation of these joint relationships seems logical considering the consistent associations identified among elevated proinflammatory cytokine activity, acute and chronic stress, depressive episodes, relapse of MDD, depressive severity, symptom profiles (e.g., neurovegetative symptoms), and a more treatment-resistant form of the disorder (Raison, 2016; Strawbridge et al., 2015; Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016; Felger et al., 2016; Raison et al, 2013). Considering the relationship between stress and depression, the relationship between stress and immune activity, and research linking immune activity to depression, it seems plausible that immune activation may be one mechanism that translates life stress into a heightened risk for depression, although these relationships have not been systematically investigated.

Second, in addition to examining the immune system in isolation, it is important to consider the relationship between the immune system and HPA axis in the context of stress vulnerability. Although immune activity may contribute to depression, this relationship is likely dependent on activity from the HPA axis. Research on neuroendoimmune activity demonstrates the possibility of two separate stress-related profiles: the first, of hypercortisolism in the presence of immune activity, and the second of hypocortisolism in the presence of immune activity. Based on research from the cancer and hepatitis C literature, there is some indication that individuals who demonstrate elevated HPA axis response under inflammatory challenge are at a much higher risk of developing an episode of depression (Capuron et al., 2003). This suggests that the HPA axis response to inflammation is more pronounced in those who are susceptible to a medical treatment-induced depressive episode. In line with this notion, higher cortisol levels are associated with higher immune activity in patients with MDD (Jehn et al., 2010; Perrin,

Horowitz, Roelofs, Zunszain, & Pariante, 2019), as well as in individuals with comorbid MDD and BPD (Kahl et al., 2006; 2009).

Although these findings indicate the possibility of hyperactive neuroendoimmune activity in relation to depression, hypocortisolism has also been identified in depressed samples. Furthermore, neuroendoimmune profiles may be significantly impacted by biological sex. Sex-specific investigations of MDD demonstrate a hypoactive HPA axis response in the presence of hyperactive immune activity and acute stress in female participants (Miller, Rohleder, Stetler, & Kirschbaum, 2005). Furthermore, similar profiles of deficient CORT/CRP ratios were related to depressive symptom severity in females, but not in males, in another study (Suarez, Sundy, Erkanli, 2015). It is well documented that stress-related alterations in the HPA axis response can disrupt the inhibitory feedback loop of cortisol to promote unabated inflammatory activity when cortisol production and signalling is disrupted (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). This may be captured in more extreme immune-HPA axis concentrations, although more research is needed to assess neuroendoimmune dynamics within the context of stress and depression. To date, the relationship between the immune system and HPA axis, and the link to stress, has not been thoroughly investigated in depressed samples.

Third, the type and timing of stressors may play a critical role in the relationship between the immune and HPA axis (Miller, Chen, & Zhou, 2007). To date, research examining the association between specific time periods of stress (e.g., across the lifespan, early life, or recent stress), depression, and neuroendoimmune activity is sparse, and the associations remain unclear. Although some research has examined neuroendoimmune activity in the presence of acute laboratory stressors in depressed samples (Miller, Rohleder, Stetler, & Kirschbaum, 2005), the role of neuroendoimmune activity in translating psychological experiences of life stress into depression is limited. More specifically, while it is well-documented that acute and chronic stressors influence neuroendoimmune activity, it is unclear how specific time frames of perceived stress may uniquely contribute to these relationships, and more specifically, to chronic levels of inflammation that are tied to depression.

These nuances are important, as the timing and severity of stressors may directly influence cortisol levels—and ultimately immune activity—to contribute to depression. Cortisol is commonly hyperactive when a stressor is recent but dampened when the source of stress is more prolonged and distant (Miller, Chen, & Zhou, 2007). In the first case, the cortisol response may be adaptive (Burke, Davis, Otte, & Mohr, 2005); in the latter, it may represent disrupted cortisol production or signaling, potentially contributing to hyperactive immune responses under continued states of stress (Raison & Miller, 2003; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). In the second case, immune activity may become elevated and lead to depressive symptoms, although more research is needed. Given that cortisol plays a critical role in regulating the magnitude and duration of immune activity, it is important for research to capture these relationships. If stress experienced across the lifespan leads to a dysregulation of neuroendoimmune system processes, individuals may experience chronic elevations of immune activity and depressive symptoms as a result of glucocorticoid resistance or deficient cortisol signaling at the hormone level. These are critical lines of investigation because they may capture important links between stress-sensitive biological systems that could partially explain how experiences of stress manifest in the body to sustain prolonged effects across the lifespan, and that ultimately leave some individuals feeling stressed, sick, and sad.

Fourth, it is well recognized that cognitive control deficits are common in depressed populations, and that cognitive control moderates the impact of acute stressors on immune activity and negative physical health outcomes (Shield, Moons, & Slavich, 2017; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016). However, less research has examined the relationship between mediating factors, such as stress appraisals and immune function, to determine whether these variables account for the relationship between cognitive control and depression. It is unclear whether there is a relationship between cognitive control and psychological experiences of stress—or stress appraisals—and specific biological and clinical outcomes related to depressive symptoms. While it appears that stress appraisals influence immune activity to predict depressive symptoms over a one-year follow-up period (Aschbacher et al., 2012), and that stress appraisals mediate the relationship between life stressors and negative health outcomes (Shield, Moons, & Slavich, 2017), whether individual variability in cognitive control is associated with stress appraisals and biological disturbances to contribute to overall depressive symptom burden has not been addressed. To date, the relationship between cognitive control and stress appraisals

has not been examined in depressed samples and in relation to immune mediators. In line with integrative theories of depression, given the fact that cognitive control deficits are commonly linked to depression, and that cognitive control may causally contribute to depressive symptoms (Siegle et al., 2014; Hoorelbeke, Faelens, Behiels, & Koster, 2017), it is possible that this relationship is mediated by stress appraisals and subsequent immune system responses to contribute to depression outcomes (Miller & Raison, 2016; Slavich & Irwin, 2014).

Lastly, elevations in immune activity are only present in a subset of individuals with MDD, and effect sizes between immune activity and depression vary between studies (Howren, Lamkin, & Suls, 2009; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). Part of this variability may be due to the heterogeneity inherent in the prevailing categorical psychiatric classification system (as there are hundreds of combinations of MDD symptoms that meet criteria for the diagnosis), and it may be indicative of a biological variant (or variants) of the disorder that relates to experiences of stress and neuroendoimmune activity. Calls for dimensional and transdiagnostic research are mounting, as these approaches to psychopathology research may help to uncover neurobiological disruptions that may exist across diagnostic categories and reflect common underlying symptom dimensions (Patrick & Hajcak, 2016; Kotov et al, 2017).

1.4.1 Present study

The aim of the present study is to examine the associations among cognitive capacities associated with stress management, psychological experiences of stress, neuroendoimmune activity, and depressive symptom severity. Based on contemporary integrative models of depression, the central theoretical question addressed in this research is whether disruptions in neuroendoimmune activity, which are postulated to be caused by psychological experiences of stress over the lifespan, are mechanistically linked to depressive symptoms. The study adopts a dimensional and transdiagnostic research approach by examining adult females with varying levels of depression, ranging from no or minimal depressive symptoms, to mild, moderate, and severe depression. Based on existing integrative models of depression, three primary variables are examined in relation to depressive symptoms (as reported over the preceding two weeks): (a) the severity of experiences of life stress (i.e., severity of cumulative life stress, which will be

further subdivided into early life stress—≤12 years of age and recent life stress—previous six months) measured by the STRAIN, and past month stress severity measured by the PSS; (b) neuroendoimmune activity (i.e., proinflammatory immune activity and free cortisol measured the morning of data collection, representing the biological response to stress); and (c) psychological variables related to stress regulation (i.e., cognitive control and stress appraisals).

1.4.1.1 Research Questions

Three main research questions are addressed in this dissertation:

1. Are proinflammatory immune activity and psychological experiences of life stress associated with depressive symptom severity?

To assess this research question, the relationship between psychological experiences of life stress (i.e., severity ratings of cumulative life stress as measured by the STRAIN) and immune activity (measured primarily via the proinflammatory cytokine, IL-6) are examined dimensionally in relation to depressive symptom severity (measured by the BDI-II) (see Hypothesis 1.1). Psychopharmaceutical and contraceptive use, BMI, and menstrual cycle are included and examined as covariates to determine whether these variables change any of the observed immune-depression relationships. The *a priori* decision to analyze IL-6 in the primary analyses is based upon findings that IL-6 appears to demonstrate the most consistent relationships with both stress and depression (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011; Mac Giollabhui et al., 2019; Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020); however, the relationships with additional proinflammatory immune markers commonly identified in depression research, including TNF-α, IL-1β, and CRP, will also be explored in secondary analyses.

Furthermore, specific symptoms of depression (neurovegetative versus cognitive/affective symptoms) are separated to determine which symptom groupings may be most strongly associated with immune activity (see Exploratory Hypothesis 1.2). To derive these symptom groupings, items from the BDI-II are divided into two categories: (a) neurovegetative symptoms, which include symptoms common to sickness behaviours (e.g., loss of pleasure, loss of energy, and changes in appetite and sleep) (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008;

Raison et al, 2010; Dantzer, 2018); and (b) cognitive and affective symptoms, which comprise symptoms oriented to thinking styles and affect common to depression (e.g., sadness, guilt, and thoughts of suicide) (see Appendix B for a comprehensive list of symptom groupings).

2. Does neuroendoimmune activity mediate the relationship between psychological experiences of cumulative life stress and depressive symptom severity; and, are specific time periods of stress more related to neuroendoimmune activity and depressive symptoms?

To address these questions, IL-6 is examined in relation to cumulative life stress (i.e., total severity ratings of cumulative life stress as measured by the STRAIN) and depressive symptom severity (as measured by the BDI-II). Furthermore, due to the critical role of cortisol in regulating immune activity (Irwin & Cole, 2011; Ménard, Pfau, Hodes, & Russo, 2017), a mediation analysis is conducted to determine how cortisol (hypercortisolism or hypocortisolism) and immune activity relate to experiences of cumulative life stress and depression severity. A loss of inhibitory control between the HPA axis and immune system (possibly due to deficient cortisol signalling or production) may lead to runaway immune activity to bolster the impact of stress on depression (see Hypothesis 2.1).

Additionally, three specific time periods of perceived stress—early life stress severity (ratings of perceived stress severity ≤12 years of age as measured by the STRAIN), recent stress severity (ratings of perceived stress severity from the previous six months as measured by the STRAIN) and past month perceived stress (as measured by the PSS)—are examined to assess whether one of these time periods accounts for more of the variance between neuroendoimmune disruptions and depression (see exploratory Hypothesis 2.2).

3. Is cognitive control associated with lower depression severity, and is this relationship mediated by levels of perceived stress severity and immune activity?

To examine this question, the association between cognitive control (measured by the CWIT), cumulative life stress (severity ratings of cumulative life stress as measured by the STRAIN), IL-6, and depressive symptom severity (measured by the BDI-II) are investigated. This analysis tests whether cognitive control is negatively associated with depressive symptom severity, and

whether this relationship is mediated by cumulative life stress severity ratings and immune activity (see Hypothesis 3.1).

1.4.1.1.1 Hypotheses

Hypothesis 1.1. (Primary) Higher proinflammatory immune activity (IL-6) and greater cumulative life stress will be correlated with higher levels of depression.

Hypothesis 1.2. (Exploratory) To explore the impact of immune activity on specific symptom groupings of depression, neurovegetative and cognitive symptoms will each be examined separately in relation to IL-6.

Hypothesis 2.1.(Primary) Neuroendoimmune activity will mediate the relationship between cumulative life stress severity ratings and depressive symptom severity. Specifically, due to the anti-inflammatory role of cortisol, it is hypothesized that lower levels of morning free cortisol and higher levels of immune activity will relate to higher levels of depression.

Hypothesis 2.2. (Exploratory) To explore the association between specific time periods of stress, neuroendoimmune activity and depression, the model tested in Hypothesis 2.1 will be examined in relation to early life stress (severity ratings from ≤12 years of age), recent life stress (i.e., severity ratings from the past six months), and past month stress (measured by the PSS) to determine whether specific time periods account for more of the variance in Hypothesis 2.1.

Hypothesis 3.1. (Primary) Higher cognitive control will be associated with lower levels of depression, and this relationship will be mediated by lower ratings of cumulative life stress severity (as measured by the STRAIN) and immune activity (IL-6). That is, higher cognitive control will share an indirect relationship with lower depression through cumulative life stress and IL-6.

Chapter 2
Methods

2.1 Participants

Female participants with varying levels of depressive symptoms, including individuals with MDD, were recruited as part of a larger study examining biological factors associated with depression and BPD. To investigate stress and inflammation across the full range of depression severity, participants with a combination of eligibility criteria were recruited to target both the low and severe ends of the depression spectrum. First, we recruited individuals within the "normal" range HAMD scores (i.e., a score less than seven on the HAMD), which allowed for a sampling of people with no or minimal current depressive symptoms, including individuals subthreshold for MDD. We also recruited participants with mild to severe levels of depressive symptoms (a score of greater than seven on the HAMD), and separately, those with comorbid depressive symptoms and symptoms of BPD (the diagnosis of which was initially screened based on a McLean Screening Instrument score of greater than seven). As previously highlighted, about 30% of all MDD patients also have a diagnosis of BPD (Rossi et al., 2001), and individuals with comorbid MDD and BPD tend to demonstrate equal, if not more severe levels of depression (Newton-Howes, Tyrer, & Johnson, 2006; Köhling, Ehrenthal, Levy, Schauenburg, & Dinger, 2015). Participant recruitment was limited to females to control for known sex differences in inflammatory and HPA axis activity (Veldhuis et al., 2009; Moieni et al., 2015). Furthermore, we included participants who were currently taking psychiatric medications and contraceptives to increase the generalizability of the study findings to resemble more typical patient populations, as well as to optimize the feasibility of participant recruitment.

Eligibility criteria for the study included medically healthy, female adults (ages 18 – 55) who were English-speaking and right-handed. Exclusion criteria for all participants included a lifetime diagnosis of a psychotic disorder, bipolar disorder, current eating disorder, serious medical or neurological illness (see page 6 of Appendix C for a list of exclusionary disorders), neurodevelopmental disorder, alcohol or substance use disorder within the past month, pregnancy, lactation, or current antibiotic or anti-inflammatory drug use. Prior to providing a blood sample, participants agreed to abstain from drug and alcohol use for two days before

testing, were instructed to avoid any anti-inflammatory medications and intense physical exercise for 24 hours prior to study participation, and were asked to aim for at least eight hours of sleep the night before the research. The control group was not permitted to have a current diagnosis of MDD, but other psychiatric diagnoses were allowed, aside from the aforementioned exclusions. The MDD group was required to have a current diagnosis of MDD, and the MDD+BPD group additionally had a diagnosis of BPD. The study was approved by the Research Ethics Board at the University of Toronto.

Figure 2 presents a study inclusion chart to detail the study recruitment process. In total, 64 participants were included and consented to the study. Prior to data collection, one participant was deemed MRI incompatible and was thus excluded from participating in the study. After data collection, three participants were removed from the final dataset due to a current substance use disorder identified during the structured clinical interview, and one due to an incidental MRI finding. After study exclusions, 59 individuals remained in the final sample for analysis. Of note, participants who were experiencing menostasis (a halting of menstruation) were not excluded from the study. In total, four participants were not menstruating on a regular basis at the time of data collection: one due to a surgery, one due to menopause, and two due to continuous birth control use. Although this is a limitation of the research, the majority of depression and immune research studies have not excluded participants based on menostasis, and most include both sexes (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). Studying an all-female sample is one advantage in the present research design to eliminate sex-related neuroendoimmune effects (Veldhuis et al., 2009; Foley & Kirschbaum, 2010). Additionally, menstrual cycle and contraceptive use are included as covariates in the analyses to consider their potential effects in the relationship to immune and depression outcomes (Bouman, Jan Heineman, Faas, 2005).

2.2 Procedures

Participants were recruited from a combination of community (e.g., online postings) and clinical (e.g., psychiatric clinics) settings. A phone screen was conducted to assess eligibility prior to study enrollment. This included an interview to assess inclusion and exclusion criteria, including

a more in-depth assessment of depression severity (measured by the HAMD) and the likely presence or absence of BPD (i.e., a score greater than or equal to seven on the MacLean's Screening Instrument for BPD; Zanarini et al., 2013). Participants provided written informed consent and completed a fasted blood draw between 8 a.m. and 9 a.m. on the day of testing. Following the blood draw, weight was measured, and height was reported to calculate BMI. Subsequently, participants provided demographic information and completed diagnostic, clinical, and neuropsychological assessments (as described below). Participants also underwent magnetic resonance imaging scanning as part of a separate component of the study. A suicide operating procedure was followed when participants endorsed current (past 24-hour) suicidal thinking on the SCID-5, HAMD, and/or BDI-II (see Appendix D). At the conclusion of testing, participants were debriefed (see Appendix E) and financially compensated for their time (see Table 3 for a schedule of measurements).

2.2.1 Demographic and Background Information

Participants were asked to provide demographic information and answer questions about their background and development. The demographic information collected included items pertaining to biological sex, age, ethnicity, educational history, financial status, occupation, family history, psychiatric treatment history, and medication use (see Appendix F).

2.2.2 Clinical Measures

2.2.2.1 Structured Interview for DSM-5 (SCID-5)

The SCID-5 was used to determine the presence of psychiatric diagnoses relevant to the study's eligibility criteria. The interview was administered by trained Ph.D. students in Clinical Psychology and supervised by a licensed psychologist. The following diagnoses were assessed: depressive disorders, including MDD and persistent depressive disorder (PDD), alcohol and non-alcohol substance use disorders, and PTSD. The SCID for Personality Disorders was administered to assess BPD. The information obtained from the diagnostic interviews was discussed in a consensus diagnostic meeting and diagnoses were arrived at with a licensed psychologist.

2.2.2.2 Hamilton Depression Rating Scale (HAMD)

The 17-item HAMD (Hamilton, 1960) was used to assess depression severity at the time of the phone screen and on the day of participant testing. The HAMD is a semi-structured interview designed to evaluate depressive symptoms over the past week. Scores range from 0 – 52 and can be categorized as normal (≤7), mild (8 – 16), moderate (17 – 23), and severe (≥24) (Sharp, 2015). The HAMD is one of the most commonly used interviews for major depression with high internal reliability estimates (Bagby, Ryder, Schuller, & Marshall, 2004). Participants who scored greater than or equal to 7.0 on the HAMD during the phone interview and day of research were considered part of the MDD or MDD+BPD group, but full episodes were assessed by the SCID-5. Internal consistency of this scale was high (Cronbach's α = .88).

2.2.2.3 Beck Depression Inventory (BDI)-II

The BDI-II (Beck, Steer, & Brown, 1996) is a 21-item self-report questionnaire used to assess depressive symptom severity (scores range from 0 – 63). Depressive symptom severity refers to the number and pervasiveness (e.g., "I do not feel sad" versus "I feel sad all the time") of reported symptoms. This inventory demonstrates high internal validity and test-retest reliability, as well as good discriminant, convergent, and predictive validity (Beck, Steer, Ball, & Ranieri, 1996). Scores from the measure were used as the primary outcome variable in the analyses to evaluate depressive symptom severity. Internal consistency of this scale was high, with a Cronbach's α of .96. To differentiate between neurovegetative versus cognitive/affective symptoms of depression, items from the BDI-II were divided into two categories as described above in Section 1.4.1.1: (a) symptoms common to sickness behaviours (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Raison et al, 2010; Dantzer, 2018); and (b) symptoms oriented to thinking styles and affect common to depression (e.g., sadness, guilt, thoughts of suicide, etc.) (see Appendix B for a list of symptom groupings). These two categories of symptoms were further assessed in relation to immune activity in Exploratory Hypothesis 2.1 to determine whether one symptom grouping is more strongly related to immune activity. Internal consistency of both scale groupings was high, with a Cronbach's α = .93 for neurovegetative symptoms and α = .94 for cognitive/affective symptoms.

2.2.2.4 Stress and Adversity Inventory for Adults (STRAIN)

The STRAIN is an adaptive (i.e., questions are tailored to each individual depending on their responses), self-report, and computer-administered questionnaire that assesses the frequency of life stressors (i.e., total count), timing of stressors (i.e., age at which stressors occurred), and perceived severity of stressors (e.g., At its worst, how stressful or threatening was this for you?). Life stressors include work stress, financial stress, loss, bereavement, medical concerns, caregiving, substance use, interpersonal strain, and divorce, among either life events (Slavich & Shields, 2018). The STRAIN provides scores on the frequency (to produce a total count score) and intensity of stressors (to produce a severity score) and categorizes stressors and perceived stress severity into clinically meaningful timelines (e.g., early life adversity, college years, adulthood exposure, recent past six months, etc.). The timing of stressful events is relevant, as, for example, research demonstrates that stressors experienced in early-life are strongly associated with later psychopathology (Danese & Baldwin, 2017), as well as biological disruptions (e.g., increased levels of inflammation, shorter telomeres, alterations in gene methylation, etc.) (Slopen, Kubzansky, McLaughlin, & Koenen, 2013). The measure takes approximately 18 minutes to complete and demonstrates good psychometric properties (Toussaint, Shields, Dorn, & Slavich, 2016; Dooley, Slavich, Moreno, & Bower, 2017). Scores on the STRAIN are associated with higher levels of physical health complaints and medical diagnoses, disrupted sleep, and lower executive functioning (Slavich & Shields, 2018).

In the present study, stress severity, as opposed to count scores, was selected for analyses because the psychological experience of stress is considered the central theoretical construct of interest in this study (as described in Section 1.2.2.1 on subjective measures of stress). The central focus of this study is how one's psychological experience of stress may relate to neuroendoimmune disruptions and depression, as opposed to the type or number of stressful life experiences. The primary hypotheses tested in this dissertation focus first on cumulative life stress severity ratings as assessed by the STRAIN. Next, exploratory analyses assess two additional time periods of stress measured by the STRAIN: childhood perceived stress severity (≤12 years of age) and past six-month perceived stress severity. These exploratory analyses help to determine whether experiences of stress during specific time periods of life have greater associations with neuroendoimmune and depression outcomes.

2.2.2.5 The Perceived Stress Scale (PSS)

The PSS was developed to measure the degree to which individuals appraise events in their life as stressful over the past month (without any specific information collected about the stressors) (Cohen, Kamarck, & Mermelstein, 2006). The PSS was used to assess current (i.e., past month) levels of subjective stress and participants' ability to cope with the stress. The PSS is a 10-item self-report inventory that presents questions to gauge how well an individual coped with, or responded to, stressors (e.g., In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?), rated from 0 (*Never*) to 4 (*Very Often*). Four items are coded in reverse and are inverted when calculating total scores, with scores ranging from 0 to 40. The PSS demonstrates high construct validity and internal consistency (Cronbach's alpha = .89; Roberti, Harrington, & Storch, 2011). High PSS scores are associated with higher rates of physical health complaints, lower health behaviours, and greater vulnerability to depressive symptoms (Hewitt, Flett, & Mosher, 1992; Roberti, Harrington, & Storch, 2011). The PSS provides a measure of current perceived stress, which is incorporated into Exploratory Hypothesis 2.2 to test specific time periods of perceived stress on immune and depression outcomes. Internal consistency of this scale was high, with a Cronbach's $\alpha = .93$.

2.2.3 Cognitive Control Measure

The Colour-Word Interference Test (CWIT) is a test that is part of the Delis-Kaplan Executive Function System (DKEFS) and is designed to measure aspects of executive function and cognitive control, including goal maintenance, inhibitory control, cognitive flexibility, and performance monitoring (Homack, Lee, & Riccio, 2005; Delis, Kaplan, & Kramer, 2006). As previously mentioned, this test asks individuals to inhibit learned behaviours (e.g., word reading) and instead exert controlled goal-oriented behaviours (e.g., saying the colour of the ink a word is printed in). There are four conditions in the DKEFS version of the test. Condition 1 ("colour naming") requires the participant to name the colours of a series of coloured boxes (e.g., red, green, blue). Condition 2 ("word reading") requires the participant to read a page of words (e.g., "red," "green," "blue") that are printed in black ink. In Condition 3 ("inhibition"), participants are asked to look at colour names that are printed in incongruent ink colours, and to name the colour of the ink instead of reading the word. This trial is based on the Stroop procedure (Stroop, 1935). Lastly, Condition 4 ("inhibition/switching") asks the participant to switch between two

different rules (reading words or naming ink colours), depending on whether the words are enclosed in boxes or not. Regardless of condition, participants are asked to work as quickly as possible without making any mistakes. In the present study, Condition 4 (inhibition/switching) was used as the primary outcome variable because it places the greatest demands on cognitive control (Homack, Lee, & Riccio, 2005; Lippa & Davis, 2010). Scores on this measure were standardized to each participant's normatively-referenced age group to account for normal age-related changes in cognitive control.

2.2.4 Biomarkers

In the present study, objective biomarkers of stress were measured via free cortisol and proinflammatory cytokines assays. To control for circadian variations in immune biomarkers, blood samples were collected fasted between 8 a.m. and 9 a.m. with BD Vacutainer® Blood Collection Tubes (K_2EDTA as anticoagulant). Plasma was separated through centrifuge and transferred into five 500µL aliquots and stored at -80 °C until analysis. IL-6, IL-1β, and TNF-α were measured by Bioplex 200 multiplex immunoassay system (BioRad, USA). The 4-plex plate was included in a kit from BioRad including all standards and reagents. The analysis was carried out following established manufacturer protocol. The quantification range for these assays are 0.27-4457 pg/mL for IL-1β, 0.4-6557 pg/mL for IL-6, and 3.16-51852 pg/mL for TNF-α, respectively. Analysis of (high sensitivity) hsCRP was conducted with a routine certified clinical assay for cardiovascular risk assessment. The low (<1.0mg/L), average (1.0-3.0mg/L) and high risk (>3.0mg/L) range was provided by the certified clinical biochemist. Analysis of total cortisol was also conducted with a routine certified clinical assay. The normal adult (193-690nmol/L) range was also provided by the certified clinical biochemist. Free cortisol assay was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology, similar to the procedure reported above (Huang, Kalhorn, Baillie, Shen, & Thummel, 2007). Briefly, plasma filtrates were obtained through ultrafiltration. Free cortisol was extracted using ethyl acetate. The organic phase was removed and dried down followed by reconstituting with HPLC mobile phase (45/55 1mM ammonium acetate/acetonitrile). The Agilent 1100 HPLC was coupled to an AB Sciex 4000 trip quadrupole mass spectrometer in MRM mode. Linear range was from 5nmol/L to 500 nmol/L.

2.3 Statistical Analyses

The analytic plan described below was developed to analyze intermediate biological and psychological factors that may facilitate and influence the relationship between psychological experiences of stress and depression outcomes. To do this, dimensional analyses using linear regression and structural equation modeling (SEM) were conducted to analyze the primary and exploratory hypotheses. Simple linear regression was used to test Primary Hypothesis 1.1 (relationships of proinflammatory immune activity and cumulative life stress with depression severity) and Exploratory Hypothesis 1.2 (relationship of proinflammatory immune activity with depression symptom groupings). The slope (*b*), Pearson correlation coefficients (*r*), and statistical significance (*p* values) are reported for each of these analyses. Then, two separate mediation models were constructed to run the mediation analyses. The statistical package lavaan in R was used to estimate SEM. The first model is a parallel mediation model (Hayes, 2017) used to test Primary Hypothesis 2.1 (neuroendoimmune activity mediating the relationship between cumulative life stress and depression severity) and Exploratory Hypothesis 2.2 (investigating different time periods of perceived psychological stress in the aforementioned relationships) (see Figure 3). The second mediation model is a serial mediation model (Hayes, 2017) used to test Hypothesis 3.1 (whether stress appraisals and immune activity mediate the relationship between cognitive control and depressive symptom severity) (see Figure 4).

