A Personalized Approach to Understanding Depression: Examining the Biological and Psychosocial Basis of Symptom Clusters

by

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Abstract

Despite the prevalence and impact of depression, effective treatments lag behind that of many physical conditions owing, in part, to the complexity of this disorder. Considering the heterogeneity of depression and comorbidities with other mental illnesses, a focus on the symptoms expressed and how these relate to psychosocial and biological factors, may inform a personalized treatment strategy. We developed transdiagnostic symptom clusters spanning boundaries of anxiety and depression that mapped onto specific psychosocial and biological factors. Namely, clusters representing the neurovegetative features of depression strongly related to inflammatory profiles, suggesting that this relationship is symptom specific. Moreover, clusters representing comorbid symptomatologies were associated with increased severity of symptoms, higher early life adversity scores and suicidal behaviours. The present study suggests distinct symptomatologies have differing biological underpinnings. Thus, shifting away from diagnostic categories and further exploring personalized approaches to better understand the neurobiology of depression and inform future treatments is warranted.

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Depression is the leading cause of disability worldwide and is projected to have the greatest burden of disease by the year 2030 (Mokdad et al., 2016; WHO, 2008; World Federation For Mental Health (WFMH), 2012). Despite its prevalence and impact, effective treatments lag far behind that of many chronic physical conditions owing to our limited understanding of the pathophysiology of depression. Moreover, depression is a heterogeneous disorder in which individuals display at least five of nine symptoms from the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V; American Psychiatric Association, 2013; Grisanzio et al., 2018). Thus, there are many combinations of ways to meet diagnostic criteria, and individuals can present vastly different symptomatologies (i.e., symptom profiles) and responses to treatment (Fried, 2017). The majority of studies to date have focused on overall depression scores, examining depression as a single entity and not taking into consideration the differences in symptoms expressed (Majd et al., 2019). This perspective may explain the inconsistencies in research examining the neurobiology of depression and in turn, the limited efficacy in pharmacological treatments (Ahmed et al., 2018).

It has been suggested that there might be multiple forms of depression, of which, the biological basis is poorly understood (Ahmed et al., 2018; Insel & Cuthbert, 2015). Recent brain imaging studies have identified depressive subtypes, and it has been maintained that biological markers could be useful in distinguishing between these subtypes (Drysdale et al., 2017; Grisanzio et al., 2018). Blood-based biomarkers, such as elevated inflammatory factors, including immune messaging molecules (e.g., pro-

inflammatory cytokines) and hypothalamic-pituitary-adrenal (HPA) axis activity have been strongly linked with depression and might be useful in differentiating individuals' symptoms as well as treatment response (Gibb et al., 2009; Miller & Raison, 2015b; Osimo et al., 2019).

It is also likely that the heterogeneity related to depression stems from diverse experiences, such as histories of childhood trauma. Stressors, such as early life trauma, are strongly linked to depression, increasing the risk of developing this disorder by approximately three times compared to individuals who have not experienced early life trauma (Afifi et al., 2014). Experiences of stress can cause dysregulation of HPA axis functioning, which is one of the main biological pathways involved in the pathogenesis of depression (Pariante & Lightman, 2008; Pariante & Miller, 2001; Slavich & Auerbach, 2018). Similarly, such stressors also result in the release of inflammatory factors (Slavich & Auerbach, 2018; Slavich & Irwin, 2014), which are thought to be centrally involved in the development of depression. In fact, depressed individuals with trauma histories have displayed more pronounced levels of pro-inflammatory cytokines compared to depressed individuals without such a history (Lu et al., 2013). These data might suggest that one mechanism linking early life stress to mental health symptomatologies is through inflammation.

Beyond the heterogeneity of depression, there is incredible overlap between mental health disorders, with 50% of individuals having concurrent anxiety and mood disorder diagnoses (Kessler et al., 2008; Slavich & Auerbach, 2018). Moreover, while not all individuals with mood disorders experience suicide ideation or display suicide behaviours, mental health issues are thought to account for a large number of suicide

attempts and/or deaths due to suicide (Navaneelan, 2012). Therefore, to better understand the biological mechanisms of these disorders and, in turn, to move towards more personalized approaches to treatment, there needs to be a shift away from diagnostic categories, and biomarker studies ought to focus on symptoms/constructs that cut across many disorders (Insel & Cuthbert, 2015). Thus, the present investigation will focus on symptomatologies, characterizing distinct clusters of depressive symptoms that capture the heterogeneity of depressive features and that span across highly comorbid disorders, including symptoms of anxiety and suicidal thoughts. Using a data-driven approach, we plan to determine specific profiles of peripheral biomarkers (i.e., cortisol, cytokines and other inflammatory markers found in the blood) that map onto and can be used to differentiate symptom clusters at a biological level. Moreover, we will gain a better understanding of the role of early life trauma in the relationship to symptom clusters and biomarker profiles.

Stressful Experiences and Mental Health

Stressful life events have consistently been linked to the development of a number of mental and physical health disorders (Cohen et al., 2019; Douglas et al., 2010; Hammen, 2016; Slavich, 2016). For example, individuals who experience early life stress, such as childhood trauma (i.e., abuse and/or neglect) are three times more likely to develop depression and are six times more likely to have attempted suicide later in life (Afifi et al., 2014). These statistics are especially concerning as experiences of childhood trauma are fairly common, with 32% of children in Canada experiencing some form of trauma or abuse early in life (Afifi et al., 2014). Studies demonstrate a dose-response relationship between early life adverse experiences and the later development of

psychopathologies. Specifically, as the number of adverse experiences increases, the symptoms of depression and anxiety, as well as suicide ideation and attempts, also increase (Afifi et al., 2014; Chapman et al., 2004; Dube et al., 2001). It may not only be the cumulative impact of trauma influencing mental health outcomes, but also the severity and type of adversity experienced (Schilling et al., 2008). Moreover, individuals who experience early life adversity are more likely to encounter greater levels of stress later on, with current life stressors being more prevalent in those who have experienced early life adverse events (McElroy & Hevey, 2014).

Stressors, including early life adversities, have been increasingly reported by university students (Gallagher, 2014) and are thought to contribute to the increases in numbers of students presenting at university health and counselling services for mental health concerns (Benton et al., 2003; Hoban & Leino, 2006; Karatekin, 2018). Recent attention has been brought to the 'mental health crisis' occurring on campuses, with reports stating that 88.8% of university students report feeling overwhelmed, and that roughly 50% of students are experiencing significantly high levels of stress presenting in the form of depression and anxiety (Craggs, 2012; Jones et al., 2018; Regehr et al., 2013). These findings are especially concerning as mental health disorders are strong predictors of productivity, educational achievement and university attrition (Auerbach et al., 2016). In a recent *Status of Mental Health in Ottawa report*, it was found that one out of nine students in Ottawa had seriously considered suicide in 2017 (Ottawa Public Health, 2018). With the average age of onset of mental health disorders, such as anxiety and depression, coinciding with the age that students begin college/university (Eisenberg et al., 2009; Kessler et al., 2005; Patten, 2017), university students have become an

important population to better understand the onset of mental health symptoms, individual differences, and underlying biological mechanisms in order to inform more effective early interventions and personalized treatments.

Biological Consequences of Stress and Links to Depression

Hypothalamic-Pituitary-Adrenal (HPA) Axis

A number of neurochemicals and hormones are released in response to stress; however, most notably, stressors immediately activate the *hypothalamic-pituitary-adrenal (HPA) axis* (Chrousos, 2009). When encountering a stressor, the hypothalamus releases corticotrophin-releasing hormone (CRH), which sends a message to the pituitary gland signalling it to secrete adrenocorticotropic hormone (ACTH) into the periphery (i.e., the bloodstream). The ACTH travels to the adrenal glands, which results in a release of the main stress hormone, cortisol, a glucocorticoid. Typically, elevated levels of glucocorticoids in the blood form a negative feedback mechanism that will shut down the stress response when it is no longer needed (i.e., the stressor is no longer a threat; Pariante & Lightman, 2008). In the case of chronic stress, the HPA axis can become hyperactive, resulting in excess amounts of glucocorticoids, such as cortisol, circulating in the bloodstream. Over time, this increase in glucocorticoids causes disruptions in the negative feedback mechanism that shuts down the HPA axis. Such abnormalities in HPA axis functioning have been demonstrated among individuals with depression, and this link has been one of the most consistent findings in psychiatry (Juruena et al., 2018; Pariante & Miller, 2001). Moreover, depressed individuals are more likely to display an increased HPA axis response to a psychosocial stressor (Bylsma et al., 2011; Pariante & Lightman, 2008). In line with these findings, it has been demonstrated that some

depressed individuals have an exaggerated cortisol response to ACTH, enlargement of the adrenal and pituitary glands and increased baseline plasma (i.e., blood), urine and cerebrospinal fluid (CSF) concentrations of cortisol (Gold et al., 1988; Gold et al., 2015; Nemeroff, 1996; Owens & Nemeroff, 1993).

Chronic stressors, such as experiences of early life stress, can cause long-term disturbances in the stress response system (Maydych, 2019). Depressed individuals with experiences of childhood trauma display an ACTH response to an acute stressor that is six times greater than depressed individuals without trauma histories (Heim et al., 2000). Biological changes as a result of early life stress (e.g., dysregulation of HPA axis functioning, most often hyperactivity resulting in glucocorticoid resistance) can impact later sensitivity to stress and ultimately lead to the development of stress-related psychopathologies (Maydych, 2019; Slavich & Auerbach, 2018). Specifically, adverse early life experiences can cause a decreased threshold stress response, resulting in an exaggerated reaction to lower level acute stressors later in life (also known as stress sensitization). This is likely one of the mechanisms through which early life adversity leads to an increased risk for the later development of depression and other mental health disorders (Claes, 2009; Espejo et al., 2006; Slavich & Auerbach, 2018).

Inflammation

The HPA axis has a bidirectional relationship with other systems that impact psychopathology, such as the immune system (Baumeister et al., 2014; Miller & Raison, 2015b). Stressful events can impact immune system functioning; specifically, experiences of acute and chronic psychological stress can result in increased levels of inflammatory factors such as C-reactive protein (CRP) as well as and pro-inflammatory

cytokines (Raison et al., 2006; Slavich & Auerbach, 2018). Moreover, inflammatory cytokines can stimulate the HPA axis through the release of glucocorticoids (Silverman et al., 2005), which in turn can supress the immune system (Dantzer et al., 2008; Irwin & Cole, 2011; Pace et al., 2007). It is suggested that the brain may interpret immune activation in the same way as it would interpret a psychological or physical stressor (Anisman et al., 2008; Anisman & Merali, 1999). Pro-inflammatory cytokines are thought to contribute to the pathogenesis of stress-related disorders, especially the prognosis and maintenance of depressive symptoms (Haapakoski et al., 2015; Khandaker et al., 2014; Maes, 2008).

A recent meta-analysis found that depressed individuals are 50% more likely to display elevated levels of inflammation compared to non-depressed controls (Osimo et al., 2019). Thus, a strong inflammation-depression link has been well-established (Miller & Raison, 2016). Multiple meta-analyses have revealed that individuals suffering from depression display elevated circulating levels of inflammatory markers (Dowlati et al., 2010; Goldsmith et al., 2016; Haapakoski et al., 2015; Osimo et al., 2019). More specifically, elevated levels of CRP and pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α, and have been reported among those with major depressive disorder (MDD; Haapakoski et al., 2015; Howren et al., 2009; Miller & Raison, 2016; Osimo et al., 2019; Valkanova et al., 2013). Moreover, some individuals with depression display lower peripheral levels of the anti-inflammatory cytokine IL-10 (Dhabhar et al., 2009). This evidence suggests that not only are pro-inflammatory cytokines related to depression, but a shift towards a pro-inflammatory state, involving

elevated pro- and/or reduced anti-inflammatory cytokines, could be associated with depressive symptoms (Audet et al., 2014).

In further support of the inflammation-depression link, administration of pro-inflammatory cytokines resulted in the development of depressive symptoms among adults that were being treated for hepatitis C and some types of cancer (Dantzer et al., 2008; West & Maes, 1999). These inflammatory-induced symptoms were attenuated when individuals were treated with antidepressant medication, a finding that suggested cytokines might not just be a marker of depression but fundamentally involved in the development of this disorder (Dantzer et al., 2008). Moreover, a recent meta-analysis examining antidepressant treatments demonstrated that peripheral levels of IL-6, IL-10 and TNF-α were significantly decreased following antidepressant therapy (Köhler et al., 2018). However, while strong links between depression and inflammation have been established, not all individuals with depression display increased inflammatory activity (Jokela et al., 2016; Majd et al., 2019; Maydych, 2019). There are a handful of studies unable to find this link (Dowlati et al., 2010; Howren et al., 2009; Maydych, 2019), and it is suggested that the inconsistencies in this relationship may be due to the notion that elevated peripheral inflammation may only be observed in a subset of individuals with MDD, and that this relation may, in fact, be symptom-specific (Ahmed et al., 2018; Jokela et al., 2016; Miller & Raison, 2015a, 2016). Thus, there is a need to better understand what specific depressive symptoms, or combination of symptoms, are characterized by elevated levels of inflammation, in order to potentially inform more targeted and novel approaches to pharmacological treatment.

Depression Heterogeneity, Comorbidity and Treatment Implications

Heterogeneity of Depression

Depression is an incredibly *heterogeneous* disorder, as it can present in many different ways and, as such, there is much variability within the diagnosis itself and in responses to treatment (Grisanzio et al., 2018). In this regard, MDD is diagnosed based on the DSM-V when the following symptoms are present for a minimum two-week period: a depressed mood or anhedonia and when four of nine possible symptoms are present, including; significant weight loss/gain, frequent insomnia or hypersomnia, visible psychomotor disturbances in the form of agitation or retardation, fatigue, feelings of worthlessness or unwarranted guilt, impaired concentration and/or decision making, or suicide ideation (American Psychiatric Association, 2013). In fact, over 1000 unique combinations of symptoms warranting a DSM diagnosis were identified among 3,703 individuals seeking treatment for MDD (Fried & Nesse, 2015). It is suggested that symptom heterogeneity may be related to external factors and individual experiences, such as psychosocial variables (i.e., perceived stress and social support; Hybels et al., 2011). Heterogeneity not only exists in symptom expression, but also in course of illness. For example, females are disproportionately impacted by depression and anxiety, not only due to their increased likeliness to experience symptoms (Acciai & Hardy, 2017; Auerbach et al., 2018; Kessler et al., 2005; Leach et al., 2008), but they are also more likely to have a younger age of onset of depressive symptoms, a lower quality of life with diagnoses, and generally present symptomatologies that are more severe, prolonged and recurrent compared to males (Kornstein et al., 2000; Sramek et al., 2016). Moreover,

comorbid diagnoses of depression and anxiety are significantly more common among females compared to men (Asher et al., 2017; McLean et al., 2011; Sramek et al., 2016).

Comorbidity Between Depression and Other Mental Health Disorders

In addition to the vast heterogeneity of depressive symptoms, depression is a highly *comorbid* disorder, often co-occurring with other mental health disorders. Comorbid disorders can share common underlying mechanisms or respond to similar environmental triggers (Anisman, 2014; Klein & Riso, 1993). Anxiety is the most common comorbid mental illness with depression, as depression co-occurs with an anxiety disorder more than 50% of the time (Fava et al., 2008; Kessler et al., 1996; Kessler et al., 2005; Slavich & Auerbach, 2018). Comorbid anxiety and depression are indicative of higher severity of illness, increased psychosocial and occupational impairment, reduced quality of life and reduced efficacy of treatment (Fava et al., 2008; Hirschfeld, 2001; Saveanu et al., 2014). In addition, mental illness is a strong risk factor for suicidal thoughts and behaviours. In fact, 90% of individuals who die by suicide have a co-occurring mental health disorder, of which depression is the most common (60%; Cavanagh et al., 2003; Navaneelan, 2012). While depression is strongly related to suicide ideation, a recent meta-analysis highlights that little is known about the characteristics of individuals with depression who have an increased risk of suicide ideation (Brådvik, 2018). This is important, particularly for youth and emerging adults as suicide is the second leading cause of death among Canadians under the age of 25 years (accounting for 35% of deaths; Statistics Canada, 2018; Navaneelan, 2012).

Treatment Efficacy, Biomarkers and Depressive Subtypes

Depression is most commonly treated by antidepressant medication, and first-line treatment antidepressants are typically selective serotonin reuptake inhibitors (SSRIs) and selective norepinephrine reuptake inhibitors (SNRI's) which increase serotonin and/or norepinephrine by inhibiting its reuptake mechanism (Dale et al., 2015; Gałecki et al., 2018; Poluzzi et al., 2013). Other novel treatments are undergoing clinical trials such as low-dose ketamine infusions (Chen et al., 2018). However, only about one-third of those receiving antidepressant treatments reach remission, and having a comorbid condition, such as co-occurring anxiety symptoms, leads to still lower rates of remission (Saveanu et al., 2014). It is suggested that the limited efficacy of antidepressants is due in part to the classification and treatment of depression as a whole, considering the symptom heterogeneity that exists (Ahmed et al., 2018; Fried, 2017).

Classifying and treating depression as a single illness may be counterproductive, and it may be more appropriate to conduct research and treat individuals based on the specific symptoms expressed and their corresponding biological markers (Anisman, 2014; Audet et al., 2013; Insel & Cuthbert, 2015; Østergaard et al., 2011). This perspective, much in line with a precision medicine approach, has been successful in treating physical health disorders. For example, in oncology, the process of diagnosing individuals based on biological subtypes has driven well-defined individualized treatments that have provided better outcomes than ever before (Collins & Varmus, 2015). It is suggested that a similar method of incorporating the measurement of both brain and behaviour will be essential to improve treatments for mental health disorders, incorporating individual needs and their specific neural pathology (Insel & Cuthbert,

2015). This method has been applied to the assessment of attention deficit hyperactivity disorder (ADHD), as three subtypes of this disorder have been identified and distinguished by cardiac response and resting-state functional brain connectivity that respond differentially to treatment with a stimulant medication (Karalunas et al., 2014).

The field of depression research has not yet experienced success in precision medicine advancements; however, there is recent brain imaging evidence characterizing depressive subtypes (Drysdale et al., 2017; Grisanzio et al., 2018). Constructs relating to depressive symptoms such as anhedonia (i.e., lack of pleasure experiences) and executive functioning can cut across many diagnostic boundaries, eluding to the idea that depressive subtypes may have the ability to be transdiagnostic (Insel & Cuthbert, 2015). It is suggested that there may be multiple forms of depression based on symptomatolagies, of which, the neurobiological basis is poorly understood (Ahmed et al., 2018; Insel & Cuthbert, 2015). A recent study was able to effectively identify robust subtypes of mental health symptom profiles that that cut across DSM-V-defined diagnoses of MDD, panic disorder, and post-traumatic stress disorder. These subtypes signified distinct, coherent and meaningful associations in cognition (working memory, cognitive control), brain function (e.g., electroencephalography, EEG) and observable real-world function including emotional resilience and social functional capacity (Grisanzio et al., 2018). Moreover, a recent brain-based biomarker study has identified four depressive subtypes according to distinct whole-brain patterns of abnormal functional connectivity in resting-state networks, and these profiles mapped onto differences in depressive symptomatologies, helping to identify which individuals would most likely benefit from neurostimulation treatment (Drysdale et al., 2017).

The importance of considering biomarkers in differentiating depressive symptom profiles can be further highlighted through the understanding of atypical versus melancholic (i.e., typical) depressive subtypes. Melancholic features of depression are characterized by anhedonia or lack of mood reactivity, loss of appetite/weight loss, insomnia, feelings of guilt and psychomotor retardation whereas atypical features involve increased mood reactivity, increased appetite/weight gain, hypersomnia, increased sensitivity to rejection and leaden paralysis (American Psychiatric Association, 2013). Due to the opposing features of these subtypes, it is suggested that they may respond differently to treatment (Harald & Gordon, 2012); however, such findings have been inconsistent (Arnow et al., 2015). Rather, recent studies indicate that inconsistencies may relate to heterogeneity within these broad categories, and suggest that biomarkers can be more useful in differentiating depressive subtypes (Ahmed et al., 2018; Juruena et al., 2018; Majd et al., 2019). Thus, biomarker studies have shown that those who display melancholic features of depression have significantly higher basal plasma levels of cortisol than those with atypical features or controls (Gold & Chrousos, 2013; Juruena et al., 2018; Lamers et al., 2013). However, these associations among melancholic and atypical subtypes appear to be more apparent and robust when studies solely focus on *neurovegetative* symptoms (Juruena et al., 2018; Majd et al., 2019). The core symptoms of atypical and melancholic depressive subtypes are based on the neurovegetative symptoms of this disorder, which involve appetite, weight, and sleep patterns (Kendler, 2016). Melancholic depression involves typical neurovegetative symptoms of reduced appetite/weight and insomnia, whereas atypical depression is characterized by reversed neurovegetative symptoms (e.g., increased appetite/weight, hyposomnia; American

Psychiatric Association, 2013; Kendler, 2016).

A proposed explanation for the development of these distinct symptom profiles could be through immune response and inflammation (Capuron et al., 2002; Jokela et al., 2016). Neurovegetative symptoms, specifically those of the melancholic type, characterize *sickness behaviour*, which an individual exhibits when exposed to an infection in order to preserve energy to promote healing in response to the infection (Jokela et al., 2016; Miller & Raison, 2016). Thus, pro-inflammatory cytokines and indicators of inflammation are markedly elevated among those displaying neurovegetative symptoms of depression, including fatigue, reduced appetite, and withdrawal (Capuron & Miller, 2004; Jokela et al., 2016; Majd et al., 2019; Miller & Raison, 2016). In fact, associations between inflammation and other depressive symptoms, such as more cognitive and emotional symptoms (e.g., anhedonia, concentration), are no longer significant after controlling for neurovegetative symptoms (Jokela et al., 2016; Majd et al., 2019). Moreover, when healthy individuals were treated with interferon (IFN)-α (i.e., a cytokine that stimulates the release of pro-inflammatory cytokines), all participants developed neurovegetative symptoms of depression within the first two weeks. However, symptoms that involve changes in mood and memory/concentration developed later in treatment and were only demonstrated in 30-50% of participants (Capuron & Miller, 2004). In addition, these mood and memory/concentration symptoms were most responsive to a prophylactic antidepressant treatment, whereas the neurovegetative symptoms were less responsive (Capuron & Miller, 2004; Maydych, 2019; Musselman et al., 2001).

These findings indicate that neurovegetative symptoms of depression appear to be

most closely tied to inflammation, which might influence treatment outcomes. However, it remains unclear whether additional depressive symptom profiles are linked to inflammation, and whether these associations will occur across diagnostic categories. Moreover, CRP has recently been found to be elevated among those with treatment-resistant depression when compared to individuals with treatment-responsive depression (Chamberlain et al., 2019). Such findings further insinuate that those displaying elevations in inflammatory markers could be a specific subset of individuals who may benefit from targeted novel treatment methods.