Mediation analyses were selected to address the central research questions because the effects of the independent variables (IV; stress and cognitive control in Figures 3 and 4, respectively) on the dependent variable (DV; depressive symptom severity) are assumed to occur via neuroendoimmune mediators (IL-6 and free cortisol). As such, it is assumed that the IVs influence the mediators, and the mediators, in turn, influence the DV. Below, the logic and equations used to test the two mediation models are outlined in detail. Tables 4 and 5 outline the steps used to examine the parallel and serial models (Hayes, 2017). Although the analyses were conducted using SEM (where all analyses were conducted in two distinct steps relying on bootstrapping to estimate the indirect effects), the Baron and Kenny (1986) notation will be used to outline and report the estimates of the various pathways of each model.

The present research study utilizes cross-sectional data to test the mediation models. It is worth noting that mediation models assume temporal causality or directionality between predictors, mediators, and outcome variables. However, to detect true mediation effects with predictive validity, experimental manipulation or longitudinal research designs are required (Hayes, 2017). As such, all effects within the present study merely report on observed associations, and do not infer or prove causality. However, the components of the models and the model parameters were selected based on theoretical rationale and prior research that demonstrates temporal sequencing of the variables (e.g., that immune activity most commonly precedes depressive symptoms; Chu et al., 2019; Huang et al., 2019; Lamers et al., 2019). Similarly, all metrics of perceived stress utilized in the present study incorporate time periods of stress appraisals (i.e., cumulative life history, early childhood, past six-month, or past month) that either precede and/or subsume the period of depression being analyzed (i.e., past two weeks) to strengthen the assumptions of the mediation models being tested. Lastly, all of the models were developed *a priori* based on theory and empirical research, and if supported, increase confidence in the hypothesized relationships.

2.3.1 Model 1: *Cumulative Stress, Neuroendoimmune Activity, and Depression* (Parallel Mediation Model)

In a parallel mediation model, it is assumed that the impact of the IV on the DV occurs via two or more mediators (Hayes, 2017). In Figure 3, the bidirectional arrow refers to any possible covariance which accounts for any overlap or relationship between the two mediators. In contrast to running simple mediation models (with only one mediator), parallel mediation models have the advantage of increasing the power to detect indirect effects of the mediators because the effects of the mediators can be combined. In Model 1, the IV is stress severity (total cumulative life; ≤12 years of age; past six months; or PSS, respectively), the mediators are neuroendoimmune activity (IL-6 and free cortisol), and the DV is depressive symptom severity (BDI-II scores; see Figure 3).

In the analysis, paths a, b, and direct effects are all calculated simultaneously using SEM. From there, the indirect effect and total effect are calculated, using the separate formulas listed below. Here, however, each pathway is described using the step notation to provide a comprehensive overview of the model. The first step of a parallel mediation model evaluates the direct effect of

the IV on the DV, while controlling for the mediators. In the case of the models presented in Figure 3, the direct effect represents whether changes in stress severity (total cumulative life; ≤12 years of age; past six months; or PSS, respectively) impact levels of depressive symptom severity. This is represented by path c' in Figure 3.

Step 2 reports on whether the IV has a significant effect on the mediator variables. This step assesses whether changes in stress severity impact levels of neuroendoimmune activity (i.e., IL-6 and free cortisol, respectively). This is represented by paths a1 and a2 in Figure 3.

Step 3 reports on whether the mediators are related to the outcome, while controlling for the IV. This step assesses whether neuroendoimmune activity (IL-6 and free cortisol) is related to depressive symptom severity while controlling for stress severity. This is represented by paths b1 and b2 in Figure 3.

Step 4 of the mediation process estimates indirect effects using SEM. The indirect effect tests how much the relationship between the IV and the DV decreases once the mediators are statistically controlled for. The below equation was used to calculate the indirect effect of perceived stress severity (total cumulative life; ≤12 years of age; past six months; or PSS, respectively) on immune activity (IL-6) and to depressive symptom severity (path ab1):

$$ab1 = a1*b1 \quad (1)$$

Similarly, the following equation was used to estimate the indirect effect of stress severity (total cumulative life; ≤12 years of age; past six months; or PSS, respectively) on free cortisol and depressive symptom severity (path ab2):

$$ab2 = a2*b2 \quad (2)$$

To examine the total indirect effect (indtot) of both mediators in the model (i.e. the *parallel* mediation), the following equation was used to capture the effect of perceived stress severity and both mediators (IL-6 and free cortisol) on depressive symptom severity:

$$indtot = ab1 + ab2 \quad (3)$$

Finally, in step 5, the total effect (i.e., the relationship between the IV and the DV without controlling for the mediators) can be recovered due to some straightforward mathematical equivalencies. The total effect is often referred to as c, but here it is referred to as "tot" to avoid confusion. In mediation modelling, the following equivalency can be proven (Hayes, 2017):

$$ab1 + ab2 = tot - c' \quad (4)$$

That is, the indirect effect equals the total effect minus the direct effect. With some simple algebra, the total effect can be recovered:

$$tot = c' + (ab1 + ab2) \quad (5)$$

Note that this differs from the traditional Baron & Kenny (1986) approach, which runs the total effect as a separate analysis. Using SEM, this parameter is recovered post hoc with some simple algebra. It is worth noting, that while a significant total effect between the IV and DV is predicted in this sample, it is generally accepted that this is not a necessary condition for an indirect effect to be found in the overall model (Zhao, Lynch, & Chen, 2010; Rockwood & Hayes, 2020).

2.3.2 Model 2: *Cognitive Control, Cumulative Life Stress, Immune Activity, and Depression* (Serial Mediation Model)

In a serial mediation model, it is assumed that one mediator influences another (in addition to the IV influencing the mediator, which in turn, influences the DV). As such, it is assumed that the IV influences the first mediator, which influences the second mediator, which influences the DV. In Model 2, the IV is cognitive control, the mediators are cumulative life stress severity and neuroendoimmune activity (IL-6), and the DV is depressive symptom severity (see Figure 4). The same basic steps outlined in Model 1 are also executed in a serial mediation model with slight modifications made to the statistical parameters to examine the serial mediation (see Table 5).

In the analysis, paths a, b, and direct effects are all calculated simultaneously using SEM. From there, the indirect effect and total effect are calculated, using the separate formulas listed below.

Here again, each pathway is described using the step notation to provide a comprehensive overview of the model. Step 1 reports the direct effect of the IV on the DV, while controlling for the mediators. In the case of the model presented in Figure 4, the direct effect represents whether changes in cognitive control impact levels of depressive symptom severity while controlling for the mediators. This is represented by path c' in Figure 4.

Step 2 is designed to determine whether the IV has a significant effect on the mediator variables. This step assesses whether changes in cognitive control impact levels of cumulative life stress severity and neuroendoimmune activity (IL-6). To do this, SEM is used to estimate the relationship between the IV and each mediator separately. This is represented by path a1 and a2 in Figure 4.

Step 3 reports on whether the mediator(s) are related to the outcome, while controlling for the IV. This step assesses whether cumulative life stress severity ratings and IL-6 are associated with depressive symptom severity while controlling for cognitive control (measured by Condition 4 of the CWIT). This is represented by paths b1 and b2 in Figure 4.

Step 4 looks at the relationship between mediators. In a serial mediation model, a 4-variable chain is proposed: IV -> m1 -> m2 -> DV. Thus, it is also important to specify the relationship between both mediators. In Figure 4, it is assumed that cumulative life stress severity will be associated with IL-6, while controlling for CWIT-4. This is represented by path d in Figure 4.

Step 5 of the serial mediation process estimates indirect effects using SEM. The indirect effect tests how much the relationship between the IV and the DV decreases once the mediators are statistically controlled for. The below equation was used to calculate the total indirect effect (indtot) of cognitive control and both mediators (IL-6 and cumulative stress severity) on depressive symptom severity:

$$indtot = a1*b1 + a2*b2 + a1*d*b2 \quad (6)$$

Finally, in step 6, the total effect of CWIT-4 on depressive symptom severity (without controlling for the mediators) is recovered with the following formula:

$$tot = c' + (a1*b1 + a2*b2 + a1*d*b2) \quad (7)$$

Bootstrapping with 10,000 resamples was used to estimate standard errors and indirect effects for all mediation analyses to make analyses more robust to potential violations of the normality assumption (Pituch & Stapleton, 2008; Yuan & MacKinnon, 2014). Additionally, 95% confidence intervals (*CI*) were computed to quantify the margin of error around effects. Correlations (*r)* and effect sizes (R^2) of the various relationships are reported. For each mediation model, the estimate (unstandardized slope, *b*), confidence intervals (95% *CI*), significance of the slope (p value, *p*), and the percentage of variance accounted for by the relationship (standardized beta coefficients, β) are reported in Chapter 3. This information will help to determine the direction of each effect and how much of the variance in depressive symptom severity is accounted for by the various independent variables and mediators. To calculate the effect size of the mediation, the estimate of the unstandardized slope of the total indirect effect is divided by the estimate of the unstandardized slope of the total effect. This yields a percentage that indicates how much the total effect is reduced by the mediators and is reported for each model.

Chapter 3
Results

3.1 Preliminary Analyses

Table 6 displays the demographic characteristics of the study participants who completed the research (n = 63). Table 7 presents the diagnostic and clinical characteristics of the sample, confirming that the recruitment procedures produced scores across the range of depression severity as intended. A boxplot of depressive symptom severity (as measured by the HAMD) on the day of study participation is displayed in Figure 5. This figure provides a visualization of the HAMD score distribution. Of note, scores on the HAMD did not differ between MDD and the MDD+BPD group, F = .28, t = .95, p = .60 (see Appendix G and H for clinical and descriptive statistics of the diagnostic groups). As such, depression severity scores appeared to be similar in both groups that were comprised of participants recruited with depressive symptoms greater than seven on the HAMD. In addition to MDD diagnoses, 19% of participants met criteria for PDD, which represents a depressed mood that occurs more days than not for at least two years. Of the 21 participants with no current MDD diagnoses, four participants met criteria for a past episode of MDD that was in full remission at the time of their study participation. The mean number of past major depressive episodes in the sample was 3.7 (SD = 6.9), indicating that a substantial number of participants experienced recurrent episodes. Similar to the HAMD, a boxplot of depressive symptom severity, as measured by the BDI-II (the main DV), can be seen in Figure 5. This figure provides a visualization of the BDI-II score distribution. There were no outliers in the data set and scores were fairly evenly distributed across the range to provide a full spectrum of depression severity ratings.

Descriptive statistics for, and correlations among, the primary study variables are presented in Table 8. Statistics provided in this table, and on all forthcoming statistics, are presented on the final sample of 59 participants. Note that one participant's plasma sample had insufficient supply to complete the morning free cortisol assay. For the descriptive statistics and correlational analyses, listwise deletion was used for incomplete samples. For SEM analyses, missing data were handled using a full information maximum likelihood approach. Although the statistical models focused on the proinflammatory cytokine IL-6, additional proinflammatory molecules

(TNFα and CRP) are listed in Table 8 to illustrate correlations with other study variables. Twenty-nine participant samples produced IL-6 levels that were below the range of detection. This means that there was an observed signal for IL-6, but it was too low to quantify. By default, these samples are given a value of .04pg/mL (the lowest detectable concentration) for data analytical purposes, as is standard practice (Maes, Mihaylova, Kubera, & Ringel, 2012; see Appendix I for analyses with these individuals removed from the dataset). Three samples of TNFα were also below detection, and thus labeled as the lower limit of 3.16pg/mL. Of note, assays for IL-1β were also conducted but were below the detectable limit in this sample. Limited detection of IL-1β and null associations have been typical in other MDD samples (Kleiner et al., 2013; Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020).

All immune marker data were positively skewed (see Figure 5 for the boxplot for IL-6). This is typical of immune biomarkers as reported in the research literature (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). Log transformations were not applied to the data because bootstrapping methods circumvent violations of normality, and this method has the advantage of providing interpretable coefficients.

It appears that normal reference ranges are not well-established for the immune markers analyzed in this study, with the exception of CRP. However, a few published studies using healthy participants have been reported, and these ranges are captured in Table 1 (Todd, Simpson, Estis, Torres, & Wub, 2013; Sekiyama, Yoshiba, & Thomson, 2008; Kleiner, Marcuzzi, Zanin, Monasta, & Zauli, 2013; Arican, Aral, Sasmaz, Ciragil, 2005; Mayo Clinic Laboratories, 2020). According to these ranges, six participants in the study demonstrated concentrations of IL-6 above normal (>1.8pg/mL). This matches the outliers noted in Figure 5. Four participants had levels of TNFα above normal (>18.5pg/mL) and 11 below normal (<3.89pg/mL); 15 participants produced sample concentrations of CRP above normal (>3.0mg/L) and 23 below normal (<.80mg/L). Levels within the normal range are expected in these samples, as otherwise healthy depressed populations typically demonstrate “low-grade” elevations in immune activity, meaning that they are slightly elevated (and more chronically so), but at statistically significant deviations compared to non-psychiatric controls (Berk, 2013;

Osimo et al., 2020). These low-grade levels are typical of other samples with otherwise healthy MDD participants (Maes, Mihaylova, Kubera, & Ringel, 2012; Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020).

A boxplot of morning free cortisol is presented in Figure 5. Certified clinical reference ranges are not well established for free cortisol, and thus, are not reported here. Generally, free cortisol is expected to be about five percent of total cortisol (Foley & Kirschbaum, 2010; Mayo Clinic Laboratories, 2020). In this sample, free cortisol concentrations appear to map onto the lab values of total cortisol (suggesting that the same participants with more extreme hyper/hypo concentrations of free cortisol also had more extreme concentrations of total cortisol). Of note, six participant samples of total cortisol registered as above the normal morning range (>690nmol/L), and five samples were below normal (<193nmol/L).

A boxplot of cumulative life stress severity scores as measured by the STRAIN is displayed in Figure 5. The boxplot indicates a fairly wide range of scores, but well within the limits of published values (Slavich & Shields, 2018). A wide distribution of scores was anticipated, as populations that experience depression and other stress-related comorbid diagnoses represented in this sample (e.g., BPD, PTSD) are known to experience high incidents of trauma (Crowell, Beauchaine, & Linehan, 2009; Monroe, Slavich, & Georgiades, 2014). In Figure 5, one cumulative life stress severity score was identified as an outlier; however, this individual reported consistently high ratings on all items of the STRAIN, suggesting that their scores are a valid representation of their life experiences; therefore, this participant's data was included in the analysis. Although the analyses conducted in the present study focus on severity measures to provide an index of the psychological experience of stress, it is worth noting that cumulative life stress severity was strongly correlated with total count of cumulative life stressors as measured by the STRAIN, $r = .94$, $p = <.001$. As such, one might expect that analyses conducted with total count of life stressors would likely yield similar results as compared to the perceived stress severity ratings. The latter construct is the basis of the present research given the theory and empirical findings reviewed in Chapter 1. Figure 5 provides visualizations of the additional stress categories and measures, including childhood stress severity, past six-month stress severity, and PSS scores (past month stress severity).

A boxplot of scores from the CWIT is also presented in Figure 5. Normatively-referenced scaled scores for the participant sample (M = 11.20 SD = 2.45) are similar to the expected level of performance based on the scales scores of the normative sample (M = 10.00, SD = 3.00), suggesting that participants performed within the average range. One participant scored 1.5 SD below the normative average, and two participants 1.5 SD above. As is evident from the correlations presented in Table 8, the CWIT did not show any significant associations with any of the other study variables. Correlations ranged from r = .01 (with past six-month stress) to r = .16 (with childhood stress), suggesting negligible to small magnitudes of relationships between the CWIT and other study variables.

3.2 Primary Analyses

Are Cumulative Stress and Immune Activity Associated with Depression?

As hypothesized, cumulative life stress severity (b = .46, r = .44, p = <.01), and IL-6 (b = 8.20, r = .39, p = <.01) were each significantly associated with depressive symptom severity. In the case of cumulative life stress severity, a one-point increase in cumulative life stress severity as measured by the STRAIN was associated with a 0.46-point increase in BDI-II scores (see Figure 6). The range of the values in the 95% CI for this parameter are quite narrow, *CI* (0.22, 0.71), indicating a high level of certainty that the true slope would lie within this range in 95% of samples.

Looking at the relationship between IL-6 and depression scores, a 1pg/mL increase in IL-6 concentration in the blood plasma is associated with an 8.20-point increase in BDI-II scores, *CI* (3.0, 13.41) (see Figure 6). When covariates of medication use (including both psychopharmaceuticals and birth control), BMI, and menstrual cycle were analyzed, the relationship between IL-6 and BDI-II scores remained significant, b = 7.96, p = <.01, *CI* (3.04, 12.88). Table 9 outlines the relationship of the variables included in the covariate analysis in relation to BDI-II scores. Psychopharmaceutical use was the only variable that was significantly associated with BDI-II (as would be expected) but did not account for a significant amount of the variance between IL-6 and BDI-II, b = 17.36, p = <.001, *CI* (10.58, 24.14). Similarly, only BMI

was significantly correlated with IL-6, $b = 1.70$, $p = <.01$, *CI* (.68, 2.71) when accounting for the shared variance of the other variables in the covariate analysis. It appears that a 1kg/m^2 increase in BMI is associated with a 1.70 pg/mL increase in IL-6 concentrations. A significant relationship between IL-6 and BMI is well documented in the literature (Mac Giollabhui et al., 2019); however, in this study, BMI did not account for a significant proportion of the variance between IL-6 and BDI-II scores. Overall, the covariate analysis suggests that the relationship between IL-6 and BDI-II was not different when covariates were considered, and that the covariates did not account for a significant portion of the variance in depression scores explained by IL-6.

Does Neuroendoimmune Activity Mediate the Relationship between Cumulative Stress and Depression?

The next set of analyses tested whether neuroendoimmune activity mediates the relationship between cumulative life stress and depressive symptom severity (Hypothesis 2.1). Accordingly, cumulative life stress severity was entered as the independent variable, IL-6 and free cortisol as the mediators, and depressive symptom severity as the dependent variable. As previously mentioned, this hypothesis was tested in a parallel mediation model and is visually represented in Figure 3. The results of the analyses are presented in Figure 7 and Table 10 and provided in more detail below.

First, examining the total effect of the parallel mediation model, cumulative life stress severity was significantly associated with depressive symptom severity, $b = .23$, $p = <.01$ (step 5 [parameter c' + (ab1+ab2)]). To examine the individual pathways in the model, the effect of cumulative life stress severity on IL-6 (path a1) was not significant, $b = .00$, $p = .21$. As such, the null hypothesis is inclusive as no relationship between cumulative life stress severity and IL-6 was found in this sample. This is represented by a grey dotted line in Figure 7. Within simple mediation models, such a finding would negate the possibility of an overall mediation. However, in a parallel mediation model, both mediators can be examined and thus it is important to look at all relationships and indirect effects. Path a2 showed that the estimate of cumulative life stress severity on free cortisol was significant, $b = -.08$, $p = <.05$. The slope of the line indicates that a one-point increase in cumulative life stress severity as measured by the STRAIN results in a

.08nmol/L decrease in free cortisol concentration. The range of the values in the 95% CI for this parameter are quite narrow, *CI* (-.14, -.02), indicating a high level of certainty that the true slope would lie within this range in 95% of samples. Here, the null hypothesis can be rejected, as there is a significant effect of cumulative life stress severity (the IV) on free cortisol concentration (the second mediator).

The relationship between IL-6 and depressive symptom severity (path b1) was significant, b = 6.51, p = <.05. The slope of the line indicates that an increase of 1pg/mL in IL-6 blood plasma concentration results in a 6.51-point increase in depressive symptom severity scores on the BDI-II. Here, the null hypothesis can be rejected, as there is a significant effect of IL-6 (the mediator) on depressive symptom severity (the DV). The range of the values in the 95% CI for this parameter are quite wide, *CI* (1.93, 11.10), indicating some degree of uncertainty about the true slope. Path b2 found that the effect of free cortisol on depressive symptom severity was trending toward significance, b = -.43, p = 05. However, the null hypothesis is inclusive as no clear relationship was found between free cortisol and depressive symptom severity.

Path ab1 of the mediation process, showed that the first mediator (IL-6), controlling for cumulative life stress severity, was not significant, b = .02, p = .25. As such, the null hypothesis is inconclusive in this sample, and cannot be rejected as there is no clear relationship between IL-6 and depressive symptom severity in this sample. Path ab2 of the mediation process, showed that the second mediator (free cortisol), controlling for cumulative life stress severity, was also not significant, b = .04, p = .12. Parameter ab1+ab2 of the indirect analyses included both mediators, but was also not significant b = .06, p = .06. Although the effect was trending toward significance, there was no total indirect effect of the mediators detected in this sample and results are inconclusive.

When the variance shared by both mediators was controlled for, the total effect size was reduced by 26.1%. Although the combined indirect effect did influence the outcome by reducing the impact of the IV, the combined indirect effect of the mediators did not reach significance. Here, the null hypothesis can be rejected, as there is a significant direct effect of cumulative life stress severity on depressive symptom severity, b = .17, p = <.01 (path c'). As such, no mediation effect was detected for Hypothesis 2.1. In this sample, it appears that the relationship between

cumulative life stress severity and depressive symptom severity is significant, and there is insufficient evidence to suggest that neuroendoimmune activity significantly mediated this relationship.

Is the Relationship between Cognitive Control and Depression Mediated by Immune Activity and Perceived Stress?

To test whether perceived stress and immune activity mediate the relationship between cognitive control and depression (Hypothesis 3.1), cognitive control was entered as the independent variable, cumulative life stress severity and IL-6 as the mediators, and depressive symptom severity as the dependent variable in a serial mediation model. This model was designed to test whether cognitive control is associated with lower depression ratings and whether this relationship is mediated by stress perceptions and immune activity. The results of the model are depicted in Figure 8. Solid black lines represent significant relationships, and dotted grey lines represent relationships that did not meet statistical significance. The coefficients of the model pathways are presented in Table 11.

Examining the total effect of the serial mediation model, cognitive control was not significantly associated with depressive symptom severity, b = -.67, p = .43 (step 6 [parameter c' + (ab1+ab2+a1db2)]). However, a significant relationship between these two variables was not explicitly predicted and a significant total effect is not a requirement of mediation (Zhao, Lynch, & Chen, 2010; Rockwood & Hayes, 2020); as such, the additional steps of the serial mediation proceeded. To examine the individual pathways in the model, the relationship between cognitive control and cumulative life stress severity was not significant, b = 1.73, p = .24 (path a1). As such, the null hypothesis is inconclusive and cannot be rejected as there is no clear relationship between cognitive control and cumulative stress severity in this sample. The effect of cognitive control on IL-6 was not significant (path a2), b = -.02, p = .45. As such, the null hypothesis is inconclusive in this sample, and cannot be rejected as there is no clear relationship between cognitive control and IL-6 in this sample. Given that cognitive control was not significantly associated with either mediator in the serial mediation model implies that a mediation effect will not be detected (Hayes, 2017). Nevertheless, the additional data analytic steps of the model are presented below to examine all a priori predictions.

A significant relationship was found between both mediators and depressive symptom severity (path b1 and b2). Cumulative life stress severity was significantly related to BDI-II scores, b = .21, p = <.01, as were IL-6 concentrations, b = 6.49, p = <.05. Path d showed that the relationship between cumulative life stress severity and IL-6 was not significant, b = .00, p = .22. As such, no relationship between the mediators was detected. Although there were significant relationships found between each of the mediators (total life stress severity and IL-6) and the dependent variable (depressive symptom severity), parameter ab1+ab2+a1db2 of the mediation process, showed that the serial mediation was not significant, b = .26, p = .54. In this sample, there is no evidence to suggest that cognitive control is directly or indirectly (via cumulative life stress and immune activity) associated with depressive symptom severity. As such, no mediation effect was detected.

3.3 Exploratory Analyses

Relationships of Other Immune Markers (TNFα and CRP) with Perceived Stress and Depression

As previously indicated, IL-6 was selected as the primary proinflammatory immune marker in this research because of its consistent relationship with both stress and depression (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011; Mac Giollabhui et al., 2019). However, additional proinflammatory immune markers that have commonly been identified in the depression and stress literature were assayed to examine in exploratory analyses based on prior related research (Kiecolt-Glaser, Gouin, et al., 2011; Jaremka et al., 2013; Slopen, Kubzansky, McLaughlin, & Koenen, 2013; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020).

The correlation matrix presented in Table 8 indicates a significant correlation between TNFα and ratings of childhood stress severity, r = .33, p = <.05. However, neither TNFα nor CRP were significantly correlated with any other IVs or the DV (depressive symptom severity) in the study. Although both TNFα, r = .53, p = <.05, and CRP, r = .60, p = <.001, were positively correlated with IL-6, they did not show statistically significant relationships with other markers of stress or depressive symptom severity. There were no significant relationships found between either

TNFα, $r = .21$, $p = .08$, or CRP, $r = .12$, $p = .32$, and BDI-II scores. The null findings with TNFα and CRP may be due to the substantial numbers of participants that demonstrated below-normal concentrations of these markers. Given these null findings, no exploratory analyses were conducted with these immune markers. While various studies have noted significant relationships with these markers and MDD (Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020), IL-6 remained the only influential immune marker on study outcomes in the present research.

Is Immune Activity Related to Neurovegetative versus Cognitive-Affective Symptoms of Depression?

To further investigate findings pertaining to the relationship between IL-6 and overall depressive symptom severity (Hypothesis 1.1), exploratory analyses were planned to investigate whether immune activity is related to specific depression symptom groupings. More specifically, the relationship of IL-6 with neurovegetative and cognitive/affective symptoms of depression (as measured by the BDI-II) were examined (Exploratory Hypothesis 1.2; see Table 8). These analyses revealed a significant relationship between IL-6 and neurovegetative symptoms, $r = .40$, $p = <.01$, and a significant relationship between IL-6 and cognitive-affective symptoms, $r = .35$, $p = <.01$. Both of these correlations were in the positive direction, suggesting that an increase in IL-6 was associated with an increase in cognitive/affective and neurovegetative symptoms. When directly comparing these two relationships, the difference between the correlations is .05, and the 95% CI of that difference is -.08 to .18. As such, it appears that IL-6 demonstrated associations with both symptom groupings (neurovegetative and cognitive/affective) that were roughly similar in magnitude. This is not surprising given how strongly correlated these two symptom groupings were in this sample, $r = .86$, $p = < .001$.

Does Neuroendoimmune Activity Mediate the Relationship between Specific Time Periods of Stress and Depression?

As described above, Hypothesis 2.1 examined whether neuroendoimmune activity mediated the relationship between cumulative life stress severity and depression. From here, the question of

whether neuroendoimmune activity mediates stress at specific time periods across the lifespan was examined (Exploratory Hypothesis 2.2). To test the relationship between specific time periods of stress and neuroendoimmune activity, three separate analyses were conducted using the same parallel mediation model presented in the primary results section above for Hypothesis 2.1, except the independent variable was changed to explore three distinct time periods of perceived stress—early life stress (≤12 years of age as measured by the STRAIN), past six-month stress (as measured by the STRAIN), and past month stress (as measured by the PSS). IL-6 and free cortisol were included as mediators, and depressive symptom severity as the dependent variable. The results are presented in Figure 9, represented as Exploratory Hypothesis 2.2a, 2.2b, and 2.2c to match each of the distinct time periods, respectively.

Childhood Stress Appraisals, Neuroendoimmune Activity, and Depression. The first model presented in Figure 9 (Hypothesis 2.2.a), examined whether neuroendoimmune activity mediates the relationship between stress experienced before the age of 12 and depressive symptoms experienced at the time of the study (i.e., during adulthood). Statistical results of this model are outlined below and summarized in Table 12. Examining first the total effect of Exploratory Hypothesis 2.2a, childhood stress severity was significantly associated with depressive symptom severity, $b = .50$, $p = <.01$ (step 5 [parameter c' + (ab1+ab2)]). The range of the 95% CI for this parameter are quite narrow, *CI* (.25, .75), indicating considerable certainty that the true slope would be comparable to the estimate of the unstandardized slope reported here in the majority of repeated samples. To examine the individual pathways in the model, the effect of childhood stress severity on IL-6 was significant, $b = .02$, $p = <.01$(path a1). In this case, the slope indicates that a one-point increase in childhood stress severity reported on the STRAIN is associated with a .02pg/mL increase in IL-6 concentration. Path a2 showed that the relationship between childhood stress severity and free cortisol was not significant, $b = -.11$, $p = .12$. As such, the null hypothesis is inconclusive, as no relationship between childhood stress severity and free cortisol was found in this sample. This is represented by a grey dotted line in Figure 9. Within simple mediation models, such a finding would negate the possibility of an overall mediation. However, in a parallel mediation model, both mediators can be examined, as outlined below, to fully describe the mediation process.

The relationship between IL-6 and depressive symptom severity was significant, $b = 5.17$, $p = <.05$ (path b1). The slope indicates that an increase of 1pg/mL in the mediator IL-6 results in a 5.17-point increase in BDI-II scores. Path b2 showed that the relationship between free cortisol and depressive symptom severity was significant, $b = -.54$, $p = <.05$. The slope indicates that an increase of 1nmol/L in free cortisol concentration results in a .54-point decrease in BDI-II scores. As such, when free cortisol concentrations are low, individuals report higher levels of depressive symptom severity.

Path ab1 of the mediation process, showed that the first mediator (IL-6), controlling for childhood stress severity, was not significant, $b = .09$, $p = .09$. The null hypothesis is inconclusive, as no relationship between IL-6 and childhood stress severity was detected in the sample. A similar conclusion can be drawn for path ab2 of the mediation process, because this parameter showed that the second mediator (free cortisol), controlling for childhood stress severity, was also not significant, $b = .06$, $p = .19$. However, parameter ab1+ab2 of the analyses combined the effect of both mediators and found a significant effect, $b = .16$, $p = <.05$, suggesting that collectively, there is a mediation effect when both mediators are included. Here, when there is a 1pg/mL increase in IL-6 *and* a 1nmol/L decrease in free cortisol, there is a .16-point increase in BDI-II scores. This indicates that there is a total indirect effect when the mediators are combined in the model.

The combined effect of the mediators significantly decreased the total effect of the model. When both mediators were added to the model, the total effect was reduced by about 32%. This indicates that the mediators reduced the overall effect of childhood stress on depressive symptom severity, but the mediation effect only occurred when both mediators were included in the model, and not each individually. As such, it can be concluded that neuroendoimmune activity mediated the relationship between childhood stress appraisals and depressive severity in adulthood.

Past Six-Month Stress Perception, Neuroendoimmune Activity, and Depression. The second model presented in Figure 9 (Hypothesis 2.2.b), examines whether neuroendoimmune activity mediates the relationship between levels of perceived stress measured over the past six months and depressive symptoms experienced at the time of data collection. Statistical results of this model are outlined below and summarized in Table 13. Examining first the total effect for

Exploratory Hypothesis 2.2b, past six-month stress severity was significantly associated with depressive symptom severity, b = .64, p = <.01 (step 5 [parameter c' + (ab1+ab2)]). To examine the individual pathways in the model, the relationship between past six-month stress severity and IL-6 (path a1) was not significant, b = .01, p = <.39. Path a2 showed that the relationship between past six-month stress severity and free cortisol was significant, b = -.19, p = <.01. In this case, the slope indicates that a one-point increase in past six-month stress severity reported on the STRAIN is associated with a .19nmol/L decrease in free cortisol concentration. Higher levels of six-month stress were associated with decreased levels of free cortisol.

The relationship between IL-6 and depressive symptom severity (path b1) was significant, b = 6.64, p = <.01. The slope indicates that an increase of 1pg/mL in the mediator IL-6 results in a 6.64-point increase in BDI-II scores. Path b2, showed that the relationship between free cortisol and depressive symptom severity was not significant, b = -.29, p = .15. As such, the null hypothesis is inconclusive as no relationship between free cortisol and depressive symptom severity was found in this sample.

Path ab1 of the mediation process, showed that the first mediator (IL-6), controlling for past six-month stress severity, was not significant, b = .04, p = .41. A similar conclusion can be drawn for path ab2 of the mediation process, which showed that the second mediator (free cortisol), controlling for past six-month stress severity, was also not significant, b = .06, p = .20. Parameter ab1+ab2 of the analyses combined the effect of both mediators and was not significant, b = .09, p = .14. As such there was no total indirect effect found to support mediation in this model. As such, there was insufficient evidence to suggest that neuroendoimmune activity mediates the relationship between past six-month stress perceptions as measured by the STRAIN and depressive symptom severity.

Current Perceived Stress, Neuroendoimmune Activity, and Depression. The third model presented in Figure 9 (Hypothesis 2.2.c), examines whether neuroendoimmune activity mediates the relationship between current (past month) levels of perceived stress and depressive symptoms experienced at the time of the study. Statistical results of this model are outlined below and summarized in Table 14. Examining first the total effect for Exploratory Hypothesis 2.2c, past month perceived stress severity was significantly associated with depressive symptom severity, b

= 1.60, p = <.001 (step 5 [parameter c' + (ab1+ab2)]). To examine the individual pathways in the model, the relationship between perceived stress severity and IL-6 was significant (path a1), b = .03, p = <.01. In this case, the slope indicates that a one-point increase in past month perceived stress severity reported on the PSS is associated with a .03pg/mL increase in IL-6 plasma concentration. Path a2 showed that the relationship between perceived stress severity and free cortisol was not significant, b = -.25, p = .06. As such, the null hypothesis is inconclusive as no relationship between past month perceived stress and free cortisol was found in this sample.

The relationship between IL-6 on depressive symptom severity (path b1) was not significant, b = 3.01, p = .09. As such, the null hypothesis is inconclusive as no relationship between IL-6 and depressive symptom severity was found in this sample. Path b2 showed that the relationship between free cortisol and depressive symptom severity was significant, b = -.34, p = <.05. The slope indicates that an increase of 1nmol/L in free cortisol concentration results in a .34 decrease in BDI-II scores. As such, when free cortisol concentrations are low, individuals report higher levels of depressive symptom severity.

Path ab1 of the mediation process, showed that the first mediator (IL-6), controlling for perceived stress severity, was not significant, b = .09, p = .16. The null hypothesis is inconclusive, as no relationship between IL-6 and past month stress severity was detected in the sample. A similar conclusion can be drawn for path ab2 of the mediation process, which showed that the second mediator (free cortisol), controlling for perceived stress severity, was not significant, b = .09, p = .16. However, parameter ab1+ab2 of the analyses combined the effect of both mediators and found a significant effect, b = .17, p = <.05, suggesting that collectively, there is a detectable mediation effect. Here, when there is a 1pg/mL increase in IL-6 *and* a 1nmol/L decrease in free cortisol, there is a .17-point increase in BDI-II scores. This indicates that there was a total indirect effect when the mediators are combined in the model.

The combined effect of the mediators significantly decreased the total effect of the model. When both mediators are added to the model, the total effect shrinks by about 10.6%. This indicates that the mediators did reduce the overall effect of past month perceived stress on depressive symptom severity, but a mediation effect only occurred when both mediators were included in the model, and not each individually.

Chapter 4
Discussion

Depression is the most widely experienced mental health condition on the planet, and one that exacts a massive toll on all affected (Lam, Kennedy, McIntyre, & Khullar, 2014; Reddy, 2010; Kessler, 2012; World Health Organization, 2018). Although enormous progress has been made in understanding the neurobiology and treatment of the disorder, this has not yet translated into a reduction in the astounding rates of people affected worldwide (Hidaka, 2012). This may be due, in part, to the heterogeneous nature of the disorder not yet accurately captured within current diagnostic nosology (Goldberg, 2011; Nelson, Strickland, Krueger, Arbisi, & Patrick, 2015; Patrick & Hajcak, 2016; Kotov et al, 2017), and it also may be due to the lack of a comprehensive theory that captures the disorder's complex nature and vast array of contributing factors (Irwin & Cole, 2011; Iwata et al., 2016; Slavich & Irwin, 2014; Miller & Raison, 2016).

The research presented in this dissertation sought to explore an integrative approach to the study of depression by adopting a biopsychosocial framework. Specifically, this dissertation investigated cognitive and psychological variables relate to stress-sensitive biological systems to examine whether such processes contribute to depressive symptom severity and specific symptom groupings. Nevertheless, it is important to consider that this work represents but a pixel of the whole picture when it comes to what truly comprehensive theories can offer (Iwata et al., 2016; Slavich & Irwin, 2014; Miller & Raison, 2016).

To explicate a biopsychosocial perspective on depression research, Chapter 1 of this dissertation began by providing a critical examination of current approaches to the classification and conceptualization of depression, followed by a comprehensive review of research on stress, neuroendoimmune activity, and depression. It concluded with a discussion of the psychological and cognitive factors involved in stress appraisal as they relate to neuroendoimmune activity and depression. Chapter 2 described the research methods used in this dissertation to investigate how immune activity might mediate the association between experiences of life stress and depression severity. Multiple models were tested, and exploratory analyses were carried out to examine the relevance of the timing of psychological experiences of stress, alternative immune markers, and theoretically-based symptom groupings as they relate to the broader research questions about the nature and relationships of these systems. Chapter 3 presented a detailed description of the

results of those analyses, which provided partial support for the research hypotheses. In this final chapter, the key findings from the research will be summarized, including their theoretical implications, followed by a discussion of the limitations of this work. Finally, future directions and clinical implications will be highlighted to advance the understanding of depression through the lens of biopsychosocial and dimensional perspectives on psychopathology.

4.1 The Translation of Experiences of Life Stress into Depression Through Neuroendoimmune Activity

The research described in this dissertation utilized a cross-sectional approach to investigate the extent to which neuroendoimmune activity mediates the relationship between stress experienced across the lifespan and depression severity in adulthood. This dissertation posed three broad questions that address how stress is impacted by cognitive and biological variables, and whether such processes contribute to specific depressive symptom groupings and severity ratings. Below, the key findings of this research are summarized and related back to the integrative theories of depression reviewed in Chapter 1.

Are Perceived Stress and Immune Activity Related to Current Depression Severity?

The first broad question posed in this research was whether proinflammatory immune activity and subjective experiences of stress are associated with depressive symptom severity. As hypothesized, the proinflammatory immune marker, IL-6, was significantly associated with depressive symptom severity. On average, individuals with higher levels of IL-6 reported higher levels of depression. It total, IL-6 accounted for 15% of the variance in depressive symptom severity in the sample. This finding is well supported by multiple studies, including several meta-analyses (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020). However, to date, the bulk of research has examined immune activity in relation to groups of individuals with MDD compared to healthy controls. Less research has examined individual differences in immune activity in relation to depressive symptom severity in a sample of individuals with varying levels of psychopathology and subclinical levels of depression, such as the sample

described in this dissertation (Zalli, Jovanova, Hoogendijk, Tiemeier, & Carvalho, 2016; Setiawan et al., 2015). Although these relationships say nothing about causality, these findings are in line with evolutionary and integrative theories of depression which incorporate macrophage perspectives that immune activity may contribute to depressive phenotypes by activating withdrawal and sickness behaviours adaptive for healing (Hart, 1988; Dantzer & Kelley, 1989; Smith 1991; D'Mello & Swain, 2017; Dantzer, 2018). If these withdrawal systems are overextended in chronically elevated states of immune activity, they may become maladaptive as one suffers from extended malaise, apathy, and low mood.

In addition to immune activity, all forms of perceived stress were associated with depressive symptom severity in this sample. Four distinct time periods of perceived stress were analyzed in the sample: cumulative life stress, childhood stress, past six-month stress, and past month stress. Past month stress demonstrated the strongest relationship with depressive severity, followed by past six-month stress, then cumulative life stress severity, and finally by childhood stress. The fact that recent (past month) levels of perceived stress are most strongly associated with levels of depression seems logical given that stress often precipitates and maintains an episode (Mazure, 1998; Harkness et al., 2010; Kendler, Thornton, & Gardner, 2000; Kessler, 2002; Monroe, Slavich, & Georgiades, 2014; Slavich, 2016). It also follows that experiencing an episode of depression will often increase levels of perceived stress when one is suffering from low mood and the functional impairment this can cause (Lam, Kennedy, McIntyre, & Khullar, 2014; Knight, Aboustate, & Baune, 2018). Although not modeled directly within this research, this bidirectional relationship may further contribute to the high correlation between these variables.

Does Neuroendoimmune Activity Mediate the Relationship Between Perceived Stress and Depression?

The second main question posed in this dissertation was whether neuroendoimmune activity mediates the relationship between psychological experiences of stress and depressive symptom severity and, if so, what time periods of stress are most relevant in terms of these outcomes. Although neither immune nor HPA axis activity mediated the relationship between stress and depression severity in isolation, the combined effect of neuroendoimmune activity (IL-6 + free cortisol) did mediate the relationship between childhood stress and depressive symptom severity,

as well as past month stress and depressive symptom severity. When both IL-6 and free cortisol were included in the model, the mediation effect was strongest for the condition of childhood stress. Neuroendoimmune activity, represented by increases in IL-6 and decreases in morning free cortisol, significantly mediated the relationship between childhood stress and depressive symptom severity.

That stress experienced in childhood was connected to depression outcomes in adulthood is an interesting finding in and of itself, and one that is well-established in the scientific literature (Green et al., 2010; Danese & Baldwin, 2017). However, the finding that neuroendoimmune activity mediated this relationship, and mediated this particular time period of stress more than any other, warrants some additional attention. Research strongly suggests that childhood stress and trauma are potent risk factors for depression in adulthood (Green et al., 2010; Danese & Baldwin, 2017). The possibility that neuroendoimmune disruptions may be one mechanism through which these vulnerabilities are sustained would have significant implications. Such findings may signal the influence of these stress-sensitive biological systems, especially during critical periods of development, on vulnerability to depression across the lifespan (Danese & Baldwin, 2017; Slopen, Kubzansky, McLaughlin, & Koenen, 2013).

Focusing in on the specific patterns of neuroendoimmune activity found within this study, it appeared that elevations in immune activity and deficits in morning levels of free cortisol contributed to the identified mediation effect between childhood stress and depression severity experienced in adulthood. Although cortisol and IL-6 activity were not significantly correlated, this pattern of high immune activity and low cortisol makes theoretical sense in relation to more severe levels of depression. One of the many functions of cortisol is to downregulate immune activity (Irwin & Cole, 2011; Ménard, Pfau, Hodes, & Russo, 2017; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). If cortisol levels are depleted, this hormone may fail to inhibit immune activity that, in turn, may ultimately contribute to depressed states (Raison & Miller, 2003; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015).

Patterns of elevated immune activity and deficient cortisol have been documented in relation to depression severity (Suarez, Sundy, Erkanli, 2015) and within the context of acute stress paradigms with MDD patients (Miller, Rohleder, Stetler, & Kirschbaum, 2005) in female

samples. It is well documented that stress-related alterations in the HPA axis response can disrupt the inhibitory feedback loop of cortisol to promote runaway immune activity when cortisol production or signaling is deficient (Raison & Miller, 2003; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019). The results of the present study indicate that lower levels of morning free cortisol contributed to this pattern. Of course, nothing can be said about the exact mechanism at play, but it may be the case that adrenal output of the hormone is depleted after extended periods of prolonged stress (Raison & Miller, 2003; Miller, Chen, & Zhou, 2007). This decreased glucocorticoid tone may result from decreased production of upstream precursors and signaling molecules such as CRH or ACTH, a deficit in hormonal production from the adrenal gland, or some other signaling disruption along the HPA axis (Raison & Miller, 2003). This makes sense given that this relationship was most robust within the context of childhood stress. If early life stress during critical periods of development leads to long-standing patterns of decreased glucocorticoid tone, and deficient cortisol output, immune pathways may become chronically elevated (Miller, Chen, & Zhou, 2007; Slavich & Irwin, 2014). These more chronic states of inflammation may then translate into depressive symptoms and vulnerability later in life, although much more research is needed to identify these mechanistic pathways.

Within cognitive models of depression, early life stress is assumed to instill negative self-schemas that are reactivated by stress experienced later in life (Beck, 1967; Hollon, 2010; Colodro-Conde et al., 2018). These negative self-perceptions then lead to negative thinking styles that predispose one to the ruminative processes and behaviours common to depression (Disner, Beevers, Haigh, & Beck, 2011). It is possible the same process occurs in parallel within the body. If the neuroendoimmune system is overactive during the formidable years of one's life, and this becomes a default state into adulthood, the dysregulation of neuroendoimmune processes may leave one more vulnerable to stress and depressive symptoms over the course of their life. As described in the section above, vulnerability in this case may be due to an underlying and over-extended immune response that activates biobehavioural symptoms of withdrawal that, overtime, manifest as symptoms of depression (D'Mello & Swain, 2017; Dantzer, 2018). From an evolutionary perspective, dampened cortisol production or signaling under prolonged periods of stress may have offered a survival advantage, as any immune and SNS responses would be left on alert to respond to ongoing and sustained threats (Iwata et al.,

2016; Slavich & Irwin, 2014; Miller & Raison, 2016). It may be the case that biological processes default to this state when early life environments are unpredictable and traumatic. If one endures significant hardship during childhood—is neglected, abused, bullied, and invalidated—and those experiences are imprinted in their biology, this searing of negative life experiences may increase susceptibly to mental health disturbances later in life. In the case of the relationship identified here, levels of childhood stress severity may predispose one to carry sustained alterations in neuroendoimmune activity that contribute to the severity of depressive symptoms in adulthood.

While there is substantial research to support hypocortisolism in depressed groups (Miller, Rohleder, Stetler, & Kirschbaum, 2005; Burke, Davis, Otte, & Mohr, 2005; Stetler & Miller, 2011), and particularly individuals who have experienced significant life stress (Miller, Chen, & Zhou, 2007), there is also contradictory evidence of hypercortisolism in the literature (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013). It is challenging to explain these mixed results; however, biological sex (Miller, Rohleder, Stetler, & Kirschbaum, 2005; Suarez, Sundy, Erkanli, 2015) and the type and timing of stress and stress biomarker measurement are important factors to consider (Woda, Picard, & Dutheil, 2016; Steptoe & Serwinski, 2016). It may be the case that biological females demonstrate deficient morning cortisol levels, and especially within the context of stress and depression (Foley & Kirschbaum, 2010; Miller, Chen, & Zhou, 2007; Suarez, Sundy, Erkanli, 2015; Miller, Rohleder, Stetler, & Kirschbaum, 2005; Ménard, Pfau, Hodes, & Russo, 2017).

Does Perceived Stress and Immune Activity Mediate the Relationship Between Cognitive Control and Depression?

The third central question posed in this dissertation was whether stress perceptions and immune activity mediate the relationship between cognitive control and depression outcomes. Contrary to expectations, there were no detectable relationships found between metrics of cognitive control measured by the CWIT and any other study variables, including stress appraisals, immune activity, and depression. This could be due to the limited range and variability of CWIT scores obtained in the study sample. Indeed, test scores did not deviate markedly from normatively-referenced scores and were in fact slightly higher. At the very least, this provides some

indication that motivational disruptions related to low mood were not present and did not impede performance outcomes on this measure. This may signal that the participants were highly motivated to participate in the research.

However, cognitive control is commonly disrupted in individuals with MDD compared to non-psychiatric controls (Rock, Roiser, Riedel, & Blackwell, 2014; Snyder, 2013), and deficits in cognitive control have predicted depressive symptoms in other samples (Pe, Brose, Gotlib, & Kuppens, 2015). Although there is research evidence suggesting that deficits in cognitive control, including inhibition, updating, and shifting are detectable in depressed groups (Snyder, 2013), the CWIT did not produce discernable differences in relation to levels of depression in this sample. In the present study, condition 4 of the CWIT was selected for analysis because it places high demands on cognitive control (Homack, Lee, & Riccio, 2005; Lippa & Davis, 2010), and also because it is one of a very few previously utilized measures of cognitive control within the context of immune activity (Krogh et al., 2014; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016). However, this measure may not be sensitive enough to capture cognitive deficits in relation to depression severity, even though this sample reported a significant range of depressive symptoms (i.e., 0 – 58 on the BDI-II).

Given that cognitive control measures one's ability to flexibly organize and manipulate information to achieve a goal (Friedman & Miyake, 2017), the present study assumed that individuals with higher levels of cognitive control may be better equipped to manage life stressors (Compton, Hofheimer, & Kazinka, 2013), may rate life stressors as less severe (Yamakawa et al., 2009; Aschbacher et al., 2012), and in turn demonstrate lower stress-related immune activity and depression (Yamakawa et al., 2009; Aschbacher et al., 2012; Pe, Brose, Gotlib, & Kuppens, 2015). However, in this sample, cognitive control was not related to stress appraisals measured across the lifespan, or more currently over the past month. While there was no evidence to support the assumption that cognitive control may influence stress management and appraisals in the present study, it may be the case that emotionally-valanced tasks, or paradigms designed to induce stress, are more powerful at detecting cognitive-related deviations in stress management that could contribute to biological disruptions and depression (Yamakawa et al., 2009; Aschbacher et al., 2012; Joormann & Gotlib, 2010; Joormann & Vanderlind, 2014;

Pe, Brose, Gotlib, & Kuppens, 2015; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016).

Research that has examined executive functioning under acute states of stress, such as the TSST, has demonstrated links to lower physical health complaints (Shield, Moons, & Slavich, 2017) and dampened immune activity (Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016). Importantly, these results were not identified under basal conditions, but only when participants were tested under pressure (Shield, Moons, & Slavich; 2017). In another study, higher levels of cognitive control under high stress conditions were found to predict depressive symptoms four and 12 months later (Pe, Brose, Gotlib, & Kuppens, 2015). This is important, as it may be the case that cognitive capacities influence stress appraisals and stress-related biological responses when stress is high, but these effects are less discernable when tested in basal conditions (Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016), as was the design of the present study. Instead, stress induction paradigms may provide more ecologically valid approaches to study nuanced aspects of cognitive capacities related to stress management and biological outcomes. Real-time assessments of both cognitive capacities and biological responses under states of acute stress may capture more subtle elements of these relationships (Kudielka, Hellhammer, & Kirschbaum, 2007; Foley & Kirschbaum, 2010). These are important lines of inquiry because they may help to uncover why some individuals are more vulnerable to the harmful impacts of stress on depression (Pe, Brose, Gotlib, & Kuppens, 2015; Rock, Roiser, Riedel, & Blackwell, 2014).

Despite the data indicating that cognitive impairment is common in depression (Trivedi & Greer, 2014; Rock, Roiser, Riedel, & Blackwell, 2014), in addition to ample evidence that inflammation is strongly associated with cognitive deficits in other populations (e.g., aging populations; Weaver et al., 2015; Solvang et al., 2019), there is limited research investigating the relationship between inflammation, cognition, and depression. In addition to the CWIT task described above, other cognitive measures that have been examined in relation to depression and immune activity include measures of short- and long-term memory using the Auditory-Verbal Learning Test (AVLT) and the Controlled Oral Word Association Test (COWAT) (Lieb et al., 2006), as well as the Wechsler Memory Scale-Revised (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011). For instance, in MDD patients, IL-6 levels were found to be positively correlated with

depressive symptoms and negatively correlated with immediate verbal recall, and delayed verbal recall on the logical memory subtests of the Wechsler Memory Scale-Revised, even after controlling for confounds such as age and BMI (Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011). Although it is beyond the scope of the present research, immune activity appears to reliably impact hippocampal systems by disrupting cellular processes, such as long-term potentiation and neurogenesis (Yirmiya & Goshen 2011; Frank, Watkins, & Maier, 2013; Ménard, Pfau, Hodes, & Russo, 2017). Given these findings, it could be the case that memory-related capacities are susceptible to the impacts of immune activity (Elderkin-Thompson, Irwin, Hellemann, & Kumar, 2012). Whether such processes contribute to depressive states and symptoms remains a question for further investigation.

4.2 Limitations

The research approach described in this dissertation has several limitations that should be considered when interpreting the findings of the investigation. First, the study was likely underpowered to fully address many of the research questions posed in the dissertation. Although the relationships that were detected and discussed above warrant consideration, they should be interpreted with caution. With increased sample size, mediation effects that do exist would be more reliably detected (Wong, 2016; Rockwood & Hayes, 2020). As a result of the limited sample size, the decision not to correct for multiple comparisons was made to bias toward controlling type II versus type I error rates. Although it would be ideal to correct for false discovery rates, the findings of this study should be considered a preliminary investigation to examine the utility of using dimensional methodologies to investigate the relationships of perceived stress and immune markers with depression. Although the mediation models did not detect the expected stress-immune-depression pathway, the pattern of neuroendoimmune biomarkers and the correlations to stress and depression, provide preliminary seed data that may be more fully realized with larger sample sizes. Similarly, although medication use, BMI, and menstrual cycle were included as covariates in the primary analysis between IL-6 and depressive symptom severity, the *a priori* decision not to include covariates in the mediation models was made based on power estimates. Future research incorporating this type of mediation analysis should aim for larger sample sizes to ensure that the models are sufficiently powered to allow for

appropriate statistical adjustments and interpretations (Schoemann, Boulton, & Short, 2017; Liu & Wang, 2019).

In the present research, additional immune markers either did not show consistent relationships with depression or were below the limits of detection. The markers examined in this study are commonly found in depressed samples (Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020), and more advanced biometrics will help to uncover the true nature of immune-depression relationships. This study utilized the Bioplex 200 multiplex immunoassay system (BioRad, USA). However, there are alternative assay kits that may permit more fine-grained analysis of markers at especially low concentration levels (e.g., Meso Scale Discovery Assay Services, Ella Automated Immunoassay Systems; Yeung et al., 2016; Dysinger, Marusov, & Fraser, 2017; Aldo, Marusov, Svancara, David, & Mor, 2016). Although these systems were not available to the present research, future studies may select methodological approaches more appropriately suited to molecules at these low quantities in both non-psychiatric and depressed samples. Furthermore, advanced functional genomic techniques that examine immune response genes may provide more fine-grained transcriptional profiles that more reliably capture the activation of the immune system (Slavich & Irwin, 2014; Almeida & Turecki, 2017). Additionally, although the current study benefitted from measuring and analyzing the bioavailable free fraction form of cortisol in a fasted state between 8a.m. and 9a.m. (to control for food-related and diurnal effects on cortisol), future research would benefit from analyzing the cortisol awakening response in the first 30 minutes after waking, or diurnal patterns across the day, to provide a more reliable assessment of the pattern of cortisol in samples with high levels of current and historical stress and depression (Steptoe & Serwinski, 2016; Woda, Picard, & Dutheil, 2016; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Dienes, Hazel, & Hammen, 2013).