There is much less literature surrounding anxiety and biomarker profiles; however, a meta-analysis showed that individuals with clinical anxiety have higher IL-6 levels compared to those without anxiety, an effect independent of depression severity (O'Donovan et al., 2010). Symptoms of generalized anxiety have been associated with elevated levels of both salivary and basal blood cortisol as well, and these associations appear to be dependent on the severity of symptoms being expressed (Mantella et al., 2008; Wang et al., 2017). Moreover, a meta-analysis suggests that specific peripheral cytokine profiles may be present in depressed individuals who are also experiencing suicidal ideation, but not among depressed individuals without suicidal ideation (Serafini et al., 2013). Considering the above, identifying transdiagnostic symptom clusters and being able to distinguish the cytokine profiles that map onto these clusters, could help clarify inconsistencies in the literature regarding depressive symptoms and biomarker profiles, as well as differential treatment response (Audet et al., 2013; Majd et al., 2019; Maydych, 2019; Slavich & Auerbach, 2018). Additionally, as psychosocial factors, such

as stressors, have a significant impact on cytokines, consideration of the impact of stress on the depression-immune link is warranted (Slavich & Irwin, 2014).

Connecting Early Life Adversity, Depression and Inflammation

Early life adversity has been associated with elevated pro-inflammatory cytokines (Hostinar et al., 2018). Specifically, females who report childhood experiences of sexual abuse display higher plasma IL-6 and CRP levels compared to females with no abuse histories (Bertone-Johnson et al., 2012). Moreover, it has been observed that individuals with depression who have experienced childhood trauma display higher IL-6 and CRP levels compared to individuals with depression who have not experienced childhood trauma (Frodl et al., 2012; Lu et al., 2013; Munjiza et al., 2018; Moreira et al., 2018). Additionally, children (ages 3-12) who experienced trauma prior to the age of 4 displayed higher peripheral IL-6 levels compared to those without experiences of trauma (Bücker et al., 2015). Despite the above, there are a handful of studies who have not found such associations between early life adversity and elevations in inflammatory markers (Carpenter et al., 2012; Gouin et al., 2012). It is possible that childhood adversity is associated with a subset of depressed individuals who display higher levels of inflammation; however, it is unclear if these individuals are also more likely to display distinct depressive symptom profiles.

Taken together, the purpose of the current study is to characterize distinct clusters of mental health symptoms that capture the heterogeneity of depressive features and that cut across highly comorbid conditions, such as symptoms of anxiety and suicidal thoughts. This will be a data-driven approach; however, in general, we expect that meaningful symptom derived subtypes will develop within our heterogeneous sample that

span diagnostic boundaries. Additionally, while specific depressive symptoms, mainly neurovegetative profiles and anxiety symptoms, have been separately examined in relation to inflammation, this has not yet been done by creating transdiagnostic symptom clusters. Thus, we will map the distinct symptom clusters that we create through a data-driven approach onto peripheral biomarkers (i.e., cortisol, cytokines and inflammatory markers found in the blood) in order to differentiate symptom clusters on a biological level. We hypothesize that certain depressive subtypes will be characterized by higher levels of cortisol and inflammation. In particular, we predict that symptom profiles associated with more neurovegetative features will be differentiated by elevated levels of pro-inflammatory cytokines as well as other inflammatory markers, such as CRP. However, we also anticipate that individuals with a more anxious/aroused symptom profile will display elevations in cortisol. Thus, we anticipate that it will not necessarily be the clusters with the highest total depression and/or anxiety scores that are marked by elevated CRP or cortisol, but rather the specific symptoms subtypes that will more importantly map onto peripheral biomarkers. The final objective is to gain a better understanding of how histories of childhood trauma will be associated with symptom clusters. As there is no evidence that trauma is linked to any one symptom profile, we expect that clusters with the greatest depressive symptoms and highest levels of peripheral inflammation will also have the highest early life trauma scores.

Methods

Participants

Participants comprised 539 students with an average age of 19.38 (*SD* = 2.15, range = 17-29 years). Of participants, 76.3% identified as female (*n* = 411), 23.2% as male (*n* = 125), and 0.6% identified as gender non-conforming (*n* = 3). Participants came from varied socioeconomic backgrounds, with 22.6% (*n* = 77) reporting a household income of less than $45,000, 39.7% had an income between $45,000 and $105,000 (*n* = 210), and 25.2% reporting a household income greater than $105,000 (*n* = 136). In addition, participants had diverse ethnicities, the majority, 59.4%, identifying as White/European (*n* = 320), followed by 10.9% Black (e.g., African, Haitian, Jamaican, Somali; *n* = 59), 7.6% Arab/West Asian (e.g., Armenian, Egyptian, Iranian, Lebanese, Moroccan; *n* = 41), 6.1% Asian (e.g., Chinese, Japanese, Korean; *n* = 33), 5.8% South Asian (e.g., East Indian, Pakistani, Punjabi, Sri Lankan; *n* = 31), and 10.1% other (*n* = 54).

Over one third of participants (35.1%; *n* = 189), self-reported having a current mental health condition. Of those who reported a current mental health condition, 42.9% (*n* = 81) reported anxiety, 30.7% (*n* = 58) reported comorbid anxiety and depression, 16.9% (*n* = 32) depression, and 7.9% (*n* = 15) other. Moreover, twenty percent of participants (*n* = 108) reported receiving treatment for a mental health condition. The specific treatments are outlined in Table 1. Suicidal behaviours were also prevalent in this student sample, with roughly half of participants reporting having experienced suicidal thoughts at some point in their lifetime (51%), and 25.4% having experienced suicidal thoughts in the past year. Moreover, 11.9% of participants reported having attempting

suicide at some point in their lifetime, and 2.6% reporting attempting suicide in the past year (Table 1).

Table 1

Mental Health-Related Demographics of Sample

Mental health variable	*n*	%
Current mental health condition	189	35.1
Currently being treated for mental health condition	108	20.9
Treatment type		
Anti-depressants	27	5.0
Therapy	26	4.8
Therapy + medication	20	3.7
Anti-anxieties	3	0.6
Other	30	5.6
Suicide ideation		
Lifetime	275	51.0
Past 12 months	137	25.4
Suicide attempts		
Lifetime	64	11.9
Past 12 months	14	2.6

Procedure

Participants were recruited through SONA, the universities online computerized system. All participants who met the age criteria, regardless of the presence of mental health concerns were able to participate in the current study to ensure a wide range of mental health symptomatologies were present, including individuals with normative mood. Upon arrival to the laboratory sessions, conducted between 1200h and 1530h to control for hormone and cytokine diurnal patterns, participants reviewed and completed an informed consent form (Appendix B). Following the informed consent, participants were provided with a questionnaire booklet assessing various demographic information, current mood including anxiety and depressive symptoms, suicide ideation and past suicide behaviours, childhood trauma and adverse experiences, which took approximately an hour and a half to complete (Appendix E). Please note that the above-mentioned scales were administered in the specific order outlined in Appendix E and blood samples were collected after their completion. At the end of the questionnaires, there were a series of questions in order to determine eligibility to provide a blood sample for cortisol and cytokine determination (Appendix C). If participants were willing to provide a small blood sample and met eligibility criteria (i.e., were not extremely nervous, have had blood drawn previously and have never had any complications, and were not taking any anti-inflammatory medication), they were deemed eligible to provide a blood sample and were given an additional blood consent form for signing (Appendix D). Upon signing the blood consent form, they were escorted to a separate room where a registered phlebotomist collected a small blood sample. All participants also provided a saliva sample upon completion of the questionnaire booklet that will be used for genetic testing

for future projects. Once complete, participants were debriefed, which included providing information on services for mental health resources/supports on campus and within the Ottawa region (Appendix F). Participants received a 2% course credit as compensation for their time. Ethical approval for all procedures was obtained from the Carleton University Research Ethics Board (B) and the Carleton University Biohazard Committee (Appendix A).

Blood Collection

Participant blood samples were collected into chilled EDTA coated Vacutainer tubes by a registered phlebotomist. Upon collection, blood samples were immediately placed on ice and centrifuged for 20 minutes at 4°C and 1000g. Plasma was then aliquoted into Eppendorf tubes and frozen at -80°C until required for cortisol and inflammatory assays (CRP and cytokines).

Inflammatory Assay

Circulating levels of CRP and IL-6 were determined in duplicate by high sensitivity human ELISA kits using high performance human CRP and IL-6 kits obtained from Life Technologies (Fisher Scientific). The assays were performed according to the manufacturer's instructions. The inter- and intra-assay variability was less than 15%.

Cortisol Assay

Plasma cortisol was determined in duplicate by radioimmunoassay (RIA) using a Cortisol Coated Tube RIA kit obtained from MP Biomedicals (Fisher Scientific). The assay was performed according to the manufacturer's instructions. The inter- and intra-assay variability was less than 10% and the minimum detectable concentration was 0.17 μg/mL.

Measures

Depressive symptoms. Depressive symptoms were assessed using the 21-item version of the Beck Depression Inventory or BDI (Beck et al., 1961) All items range from low (score of 0) to high (score of 3) depressive symptoms. This includes one item assessing suicide ideation. Moreover, 5 additional items assessing atypical depressive symptoms, such as, increased sleep, fatigue, increased eating, changes in diet, were added to the BDI for inputting into the PCA. Items in this scale were used separately to determine symptom clusters. In addition to being used on an item-level, BDI items were summed for scores to assess relationships (α = .89).

Anxiety symptoms. Anxiety symptoms were assed using the 21-item version of the Beck Anxiety Inventory or BAI (Beck & Steer, 1990). The BAI is a scale used to assess symptoms of anxiety that have caused disturbances in the past week (i.e., current symptoms of anxiety). All 21 items are scored on a range from 0-3, 0 indicating that they have not experienced that symptom in the past week and 3 suggesting that they experienced that symptom frequently. Items in this scale were used separately to determine symptom clusters in addition to being summed in order to obtain a total anxiety score (α = .90).

Depression anxiety stress scale. Depression, anxiety and stress symptoms were assessed using the 21-item Depression, Anxiety and Stress Scale, version 21 or DASS-21 (Lovibond & Lovibond, 1995). The DASS-21 assesses 3 areas of symptoms that are common to mood, anxiety and stress disorders. The 21 items are separated into 3 sub-scales. The first depression sub-scale assesses negative emotional states associated with

depression (e.g., dysphoria, hopelessness, devaluation of life, self-depreciation, lack of interest/involvement, anhedonia, and inertia). The second anxiety sub-scale assesses negative emotional states associated with anxiety (e.g., autonomic arousal, skeletal muscle effects, situational anxiety, and the subjective experience of anxious affect). The third stress sub-scale assesses negative emotional states associated with stress by asking questions regarding levels of chronic non-specific arousal (e.g., difficulty relaxing, nervous arousal, impatience, irritability and agitation). Items in this scale were used separately to determine symptom clusters.

Anhedonia. Anhedonia, a common feature of depression that is characterized by a lack of pleasure, was measured using the 14-item Snaith-Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995). The SHAPS is used to assess an individual's ability to experience pleasure in the last few days (i.e., current symptoms of anhedonia). The SHAPS includes 14 items that describe pleasurable experiences (e.g., 'I would enjoy being with family or close friends'), these items are scored on a range from 1-4, 1 indicating that they strongly agree with the statement and 4 indicating that they strongly disagree with the statement. Responses to items in this scale were summed to represent a total anhedonia score (α = .90).