The present study examined cognitive control using the CWIT because it is one of a very few previously utilized measures of cognitive control studied within the context of immune activity (Krogh et al., 2014; Shields, Kuchenbecker, Pressman, Sumida, & Slavich, 2016). However, this measure may not be sensitive enough to capture cognitive deficits in depressed samples (Rock, Roiser, Riedel, & Blackwell, 2014; Grahek, Shenhav, Musslick, Krebs, Koster, 2019). Other well-validated measures of cognitive control in depressed samples include the Go/No-go task, n-

back task, Tower Test, Trail Making Test (Part B), Digit Symbol Substitution Test, and WCST (Snyder, 2013). Future research would be well-advised to explore alternative models and metrics of this capacity to fully explore whether cognitive control influence stress appraisals and immune disruptions in stress-susceptible populations.

An additional limitation of the present study is that the methods were cross-sectional in nature, and thus causality could not be inferred. Despite the substantial research literature supporting an exchange of information between the immune system and HPA axis (McEwen, Gray, & Nasca, 2015; Ménard, Pfau, Hodes, & Russo, 2017), statistically significant correlations between IL-6 and free cortisol were not detected in this study. This null finding simply indicates that there was no notable correlation between these markers; it does not, however, reveal anything about whether these two systems are intricately linked or causally influence one another. As much as it may be the case that inhibited levels of cortisol contribute to elevations in immune activity (Raison & Miller, 2003; Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013; Suarez, Sundy, Erkanli, 2015; Perrin, Horowitz, Roelofs, Zunszain, & Pariante, 2019)—as indicated by the pattern in the data—an overt signal is not provided in this dataset. Ideally, neuroendoimmune markers and depression levels would be examined at multiple time points to examine the pattern of neuroendoimmune fluctuations and to determine whether changes in neuroendoimmune activity directly influence depression outcomes (Huang et al., 2019; Chu et al., 2019). While the present study analyzed free cortisol and immune biomarkers in basal states, stress-induction paradigms offer superior conditions to truly capture the relationships between these stress-sensitive systems and depression (Burke, Davis, Otte, & Mohr, 2005; Miller, Rohleder, Stetler, & Kirschbaum, 2005). Furthermore, an important feature of depression is that it is most commonly episodic in nature (De Zwart, Jeronimus, & De Jonge, 2019). This presents the opportunity to follow individuals in and out of remission from depressive episodes to more accurately examine the nature of these dynamic systems.

Lastly, the analyses of this research relied on subjective reports of stress severity that are based upon retrospective accounts of stress experienced across the lifespan. Although the STRAIN has demonstrated excellent test-retest reliability (Slavich & Shields, 2018), these reports are not immune to the fallibility and mood-susceptibility of human memory. These reports are subject to simple forgetting, non-disclosure, and reporting biases due to current levels of negative affect

and depression (Gorwood, Corruble, Falissard, & Goodwin, 2008). However, when self-reported stress and trauma accounts from childhood have been cross-examined with external records (e.g., police records, court records, school records), stress reports of moderate to severe stressors (as assessed by the STRAIN) have shown high reliability of reporting (Hardt & Rutter, 2004; Danese & Baldwin, 2017; Slavich & Shields, 2018). While the types of stressors may increase the reliability of the STRAIN reports, these represent a subjective report, and it is likely that results will have been influenced by mood states, which may artificially increase the strength of the correlations of some of the variables (e.g., depressive symptom severity and stress scores).

4.3 Future Directions

The field of clinical neuroendoimmunology is in its infancy and there is much to be excited about in this emerging area of study. Conducting research at the intersection of so many disciplines is no easy task, but one that will undoubtedly move research on human health forward. However, to do so will require comprehensive theoretical models, savvy methodological designs, and advanced analytic approaches that can more accurately capture the complex nature of human experience, which includes a continuum of mental health states from pathology to resiliency.

As the field advances in the direction of dimensional assessments of psychopathology, more finely developed dimensional measures will be needed to capture symptom presentations. To date, dimensional measures of depressive symptoms (such as the BDI-II and HAMD) are broad and are not specifically targeted to analyze unique symptom presentations (Ruscio & Ruscio, 2000; Fried & Nesse, 2015b). Although the present research attempted to examine specific symptom groupings of depression in relation to immune activity (e.g., neurovegetative versus cognitive/affective symptoms), research also points toward specific symptoms, such as anhedonia (Capuron et al., 2012; Michopoulos, Powers, Gillespie, Ressler, & Jovanovic, 2017; Felger et al., 2016) and psychomotor impairment (Goldsmith et al., 2016) as being two particularly immune-susceptible symptoms. While the most commonly used measures of depression—the BDI-II and HAMD—assess these symptoms with a dimensional score (Beck, Steer, & Brown, 1996; Hamilton, 1960), these measures were not developed for the examination of single items and may not provide sufficiently detailed assessments of individual symptoms or

symptom groupings (Fried et al., 2016). Instead, they offer a single-item question with a range of 0 to 3 or 4 for each symptom. While these inventories are superior to cut scores (i.e., present or absent—as items on the SCID-5 are tallied), they may not sufficiently flesh out the nuances of specific symptom dimensions to accurately capture the range of variability between individuals or fluctuations in symptoms over time (Vrieze et al., 2014; Rizvi et al., 2015; Fried et al., 2016). Individual symptom inventories may be needed to explore symptom dimensions with more specificity, as is the thrust of dimensional approaches to psychopathology (Vrieze et al., 2014; Rizvi et al., 2015; Patrick & Hajcak, 2016; Kotov et al, 2017).

Writing about dynamic systems, such as the neuroendoimmune system is, at times, both a delight and a challenge. Because of the fluid and reciprocal nature of these systems, it becomes difficult to capture their true nature in both language and in scientific modeling. The core analyses implemented in this research relied upon linear regression and structural equation modeling, which operate on the assumption that variable relationships are unidirectional (Edwards & Lambert, 2007; Hayes, 2017). However, there is strong research evidence to suggest that relationships between all of the relevant variables included in this dissertation are multidirectional and fully integrated (Fried, Epskamp, Nesse, Tuerlinckx, & Borsboom, 2016; Dantzer, 2018; Leonard, 2018). In truth, such a dynamic system would be well-suited to statistical network approaches (Miho, Roškar, Greiff, Reddy, 2019). In network analyses, models are compiled from the causal interplay between symptoms (Borsboom & Cramer, 2013; Fried, Epskamp, Nesse, Tuerlinckx, & Borsboom, 2016). In these models, variables are assumed to influence one another and to cluster into patterns across time and individuals. Instead of assuming that stress causes neuroendoimmune activity which leads to depression, as was the case in the present study, these variables, and the individual symptoms of depression, are likely to reinforce one another to create a specific network pattern. In network models, variables are assumed to cluster together because they are causally related and contribute to one another, not because they are assumed to be caused by some latent variable (i.e., MDD) (Borsboom & Cramer, 2013; Beard et al., 2016). Thinking about this within the framework of the present study, experiences of stress and trauma, both past and present, likely alter and activate neuroendoimmune pathways that contribute to common symptoms of depression, and these symptoms likely also feed back into experiences of stress and biological responses. In this way, the interrelated network of variables informs what the complex experience of depression is, not

that these variables predict depression, or are caused by it (Borsboom & Cramer, 2013). Statistically speaking, within network analyses individuals no longer need to fit into a model, rather they compile their own network that can be amassed with other individuals sharing a similar experience to more accurately capture common elements of psychopathology and mental health. Although these kinds of analytic approaches can be more challenging to orchestrate with clinical samples because they require much larger sample sizes (Epskamp, Borsboom, & Fried, 2018), future research in the field of clinical neuroendoimmunology would be well-advised to adopt such approaches.

Contrary to dimensional initiatives, medical patient groups have historically been excluded from research studies that examine the relationship between immune activity and depression (Raison & Miller, 2011; Howren, Lamkin, & Suls, 2009; Dowlati et al., 2010; Liu, Ho, & Mak, 2012; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020), as was the case in the research presented in this dissertation. This methodological approach is typically employed to assess whether there is an organic relationship between immune activity, depression, and other study variables, and to eliminate confounds related to disease states (Miller, Buckley, Seabolt, Mellor, & Kirkpatrick, 2011; Modabbernia, Taslimi, Brietzke, & Ashrafi, 2013; Grassi-Oliveira et al., 2009; Foley & Kirschbaum, 2010). In the present study, immune activity was hypothesized to be activated by states of stress to then increase levels of depressive symptom severity. Although no stress-immune-depression pathways were uncovered, there was a significant relationship found between childhood stress and immune activity, as well as past month stress and immune activity. These are important findings, and ones that would be difficult to interpret in a medical patient sample (i.e., how much of immune activity was caused by states of stress, and how much was caused by the disease itself) (Grassi-Oliveira et al., 2009; Foley & Kirschbaum, 2010). While psychological experiences of stress may be one causal factor of immune activity, and a relevant one in the depression literature (Slavich & Irwin, 2014; Seo et al., 2017), the fact that disease states may also contribute to immune activation to bolster states of depression is equally worthy of study, and one that should not be ignored (Cohen et al., 2012; Leonard, 2007; Slavich, 2016). The fact that medical patient populations carry high levels of inflammatory burden and rates of clinical depression at 50% is an urgent state of affairs (Irwin & Miller, 2007), and one that could further inform immune-depression research. The contribution of medical disease to states of immune activity and depression may be one of the major sources

of depressive phenotypes (Berk, 2013). Broadening depressed clinical populations to include individuals with medical disease could be an important contribution to the field.

In the present study, it was hypothesized that individual differences in cognitive control would be associated with stress management capacities and appraisals that may contribute to biological responses and depression outcomes. In contrast to the model presented here, there exist at least two competing models for consideration in future research. The first competing model examines whether cognitive control moderates the relationship between stress, immune activity, and depression outcomes. This is the approach adopted by many stress-induction paradigms (Yamakawa et al., 2009; Aschbacher et al., 2012; Shield, Moons, & Slavich, 2017; Shields, Kuchenbecker, Pressman, Sumida, and Slavich, 2016). Although it would have been ideal to test such a competing model, the present research did not implement a stress-induction paradigm, and instead focused on the mediating role of internal psychological stress appraisals in relation to cognitive control. Although it would have been interesting to test a competing mediated-moderation analysis (where stress appraisals depend on cognitive control—the moderator—and this interaction changes immune mediated pathways associated with depression), based on G*Power analysis, to detect a medium effect size with a minimum of three predictors, an alpha of .05 and power of .90, a sample of 130 participants would be required to sufficiently address moderating factors within the present study. Future research should aim for double or triple the sample size reported here to consider these possible relationships.

A second model worthy of future investigation would be one in which stress appraisals and immune activity predict cognitive deficits, including deficits in cognitive control (and possibly memory), to contribute to depression outcomes (Bortolato, Carvalho, Soczynska, Perini, & McIntyre, 2015). There is ample evidence to suggest that cognitive capacities are impacted by immune activity (Maes et al., 2009), in addition to brain regions, such as the hippocampus, that are especially sensitive to imbalances of glucocorticoid and immune proteins (Ben Menachem-Zidon et al., 2008; Goshen et al., 2008). It is possible that immune system disruptions directly impact cognitive performance, and if so, this would be a relevant pathway to explain many of the cognitive disruptions common to depressed states that also predict poor outcomes and depression over time (Pe, Brose, Gotlib, & Kuppens, 2015; Rock, Roiser, Riedel, & Blackwell, 2014). Although the research presented here did not find associations between immune activity and

cognitive control to support such an investigation, expanding cognitive testing to capture a range of cognitive deficits in populations that carry high levels of inflammation is well-supported by the preclinical literature (Cunningham et al., 2009; Goshen & Yirmiya, 2009). Future research would be well-advised to continue to explore models of cognitive impairment in the presence of inflammation. In addition to immune-cognition research already underway in aging populations, depressed populations may be well-suited to this type of investigation given the reliable elevations in immune activity (Holmes et al., 2018; Maes et al., 2009; Grassi-Oliveira, Bauer, Pezzi, Teixeira, & Brietzke, 2011; Krogh et al., 2014).

There remains a tremendous amount to learn about the human spectrum of suffering and resiliency. Although the research presented in this dissertation only captured the narrowest of views of some of the most complex models being presented from evolutionary and integrative theorists (Iwata et al., 2016; Slavich & Irwin, 2014; Miller & Raison, 2016), it is still remarkable to be able to capture any element of such a complex system. It is exciting to think about what more advanced metrics and methodological approaches will bring to these emerging and more expansive approaches to conceptualizing the nature of mental and physical health as a whole.

4.4 Clinical Implications

There is something both elegant and horrific about the concept that our life experiences are written in our body at the level of our DNA, our biology, physiology, and ultimately our behaviour. Elegant in that we are, to some degree, a living breathing construct of our lives—shaped and informed, and nurtured by it. Horrific, on the other hand, when one's life experiences are riddled with mistreatment, neglect, disaster, and chaos. The translation of the social and physical environment into the internal organism is studied at the most fundamental level in epigenetics (Nöthling, Malan-Müller, Abrahams, Joanna Hemmings, & Seedat, 2019); however, as was highlighted throughout this dissertation, the fluid nature of our reality, and the embodiment of our lives, may be pronounced at the level of our biology and behaviour to serve as suitable intermediary targets for assessment and treatment.

Dimensional analyses that move past categorical boundaries may provide new insights into the nature of biological components of psychopathology and could radically inform treatment (Cuijpers, Ebert, Acarturk, Andersson, Gerhard, Cristea, 2016; Patrick & Hajcak, 2016; Kotov et

al, 2017; Wright & Woods, 2020). To this end, examinations of the dynamics of the neuroendoimmune system may lead to the identification of relevant targets for treatment, especially as they relate to vulnerability created by stress (Slavich & Irwin, 2014; Miller & Raison, 2017). Importantly, to address stress-linked neuroendoimmune disturbances will likely require treating the individual as a whole through psychological, medical, and lifestyle approaches in combination with social interventions to alter environmental circumstances that maintain elevated levels of stress (Opie et al., 2017; Harvey et al., 2018; Moriarty et al., 2020; Taylor & Holscher, 2020). Such advanced integrative approaches to care may ultimately be critical to curbing rates of depression (Norcross & Lambert, 2019; Wright & Woods, 2020).

Existent intervention approaches can serve as a tool to learn more about the mailability or stubborn persistence of the themes written in our biological and genetic code. For instance, there is already work underway to examine whether cognitive shifts that may result from clinical interventions, such as CBT, or mindfulness-based interventions, may interact with biological components of the stress response and depressive symptoms (Jacobs et al., 2011; Creswell et al., 2012; Gallegos, Lytle, Moynihan, & Talbot, 2015; Rådmark, Sidorchuk, Osika, & Niemi, 2019). Although the research presented here did not have the opportunity to examine intervention-based changes in biological disruptions common to depression, adopting this kind of protocol to a pre/post intervention design would be incredibly valuable, and could largely inform theory. This may help to determine whether top-down shifts in dysfunctional thinking and perception alter one's internal biology; and if not, whether these persistent neuroendoimmune disruptions contribute to vulnerability even after intervention, as one succumbs to further relapse (Raison, 2016; Osimo et al., 2020; Fischer, Strawbridge, Vives, & Cleare, 2017). If current interventions work to rewrite or edit some of the dark and dreary biological transcripts of our lives, identifying individual response to treatment at the level of one's biology may help to identify why some individuals respond to treatment while others continue to fall through the cracks.

Substantial progress has been made to advance psychological intervention, and there is much to be optimistic about in the future of treatment (Norcross & Lambert, 2019; Flynn, Moran, Rash, & Campbell, 2019; Wright & Woods, 2020). However, the field can devote all of its time and energy developing the most advanced intervention, and this is still not likely to address the staggering rates of depression and other mental health pathologies that follow in its wake

(Reddy, 2010; Hidaka, 2012; Kessler, 2012; Hung, Liu, & Yang, 2019). If we are to address the global "pandemic" that is the ongoing mental health crisis, more needs to be done to discover a metaphorical vaccine. To focus on treatment without equal, if not more, attention on prevention is putting the cart-before-the-horse, symbolically speaking (Ormel, Cuijpers, Jorm, & Schoevers, 2019). Importantly, integrative theory and research, such as that presented here, can inform preventative efforts in a substantial manner. At its most basic level, appreciating the significance of early life stress on long-term health, and the way in which the branding iron may be cast at a young age, is a remarkable demonstration of biopsychosocial principles in action. If the stresses of life are imprinted in our peripheral and central biology, and especially so during the most formative years of development, responding with early intervention, care, support, and protection to foster healing will be paramount to reducing the future burden of depression on individuals and society (Bartlett & Smith, 2019; Ormel, Cuijpers, Jorm, & Schoevers, 2019; Tabone, Rishel, Hartnett, & Szafran, 2020). Using integrative perspectives to inform policy change to support families and society—and to put the horse-before-the-cart—may be powerful in adverting the full calamity of the mental health crisis and pandemic on our hands.

4.5 Conclusion

In summary, the research presented in this dissertation provides evidence to support a link between stress, neuroendoimmune activity, and depression. All time periods of perceived stress were related to depressive symptom severity, and childhood stress and stress experienced in the past month were each related to both immune activity and depression severity. In turn, immune and HPA axis disruptions were associated with depression and mediated the relationship between childhood stress and current levels of depression in adults. Importantly, these findings were identified in a transdiagnostic sample of participants who carried diagnoses of many stress-linked disorders (e.g., MDD, BPD, and PTSD). This signals the possibility that there are common neuroendoimmune pathways that may lead to these biobehavioural relationships, although much more research is needed. Research adopting transdiagnostic and dimensional approaches is important to advance the understanding of the neurobiology of depression. This may help to elucidate common disruptions that exist across diagnostic groups to contribute to symptom presentations, including individuals whom do not meet criteria for a diagnosis but experience some level of depressive symptoms. Overall, this study provides new insights into potential

pathways among stress, the neuroendoimmune system, and depression, shedding light on how early life stress may be translated into depression across the lifespan.

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Tables

Biomarker	Normal Range	Function
CRP	0.8 – 3.0 mg/L	A pattern recognition receptor secreted by the liver in response to IL-6 to remove damaged or dying cells and bacteria.
IL-6	≤ 1.8 pg/mL ***	Described as both a pro- and anti-inflammatory molecule, activated by cortisol production, modulates plasma cell development and fever response via the hypothalamus.
TNFα	3.9 – 18.5 pg/mL***	Proinflammatory molecule that suppresses appetite and initiates fever response via the hypothalamus, increases CRH production, induces swelling, redness, pain, shock.
IL-1β	<0.3 – 1.4 pg/mL*	Proinflammatory molecule involved in fever response, cell proliferation, differentiation, and apoptosis.
Cortisol	193 – 690 nmol/L**	Steroid hormone produced by the adrenal gland to mobilize the stress response, suppress the immune system, and regulate blood sugar and metabolism.
Free cortisol	NCCRRE***	
ACTH	10 – 50 pg/mL**	A polypeptide tropic hormone produced by the pituitary gland produced in response to stress to increase cortisol production.

Table 1. Function and Ranges of Stress Biomarkers. CRP, C-reactive protein; IL, interleukin; TNF-α, tumor necrosis factor-α; ACTH, adrenocorticotropic hormone; pg, picograms; ug, micrograms; dl, deciliter; nmol/L, nanomoles per litre. *May often be below the lower limit of detection in healthy individuals because it is very low or not produced (Kleiner et al., 2013); **Indicates morning sample ranges; ***No certified clinical reference range established; ranges provided in the table come from published data on healthy controls (Todd, Simpson, Estis, Torres, & Wub, 2013; Sekiyama, Yoshiba, & Thomson, 2008; Kleiner, Marcuzzi, Zanin, Monasta, & Zauli, 2013; Arican, Aral, Sasmaz, Ciragil, 2005; Mayo Clinic Laboratories, 2020)

Factors to measure	*Methodological recommendations*
Biological sex	Recruit samples of the same sex and age range
BMI	Exclude individuals with substance use disorders
Age	Measure biomarkers at the same time of day
Medication use	Exclude moderating medical conditions
Exercise	Limit excessive exercise or monitor activity level
Sleep	Prescribe or monitor sleep habits and quality
Menstrual cycle and oral contraceptiv	Sample during follicular phase of menstrual cycle

Table 2. Biobehavioural Factors to Consider in Research Methodology. Adapted from Graham-Engeland, Smyth, & Engeland (2015).

Measure	Instrument	Variable
Consent	Informed Consent Form	Ethics
Phone Screen		
	Eligibility Interview	Inclusion/Exclusion
Self-report		
Depressive symptoms	Beck Depression Inventory, 2nd Ed. (BDI-II)	DV[a]
Perceived stress	Stress and Adversity Inventory for Adults (STRAIN)	IV[b]
	Perceived Stress Scale (PSS)	IV[b]
Clinical Interview		
Demographics	Demographic history interview	Descriptive/Covariate
MDD	Hamilton Depression Rating Scale (HAMD)	Descriptive
	Structured Interview for DSM-5 (SCID-5)	Descriptive
Suicide Risk Assessment	Suicide Operating Procedure	Ethics
Neuropsychological Tests		
Reading Comprehension	Wide Range Achievement Test 4 (WRAT)	Control
Inhibition	Colour Word Interference Test	IV[b]
Physical Dimensions		
Weight	Scale	Covariate
Height	Self-report	Covariate
Biological Markers		
Inflammatory Markers		
	TNFα	IV
	IL-6	IV
	IL-1β	IV
	CRP	IV
	Free & Total Cortisol	IV
Debriefing	Debriefing Form	Ethics

Table 3. Schedule of Measurements. [a]Dependent variable; [b]Independent variable.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1	Direct effect				
i	c'				
2	IV & mediator relationship				
i	a1				
ii	a2				
3	Mediator & DV relationship				
i	b1				
ii	b2				
4	Indirect effects				
i	ab1				
ii	ab2				
iii	ab1+ab2 (indtot)				
5	Total effect (tot)				
i	c' + (ab1+ab2)				

Table 4. Step Method Adapted for Parallel Mediation Models. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path. IV = independent variable; DV = dependent variable.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1	Direct effect				
i	c'				
2	IV and mediator relationship				
i	a1				
ii	a2				
3	Mediator & DV relationship				
i	b1				
ii	b2				
4	Relationship between mediators				
i	d				
5	Indirect effects				
i	ab1+ab2+a1db2 (indtot)				
6	Total effect (tot)				
i	c' + (ab1+ab2+a1db2)				

Table 5. Step Method Adapted for Serial Mediation Models. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path. IV = independent variable; DV = dependent variable.

Characteristic	Quantity and standard deviation
Age	28.7 (9.1)
Sex (female)	100%
Education Levels	
High School or GED	12.7%
College or University	61.9%
Graduate/Professional school	23.8%
<High School	1.6%
Ethnicity	
Caucasian	53.9%
Chinese	11.1%
South Asian	6.3%
Black	3.2%
Southeast Asian	3.2%
West Indian	3.2%
Japanese	1.6%
Korean	1.6%
Mixed race	12.6%
Other	3.2%
Income	
<$5000	3.2%
$5000-$10,000	1.6%
$10,000-$15,000	3.2%
$15,000-$20,000	4.8%
$20,000-$25,000	3.2%
$25,000-$35,000	9.5%
$35,000-$50,000	23.8%
$50,000-$75,000	3.2%
$75,000-$100,000	12.7%
$100,000-$150,000	19.0%
$150,000-$200,000	4.8%
>200,000	3.2%
Employment	
Full-time employment	33.3%
Part-time employment	30.2%
Unemployed	9.5%
Student	33.3%
Disabled	6.3%
Homemaker	1.6%
BMI	
Underweight (<18.5)	1.6%
Normal (18.5 – 24.9)	55.6%
Overweight (25.0 – 29.9)	27.0%
Obese (>30.0)	15.8%

Table 6. Participant Demographic Information Collapsed Across Recruitment Groups (n = 63).

Characteristic	Quantity and standard deviation
Current MDD	63.5%
2 previous episodes of MDD	17.5%
≥3 previous episodes of MDD	27.0%
Mean number of MDD episodes	3.7 (6.9)
PDD	19.0%
HAMD Scores	
Normal (≤7)	31.7%
Mild (8 – 16)	11.1%
Moderate (17 – 23)	9.5%
Severe (≥24)	47.6%
BPD	31.7%
PTSD	15.9%
Current alcohol use disorder	6.3%
Past alcohol use disorder	12.7%
Current substance use disorder	1.6%
Past substance use disorder	17.5%
Current nicotine/tobacco use	
Daily cigarette use	3.2%
Daily vape use	1.6%
Age of first contact with mental health services	18.3 (8.2)
Past hospitalization	33.3%
Suicide attempts	31.7%
Reported family history of mental illness	61.9%
Previous or current psychotherapy	71.4%
Currently taking psychoactive medication	50.8%
Antidepressant	46.0%
Antipsychotic	15.9%
Tranquilizer	11.1%
Stimulant	7.9%
Anticonvulsant	4.8%
Antiparkinsonian	3.2%
Sedative	3.2%
Cannabinoid (prescribed)	3.2%
Opioid antagonist	1.6%
Opioid (prescribed)	1.6%
Contraceptives	33.3%

Table 7. Diagnostic and Clinical Characteristics Collapsed Across Recruitment Groups (n = 63). HAMD = Hamilton Depression Rating Scale; MDD = major depressive disorder; PDD = persistent depressive disorder; OCD = obsessive compulsive disorder; BPD = borderline personality disorder; PTSD = posttraumatic stress disorder.

Variable	*M*	*SD*	1	2	3	4	5	6	7	8	9	10	11	12	13
1. BDI	25.2	17.0	1	.97***	.96***	.41**	.44*	.43***	.59***	.77***	-.11	.39**	-.35**	.21	.12
2. BDI Cognitive	13.3	9.5		1	.86***	.40**	.44***	.41**	.58***	.78***	-.11	.35**	-.35**	.21	.07
3. BDI Neurovegetative	9.5	8.0			1	.39**	.41**	.42**	.55***	.70***	-.10	.40**	-.33*	.18	.16
4. Total Stress Count[x]	27.1	15.9				1	.94***	.56***	.72***	.39**	.03	.15	-.32*	.19	.02
5. Total Stress Severity[x]	66.6	35.4					1	.58***	.75***	.45***	.11	.18	-.33*	.12	.06
6. Childhood Stress[x]	20.6	15.3						1	.33**	.35**	.16	.37**	-.19	.33*	.28
7. Six Month Stress[x]	18.3	15.8							1	.48***	.01	.12	-.35**	-.04	.07
8. PSS	22.3	8.3								1	-.04	.33*	-.23	.23	.13
9. CWIT	11.2	2.4									1	-.05	-.11	.03	.14
10. IL-6	0.7	0.7										1	-.10	.53*	.60**
11. Free Cortisol	21.6	8.6											1	0	.19
12. TNFα	10.1	7.1												1	.32
13. CRP	3.1	4.4													1

Table 8. Means, standard deviations, and bivariate correlations. Note n =59 with the exception of correlations with free cortisol where *n = 58.*

**p* < .05. ***p* < .01. ****p* < .001; [x]measured via the Stress Adversity Inventory for Adults (STRAIN); BDI, Beck Depressive Inventory-II; PSS, Perceived Stress Scale; CWIT, Colour-Word Interference Task; IL-6, interleukin-6; CRP, C-reactive protein.