Suicidal behaviours. To assess lifetime and past 12-months suicide ideation and attempts, four questions were added to the demographic section of the questionnaire. These items warranted dichotomous yes/no responses to the following questions "have you ever had thoughts of suicide in your lifetime/the past 12 months?" and "have you ever attempted suicide in your lifetime/the past 12 months?". Responses to these items

were coded, 1 = yes and 0 = no, and used to determine the prevalence of suicide ideation and attempts in this sample in addition to assessing which symptom clusters were associated with a higher prevalence of suicidal thoughts/behaviours.

Early life adversity. Early life adversity was assessed using the 29-item Early Trauma Inventory Self Report – Short Form or ETISR-SF (Bremner et al., 2007). The ETISR-SF assesses adverse experiences occurring prior to the age of 18 warranting dichotomous yes/no responses. The 29 items are separated into 4 categories. The first category comprises 11 items and assesses general traumatic events (e.g., experiencing natural disasters, illness or death of a family member or friend, divorce, witnessing violence, addictions or mental breakdowns). The following categories assess alternate forms of trauma such as experiences of physical (5 items), emotional (5 items) and sexual abuse (6 items). Trauma was assessed through summing total scores to represent an overall total trauma score (α = .81) in addition to each separate subscale score of physical abuse (α = .74), emotional abuse (α = .78) and sexual abuse (α = .83).

Statistical Analyses

Statistical analyses were performed using SPSS for Windows 24.0 (SPSS Science, Chicago, Illinois, USA). Statistical significance was determined at $p < .05$ (two-tailed). In order to clean data, all items were checked for out of range scores due to human error in data entry as well as all outliers (+/- 3.29) were brought into range for the analyses. In order to address missing data in this study, single imputation was performed in the form of a stochastic regression method on the basis of a non-significant Little's MCAR (.23) signifying that these data were missing completely at random (MCAR) and thus, indicating that single imputation is an appropriate method to handle missing data.

Pearson correlations were conducted to assess relationships between total BDI, BAI and SHAPS scores as well as early life trauma and biomarkers. To begin the data-driven clustering approach, we performed a principle component analysis (PCA) with the Promax rotation, including items reflecting symptoms of anxiety (BAI and DASS), depression (BDI and DASS), and stress (DASS). This process was completed prior to clustering in order reduce these often over-lapping items into dimensions of symptoms that most commonly appear together in this sample while maintaining variance. We then performed a hierarchical cluster analysis with the Ward's clustering algorithm and the squared Euclidean distance measure of proximity in SPSS using the reduced symptom dimensions obtained from the PCA as inputs. This determined how participants differentially express symptoms, by identifying clusters of individuals with the same symptom profiles. Chi-square tests were conducted between the symptom clusters and categorical variables, such as, gender, lab session time, suicide ideation and mental health-related variables, in order to determine any significant differences according to cluster. Analysis of variance (ANOVAs) and multivariate analysis of variance (MANOVAs) were then performed between the symptom clusters and total BDI, BAI and SHAPS scores, biological profiles, as well as in relation to additional measures such as early life adverse experiences including specific forms of trauma. ANOVAs and MANOVAs were followed-up with corresponding Tukey post hoc tests in order to determine where significant differences occur between clusters in relation to the above-mentioned factors.

Results

Correlations

Relationships between total scores of depression (BDI), anxiety (BAI), anhedonia (SHAPS), early life adversities (ETISR-SF) and biomarkers (i.e., cortisol, CRP, IL-6) are outlined in Table 2. As expected, depression, anxiety and anhedonia were strongly related to one another. However, of the biomarkers, only weak associations were found between cortisol and CRP and the mood and psychosocial scores. Specifically, cortisol was weakly related to depression, anxiety, as well as specific forms of early life trauma. While CRP was weakly associated with depressive symptoms, inflammatory factors were not significantly related to any other variables.

Table 2

Zero-Order Pearson Correlations Between Mood, Psychosocial and Biological Measures

	1	2	3	4	5	6	7	8	9
1. Depression	___								
2. Anxiety	.64**	___							
3. Anhedonia	.36**	.19**	___						
4. Total trauma	.37**	.35**	.11*	___					
5. Physical abuse	.06	.11**	.06	.66**	___				
6. Emotional abuse	.38**	.32**	.14**	.71**	.34**	___			
7. Sexual abuse	.29**	.28**	.08	.66**	.19**	.30**	___		
8. Cortisol	.14*	.18**	-.05	.07	-.19**	.01	.18**	___	
9. CRP	.12*	.04	-.07	.10	-.07	.08	.11	.21**	___
10. IL-6	.02	-.06	-.04	.09	.10	.07	.003	-.09	.13*

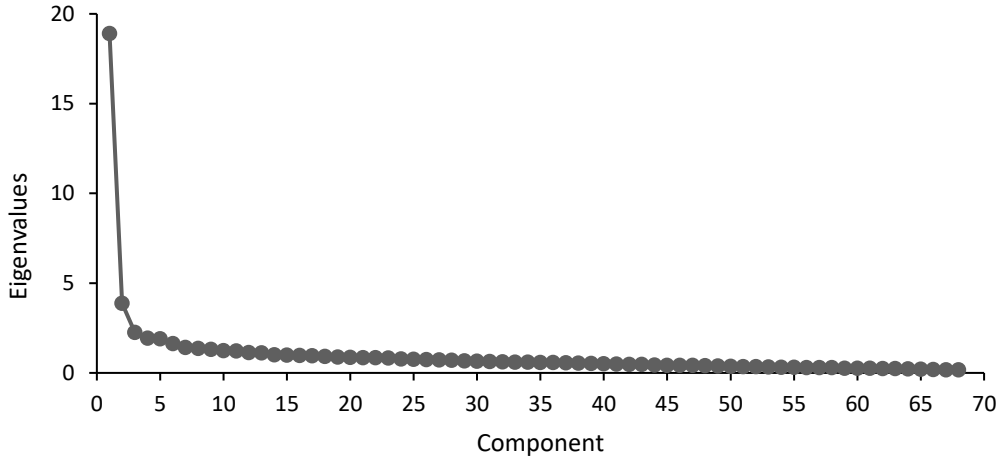
*$p < .05$, **$p \leq 0.05$

Principal Component Analysis

For the purpose of data reduction, a principal component analysis was performed on the 63 combined items of the BDI, BAI, and the DASS with five additional BDI items that assess atypical aspects of depression. The final factor structure accounted for 39.7% of the total variance. The Kaiser-Meyer-Olkin (KMO) measure of .94, and Bartlett's Test of Sphericity, $p < .001$, indicate that the factor structure was appropriate. A four-factor final solution was retained based on the interpretation of a scree plot (Figure 1), which is a more conservative method appropriate for larger sample sizes (Hair et al., 2010; Sarstedt & Mooi, 2019). Considering the underlying factors of depression, anxiety, and stress, are ultimately correlated (Field, 2005; Hair et al., 2010), the oblique rotation, Promax, was used. This is also the method used in a recent and similar investigation when combining the BDI and BAI items (Lee et al., 2018).

Figure 1

Scree Plot of Eigenvalues Against Component Scores



Note. A four-factor solution was retained based on point of inflection.

The four-factor solution loadings can be found in Table 3. Factor 1 (F1; anhedonia) explained 18.9% of variance and contained items from the BDI and the DASS depression sub-scale, including BDI item 9, which measures suicidal ideation. This factor most highly loaded items that reflected feelings of worthlessness, hopelessness, and negative/sadness related emotions. Factor 2 (F2; somatic anxiety) accounted for 3.9% of variance and included items from the BAI and the DASS anxiety subscale. This factor included items relating to the physiological experiences of anxiety, including symptoms like faintness, difficulty breathing, shakiness sweating, etc. Factor 3 (F3; generalized anxiety) explained 2.3% of variance and included items from the BAI, and both the DASS anxiety and stress subscales. This factor loaded items generally relating to fear, panic, worry, and nervousness. Factor 4 (F4; neurovegetative) explained 1.9% of variance included items from the BDI and the two additional atypical depressive

symptom items. This factor included items relating to physiological symptoms of depression, including weight gain/increased appetite, and fatigue.

Table 3

Factor Structure and Loadings of Combined BDI, BAI and DASS Items

Items	Factor loading			
	1	2	3	4
Factor 1: Anhedonia				
DASS21: I felt that life was meaningless (D)[a]	**0.85**			
DASS10: I felt that I had nothing to look forward to (D)	**0.79**			
BDI2: I feel that the future is hopeless and things cannot improve	**0.78**			
DASS17: I felt I wasn't worth much as a person (D)	**0.77**			
BDI3: I feel I am a complete failure as a person	**0.74**			
BDI1: I am so sad or unhappy that I can't stand it	**0.71**			
BDI7: I hate myself/disgusted/disappointed with self	**0.71**			
BDI9: Suicide Ideation	**0.68**			
DASS13: I felt down-hearted and blue (D)	**0.66**			
DASS3: I couldn't seem to experience any positive feeling at all (D)	**0.64**			
BDI4: I am dissatisfied with everything	**0.63**			
DASS16: I was unable to become enthusiastic about anything (D)	**0.62**			
BDI15: I can't do any work at all/productivity	**0.61**			
BDI5: I feel as though I am very bad or worthless/unworthy/guilty	**0.55**			

Items	Factor loading			
	1	2	3	4
DASS5: I found it difficult to work up the initiative to do things (D)	**0.55**			
BDI8: I feel I have many bad faults/critical of myself/blame myself	**0.53**			
BDI14: I feel that I am ugly or repulsive looking	**0.42**			
BDI12: I have lost all my interest in other people and don't care about them at all	**0.42**			
BDI13: I can't make decisions at all anymore	**0.41**			
BDI11: I am irritable all the time	**0.39**			
BDI6: I feel something bad may happen to me/I feel I am being punished/I deserve to be	**0.38**			
BDI10: Uncontrollable crying/cant cry even though want to	**0.38**			
BDI23: I feel more fatigued than usual lately, and it significantly interferes with my daily functioning	**0.37**			
Factor 2: Somatic Anxiety				
BAI19: Faint		**0.69**		
DASS4: I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion) (A)		**0.69**		
BAI12: Hands trembling		**0.69**		
BAI13: Shaky		**0.68**		
DASS7: I experienced trembling (eg, in the hands) (A)		**0.66**		
BAI6: Dizzy or light-headed		**0.66**		

Items	Factor loading			
	1	2	3	4
BAI3: Wobbliness in legs		**0.66**		
BAI15: Difficulty breathing		**0.62**		
BAI1: Numbness or tingling		**0.60**		
BAI21: Sweating (not due to heat)		**0.52**		
BAI8: Unsteady		**0.52**		
BAI11: Feelings of choking		**0.51**		
BAI2: Feeling hot		**0.51**		
DASS19: I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat) (A)		**0.49**		
BAI20: Face flushed		**0.46**		
BAI18: Indigestion or discomfort in abdomen		**0.45**		
DASS2: I was aware of dryness in my mouth (A)		**0.36**		
Factor 3: Generalized Anxiety				
BAI5: Fear of the worst happening			**0.80**	
DASS20: I felt scared without any good reason (A)			**0.75**	
BAI17: Scared			**0.75**	
BAI10: Nervous			**0.75**	
BAI9: Terrified			**0.71**	

Items	Factor loading			
	1	2	3	4
DASS8: I felt that I was using a lot of nervous energy (S)			**0.67**	
DASS12: I found it difficult to relax (S)			**0.67**	
BAI14: Fear of losing control			**0.65**	
DASS15: I felt I was close to panic (A)			**0.64**	
BAI4: Unable to relax			**0.61**	
DASS9: I was worried about situations in which I might panic and make a fool of myself (A)			**0.61**	
DASS1: I found it hard to wind down (S)			**0.57**	
BAI7: Heart pounding or racing		0.37	**0.48**	
DASS6: I tended to over-react to situations (S)			**0.46**	
Factor 4: Neurovegetative				
BDI19b: I have gained more than 15 pounds				**0.70**
BDI18b: I am eating a lot more than usual				**0.64**
BDI18c: I have had irresistible craving for sweets and starches lately				**0.49**
BDI19a: I have lost more than 15 pounds				**-0.40**
BDI17: I get too tired to do anything				**0.35**

Note. Rotation: Promax. Items loadings below .35 were suppressed.

[a]DASS sub-scales, (D) represents depression, (A) represents anxiety and (S) represents stress items.

Hierarchical Cluster Analysis

Next, a hierarchical cluster analysis was conducted to determine how individuals differ on the basis of the four PCA components and, more specifically, to identify groups of individuals who share similar symptomatologies. For the hierarchical cluster analysis, regression factor component scores for the four factors were used. This approach weights component scores based on the size of an items loading (i.e., the importance of the item in relation to the component), therefore providing a more accurate representation of the factor (Hair et al., 2010). Moreover, this approach provides standardized output variables that are ideal inputs for a cluster analysis, as demonstrated by existing literature on symptom clusters (Grisanzio et al., 2018). An agglomerative hierarchical cluster analysis with Ward's method clustering algorithm and the squared Euclidian distance measure of proximity was used as it is one of the most commonly used forms of cluster analysis.

An eight-cluster final solution was retained based on the percentage changes in heterogeneity at each final agglomeration stage (Table 4; Hair et al., 2010; Sarstedt & Mooi, 2019). Using the agglomeration coefficients, which measure the within-cluster sum of squares, a marked jump (represented in bold in Table 4) in within-cluster heterogeneity was revealed in agglomeration stage 531, suggesting that the number of clusters retained should be 8 (calculated by N (539) – agglomeration stage (531)). The marked increase in within-cluster heterogeneity is indicative that combining another cluster would significantly increase within-cluster heterogeneity. It is important to note, however, that agglomeration coefficients continue to rise exponentially as the number of clusters approach 1, therefore larger increases in heterogeneity are always the case as a lower cluster solution approach. Thus, the final decision to retain an 8 cluster solution

was also made for the manageability of data in this study and on the basis of existing literature using this approach while adjusting for the number of input variables (Grisanzio et al., 2018). The eight-cluster solution was visually confirmed through the interpretation of a scree-plot representing agglomeration coefficients and their respective stages (Figure 2).

Table 4

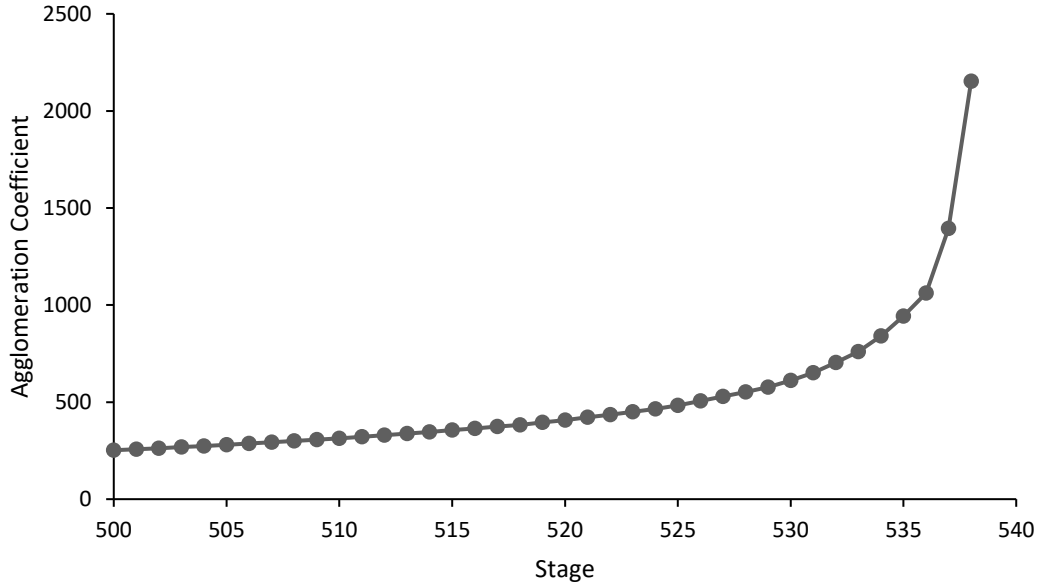
Percentage Change in Within-Cluster Heterogeneity Based on Number of Factors Retained by Cluster Analysis

Stage	Number of clusters retained	Agglomeration coefficient	Percentage increase in heterogeneity to next stage
530	9	611.90	6.34
531	**8**	**650.71**	**8.22**
532	7	704.19	7.97
533	6	760.32	10.51
534	5	840.24	12.21
535	4	942.82	12.66
536	3	1062.18	31.28
537	2	1394.45	54.36
538	1	2152	

Note. Bolding indicates the stage at which clusters were determined to be retained.

Figure 2

Scree Plot of Agglomeration Coefficients Against Agglomeration Stage in the Clustering Process



Cluster Characterization

As visualized in Figure 3, the cluster analysis resulted in the following eight clusters: *asymptomatic* (*n* = 129; 23.9%), represented by the lowest scores on all symptom dimensions; *low-grade symptomatology* (*n* = 101; 18.7%), in which evidence of mild symptoms are present but none are particularly higher than others and they are relatively low; *generalized anxiety* (*n* = 83; 15.4%), showing general worry, including nervousness and difficulty relaxing, with no physiological symptoms of anxiety; *anxious arousal with anhedonia* (*n* = 55; 10.2%), characterized by both somatic and generalized anxiety with loss of pleasure; *neurovegetative depression with generalized anxiety* (*n* = 40; 7.4%), displaying neurovegetative symptoms of depression, namely, weight-gain, increased eating and fatigue, in addition to general worry; *anhedonia* (*n* = 35; 6.5%), a loss of pleasure in the absence of other symptoms; *somatic anxiety* (*n* = 52; 9.6%),

displaying only the physiological symptoms of anxiety, such as, feeling faint, difficulty breathing, sweating, etc.; and *neurovegetative depression* (n = 44, 8.2%), symptoms of depression that are not directly mood-related, but neurovegetative, as described above. Each cluster displayed a distinct symptom profile and differed significantly on the components of the PCA including anhedonia, somatic anxiety, generalized anxiety and neurovegetative symptoms (p's < .001; Table 5).

Figure 3

Profile Plot of Symptom Clusters Among Mean Standardized Factor Component Scores

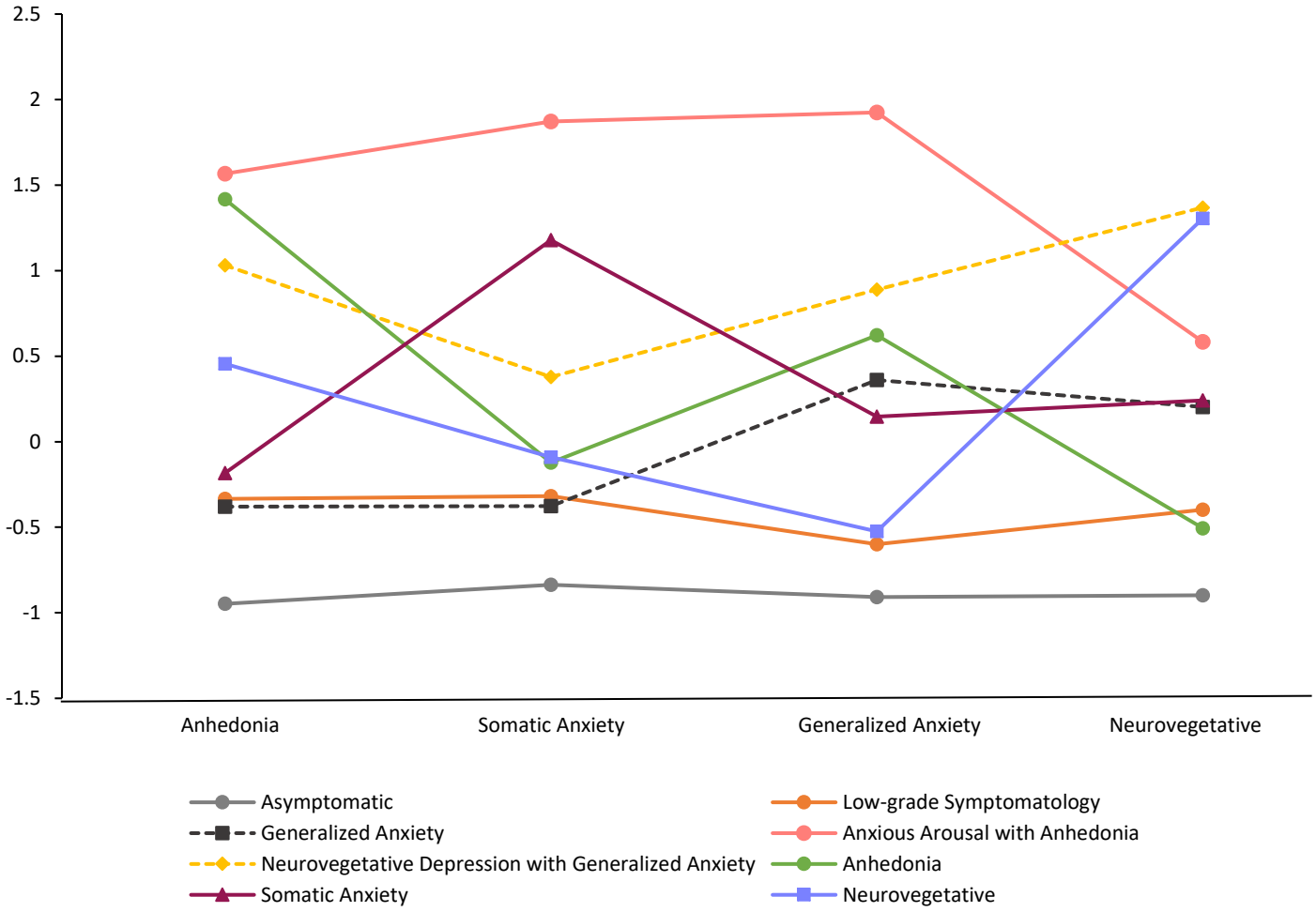


Table 5

Distinction Between Clusters on the Basis of Symptom Component Means

	Type Cluster, Mean Z-score (Standard Error; SE)								Test of Difference	
PCA Factors	Asymptomatic	Low-grade Symptomatology	Generalized Anxiety	Anxious Arousal with Anhedonia	Neurovegetative Depression with Generalized Anxiety	Anhedonia	Somatic Anxiety	Neurovegetative	$F_{(7,538)}$	*P* Value
Anhedonia	-0.95 (.02)	-0.33 (.05)	-0.38 (.04)	1.57 (.12)	1.03 (.09)	1.42 (.10)	-0.18 (.10)	0.46 (.06)	214.92	< .001
Somatic Anxiety	-0.84 (.03)	-0.32 (.05)	-0.38 (.05)	1.87 (.13)	0.38 (.08)	-0.12 (.11)	1.18 (.08)	-0.09 (.07)	188.51	< .001
Generalized Anxiety	-0.91 (.03)	-0.60 (.05)	0.36 (.06)	1.93 (.08)	0.89 (.07)	0.62 (.11)	0.15 (.08)	-0.52 (.07)	258.21	< .001
Neurovegetative	-0.90 (.04)	-0.40 (.06)	0.20 (.07)	0.58 (.13)	1.37 (.11)	-0.51 (.11)	0.24 (.10)	1.31 (.13)	98.68	< .001