Variable	Estimate	95% CI	Beta	*p*
Independent Variable				
IL-6	7.96	3.04, 12.88	.36	<.01
Covariates				
Psychopharmaceuticals	17.36	10.58, 24.14	.51	<.001
Birth Control	3.19	-4.11, 10.48	.09	.39
BMI	.02	-.79, .84	.01	.96
Menstrual Cycle	.22	-7.37, 7.80	.01	.96

Table 9. Covariate analysis of IL-6 and Beck Depressive Inventory (BDI)-II Scores (n = 59). This table presents the simple linear regression relationship between each variable and BDI-II scores while controlling for all other variables listed in the table to permit an analysis of covariance. IL-6 is considered the main independent variable. Psychopharmaceutical medication and birth control (coded 1 = present; 0 = absent), body mass index (BMI) (coded as a continuous variable), and menstrual cycle phase (coded 1 = follicular; 0 = luteal) were analyzed as covariates. All variables were analyzed with the final sample of 59 participants, with the exception of menstrual cycle, which had an $n = 55$ (due to four participants that were excluded with menostasis). Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.17	.00, .06	.35	<.01
2					
i	a1	.00	.00, .01	.16	.21
ii	a2	-.08	-.14, -.02	-.34	<.05
3					
i	b1	6.51	1.93, 11.10	.30	<.05
ii	b2	-.43	-.86, .01	-.22	.05
4					
i	ab1	.02	-.02, .06	.05	.25
ii	ab2	.04	-.01, .08	.07	.12
iii	ab1+ab2	.06	.00, .12	.12	.06
5					
i	c' + (ab1+ab2)	.23	.12, .33	.47	<.01

Table 10. Beta Coefficients of Cumulative Life Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.1. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	-.93	-2.44, 1.01	-.13	.29
2					
i	a1	1.73	-1.29, 4.54	.12	.24
ii	a2	-.02	-.08, .04	-.07	.45
3					
i	b1	.21	.09, .31	.44	<.01
ii	b2	6.49	.87, 10.78	.30	<.05
4					
i	d	.00	.00, .01	.17	.22
5					
i	ab1+ab2+a1db2	.26	-.52, 1.17	.04	.54
6					
i	c' + (ab1+ab2+a1db2)	-.67	-2.13, 1.20	-.10	.43

Table 11. Beta Coefficients of Cognitive Control, Cumulative Life Stress Severity, IL-6, and Depressive Symptom Severity of Hypothesis 3.1. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.34	.09, .06	.31	<.05
2					
i	a1	.02	.01, .03	.36	<.01
ii	a2	-.11	-.26, .03	-.20	.12
3					
i	b1	5.17	0.23, 10.12	.24	<.05
ii	b2	-.54	-.96, -.11	-.27	<.05
4					
i	ab1	.09	-.02, .02	.08	.09
ii	ab2	.06	-.03, .15	.05	.19
iii	ab1+ab2	.16	.01, .30	.14	<.05
5					
i	c' + (ab1+ab2)	.50	.25, .75	.45	<.01

Table 12. Beta Coefficients of Childhood Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2a *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.55	.34, .77	.51	<.01
2					
i	a1	.01	-.01, .02	.11	.39
ii	a2	-.19	-.33, -.06	-.35	<.01
3					
i	b1	6.64	2.53, 10.76	.30	<.01
ii	b2	-.29	-.69, .11	-.15	.15
4					
i	ab1	.04	-.05, .12	.03	.41
ii	ab2	.06	-.03, .14	.05	.20
iii	ab1+ab2	.09	-.03, .22	.09	.14
5					
i	c' + (ab1+ab2)	.64	.42, .86	.60	<.01

Table 13. Beta Coefficients of Past Six-Month Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2b. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	1.42	1.09, 1.75	.70	<.01
2					
i	a1	.03	.01, .05	.31	<.05
ii	a2	-.25	-.51, .01	-.24	.06
3					
i	b1	3.01	-.45, 6.46	.14	.09
ii	b2	-.34	-.65, -.04	-.18	<.05
4					
i	ab1	.09	-.03, .21	.04	.16
ii	ab2	.09	-.03, .21	.04	.16
iii	ab1+ab2	.17	.00, .34	.90	<.05
5					
i	c' + (ab1+ab2)	1.60	1.27, 1.92	.78	<.01

Table 14. Beta Coefficients of Perceived Stress Scale (PSS), IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2c. *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Figures

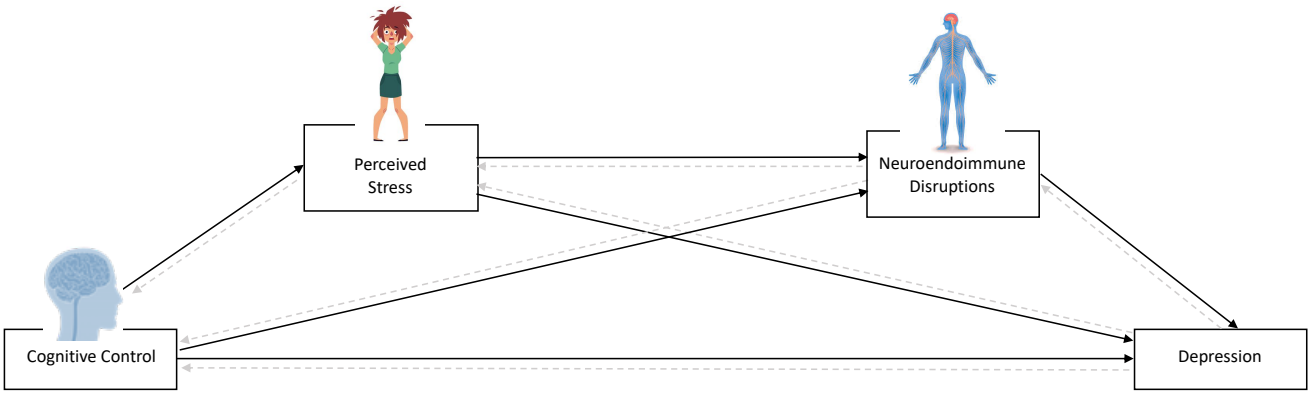


Figure 1. Integrative Model of Depression. This figure presents a basic integrative model of depression to be tested in the current dissertation. Psychological experiences of stress, and stress-related changes in immune activity, are considered a central pathway through which neurobiological changes may occur to influence depressive phenotypes. Whether individual differences in executive functioning and cognitive control interact with neuroendoimmune activity to contribute to, or buffer against, the impacts of stress on depression remain to be determined. Although the constituent parts of the model are all assumed to be bidirectional, the bold arrows indicate the directions of relationships assumed and analyzed within the current research. Stressed out women by Sabelskaya copyright 2020 Shutterstock; Brain scan image copyright 2020 iStock/Bubaone; brain image and nervous system image copyright 2020 iStock/Metamorworks.

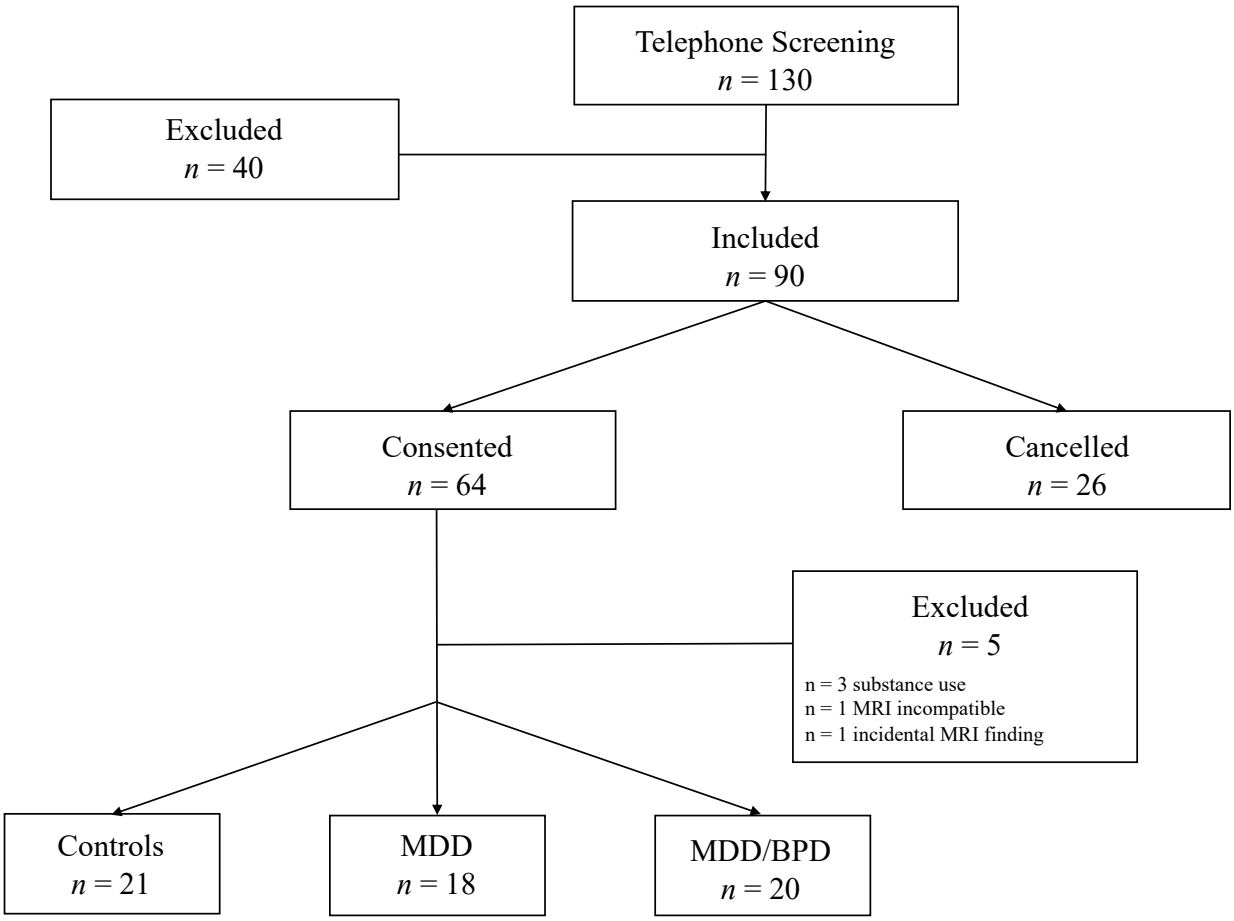


Figure 2. Participant Recruitment. This flowchart depicts of the study recruitment and inclusion process.

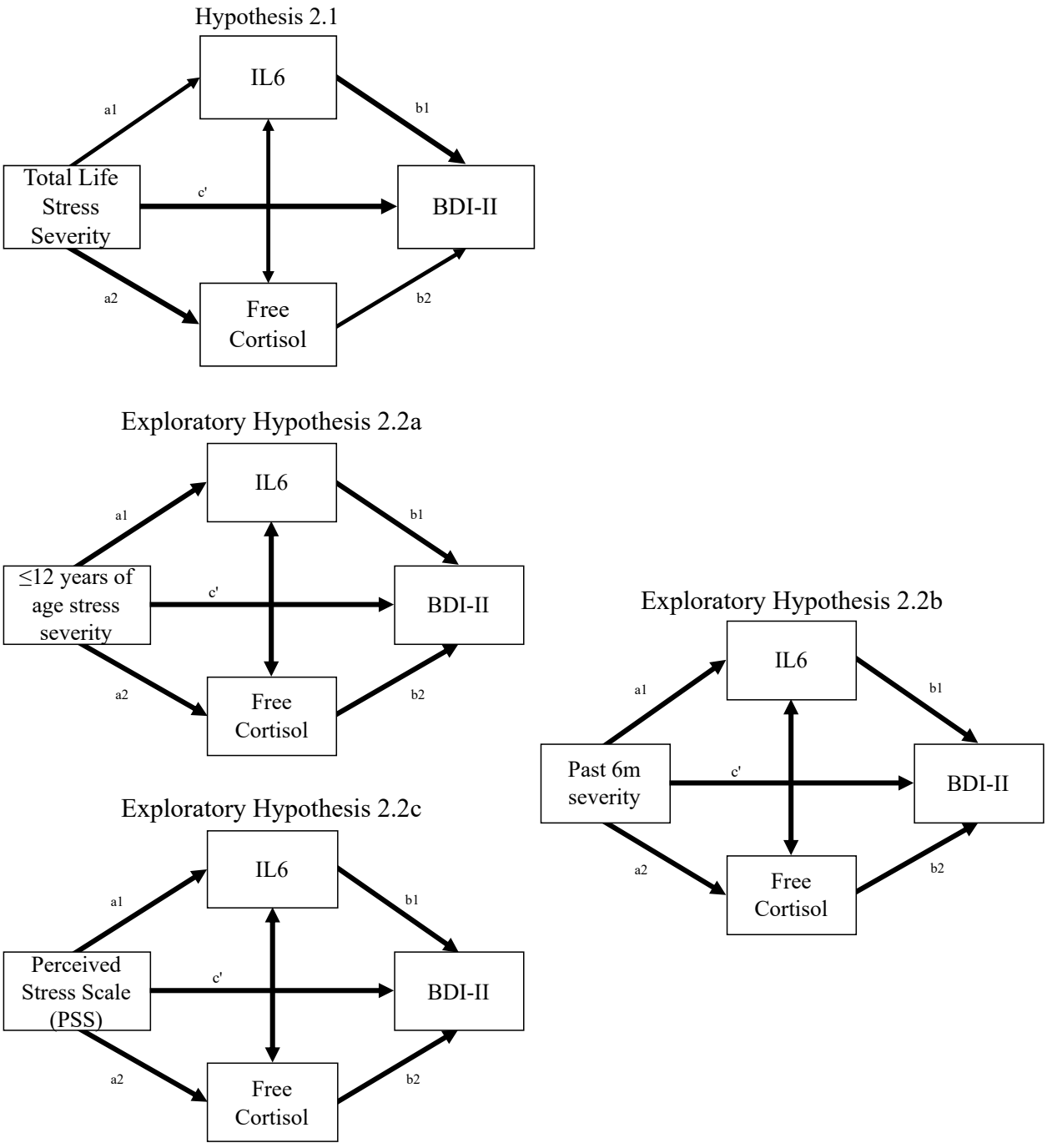


Figure 3. Parallel Mediation Model. This figure depicts the parallel mediation model used to test Hypothesis 2.1 and Exploratory Hypothesis 2.2. The arrows indicate the direction of the individual regression pathways to be tested. Exploratory Hypothesis 2.2a, 2.2b, and 2.2c represent each of the distinct time periods being examined. IL-6 represent immune activity; hypothalamic-pituitary-adrenal (HPA) axis activity measured via free cortisol; total life stress, ≤12 years of age stress, and past six-month stress is measured via the Stress and Adversity Inventory for Adults (STRAIN); depressive symptom severity measured via the Beck Depression Inventory (BDI)-II.

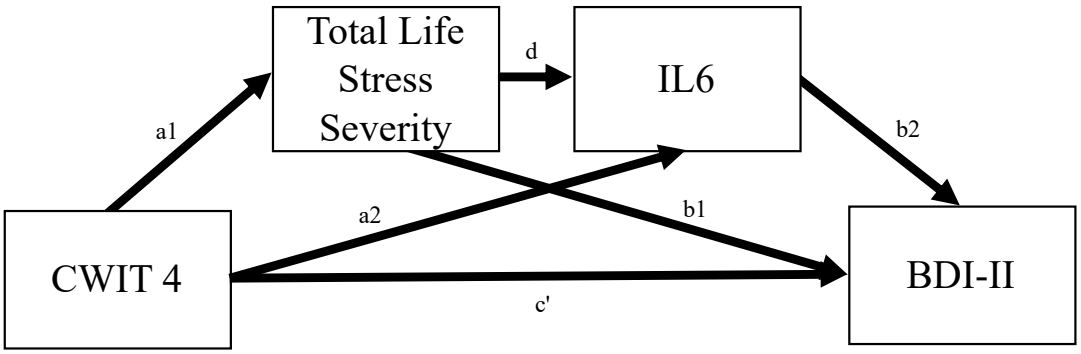
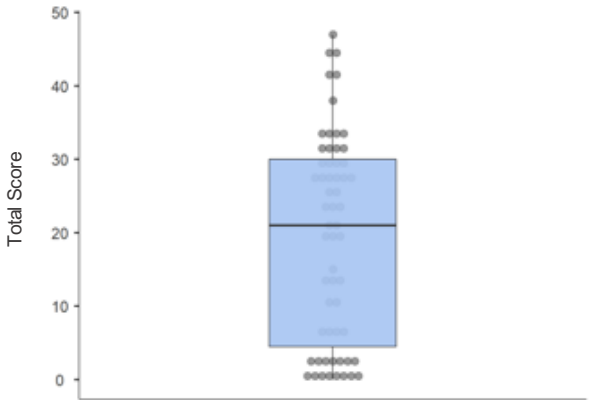
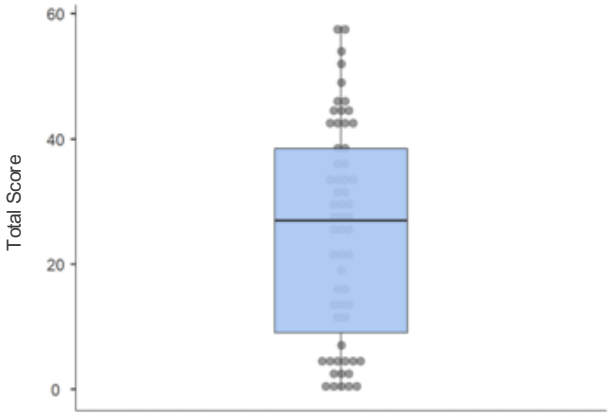


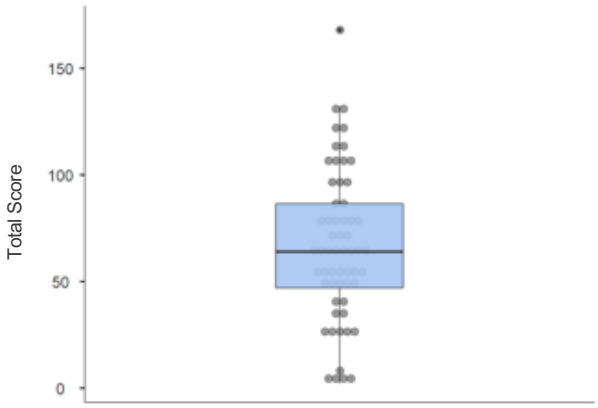
Figure 4. Serial Mediation Model. This figure depicts the serial mediation model used to test Hypothesis 3.1. The arrows indicate the direction of the individual regression pathways to be tested. Total cumulative life stress severity measured by the Stress and Adversity Inventory for Adults (STRAIN) and immune activity (IL-6) are hypothesized to mediate the relationship between cognitive control as measure by condition 4 of the Colour-Word Interference Task (CWIT 4) and depressive symptom severity measured by the Beck Depression Inventory (BDI)-II.



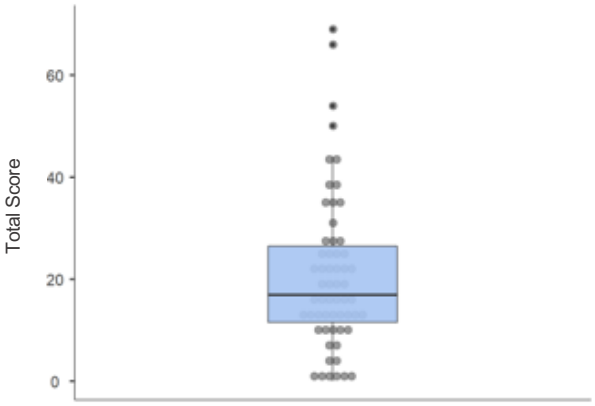
n = 59; range 0 – 47



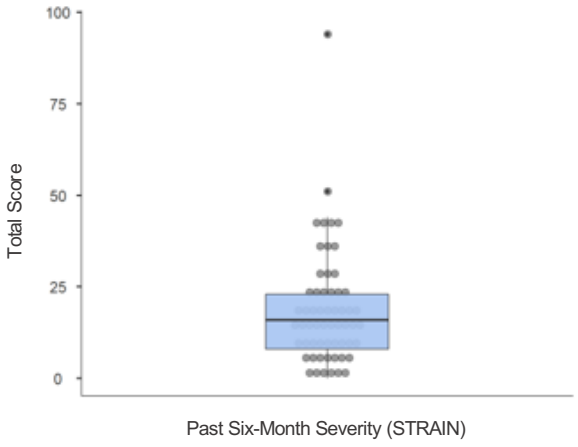
n = 59; range 0 - 58



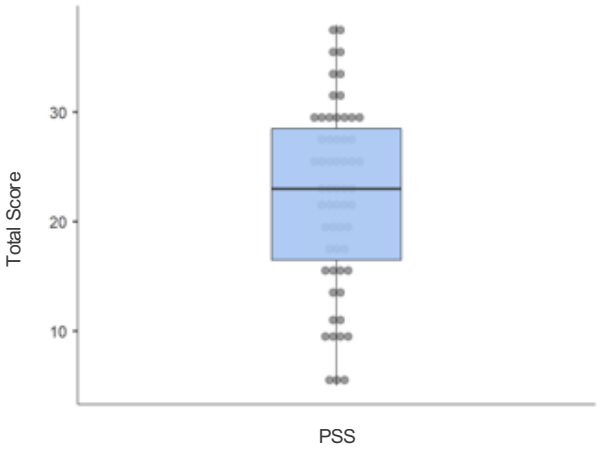
n = 59; range 2 - 168



n = 59; range 0 - 69



n = 59; range 0 - 94



n = 59; range 5 - 38

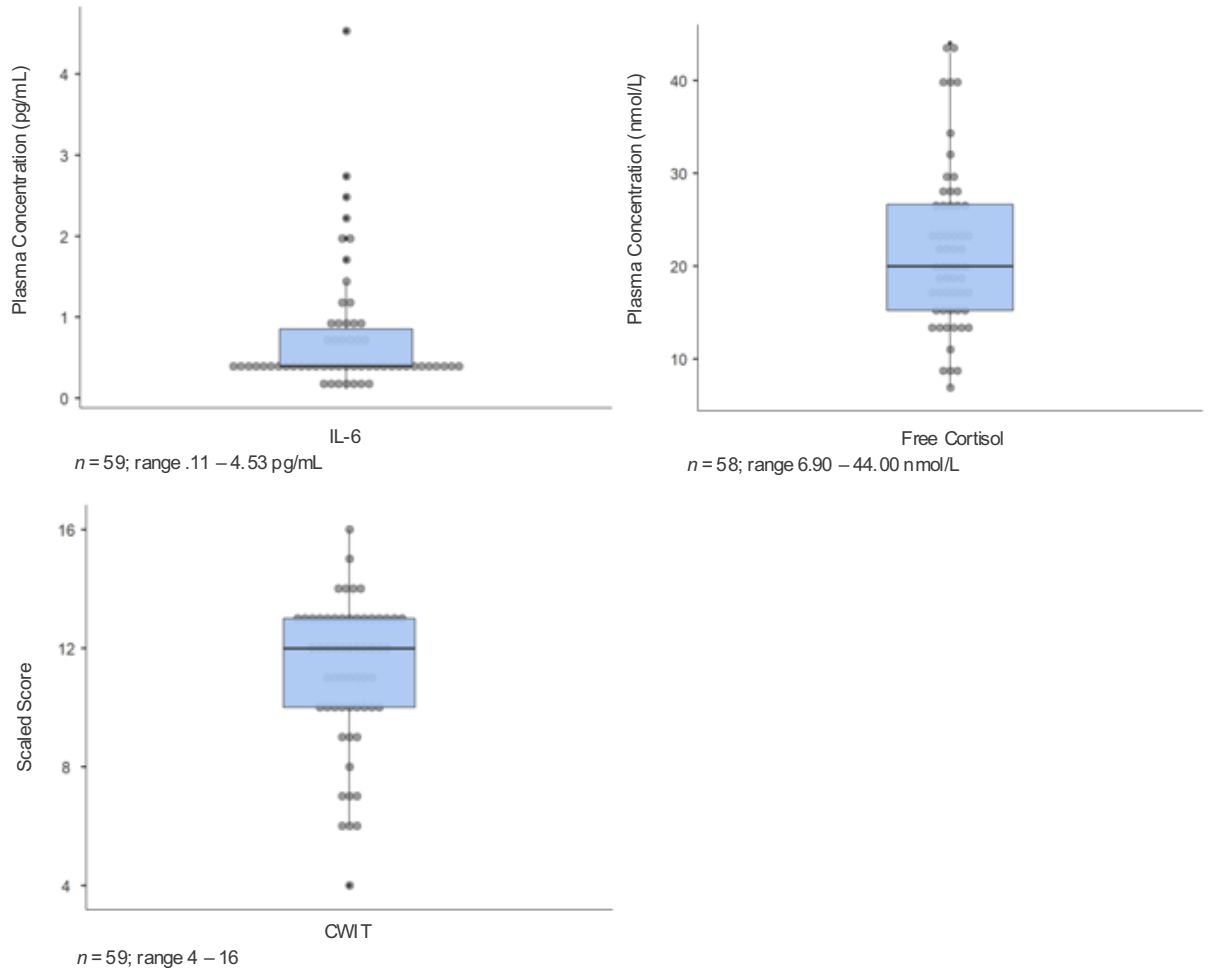


Figure 5. *Boxplots of the Main Study Variables*. The line in the box represents the median score of the sample, and the ends of the box indicate where the first and third quartile end. Data points beyond the whiskers are considered outlier values. HAMD = Hamilton Depression Rating Scale; BDI-II = Beck Depression Inventory-II; STRAIN = Stress and Adversity Inventory for Adults; PSS = Perceived Stress Scale; CWIT 4 = Colour-Word Interference Task; pg/mL: picograms/millilitre; nmol/L: nanomoles/Litre.