In addition to determining how clusters differ based on the PCA factors, it was of interest to profile the cluster solution based on other relevant variables. Clusters did not differ significantly according to age, $p = .239$, lab session time, $p = .173$, whether they were currently receiving treatment for a mental health condition, $p = .097$, and the specific type of treatment they were receiving, $p = .875$. Symptom clusters did, however, significantly differ according to gender, X^2 (14, N = 539) = 68.88, $p < .001$. Please note that follow-up tests were not conducted to analyses relating to Table 6, therefore the below descriptions are not pertaining to significant differences. When viewing Table 6, very few males were found within the clusters that were expressing high mood scores. However, females were highly represented within in the *anxious arousal with anhedonia*, *neurovegetative depression with generalized anxiety* and *somatic anxiety* clusters. Moreover, there were two individuals who were gender non-conforming in the *anhedonia* cluster and one individual who was gender non-conforming in the *anxious arousal with anhedonia* category. Clusters also differed according to self-reported current mental health condition[1], X^2 (14, N = 535) = 127.71, $p < .001$, with a much lower proportion of individuals within the *asymptomatic* and *low-grade symptomatology* clusters reporting having a mental health condition (Table 6). Moreover, type of self-reported mental health condition also differed by cluster X^2 (28, N = 535) = 201.20, $p < .001$. As outlined in Table 6, the proportion of participants in the *neurovegetative* and *anhedonia* clusters who reported a depression condition were much greater compared to the other clusters, whereas a greater proportion of individuals in the *generalized anxiety*, *anxious arousal with anhedonia* and *somatic anxiety* clusters reported anxiety conditions. Depression with

[1] Current mental health condition, diagnoses and treatment specifications are not confirmed diagnoses as they are self-reported by participants and should therefore be interpreted accordingly.

comorbid anxiety was the second most prevalent self-reported condition among our sample and within clusters that had both anxiety and depression (i.e., the *anxious arousal with anhedonia* and the *neurovegetative depression with generalized anxiety* clusters), this was much more commonly reported.

Table 6

Characteristics of Clusters According to Gender and Mental Health-Related Factors

	Asymptomatic	Low-grade Symptomatology	Generalized Anxiety	Anxious Arousal with Anhedonia	Neurovegetative Depression with Generalized Anxiety	Anhedonia	Somatic Anxiety	Neurovegetative
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
	129	101	83	55	40	35	52	44
Gender								
Female	76 (58.9)	73 (72.3)	68 (81.9)	52 (94.5)	37 (92.5)	25 (71.4)	47 (90.4)	33 (75.0)
Male	53 (41.1)	28 (27.7)	15 (18.1)	2 (3.6)	3 (7.5)	8 (22.9)	5 (9.6)	11 (25.0)
Suicide Ideation								
Lifetime	32 (24.8)	52 (51.5)	34 (41.0)	43 (78.2)	33 (82.5)	28 (80.0)	28 (53.8)	25 (56.8)
Past 12 months	6 (4.7)	16 (15.8)	13 (15.7)	34 (61.8)	21 (52.5)	22 (62.9)	12 (23.1)	13 (29.5)
Suicide attempts								
Lifetime	4 (3.1)	4 (4.0)	7 (8.4)	24 (43.6)	5 (12.5)	10 (28.6)	5 (9.6)	5 (11.4)
Current mental health condition	12 (9.3)	19 (18.8)	32 (38.6)	43 (78.2)	25 (62.5)	16 (45.7)	23 (44.2)	18 (40.9)
Self-reported diagnosis								
Depression	2 (1.6)	5 (5.0)	3 (3.7)	2 (3.7)	3 (7.5)	7 (20.0)	2 (3.8)	8 (18.2)
Anxiety	7 (5.5)	6 (6.0)	19 (23.2)	18 (33.3)	9 (22.5)	3 (8.6)	16 (30.8)	3 (6.8)
Depression and Anxiety	0	4 (4.0)	8 (9.8)	23 (42.6)	12 (30.0)	4 (11.4)	3 (5.8)	4 (9.1)

Clusters differed significantly between lifetime suicide ideation, X^2 (21, N = 528) = 81.42, $p < .001$; suicide ideation in the past 12 months, X^2 (21, N = 528) = 116.72, $p < .001$; and lifetime suicide attempts, X^2 (21, N = 528) = 78.46, $p < .001$. Too few individuals reported suicide attempts in the past 12 months to examine according to cluster. These specific differences are listed in Table 6. Notably, 82.5% of participants in the *neurovegetative depression with comorbid anxiety* cluster reported lifetime experiences of suicide ideation, followed by 80% of those in the *anhedonia* cluster and 78.2% of individuals in the *anxious arousal* cluster. This compares to only 24.8% of individuals expressing suicide ideation in the *asymptomatic* group. In terms of more recent suicidal thoughts, 62.9% of individuals in the *anhedonia* cluster, 61.8% of individuals in the *anxious arousal* cluster and 52.5% of individuals in the *neurovegetative depression with comorbid anxiety* cluster endorsed suicidal thoughts in the past 12 months. In comparison, only 4.7% of those who were in the *asymptomatic* cluster reported suicidal ideation in the past 12 months. The highest prevalence of lifetime suicide attempts occurred among the *anxious arousal* cluster, in which 43.5% participants in this cluster reported attempting suicide in their lifetime compared to only 3.1% of individuals in the *asymptomatic* cluster.

Confirming Cluster Characterization by Mood and Anxiety Scores

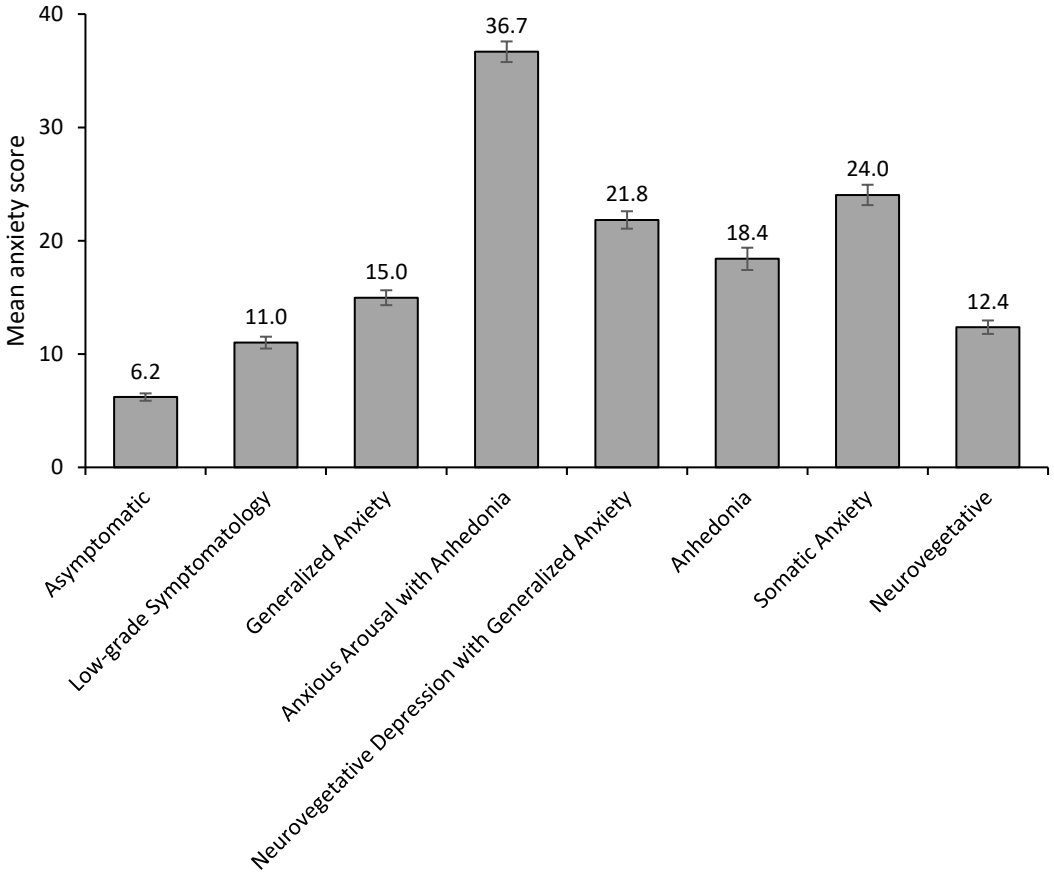
It was of interest to confirm that the clusters scoring highest on mood and anxiety PCA loadings would also display the highest total BAI and BDI scores. It was also of interest to establish the basis for predictive validity by comparing the clusters across a variable that was not used to form the cluster solution but is theoretically known to vary across clusters (Field, 2005; Hair et al., 2010). In this case, we used the SHAPS scale to

assess anhedonia, as anhedonia is a construct present in the input scales for the cluster solution but this scale is independent of the original analyses.

Not surprisingly, one-way ANOVAs revealed that the clusters differed on total anxiety scores, $F_{(7, 531)} = 225.35$, $p < .001$, $\eta^2 = .75$. ANOVAs were followed by Tukey post hoc tests in the interest of determining specific between-cluster differences. As shown in Figure 4, the *anxious arousal with anhedonia* subtype had the highest mean anxiety score and significantly differed from all of the other clusters (*p's* < .001). The *somatic anxiety* cluster had the next highest levels of anxiety, which significantly differed from all clusters (*p's* < .001) except for *neurovegetative depression with generalized anxiety* (*p* = .490), as this group had the third highest anxiety scores. As expected, the *asymptomatic* subtype had the lowest mean anxiety scores, which differed significantly from all other clusters (*p's* < .001).