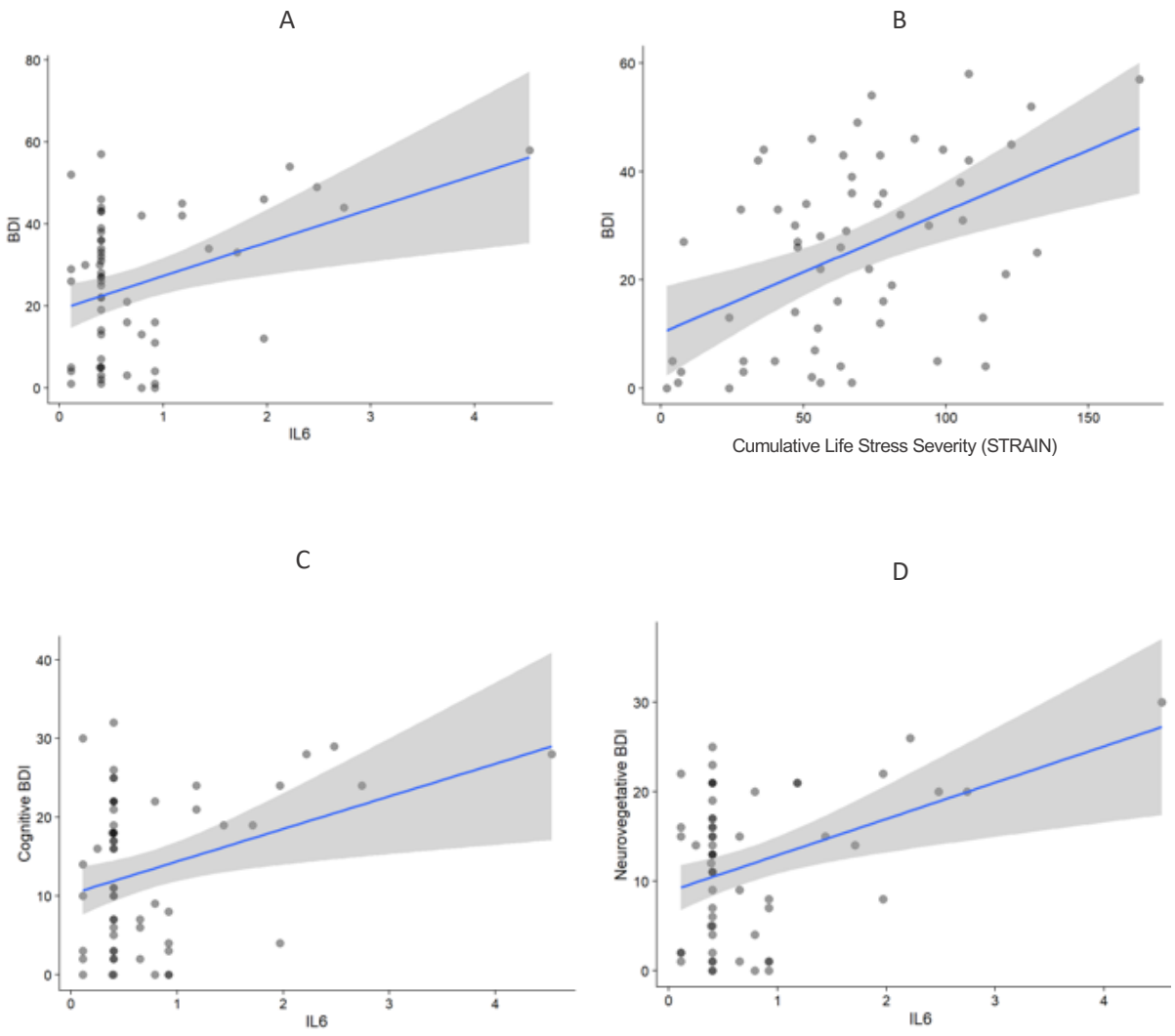


Figure 6. *Linear Regression Scatterplots (n = 59)*. This figure depicts scatterplots of the simple linear regression relationships of the main study variables. The blue line represents the slope of the model. The grey area around the line represents the 95% confidence intervals of the model. "A" depicts the relationship between IL-6 concentrations (picograms/millilitre—pg/mL) and Beck Depression Inventory-II scores (BDI-II). "B" depicts the relationship between cumulative life stress severity measured by the Stress and Adversity Inventory for Adults (STRAIN) and BDI-II scores. "C" depicts the relationship between IL-6 (pg/mL) and cognitive and affective symptoms measured via the BDI-II. "D" depicts the relationship between IL-6 (pg/mL) and neurovegetative symptoms measured via BDI-II.

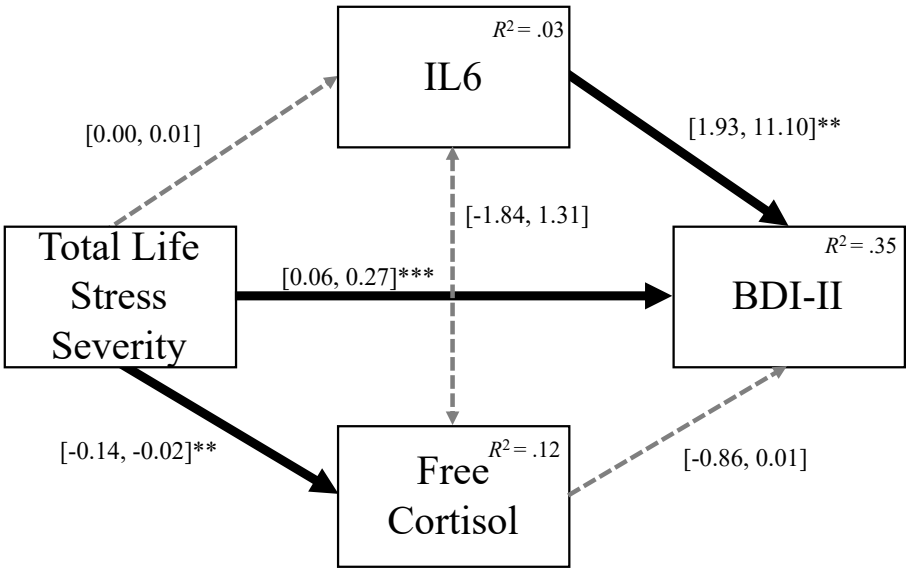


Figure 7. Results of Hypothesis 2.1. This figure provides a visual of the parallel mediation model tested to examine whether neuroendoimmune activity (IL-6 and free cortisol) mediates the relationship between cumulative life stress severity as measure by the Stress and Adversity Inventory for Adults (STRAIN) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by cumulative life stress severity.

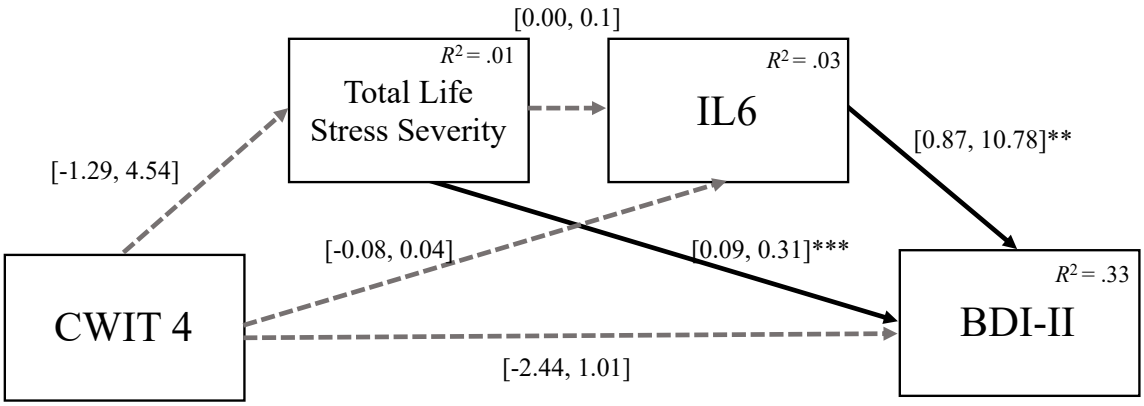


Figure 8. Results of Hypothesis 3.1. This figure provides a visual of the serial mediation model tested to examine whether perceived stress severity measured by the Stress and Adversity Inventory for Adults (STRAIN) and immune activity (IL-6) mediate the relationship between cognitive control as measure by condition 4 of the Colour-Word Interference Task (CWIT 4) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by cumulative life stress severity.

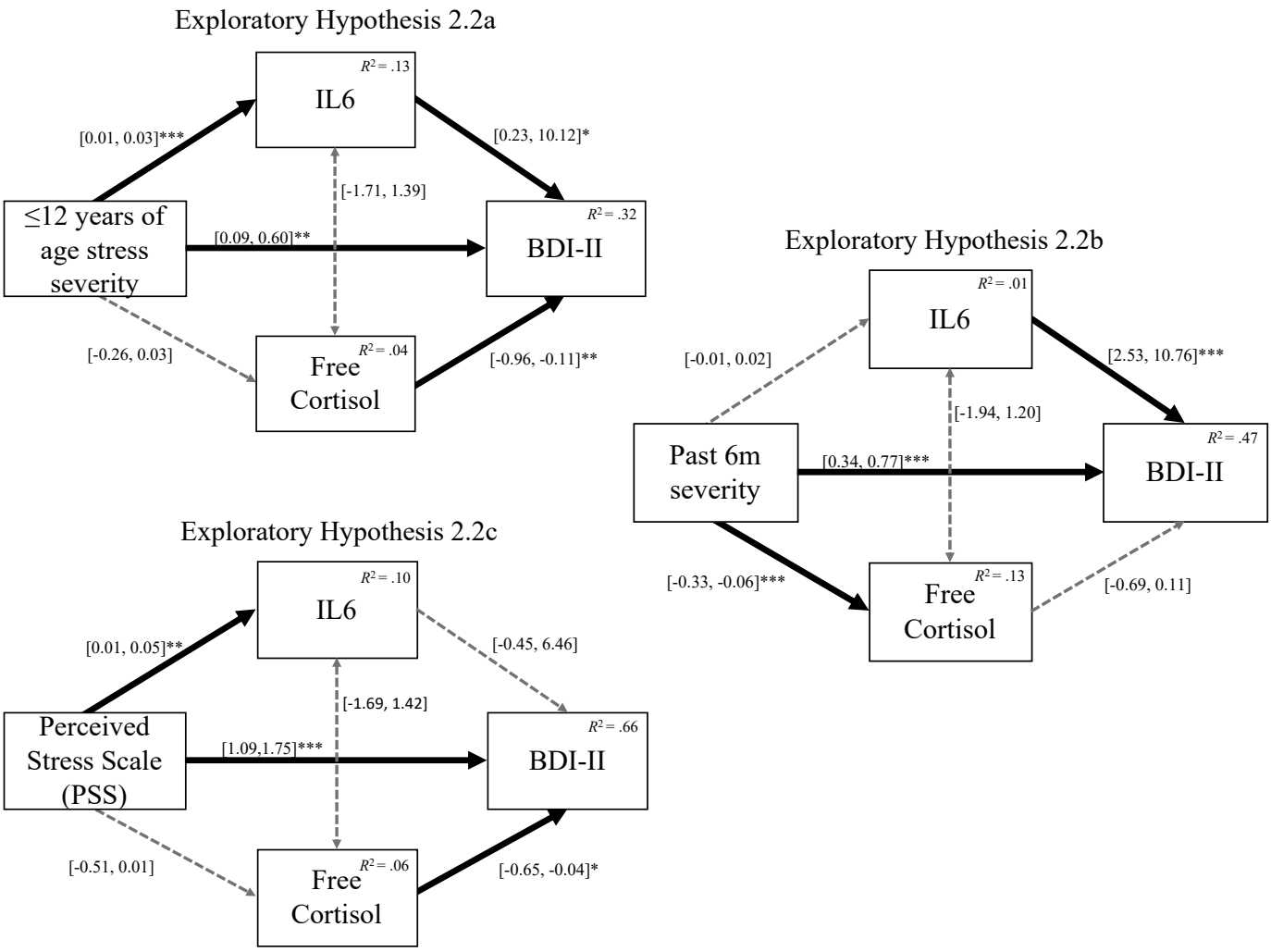


Figure 9. Results of Exploratory Hypothesis 2.2. This figure provides a visual of the parallel mediation models used to examine whether neuroendoimmune activity mediates the relationship between distinct time periods of perceived stress severity (≤12 years of age, past month, and past six months) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by stress severity and perceived stress measures.

Appendices

Appendix A

List of Abbreviations

ACTH: adrenocorticotropic hormone
ANS: autonomic nervous system
APA: American Psychiatric Association
BDI-II: Beck Depression Inventory-II
BPD: borderline personality disorder
CBG: corticosteroid binding globulin
CRH: corticotrophin-releasing hormone
CRP: C-reactive protein
CWIT: Colour-Word Interference Task
dl: deciliter
DSM: Diagnostic and Statistical Manual of Mental Disorders
GAD: generalized anxiety disorder
HAMD: Hamilton Depression Rating Scale
HPA: hypothalamic-pituitary-adrenal
ICD: International Classification of Diseases
IL: interleukin
INF-α: interferon-alpha
MDD: major depressive disorder
nmol: nanomoles
pg: picograms
PDD: Persistent depressive disorder
PSS: Perceived Stress Scale
PTSD: posttraumatic stress disorder
SCID: Structured Interview for DSM-5
SES: socioeconomic status
STRAIN: Stress and Adversity Inventory for Adults
TNF-α: tumor necrosis factor-alpha
TSST: Trier Social Stress Test
WCST: Wisconsin Card Sorting Test
WHO: World Health Organization

Appendix B

Beck Depression Inventory (BDI)-II Symptom Groupings

Neurovegetative Symptoms
#4. Loss of Pleasure
#11. Agitation
#12. Loss of Interest
#15. Loss of Energy
#16. Changes in Sleeping Pattern
#17. Irritability
#18. Changes in Appetite
#19. Concentration Difficulty
#20. Tiredness or Fatigue
#21. Loss of Interest in Sex

Cognitive/Affective Symptoms
#1. Sadness
#2. Pessimism
#3. Past Failure
#5. Guilty Feelings
#6. Punishment Feelings
#7. Self-Dislike
#8. Self-Criticalness
#9. Suicidal Thoughts or Wishes
#10. Crying
#13. Indecisiveness
#14. Worthlessness

Item numbers from the BDI-II are listed beside each symptom. To derive these symptom groupings, items were divided into two categories: (a) neurovegetative symptoms, which include symptoms common to sickness behaviours (e.g., loss of pleasure, loss of energy, and changes in appetite and sleep) (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Raison et al, 2010; Dantzer, 2018); and (b) cognitive and affective symptoms, which comprise symptoms oriented to thinking styles and affect common to depression (e.g., sadness, guilt, and thoughts of suicide).

TELEPHONE SCREENING SCRIPT

"Biological Markers of Depression and Personality"

Instructions:

When initially speaking with a potential research participant for screening purposes, read the following script in its entirety unless the potential participant answers "No" to the first question. Record each response verbatim.

My name is [*Staff Member*] and I am a member of the research team in the Clinical Neurosciences Laboratory at the University of Toronto. We are a group of researchers that carry out research on mental health.

I am phoning you because you responded to an advertisement about participating in mental health research. Are you still interested in our study? (*If yes, continue. If not, thank them for their time and ask them if they would like to join the research registry.)*

The purpose of this phone call is to determine whether or not you are eligible to participate in a research study examining genetics and biological markers in people with and without depression and borderline personality disorder. If you are eligible for the study, you will be asked to visit the University of Toronto and St. Michael's Hospital to answer more questions about your mental health history, provide a sample of your blood and saliva, complete tests of thinking abilities (such as attention and memory), and undergo a magnetic resonance imaging ("MRI") scan of your brain. You would be compensated $160 for your participation. The research would take approximately one day to complete (6 – 8 hours).

To make sure that you are eligible to participate in the study, I need to ask you some questions. Some of these questions are personal in nature. Although your answers will be kept strictly confidential, you can refuse to answer them at any time and/or choose not to participate in the study.

No information about you, or provided by you during the research, will be disclosed to others without your written permission, except in situations where it is necessary to protect your rights or welfare (for example, if you are injured and need emergency care); to protect the welfare of another person or vulnerable individual (for example, a patient abused by a regulated health professional); or when the University of Toronto Office of Research Ethics or sponsor monitor the research or consent process; or if required by law (for example, if our records of your responses to these question are subpoenaed by a court of law). As part of this research, you will be asked questions about illegal activity (e.g., drug use). This information will potentially be linkable to your personal information until identifying information is destroyed. This presents a legal risk if your information is subpoenaed. However, information gathered during this telephone screen will be destroyed immediately after you complete the study. Additionally, as part of the Research Services Quality Assurance role at CAMH, studies may be audited by the Manager of Quality Assurance. Our record of your responses may be reviewed during which confidentiality will be maintained as per CAMH policies and to the extent permitted by law.

This phone questionnaire is extensive and may take up to 20 minutes to finish. If you do not end up qualifying to participate, you will not be reimbursed for this time.

Do you want to go on? Yes No

May I ask you these personal questions now? Yes No

Record person's first name and initial of last name for screening log: _____

[If not from registry: How did you hear about our study? _____

PROBE:	RESPONSE:		ELIGIBILITY CRITERION:	MEETS ELIGIBILITY CRITERION?	
What is your age?	_____	years	Between 18-55 years old?	Yes	No
Will you be in the Toronto area for the next 6 months?	Yes	No	Available to participate?	Yes	No
Are you willing to fast after dinner the night before our research, and complete a blood draw the morning you arrive for your appointment?	Yes	No	Willing to provide fasted morning blood draw?	Yes	No
Do you identify as male, female, or another sex?	Male Other: _____	Female	Female?	Yes	No
Are you primarily right or left handed, or ambidextrous? ***If unsure, mixed, or ambidextrous, administer Edinburgh Handedness Inventory.***	Right Yes	Left No	Right handed?	Yes	No
Have you ever had any serious physical illness? ***If yes:* What illness?** _____			No history of major physical illness? *(If unsure, consult with Principal Investigator)*	Yes	No
Do you know of any family member who is/has been/plans to be enrolled in the study?	Yes	No	No knowledge of family member being enrolled in study	Yes	No
Do you have any physical disability or physical limitations? For example, do you use a wheelchair or cane?	Yes	No	*[Communicate any physical disabilities to testers so that they can be prepared for appropriate accommodations, e.g., elevator]*		
Do you have any significant visual or hearing impairment?	Yes	No	No serious visual or hearing disorder that would affect performance on the laboratory procedures?	Yes	No
Do you have any major difficulties in using your hands to write or use a computer?	Yes	No	No major manual limitations that would affect performance on the laboratory procedures?	Yes	No
Have you ever had any neurological illness or disorder, such as meningitis, encephalitis, or stroke?	Yes	No	No history of neurologic illness? *(If unsure, consult with Principal Investigator)*	Yes	No

If yes: **Please describe it:**

_____

PROBE:	**RESPONSE:**		**ELIGIBILITY CRITERION:**	**MEETS ELIGIBILITY CRITERION?**	
Have you ever had any sort of head injury?	Yes	No	No history of significant head trauma (≥ 20 min loss of consciousness and/or > 24 hrs posttraumatic amnesia or positive brain imaging findings)?	Yes	No
If yes: **Did you lose consciousness afterward?**	Yes	No			
If yes: **For how long?**	_____				
If yes: **Are there any gaps in your memory that occurred right after you hit your head?**	Yes	No			
If yes: **What is the last event you can remember <u>before</u> the injury?**	_____		*[Calculate the number of hours elapsed between these two events to obtain an estimate of Posttraumatic Amnesia]*		
If yes: **What is the first event you can remember <u>after</u> the injury?**	_____				
If yes: **Did you have a brain scan soon after the injury (like a CT or MRI) that showed any type of physical injury to the brain (like a bleed)?**	Yes	No			
Are you currently pregnant or do you intend to get pregnant at some point over the next 6 months?	Yes	No	*Not pregnant or intending to get pregnant over the next 6 months*	Yes	No
How many alcoholic drinks have you had in the last month?	_____				
Have you had any problems with alcohol in the past month (like drinking much more than you were planning, or having difficulty cutting down on your drinking)?	Yes	No	No problematic alcohol use in the last month? *(If unsure, consult with Principal Investigator)*	Yes	No

PROBE:	RESPONSE:		ELIGIBILITY CRITERION:	MEETS ELIGIBILITY CRITERION?	
Do you use any drugs recreationally, such as marijuana, LSD or cocaine?	Yes	No			
If yes: **How often do you use them?** _____					
When was the last time? _____					
Have you had any problems with drugs in the past month (like using much more than you were planning, or having difficulty cutting down using the drug [or drugs])?	Yes	No	No problematic substance use in the last month? *(If unsure, consult with Principal Investigator)*	Yes	No
Are you willing to abstain from using _____ two days before the research would take place?	Yes	No	*Willing to abstain prior to research.*	Yes	No
Are you currently taking any medications?	Yes	No			
If yes: **Which medications are you currently taking?** _____					

Name of Current Medication & Supplements	Dosage (mg)	Frequency (e.g. once/day, as needed; if as needed, indicate average taken per day or week, as relevant) and Duration

Have you ever been diagnosed with one of the following medical conditions?			No current diagnosis permitted. Past, remitted diagnosis is OK.	MEETS ELIGIBILITY CRITERION?	
alopecia areata	Yes	No		Yes	No
autoimmune hemolytic anemia	Yes	No		Yes	No
autoimmune hepatitis	Yes	No		Yes	No
cancer	Yes	No		Yes	No
cardiovascular disease	Yes	No		Yes	No
dermatomyositis	Yes	No		Yes	No
diabetes (type 1)	Yes	No		Yes	No
emphysema	Yes	No		Yes	No
glomerulonephritis	Yes	No		Yes	No
Graves' disease	Yes	No		Yes	No
Guillain-Barré syndrome	Yes	No		Yes	No
idiopathic thrombocytopenic purpura	Yes	No		Yes	No
liver disease	Yes	No		Yes	No
myasthenia gravis	Yes	No		Yes	No
some forms of myocarditis	Yes	No		Yes	No
multiple sclerosis osteoarthritis	Yes	No		Yes	No
pemphigus/pemphigoid	Yes	No		Yes	No
pernicious anemia	Yes	No		Yes	No
polyarteritis nodosa	Yes	No		Yes	No
polymyositis	Yes	No		Yes	No
primary biliary cirrhosis	Yes	No		Yes	No
psoriasis	Yes	No		Yes	No
rheumatoid arthritis	Yes	No		Yes	No
scleroderma/systemic sclerosis	Yes	No		Yes	No
Siögren's syndrome	Yes	No		Yes	No
lupus erythematosus	Yes	No		Yes	No
thyroiditis	Yes	No		Yes	No
uveitis	Yes	No		Yes	No
vitiligo	Yes	No		Yes	No

Note: The above list highlights common inflammatory medical conditions probed at the time of screening. However, participant's medical history was evaluated individually to determine if they had any health conditions beyond those listed above that were of potential confound to analyses to warrant participatory exclusion.

PROBE:	RESPONSE:		ELIGIBILITY CRITERION:	MEETS ELIGIBILITY CRITERION?	
Have you ever seen a counsellor, therapist or family doctor about things like depression, anxiety, or other mental health problems?	Yes	No			
Have you ever been diagnosed with a mental health disorder?	Yes	No			
***If yes*: What diagnoses have your received?** _____					
Do you still experience symptoms related to these diagnoses? _____	Yes	No			
***If no*: When was the last time you experienced symptoms?** _____					
Have you ever been diagnosed with a psychotic disorder like schizophrenia or schizoaffective disorder?	Yes	No	No history of psychotic symptoms.	Yes	No

PROBE:			ELIGIBILITY CRITERION:	MEETS	CRITERION?
Have you ever been diagnosed with bipolar disorder (type I or II)? **To clarify: Has there ever been a period of time when you were feeling so good, "high", excited or hyper that other people thought you were not your normal self or you were so hyper that you got into trouble? If YES: What was it like? Did anyone say you were manic? Was it more than just feeling good?**	Yes	No	No history of bipolar disorder.	Yes	No
If YES: How long did it last? As long as one week? Did you have to go to the hospital?	Yes	No	No current symptoms (past three months) of anorexia, bulimia, or binge eating disorder.	Yes	No
Are you currently suffering from an eating disorder? ***If yes*: What symptoms do you experience?** _____					
Have you ever been diagnosed with autism, Asperger's or another developmental disorder (such as Down Syndrome)? ***If yes:* Please explain**: _____ _____	Yes	No	No history of developmental disorder	Yes	No

PROBE:	**RESPONSE:**	**ELIGIBILITY CRITERION:**	**MEETS ELIGIBILITY CRITERION?**
Administer MRI safety screen: **Now, I'm going to ask you some questions to make sure that you are safe to undergo an MRI scan of your brain.**	[See MRI safety screen form]	Participant MRI safe?	Yes No
Are you currently suffering from low mood or depression?	Yes No	*If no, qualifies as control, and continue with McLean. If yes, administer the HRSD*	
Administer HRSD: **Now, I'm going to ask you some questions about how your mood.**	[See HRSD form]	***If*** **HRSD score < 7**, then may qualify as control; ***If MDD or BPD+MDD:*** at least mild depression in past week (HDRS score > 7)	Yes No
[Administer McLean Screening Instrument (MSI-BPD) for Borderline Personality Disorder--below]	[See total number of items endorsed below]	***If control or MDD:*** No BPD (MSI-BPD total score < 7) ***If BPD+MDD:*** BPD present (MSI-BPD total score ≥ 7)	Yes No

McLean Screening Instrument

Thinking about most of your adult life, please answer yes or no to the following questions.

Have any of your closest relationships been troubled by a lot of arguments or repeated breakups?
YES / NO

Have you deliberately hurt yourself physically (e.g., punched yourself, cut yourself, burned yourself)?
YES / NO

Have you had at least two other problems with impulsivity (e.g., eating binges and spending sprees, drinking too much and verbal outbursts)? YES / NO

Have you been extremely moody? YES / NO

Have you felt very angry a lot of the time? How about often acted in an angry or sarcastic manner? YES / NO

Have you often been distrustful of other people? YES / NO

Have you frequently felt unreal or as if things around you were unreal? YES / NO

Have you chronically felt empty? YES / NO

Have you often felt that you had no idea of who you are or that you have no identity? YES / NO

Have you made desperate efforts to avoid feeling abandoned or being abandoned (e.g., repeatedly called someone to reassure yourself that he or she still cared, begged them not to leave you, clung to them physically)? YES / NO

If 7 or more items answered "yes", then probable BPD.

TO DETERMINE PARTICIPANT GROUP STATUS (CHECK OFF APPLICABLE BOXES):

IF CONTROL:	**□ NO MDD**	**□ NO PROBABLE BPD**
IF MDD:	**□ CURRENT MDD**	**□ NO PROBABLE BPD**
IF BPD+MDD:	**□ CURRENT MDD**	**□ PROBABLE BPD**

Instructions:

If any "NO" in third column labelled "Meets Inclusion Criterion?" then the individual does not appear to qualify for the study. Proceed with Script A below.
If all "YES" in third column labelled "Meets Inclusion Criterion?" then the individual appears to qualify for the study. Proceed with Script B below.

A) If individual does NOT qualify for the study: **Thank you very much for taking your time to answer my questions. Unfortunately, it appears that you are not eligible to participate in the study. All the answers you gave me to these questions will be destroyed. Do you have any questions? In case you have any questions at a later point in time, please feel free to contact me. Again, my name is (*Staff Member*) and you can reach me at (416) 208-2764. Thank you very much for your interest and your time. If you are interested in joining our research registry, please visit www.researchregistry.ca.**

B) If individual does qualify to participate in the study: **Thank you very much for taking your time to answer my questions. You do appear to qualify for the study. A member of the research team will contact you soon to schedule an appointment and provide further details. We would like to send you a copy of the consent form before you visit the university. The consent form describes the study in detail, including what you will be asked to do, risks and benefits, and compensation. Where would you like us to send the consent form? We can email or fax it to you—remember that email and fax are not secure. If you would like it mailed to you instead, please let us know.**

PLEASE CIRCLE: EMAIL / FAX / POSTAL MAIL CONTACT INFORMATION: _____

On the day that you come to participate in this research study, you will first complete an informed consent session. During this time, a member of our research team will explain the study to you and you can ask any questions you might have. If you choose to participate, we will take a sample of your blood first thing in the morning after you have fasted the night before. Following this, you will be provided time and compensation for breakfast, and the remainder of the day will be devoted to collecting psychological, cognitive, and fMRI data. We will keep the information you have just given us today until you arrive for your appointment. At that point, if you decide not to participate in the study, we will destroy your answers to these questions. Do you have any questions regarding this process or the research that I can answer for you now?

Please provide us with the following information:

Name: _____
Phone Number: _____
General Availability: _____

Thank you very much for your interest in our research. In the case that you might have any further questions or concerns, please feel free to contact me. Again, my name is *(Staff Member)* and you can reach me (416) 208-2764. Thank you for your time!

Appendix D

Standard Operating Procedure: Suicide Risk Assessment and Actions Taken/Intervention Strategies (if required)

This standard operating procedure is to be used to systematically assess suicide risk and determine any follow-up actions to be taken for the protocol titled "Biological Markers in Depression and Personality". It is intended to be implemented solely by graduate students in clinical psychology and doctoral-level psychologists involved in the research protocol.

The following items on the respective interviews/scales will be examined to determine whether the participant reports current suicide ideation.

	Circle for each risk factor	
SCID-5 (A17, A43m, and PD86): If the participant responds to the question "have you thought about taking your own life" with current (within the past 24 hours) thoughts and plans of suicide, then the SOP will be administered. If responses are not about suicide, specifically, but are general thoughts of death (with no reference to suicide), this will not trigger a suicide assessment. If the participant provides additional information to the question "have you tried to hurt or kill yourself" suggesting the presence of current suicidal intent, then the SOP will be administered.	**Present**	**Absent**
Youth Risk Behaviour Survey (#3): If the participant provides additional information to the question "are you currently suicidal" suggesting the presence of current suicidal intent, then the SOP will be administered.	**Present**	**Absent**
BDI-II (#9): If the participant responds to the self-report question about "suicidal thoughts or wishes" in the past week with a 2 ("I would like to kill myself") or a 3 ("I would kill myself if I had the chance") then the SOP will be administered.	**Present**	**Absent**
ISAS Part 2 (#6, 19, 32): If the participant responds to any of these self-report questions with a 1 ("somewhat relevant") or a 2 ("very relevant"), then the SOP will be administered.	**Present**	**Absent**
HRSD (#11): If the participant responds to the question "This past week, have you had any thoughts that life is not worth living?" with a 3 (suicide ideas or gestures) or 4 (attempts of suicide), then the SOP will be administered.	**Present**	**Absent**

Checked suicide risk: _____Yes Initials of administrator:_____ Date:_____

Step 1: Risk Assessment

Questions for Participant (risk)

1. On a scale of 1 to 7, what is your intent to kill yourself right now?

Low 1 2 3 4 5 6 7 High

2. How many times have you attempted suicide in the past month? _____

3. What method would you use to commit suicide? _____

Do you have this method available? Yes No

4. Do you have a current plan and/or preparation in place (i.e., including specific time and method)?

Yes No

Interviewer Assessment

If unsure of correct response, ask participant. * indicates resilience question

1. Severe hopelessness No Somewhat Yes

2. Severe anhedonia No Somewhat Yes

3. Current psychosis, voices telling him/her to commit suicide.

No Somewhat Yes

*4. Hope for the future. No Somewhat Yes

*5. Responsibility to family, pets, or others, who the participant would not abandon

No Somewhat Yes

*6. Good working alliance with therapist. No Somewhat Yes

*7. Protective social network of family or friends.

No Somewhat Yes

*8. Fear of dying or no acceptable method available.

No Somewhat Yes

*9. Belief that suicide is immoral. No Somewhat Yes

*10. Committed to following a crisis plan No Somewhat Yes

If yes, what crisis plan is in place for the client? _____

Step 2: Conclusion of Assessment

_____ LOW RISK (Intent to kills self is below 4; no obvious plans or availability of means; indicates at least 3-4 resilience factors)

_____ MODERATE RISK (Intent to kill self is 4 or above; some access to means, some plan in place; endorses some hopelessness/anhedonia; indicates only 1-2 resilience factors)

_____ HIGH RISK (Intent to kill self is 4 or above; clear means and intent; endorses hopelessness, anhedonia, and/or psychosis; no clear resilience factors)

Notes:

If scoring moderate or high risk, proceed to Step 3.

Step 3: Intervention Strategies- what action did you take? (please check all that apply)

_____ Get commitment for participant to call/see individual therapist or family physician (either in the room or immediately after leaving the assessment)

_____ Get commitment for participant to go to any emergency room.

_____ They will call a distress hotline (Refer to debriefing form for complete list, and select appropriate option. Provide them with a copy of withdraw debriefing form.).

_____ Get commitment for participant to talk to a close family member, friend, or clergy

_____ Assure that the participant will not be left alone until he/she is out of danger

_____ Participant will go to a walk-in clinic for counselling.

_____ Call Dr. Ruocco's mobile phone (416-573-8544) for further assessment

_____ Call 911

_____ Get participant commitment to follow an in-place commitment strategy

Describe commitment strategy:

_____ _____

Print Name of SOP Administrator Date Administered

_____

Signature of SOP Administrator

_____ _____

Signature of PI Date

Appendix E

Debriefing Form

Thank you for participating in research with the Clinical Neurosciences Laboratory!

The purpose of the study that you have participated in is to help understand how genes, brain functioning, and the immune system are related to important symptoms of mental illness. Specifically, we are investigating how symptoms of depression and borderline personality disorder are related to activation in the immune system and changes is certain mental processes. We will examine these processes at a behavioural level (using performance on cognitive tasks) and a biological level (using functional magnetic resonance imaging (fMRI). We anticipate that the results of this work will help contribute to a better understanding of the neurobiology underlying symptoms of depression and borderline personality disorder as well as characteristic which may be present across many forms of mental illness.

Up to a certain point in time, you may choose to withdraw your data from the study without negative consequences. Should you choose to withdraw your data from the study, please send a request via email to cnl@utsc.utoronto.ca. Once the data collection and statistical analyses phases of the study are complete, you will no longer be able to withdraw your data.

If you would like to receive a summary of the main findings of this study after it is complete, please visit our website http://www.utsc.utoronto.ca/~aruocco/ for instructions.

Sometimes participating in a research study on mental health brings up questions about how to access mental healthcare and community resources. For people with mental health concerns looking to access resources in Ontario, the following information may be helpful:

Mental Healthcare access in Ontario

- Contact your family doctor to talk about any symptoms you are experiencing. Your doctor might be able to assist you directly, or they will provide a referral to appropriate services (e.g., psychologist or psychiatrist).
- If you are employed, try contacting your workplace human resource department to determine if you can receive any employee assistance for mental health services.
- ConnexOntario (http://www.connexontario.ca) offers a mental health helpline (1-866-531-2600) that can provide information about affordable care resources. Similarly, the Toronto branch of the Canadian Mental Health Association (http://www.cmha.ca) offers information about programs available across the Greater Toronto Area.
- If you're having an urgent mental health crisis, call Toronto's 408 Help Line at 416-408-HELP (4357) or the Gerstein Crisis Centre (416-929-5200) 24 hours a day.

Additional Distress and Crisis Lines:

- Distress and Crisis Line: 416-408-HELP (24-hour urgent mental health crisis line in Toronto area)

- Kids Help Line: 800-668-6868 (A bilingual national telephone counselling service for children and youth. Lines are open 24 hours a day.)
- Lesbian, Gay, Bi Youth Hotline: 800-268-9688 (A provincial hotline for gay, lesbian, bisexual, transsexual, transgendered, two-spirited and unsure youth.)
- Parent Help Line: 888-603-9100 (24-hour telephone counselling and referral line providing parents with information and support related to parenting issues.)
- Assaulted Women's Hotline: 866-863-0511 (An anonymous and confidential crisis line for abused and assaulted women in Ontario. They provide crisis counselling, emotional support, safety plans and referrals (e.g., for shelters, rape crisis centres, housing, legal services), and interpretation services.)
- Gerstein Centre Crisis Line: 416-929-5200 (24-hour Toronto-based crisis line to discuss current problems and access to care.)
- (York Region) Crisis Line: 905-310-COPE (24-hour urgent mental health crisis line in the York Region).

Appendix F

CNL ID: __ __ __ __ 1

DEMOGRAPHIC HISTORY INTERVIEW

Date of Interview:	__ __ / __ __ / __ __*(MM/DD/YY)*			[dateint]
Interview Format:	1=Face-to-Face	2=Telephone		[intfor]
Subject Type:	1=Healthy Control	2=Proband/Patient		[subtype]
Subject Age:	Age _____			[age]
Biological Sex at Birth:	1=Male	2=Female	Other: _____	[sex]
Gender Identity:	1=Man	2=Woman	Other: _____	[sex]
Handedness:	1=Right	2=Left	3=Ambidextrous	[hand]
Weight:	_____			
Height:	_____			

Race/Ethnicity:

Are you an Aboriginal person, that is, North American Indian, Metis, or Inuit (Eskimo)? 1=Yes 2=No [aboriginal]

What is your ethnicity? *(Check all that apply)* [ethnicity]

1 = North American Indian
2 = Metis
3 = Inuit (Eskimo)
4 = White
5 = Chinese
6 = South Asian (e.g., East Indian, Pakistani, Sri Lankan, etc.)
7 = Black
8 = Filipino
9 = Latin American
10 = Southeast Asian (Cambodian, Indonesian, Laotian, Vietnamese)
11 = Arab
12 = West Asian (e.g., Afghan, Iranian, etc.)
13 = Japanese
14 = Korean
15 = West Indian (Cuba, Haiti, Dominican Republic, Jamaica, Guyana, Bahamas, etc)
16 = Mixed
Specify: _____
17 = Other
Specify: _____

Educational History:

How far did you go in school? Record years of education*: __ __ [edusub]

Level of education	***Equivalent years**
High School or GED (General Equivalency Diploma)	12
Associate's degree	14
College/University graduate (i.e., Bachelor's degree)	16
Master's degree	18
Doctoral degree	20

CNL ID: __ __ __ __ 2

Annual Family Income:

What is your family's annual income from all sources combined, before taxes? If you are a full-time student receiving financial support from your parents, please give your parents' income. Do not include loans.

Estimate for most recent year:

<$5000
$5000 -$10 000
$10 000 - $15 000
$15 000 - $20 000
$20 000 - $25 000
$25 000 - $35 000
$35 000 - $50 000
$50 000 - $75 000
$75 000 - $100 000
$100 000 - $150 000
$150 000 - $200 000
$200 000 - $250 000
>$250 000

2 = I don't know/I don't want to answer

[famincome]

Occupational History:

Which of the following best describes your current employment status?

1 = Unemployed
2 = Full-Time Employed
3 = Part-Time Employed
4 = Full-Time Student
5 = Disabled
6 = Receiving public assistance
7 = Homemaker

[empcurr]

If on disability: **Is your disability of a psychiatric or physical nature?**

1 = Psychiatric
2 = Physical
3 = Both
4 = Unknown/Refused

[disability]

What is your current (or last) occupation? _____ [subocc]

Marital History:

What is your current marital status?

1 = Single, never married
2 = First marriage
3 = Divorced
4 = Divorced, remarried
5 = Widowed
6 = Widowed, remarried
7 = Separated
8 = Common law

[marstat]

If currently married or in a relationship: **What is the length of your current marriage (or relationship)?**

__ __ *(total months)* [lengthrel]

Family History:

CNL ID: __ __ __ __ 3

Were you adopted?	0 = No	1 = Yes	2 = Don't know	[adopt]

Developmental History:

Did your mother ever smoke, take drugs, or use alcohol during her pregnancy with you?	0 = No	1 = Yes	2 = Don't know	[drugpreg]
Were there any problems or complications during the pregnancy or delivery?	0 = No	1 = Yes	2 = Don't know	[comppreg]

Psychiatric Treatment History:

Have you ever received treatment on an inpatient or outpatient basis? (Please describe)

Have you ever been hospitalized, that is, been checked into a hospital, whether voluntary or otherwise, for psychiatric problems or a nervous breakdown?	0 = No	1 = Yes	2 = Unknown	[hosp]
If yes: **How many times have you been hospitalized?**	__ __*(record number of hospitalizations)*			[hospnum]
If you have ever been hospitalized for psychiatric reasons: **In total, how long have you spent in inpatient psychiatric treatment (across all hospitalizations)?**	0 = No history of inpatient treatment 1 = Less than one week 2 = Less than one month 3 = Less than three months 4 = Three months to one year 5 = More than one year -2 = Don't know / I don't want to say *Estimate number of weeks of inpatient hospitalization:* __ __ __			[intreat]
If history of inpatient treatment: **How old were you when you were first hospitalized?**	__ __ *(indicate age in years)*			[inage]
Have you ever received ECT or electric shock therapy?	0 = No	1 = Yes	2 = Don't know	[ECT]
Have you ever been seen on an <u>outpatient</u> basis for emotional, psychiatric, alcohol, or drug problems?	0 = No	1 = Yes	2 = Don't know	[outtreat]
If yes: **What type of treatment did you receive?**	0 = No history of outpatient 2 = Continuous outpatient treatment (including medication and/or therapy) lasting one year or more, or numerous brief periods			[outtype]

CNL ID: __ __ __ __ 4

3 = Continuous treatment for 6 months or several brief periods
4 = Consultation or brief period of treatment (i.e., < 6 months)
5 = No history of inpatient or outpatient psychiatric treatment
-2 = Don't know / refused

If you have been seen on an outpatient basis:
How old were you when you first sought outpatient treatment? __ __ *(indicate age in years)* [outage]

Family History of Psychiatric Illness:

In box (☐) record number of relatives diagnosed or strongly suspected of each disorder.

Please specify if a relative has been formally diagnosed of an illness, or if it is strongly suspected that the relative has the illness but not diagnosed.

First-Degree Biological Relatives

Schizophrenia or Schizoaffective Disorder: [schizrel]
Mother ☐
Father ☐
Sibling ☐
Child ☐

Bipolar Disorder: [bipolrel]
Mother ☐
Father ☐
Sibling ☐
Child ☐

Depressive Disorder: [deprel]
Mother ☐
Father ☐
Sibling ☐
Child ☐

Suicide or Suicide Attempt [suicrel]
Mother ☐
Father ☐
Sibling ☐
Child ☐

Medication Dosage and Frequency:

Are you currently taking any of the following medications by prescription?

CNL ID: __ __ __ __ 5

Write name of medication(s) below:	Dosage (mg per pill)	Frequency (how many pills per day?)
Anxiolytics or Sedatives:		
_____	_____	_____
_____	_____	_____
_____		
Stimulants:		
_____	_____	_____
_____	_____	_____
Minor Tranquilizers:		
_____	_____	_____
_____	_____	_____
Neuroleptics / Major Tranquilizers:	_____	_____
_____	_____	_____
_____		
Antidepressants:		
_____	_____	_____
_____	_____	_____
Mood Stabilizer:		
_____	_____	_____
_____	_____	_____
Antiparkinson Agents:		
_____	_____	_____
_____	_____	_____
Antipsychotic Drugs:		
_____	_____	_____
_____	_____	_____
Birth Control:		
_____	_____	_____
_____	_____	_____
Other *Prescribed* Drugs:		
_____	_____	_____
_____	_____	_____

CNL ID: __ __ __ __ 6

Medication History:

Have you ever taken any of the following medications by prescription to help you sleep or to change your mood (i.e., sleeping pills, tranquilizers, stimulants, or other such drugs?)

	No	Yes	
Sedatives for insomnia or calming nerves (Amytal, Ativan, Klonopin, Halcion, Nembutal, Phenobarbital, Restoril, Seconal, Clonazepam/Klonopin)	0	1	[sedever]
Stimulants for energy, staying awake, weight reduction (amphetamine, Benzedrine, Biphetamine, Dexedrine, Methedrine, Preludin, Ritalin)	0	1	[stimever]
Minor Tranquilizers(Miltown, Librium, Valium, Buspar, Xanax, Vistaril)	0	1	[mintever]
Neuroleptics / Major Tranquilizers(Thorazine, Stelazine, Mellaril, Haldol, Clozaril, Prolixin, Sparine, Trilafon, Resperidol)	0	1	[majtever]
Antidepressants (Cipralex, Citalopram/Celexa, Zoloft, Lexapro, Prozac, Wellbutrin, Paxil, Effexor, Celexa, Elavil, Trazodone, Cymbalta, Imipramine)	0	1	[adever]
Mood Stabilizer(Lithium, Depakote, Tegretol, Lamictal, Neurontin, Tripleptal, Topamax)	0	1	[msever]
Antiparkinson Agents (Artane, Benadryl, Cogentin, Eldepryl, Parlodel, Sinemet)	0	1	[aaever]
Antipsychotic Drugs (Abilify, Clozaril, Geodon, Risperdal, Seroquel, Zyprexa, Haldol, Thorazine)	0	1	[apever]
Other *Prescribed* Drugs (Marijuana/cannabis) *Specify*: _____	0	1	[odever]

Appendix G

Participant Demographic Information Across Recruitment Groups

Characteristic	Controls	MDD	MDD+BPD
Age	27.3 (8.1)	30.0 (9.3)	28.8 (9.8)
Education Levels			
High School or GED	10%	13.0%	20%
College or University	60%	60.8%	60%
Graduate/Professional school	30%	21.7%	20%
<High School		4.3%	
Ethnicity*			
Caucasian	20.0%	47.8%	85.0%
Chinese	10.0%	17.4%	
South Asian	15.0%	4.3%	
Black	20.0%		
Southeast Asian	5.0%		5.0%
West Indian		4.3%	5.0%
Japanese		4.3%	
Korean		4.3%	
Mixed race	25.0%	13.0%	
Other	5.0%	4.6%	5.0%
Income			
<$5000			5.0%
$5000-$10,000			
$10,000-$15,000	5.0%	5.0%	
$15,000-$20,000			5.0%
$20,000-$25,000			10.0%
$25,000-$35,000	10.0%	10.0%	5.0%
$35,000-$50,000	30.0%	30.0%	25.0%
$50,000-$75,000	5.0%	5.0%	
$75,000-$100,000	15.0%	15.0%	15.0%
$100,000-$150,000	25.0%	25.0%	20.0%
$150,000-$200,000			5.0%
>200,000	10.0%	10.0%	
Employment*			
Full-time employment	20.0%	39.1%	40.0%
Part-time employment	40.0%	26.1%	25.0%
Unemployed	15.0%	4.3%	10.0%
Student	45.0%	26.1%	25.0%
Disabled		13.0%	15.0%
Homemaker	5.0%		
BMI			
Underweight (<18.5)	5.0%	4.4%	10.0%
Normal (18.5 – 24.9)	55.0%	56.6%	40.0%
Overweight (25.0 – 29.9)	30.0%	26.0%	25.0%
Obese (>30.0)	10.0%	13.0%	25.0%

n = 22 controls; 21 MDD; 20; *Note.* Groups were not found to statistically differ on variables of age, education, income, or BMI.

Appendix H

Diagnostic and Clinical Characteristics Across Recruitment Groups

Characteristic	Controls	MDD	MDD+BPD
Current MDD		100%	95.0%
2 previous episodes of MDD		23.8%	20%
≥3 previous episodes of MDD	4.5%	66.6%	65.0%
Mean number of MDD episodes	0.4(1.1)	6.6 (10.2)	4.2 (4.5)
PDD		28.5%	30.0%
HAMD Scores			
Normal (≤7)	86.3%	4.7%	
Mild (8 – 16)	9.1%	14.4%	10.0%
Moderate (17 – 23)		9.5%	20.0%
Severe (≥24)	4.5	71.4%	70.0%
BPD			100.0%
PTSD		14.3%	35.0%
Current alcohol use disorder	4.5%	9.0%	5.0%
Past alcohol use disorder	4.5%		35.0%
Current substance use disorder		4.7%	
Past substance use disorder	4.5%	4.7%	45.0%
Current nicotine/tobacco use			
Daily cigarette use			10.0%
Daily vape use			10.0%
Age of first contact with mental health services	22.5 (6.5)	19.6 (8.8)	16.5 (6.1)
Past hospitalization	4.5%	38.1%	55.0%
Suicide attempts	0.04 (0.2)	0.8 (1.3)	1.7 (2.7)
Reported family history of mental illness	36.4%	80.9%	70.0%
Previous or current psychotherapy	27.2%	100%	95.0%
Currently taking psychoactive medication	0.0%	76.2%	80.0%
Antidepressant		71.4%	70.0%
Antipsychotic		9.5%	35.0%
Tranquilizer		9.5%	5.0%
Stimulant		9.5%	15.0%
Anticonvulsant			5.0%
Antiparkinsonian		9.5%	
Sedative		9.5%	30.0%
Cannabinoid (prescribed)		4.7%	5.0%
Opioid antagonist			5.0%
Opioid (prescribed)		4.7%	
Contraceptives	36.4%	33.3%	35.0%

n = 22 controls; 21 MDD; 20 MDD+BPD; HAMD = Hamilton Depression Rating Scale; MDD = major depressive disorder; PDD = persistent depressive disorder; OCD = obsessive compulsive disorder; BPD = borderline personality disorder; PTSD = posttraumatic stress disorder. Note, one participant with BPD did not meet diagnostic criteria for MDD at the time of data collection. Additionally, the MDD and MDD+ BPD group did not differ in depressive severity scores measured by the HAMD, $t = .95$, $p = .60$.

Appendix I

Supplementary Analyses Excluding Participants with IL-6 Concentrations Below the Limits of Detection

Statistical Analyses with IL-6 Concentrations within Detectable Limits

Additional analyses were conducted to re-examine the correlations and mediation models of the main study findings (see Chapter 2 and 3 for main study analytic plan and results) with the exclusion of participants who had IL-6 concentrations below the limits of detection (i.e., removal of $n = 29$). Furthermore, sensitivity analyses of both samples (with and without IL-6 exclusions) were conducted to determine potentially appropriate sample sizes for future research.

After omitting participants with IL-6 concentrations below the limits of detection from the main study analyses, the majority of relationships remained the same. In this section, any notable differences from the main study findings are highlighted and summarized.

Supplemental Preliminary Analyses

Boxplots of the main study variables are presented in Supplemental Figure 1 (see Appendix K for all supplementary figures). Descriptive statistics for, and correlations among, the primary study variables are presented in Supplemental Table 1 (see Appendix J for all supplementary tables). Statistics provided in this table, and for all forthcoming statistics, are presented on the final sample of 30 participants after the exclusion of participants with IL-6 concentrations below the detectable limit.

Supplemental Primary Analyses

Are Cumulative Stress and Immune Activity Associated with Depression?

As was reported in the main study results section in Chapter 3, cumulative life stress severity (r = .43, p = <.01) and IL-6 (r = .57, p = <.01) remained significantly associated with depressive symptom severity.

Does Neuroendoimmune Activity Mediate the Relationship between Cumulative Stress and Depression?

Analyses that examined whether immune activity and free cortisol mediate the relationship between cumulative life stress severity ratings and depression (Hypothesis 2.1) were examined. In this mediation analyses, the relationships between cumulative life stress severity ratings, IL-6, free cortisol, and BDI-II scores were found to be similar to the main study findings with the exception of the direct effect between cumulative life stress severity and depressive symptom severity (path c'), which was no longer found to be significant, b = .15, p = .07 (see Supplemental Table 2 in Appendix J, and Supplemental Figure 2 in Appendix K for a summary of results).

To examine the parallel mediation effect, when the variance shared by both mediators was controlled for, the total effect size of the model was reduced by 42%. Although this represented a much stronger mediation effect compared to the main study findings (42% versus 26%), the combined indirect effect of the mediators did not reach statistical significance (similar to the main study findings reported in Chapter 3). As such, no mediation effect was detected for Hypothesis 2.1 with IL-6 samples removed from the analyses. In sum, as concluded in the main study findings, there was no mediation effect detected to suggest that neuroendoimmune activity mediates the relationship between cumulative life stress severity and depressive symptoms severity.

Is the Relationship between Cognitive Control and Depression Mediated by Immune Activity and Perceived Stress?

After removing participants from analyses with IL-6 concentrations below the limits of detection, analyses that examined whether perceived stress and immune activity mediate the relationship between cognitive control and depression (Hypothesis 3.1) were found to be very similar to the results presented in Chapter 3 (see Supplemental Table 3 in Appendix J and

Supplemental Figure 3 in Appendix K for a summary of model results). The relationship between IL-6 and BDI-II scores, as well as the relationship between cumulative life stress and BDI-II scores, remained significant. No new significant effects were found to support a serial mediation model. In this sample, there is no evidence to suggest that cognitive control is directly or indirectly (via cumulative life stress and immune activity) associated with depressive symptom severity. As such, no mediation effect was detected.

Supplemental Exploratory Analyses

Does Neuroendoimmune Activity Mediate the Relationship between Specific Time Periods of Stress and Depression?

As described above, Hypothesis 2.1 examined whether neuroendoimmune activity mediated the relationship between cumulative life stress severity and depression. From here, the question of whether neuroendoimmune activity mediates stress at specific time periods across the lifespan was examined (Exploratory Hypothesis 2.2). To test the relationship between specific time periods of stress and neuroendoimmune activity, three separate analyses were conducted using the same parallel mediation model presented in the primary results section above for Hypothesis 2.1, except the independent variable was changed to explore three distinct time periods of perceived stress—early life stress (≤12 years of age as measured by the STRAIN), past six-month stress (as measured by the STRAIN), and past month stress (as measured by the PSS). IL-6 and free cortisol were included as mediators, and depressive symptom severity as the dependent variable. The results are presented in Supplemental Figure 4 (Appendix K), represented as Exploratory Hypothesis 2.2a, 2.2b, and 2.2c, to match each of the distinct time periods, respectively.

Childhood Stress Appraisals, Neuroendoimmune Activity, and Depression. The first model, presented in Supplemental Figure 4 (Hypothesis 2.2.a), examined whether neuroendoimmune activity mediates the relationship between stress experienced before the age of 12 and depressive symptoms experienced at the time of the study (i.e., during adulthood). Statistical results of this model are outlined below and summarized in Supplemental Table 4

(Appendix J). After removing participants with IL-6 concentrations below the limits of detection, the relationships of the various paths remained significant with the exception of path b2. Here, the relationship between free cortisol and BDI-II while controlling for the other model variables was no longer significant, b = -.61, p = .07.

Even after the removal of IL-6 participants, the combined effect of the mediators still significantly decreased the total effect of the model (as was reported in the main study findings). When both mediators were added to the model, the total effect was reduced by about 42%. This represented a stronger overall mediation effect (i.e., 42% compared to 32% in the main study analyses). This indicates that when both mediators (IL-6 and free cortisol) were combined, the mediators reduced the overall effect of childhood stress on depressive symptom severity. As such, it can be concluded that neuroendoimmune activity mediated the relationship between childhood stress appraisals and depressive severity in adulthood in this sample. This supplementary analysis provides more confidence in the mediation model detected in the main study findings (i.e., that the findings were not primarily driven by the IL-6 scores that were below the limits of detection and assigned a concentration of .4pg/mL).