Figure 4

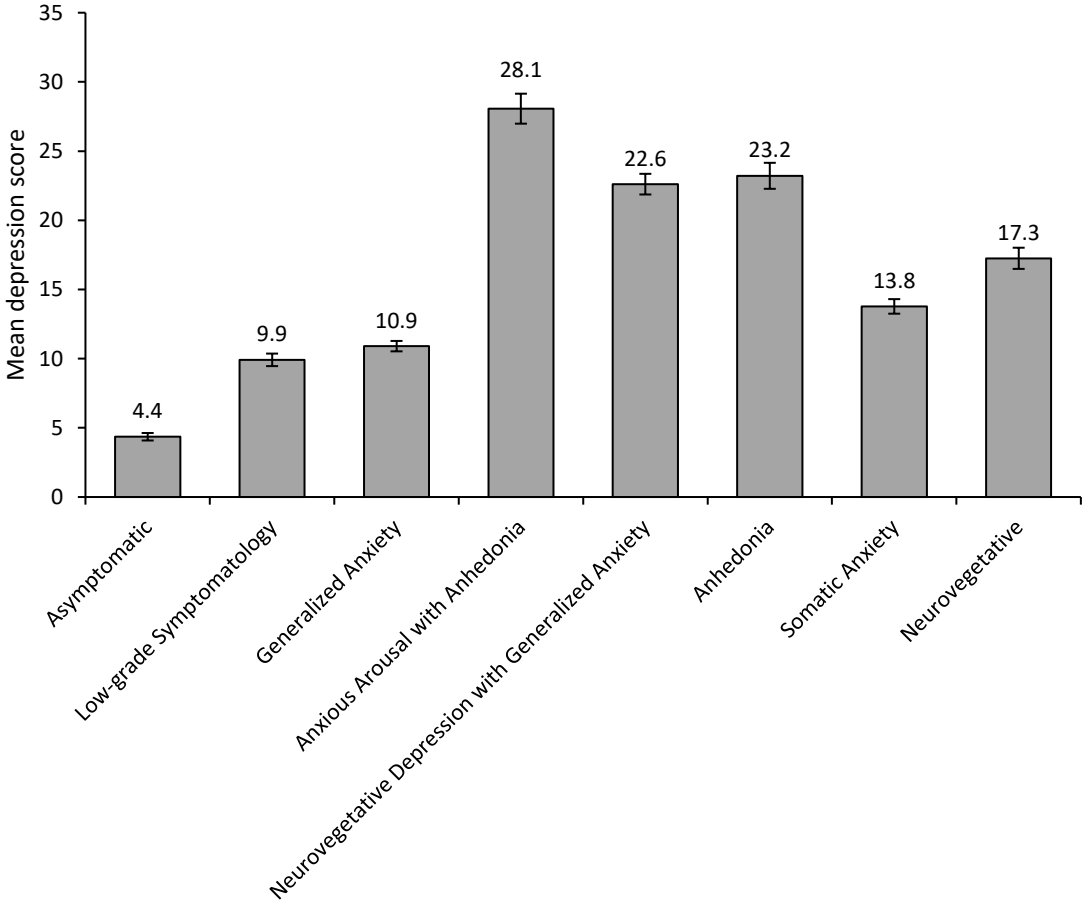
Anxiety Scores by Symptom Cluster (M ± SE)



As expected, clusters also significantly differed according to total depression scores, $F_{(7, 531)} = 208.23$, $p < .001$, $\eta^2 = .73$. Once again, the *anxious arousal with anhedonia* subtype also had the highest mean BDI score and significantly differed from all other clusters (*p's* < .001). Following this, the *anhedonia* cluster *and neurovegetative depression with generalized anxiety* cluster had the next highest levels of depression scores and while they did not differ from one another, they significantly differed from the remainder of clusters (*p's* < .001). As expected, the *asymptomatic* group had the lowest depressive scores (Figure 5).

Figure 5

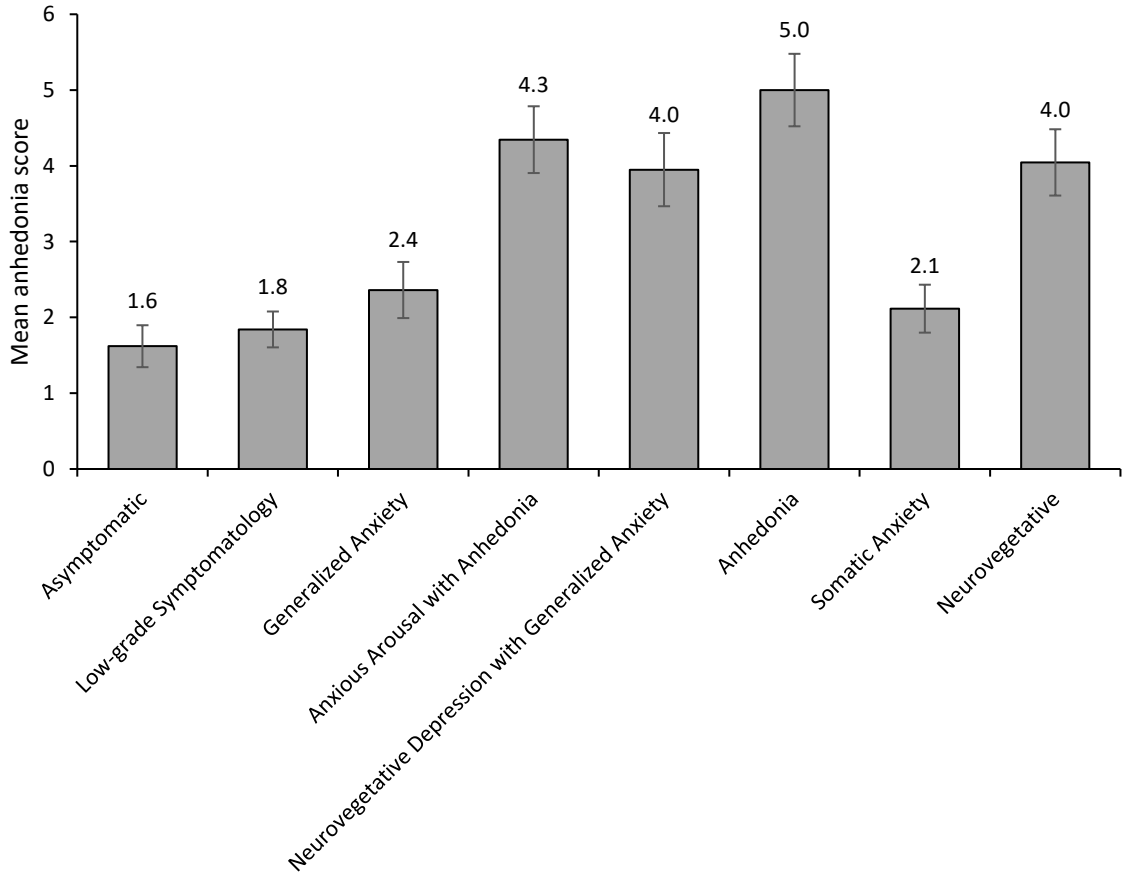
Depression Scores by Symptom Cluster (M ± SE)



In addition, the clusters differed significantly on the SHAPS anhedonia scores $F_{(7, 531)} = 12.00$, $p < .001$, $\eta^2 = .14$. In this instance, the *anhedonia* cluster had the highest mean SHAPS score, followed by the *anxious arousal with anhedonia cluster*. These clusters did not significantly differ from one another; however, they did differ from the subtypes with the lowest anhedonia scores, which were the *asymptomatic* and *low-grade symptomatology* clusters (*p's* < .001). (Figure 6).

Figure 6

Anhedonia Scores by Symptom Cluster (M ± SE)

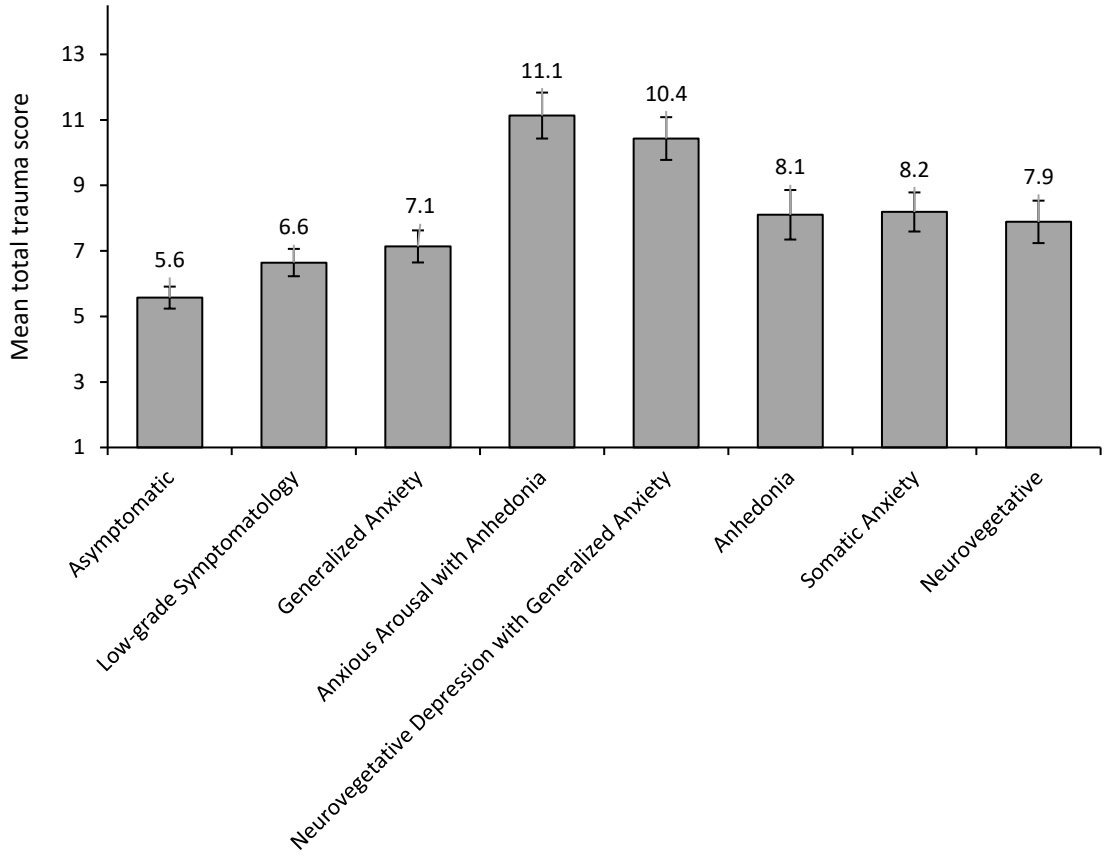


Clusters and Trauma Scores

Clusters also significantly differed according to total childhood trauma, $F_{(7, 531)} = 12.97$, $p < .001$, $\eta^2 = .15$. As shown in Figure 7, the *anxious arousal with anhedonia* and *neurovegetative depression with generalized anxiety* clusters had the highest total trauma scores, significantly differing from the clusters with the lowest total trauma scores, such as *generalized anxiety* (*p's* < .005 - .001), *low-grade symptomatology* (*p's* < .001) and *asymptomatic* clusters (*p's* < .001).