Past Six-Month Stress Perception, Neuroendoimmune Activity, and Depression. The second model, presented in Supplemental Figure 4 (Hypothesis 2.2.b), examines whether neuroendoimmune activity mediates the relationship between levels of perceived stress measured over the past six months and depressive symptoms experienced at the time of data collection (see Supplemental Table 5 in Appendix J for a summary of model results). Following the removal of participants with IL-6 concentrations below the limits of detection, the various paths and direct and indirect effects remained similar in magnitude. In keeping with the main study analyses, there was insufficient evidence to suggest that neuroendoimmune activity mediates the relationship between past six-month stress perceptions as measured by the STRAIN and depressive symptom severity.

Current Perceived Stress, Neuroendoimmune Activity, and Depression. The third model, presented in Supplemental Figure 4 (Hypothesis 2.2.c), examines whether neuroendoimmune activity mediates the relationship between current (past month) levels of perceived stress and depressive symptoms experienced at the time of the study (see Supplemental Table 6 in

Appendix J for a summary of model results). After removing participants with IL-6 concentrations below the limits of detection, the relationships of the various paths remained significant with the exception of path b2. Here, the relationship between free cortisol and BDI-II, while controlling for the other model variables, was no longer significant, b = -.50, p = .05.

Even after the removal of IL-6 participants, the combined effect of the mediators still significantly decreased the total effect of the model (similar to the results reported in the main study findings). When both mediators were added to the model, the total effect was reduced by about 26%. This represented a stronger overall mediation effect (i.e., 26% compared to 10.6% in the main study analyses). This indicates that when both mediators (IL-6 and free cortisol) were combined, the mediators reduced the overall effect of past month perceived stress on depressive symptom severity. As such, it can be concluded that neuroendoimmune activity mediated the relationship between past month stress appraisals and depressive severity in adulthood in this sample.

Power and sensitivity analysis

To assess appropriate sample size requirements post-hoc, a sensitivity analysis was conducted using the software G*Power. With power of .80 and a sample size of 59, we could expect to detect correlations of approximately r = .35 or larger. If we remove the 29 participants with IL-6 concentrations below the limits of detection, with power of .80 and a sample size of 30, we could expect to detect correlations of approximately r = .49 or larger. As such, given the current sample size, it is unlikely that we would have sufficient power to detect any weak to medium effect sizes. Examining the power plot presented in Supplemental Figure 5 (Appendix K), it appears that a total sample size of approximately 100 participants would have been appropriate to detect medium effect sizes common in depression and immune research (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Osimo et al., 2020).

Appendix J

Supplementary Tables Excluding Participants with IL-6 Concentrations Below the Limits of Detection

Variable	*M*	*SD*	1	2	3	4	5	6	7	8	9	10	11	12	13
1. BDI	24.2	19.0	1	.98***	.98***	.41	.43**	.55**	.60**	.77***	.01	.57**	-.42	.47**	.25
2. BDI Cognitive	12.5	10.5		1	.93***	.39	.41	.53**	.61***	.78***	.00	.54**	-.41	.47**	.21
3. BDI Neurovegetative	11.7	8.9			1	.42**	.43**	.56**	.57***	.74***	.01	.58**	-.42	.45**	.29
4. Total Stress Count[x]	29.6	15.9				1	.94***	.58**	.74***	.52**	.16	.11	-.43	.27	-.01
5. Total Stress Severity[x]	71.4	34.9					1	.63**	.76***	.58***	.27	.16	-.47	.23	.03
6. Childhood Stress[x]	23.5	18.7						1	.51**	.51**	.29	.36	-.38	.36	.25
7. Six Month Stress[x]	17.8	13.7							1	.54**	.03	.22	-.57**	.12	.12
8. PSS	21.5	9.2								1	-.01	.52**	-.03	.52**	.26
9. CWIT	10.9	2.3									1	-.02	.02	.02	.19
10. IL-6	1.1	1.0										1	-.12	.53*	.61**
11. Free Cortisol	20.9	7.9											1	.05	.13
12. TNFα	12.3	8.3												1	.32
13. CRP	4.52	5.2													1

Supplemental Table 1. Means, standard deviations, and bivariate correlations with participants with IL-6 below the limits of detection removed.

Note n =30 with the exception of correlations with free cortisol where $n = 29$. *$p < .05$. **$p < .01$. ***$p < .001$; [x]measured via the Stress Adversity Inventory for Adults (STRAIN); BDI, Beck Depressive Inventory-II; PSS, Perceived Stress Scale; CWIT, Colour-Word Interference Task; IL-6, interleukin-6; CRP, C-reactive protein.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.15	-.01, .31	.27	.07
2					
i	a1	.00	-.01, .01	.16	.37
ii	a2	-.11	-.19, -.04	-.50	<.01
3					
i	b1	9.37	4.50, 14.24	.49	<.01
ii	b2	-.60	-1.28, .09	-.25	.09
4					
i	ab1	.04	-.05, .14	.08	.38
ii	ab2	.07	-.02, .16	.13	.15
iii	ab1+ab2	.11	.02, .24	.20	.10
5					
i	c' + (ab1+ab2)	.26	.09, .43	.47	<.01

Supplemental Table 2. Beta Coefficients of Cumulative Life Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.1 with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30). *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*		Path or Parameter	Estimate	95% CI	Beta	*p*
1						
	i	c'	-.71	-3.36, 1.93	-.09	.60
2						
	i	a1	4.17	-.73, 8.53	.28	.07
	ii	a2	-.03	-.15, .12	-.06	.69
3						
	i	b1	.23	.04, .38	.42	<.01
	ii	b2	9.45	4.01, 16.14	.49	<.01
4						
	i	d	.01	.00, .02	.18	.34
5						
	i	ab1+ab2+a1db2	.90	-.91, 2.96	.11	.36
6						
	i	c' + (ab1+ab2+a1db2)	-.19	-2.31, 3.10	.02	.89

Supplemental Table 3. *Beta Coefficients of Cognitive Control, Cumulative Life Stress Severity, IL-6, and Depressive Symptom Severity of Hypothesis 3.1 with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30).* *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.34	.05, .63	.34	<.05
2					
i	a1	.02	.00, .04	.35	<.05
ii	a2	-.17	-.31, -.02	-.39	.02
3					
i	b1	7.93	2.90, 12.95	.41	<.01
ii	b2	-.61	-1.25, .04	-.25	.07
4					
i	ab1	.15	-.02, .32	.15	.08
ii	ab2	.10	-.04, .24	.10	.16
iii	ab1+ab2	.25	.03, .47	.24	<.05
5					
i	c' + (ab1+ab2)	.59	.29, .88	.58	<.01

Supplemental Table 4. Beta Coefficients of Childhood Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2a with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30). *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	.64	.24, 1.04	.46	<.01
2					
i	a1	.02	-.01, .04	.22	.21
ii	a2	-.34	-.51, -.16	-.58	<.01
3					
i	b1	8.52	3.99, 13.06	.44	<.01
ii	b2	-.28	-.96, .40	-.12	.42
4					
i	ab1	.14	-.09, .37	.10	.23
ii	ab2	.09	-.14, .33	.07	.44
iii	ab1+ab2	.23	-.10, .56	.17	.16
5					
i	c' + (ab1+ab2)	.87	.49, 1.26	.63	<.01

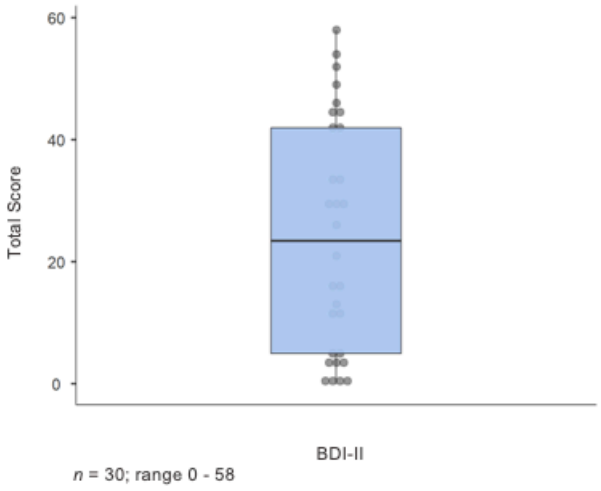
Supplemental Table 5. Beta Coefficients of Past Six-Month Stress Severity, IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2b with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30). *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

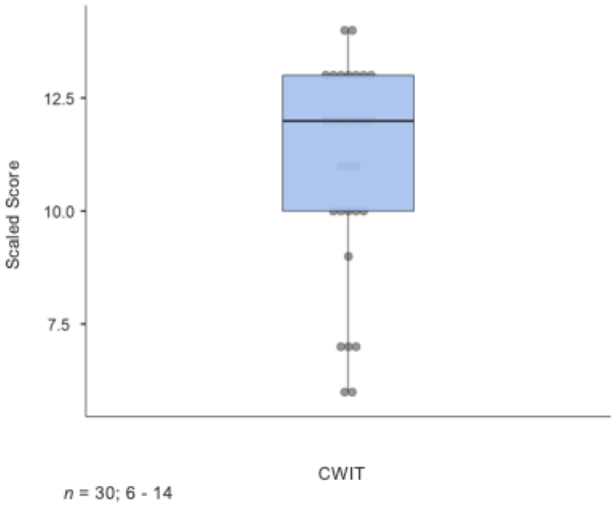
Step*	Path or Parameter	Estimate	95% CI	Beta	*p*
1					
i	c'	1.25	.75, 1.76	.60	<.01
2					
i	a1	.05	.02, .09	.51	<.01
ii	a2	-.27	-.57, .03	-.32	.07
3					
i	b1	4.37	-.09, 8.82	.23	.05
ii	b2	-.50	-1.01, .01	-.21	.05
4					
i	ab1	.24	-.04, .52	.12	.10
ii	ab2	.14	-.07, .34	.07	.19
iii	ab1+ab2	.38	.02, .73	.18	<.05
5					
i	c' + (ab1+ab2)	1.63	1.17, 2.08	.79	<.01

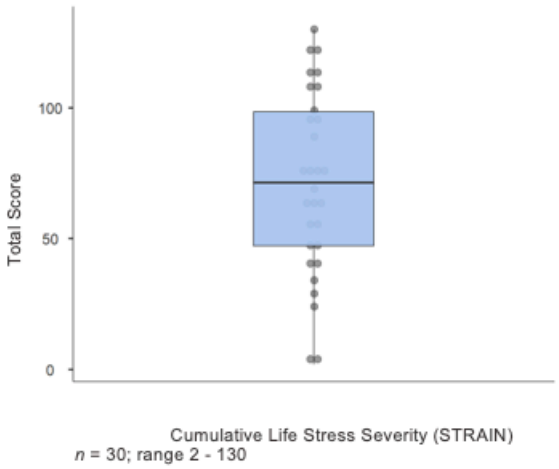
Supplemental Table 6. Beta Coefficients of Perceived Stress Scale (PSS), IL-6, Free Cortisol, and Depressive Symptom Severity of Hypothesis 2.2c with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30). *Baron and Kenny (1986) mediation step notation. Estimate = unstandardized regression coefficient for specified path. Beta = standardized regression coefficient for specified path.

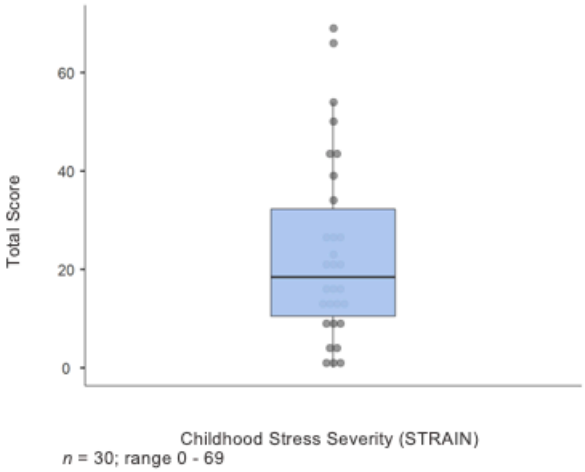
Appendix K

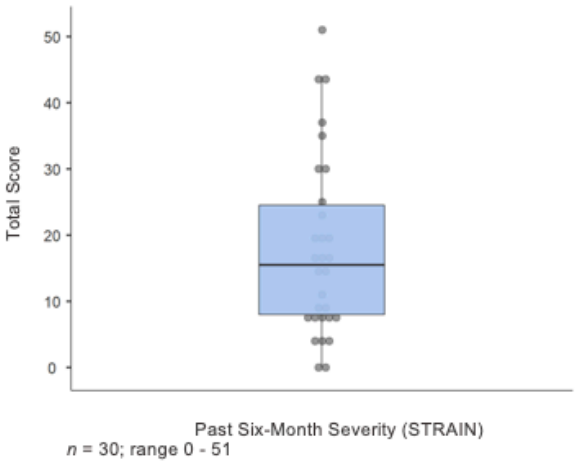
Figures for Supplementary Analyses with Participants with IL-6 Concentration Below the Limits of Detection Omitted

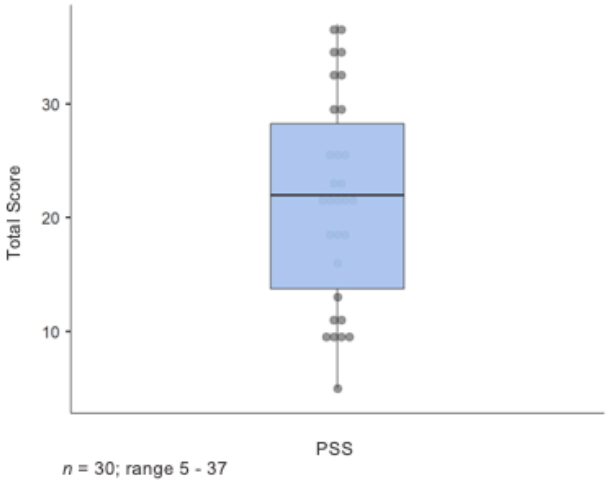


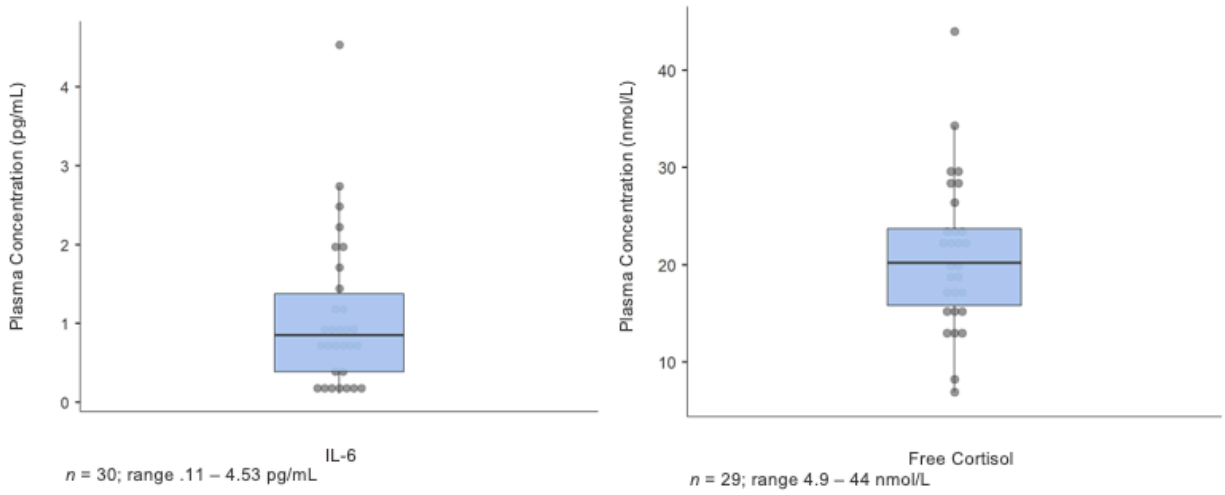




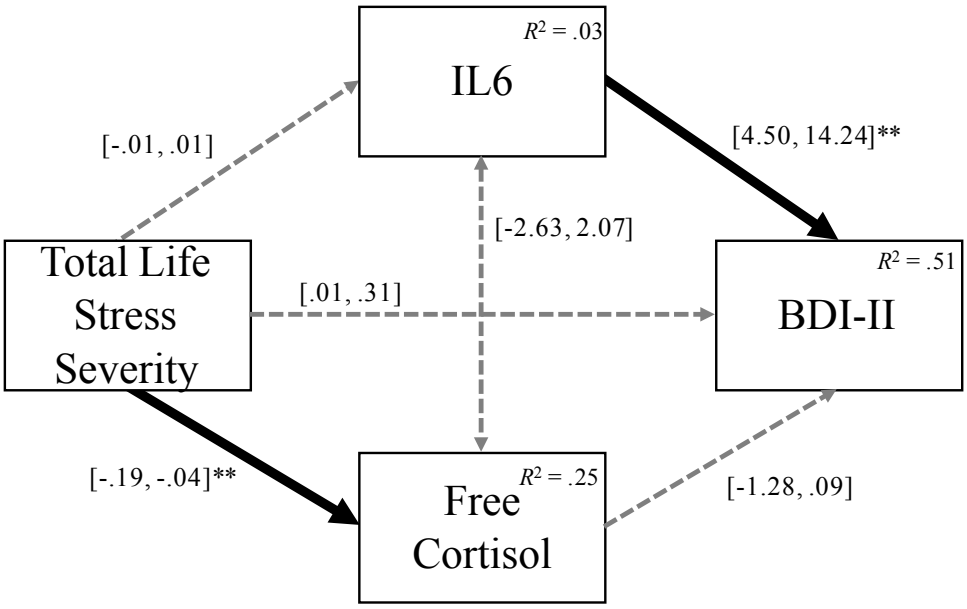




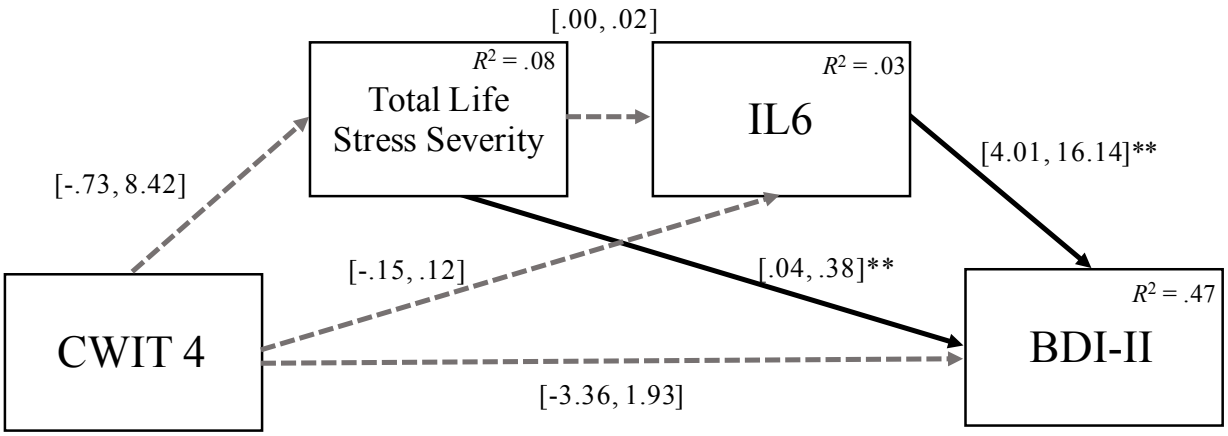




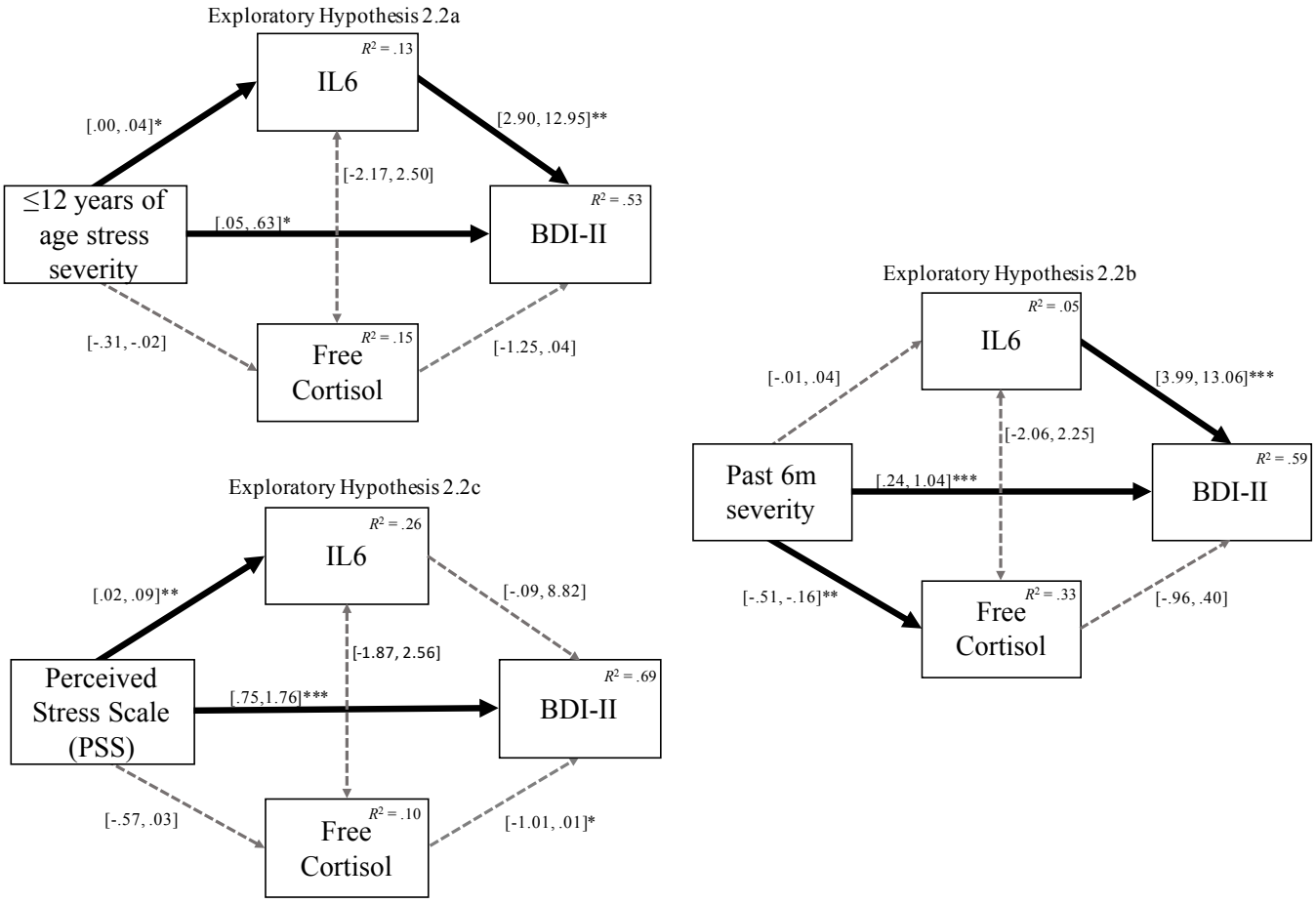
Supplemental Figure 1. *Boxplots of the Main Study Variables with Participants with Subthreshold IL-6 Concentrations Omitted (n = 30)*. The line in the box represents the median score of the sample, and the ends of the box indicate where the first and third quartile end. Data points beyond the whiskers are considered outlier values. BDI-II = Beck Depression Inventory-II; STRAIN = Stress and Adversity Inventory for Adults; PSS = Perceived Stress Scale; CWIT 4 = Colour-Word Interference Task; pg/mL: picograms/millilitre; nmol/L: nanomoles/Litre.



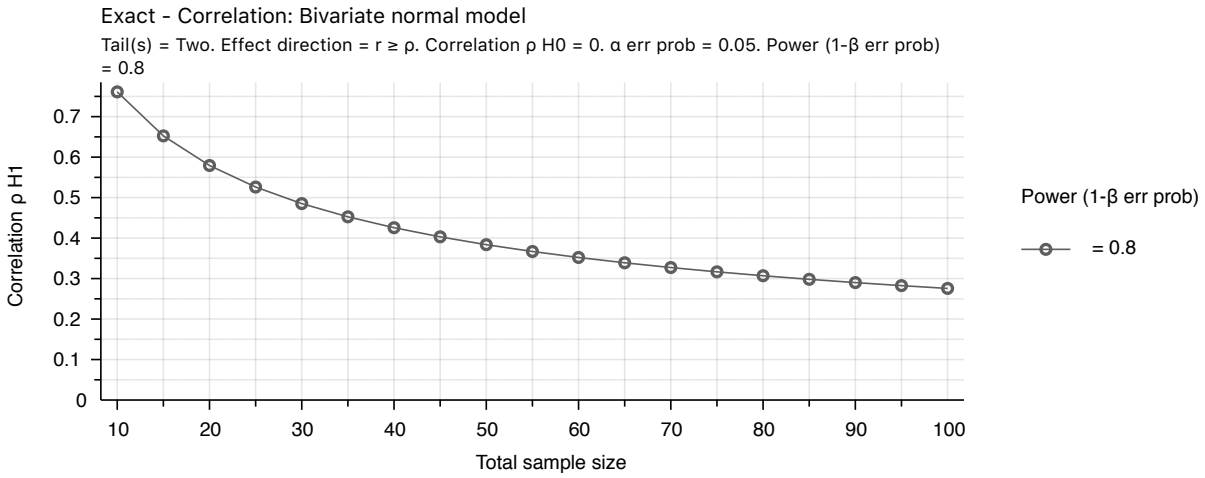
Supplemental Figure 2. Results of Hypothesis 2.1 with Participants with IL-6 Concentrations Below the Limits of Detection Omitted. This figure provides a visual of the parallel mediation model tested to examine whether neuroendoimmune activity (IL-6 and free cortisol) mediates the relationship between cumulative life stress severity as measure by the Stress and Adversity Inventory for Adults (STRAIN) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by the predictor variables.



Supplemental Figure 3. Results of Hypothesis 3.1 with Participants with IL-6 Concentrations Below the Limits of Detection Omitted. This figure provides a visual of the serial mediation model tested to examine whether perceived stress severity measured by the Stress and Adversity Inventory for Adults (STRAIN) and immune activity (IL-6) mediate the relationship between cognitive control as measure by condition 4 of the Colour-Word Interference Task (CWIT 4) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by cumulative life stress severity.



Supplemental Figure 4. Results of Exploratory Hypothesis 2.2 with Participants with IL-6 Concentrations Below the Limits of Detection Omitted. This figure provides a visual of the parallel mediation models used to examine whether neuroendoimmune activity mediates the relationship between distinct time periods of perceived stress severity (≤12 years of age, past six months, and past month) and depressive symptom severity measured by the Beck Depression Inventory (BDI-II). Bracketed numbers represent 95% confidence intervals; solid lines indicate significant regression pathways; dotted grey lines indicate relationships that were not significant; R^2 is the partial correlation coefficient which represents effect sizes that can be interpreted as the percentage of variance explained by the predictor variables.



Supplemental Figure 5. Sensitivity and Power Analysis. This figure provides a power plot of a sensitivity analysis conducted to determine appropriate sample sizes for depression severity and neuroendoimmune activity research.