Figure 7

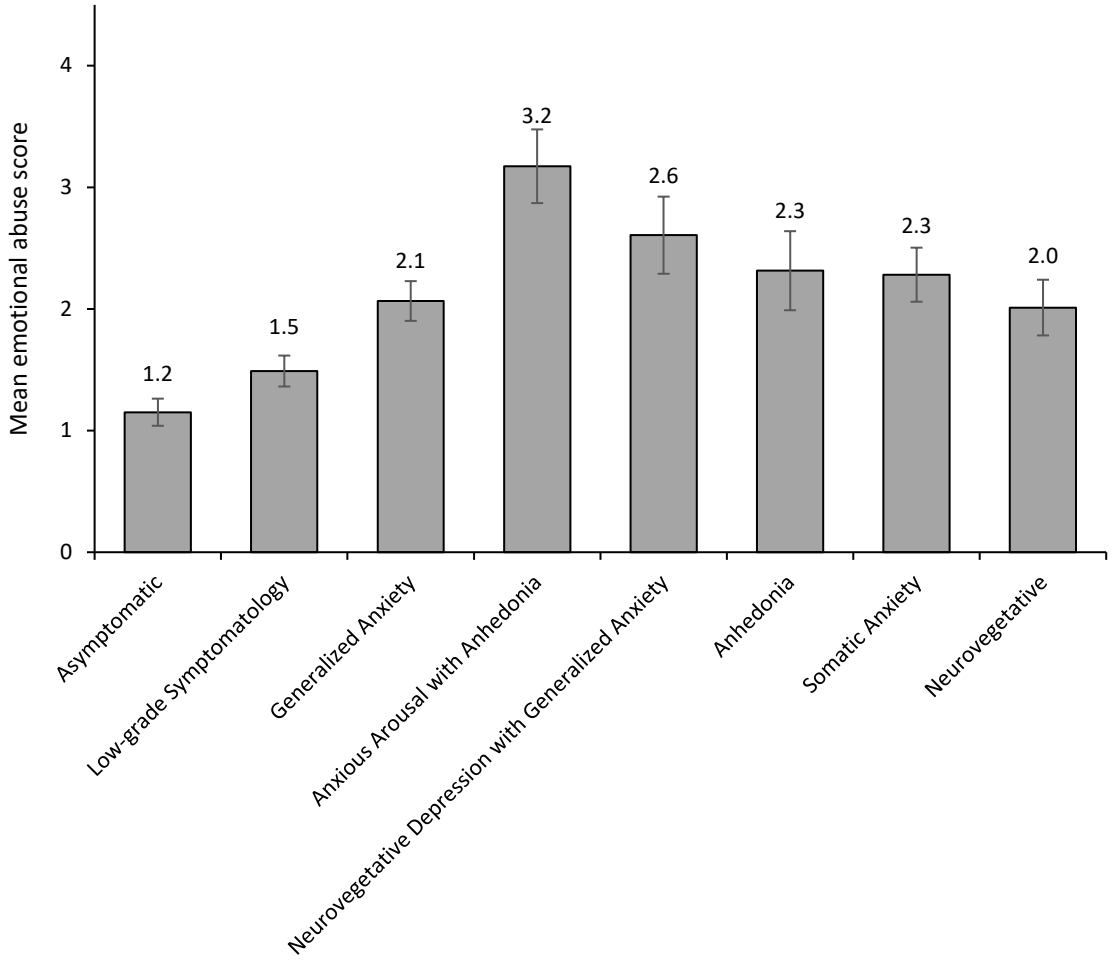
Total Trauma Scores by Symptom Cluster (M ± SE)



A MANOVA revealed that childhood trauma subscales also differed by cluster, *Pillai's Trace,* $F_{(7,531)} = 5.70$, $p < .001$, $\eta_p^2 = .07$. Univariate ANOVA's demonstrated that specific traumas, including emotional, $F_{(7, 531)} = 11.68$, $p < .001$, $\eta^2 = .13$, and sexual abuse, $F_{(7, 531)} = 8.71$, $p < .001$, $\eta^2 = .10$, differed between clusters. However, clusters did not significantly differ in relation to childhood physical abuse $F_{(7, 531)} = .95$, $p = .471$, $\eta^2 = .01$. As with total trauma, Tukey follow-up comparisons revealed the *anxious arousal with anhedonia* cluster to have the highest score on the emotional abuse subscale, followed by the *neurovegetative depression with generalized anxiety* cluster (Figure 8). These clusters differed significantly from the clusters yielding the lowest scores on emotional abuse, such as the *low-grade symptomatology* (*p's* < .01 - .001) and *asymptomatic* clusters (*p's* < .001).

Figure 8

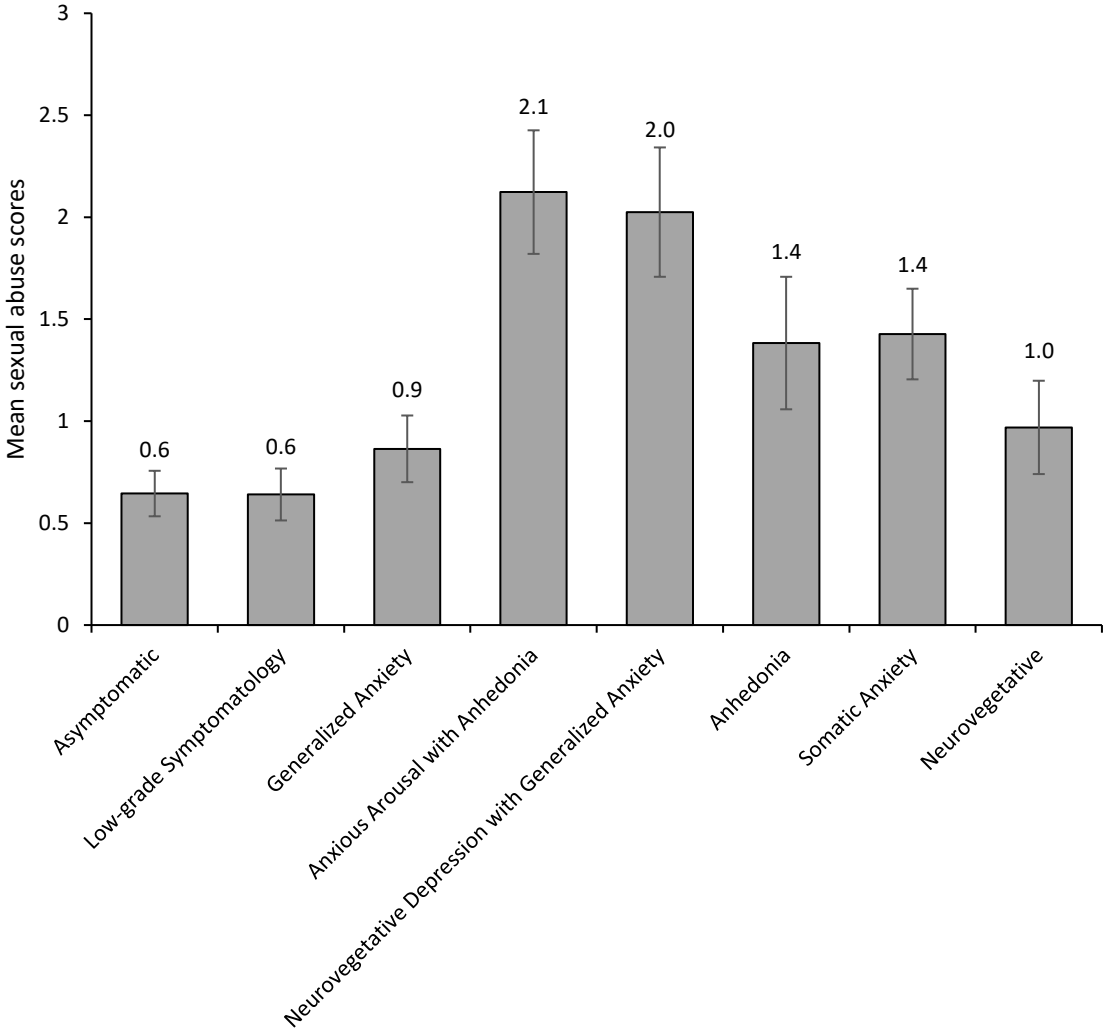
Emotional Abuse Scores by Symptom Cluster (M ± SE)



Sexual abuse scores were also highest among the *anxious arousal with anhedonia* and *neurovegetative depression with generalized anxiety* clusters, differing significantly from the *generalized anxiety* (*p's* < .005 - .001), *asymptomatic* (*p's* < .001) and *low-grade symptomatology* (*p's* < .001) clusters, who scored lowest on sexual abuse (Figure 9).

Figure 9

Sexual Abuse Scores by Symptom Cluster (M ± SE)

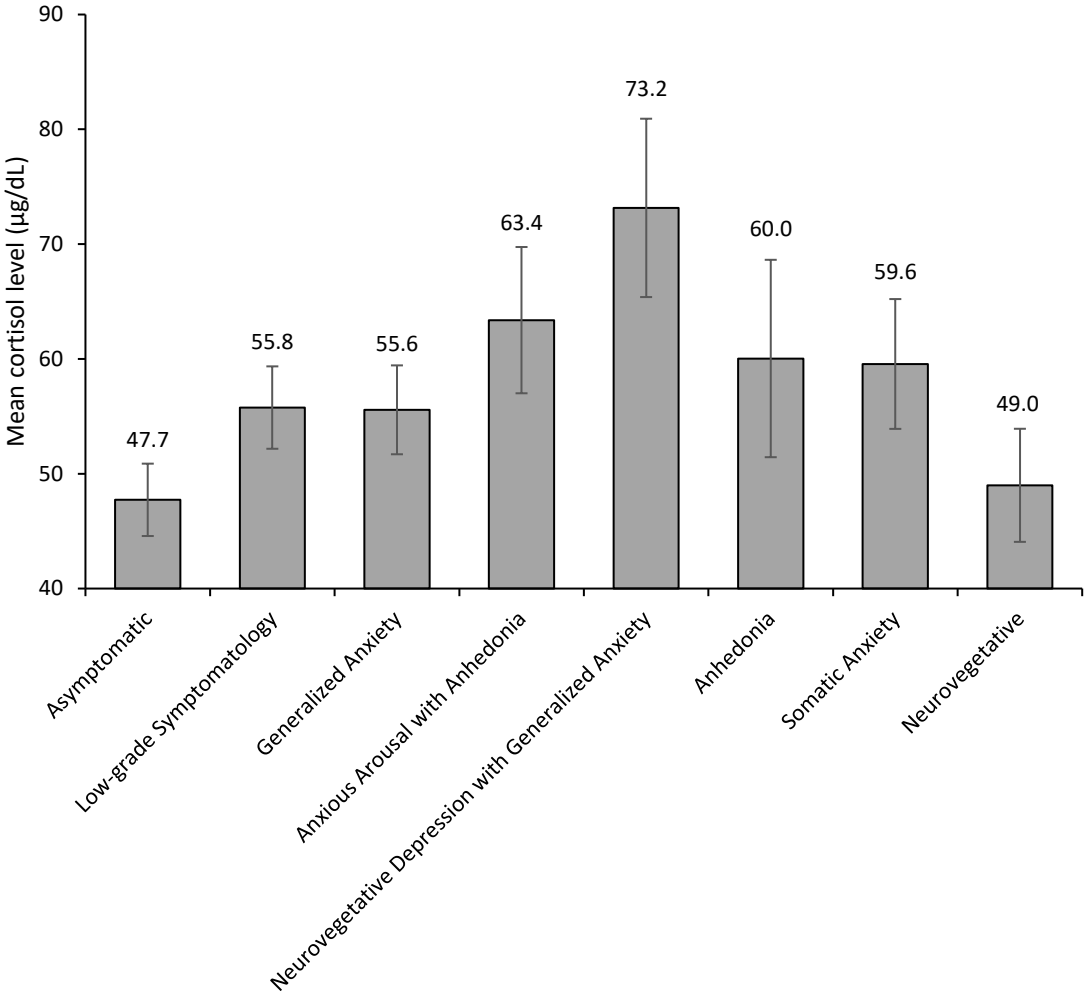


Clusters and Biological Factors

A one-way ANOVA revealed that symptom clusters differed according to basal cortisol levels, $F_{(7,253)} = 2.61$, $p = .013$, $\eta^2 = .07$. Cortisol levels, as shown in Figure 10, were highest among the *neurovegetative depression with generalized anxiety* cluster. Tukey post hoc tests revealed that cortisol levels were significantly higher for the *neurovegetative depression with generalized anxiety* cluster compared to the *asymptomatic* subtype ($p = .004$) and tended to be higher, while not significant, compared to the *neurovegetative subtype* ($p = .066$). As visualized in Figure 10, the *neurovegetative* cluster and the *asymptomatic* cluster had the lowest cortisol levels. The remainder of the clusters did not significantly differ from one another in cortisol levels.

Figure 10

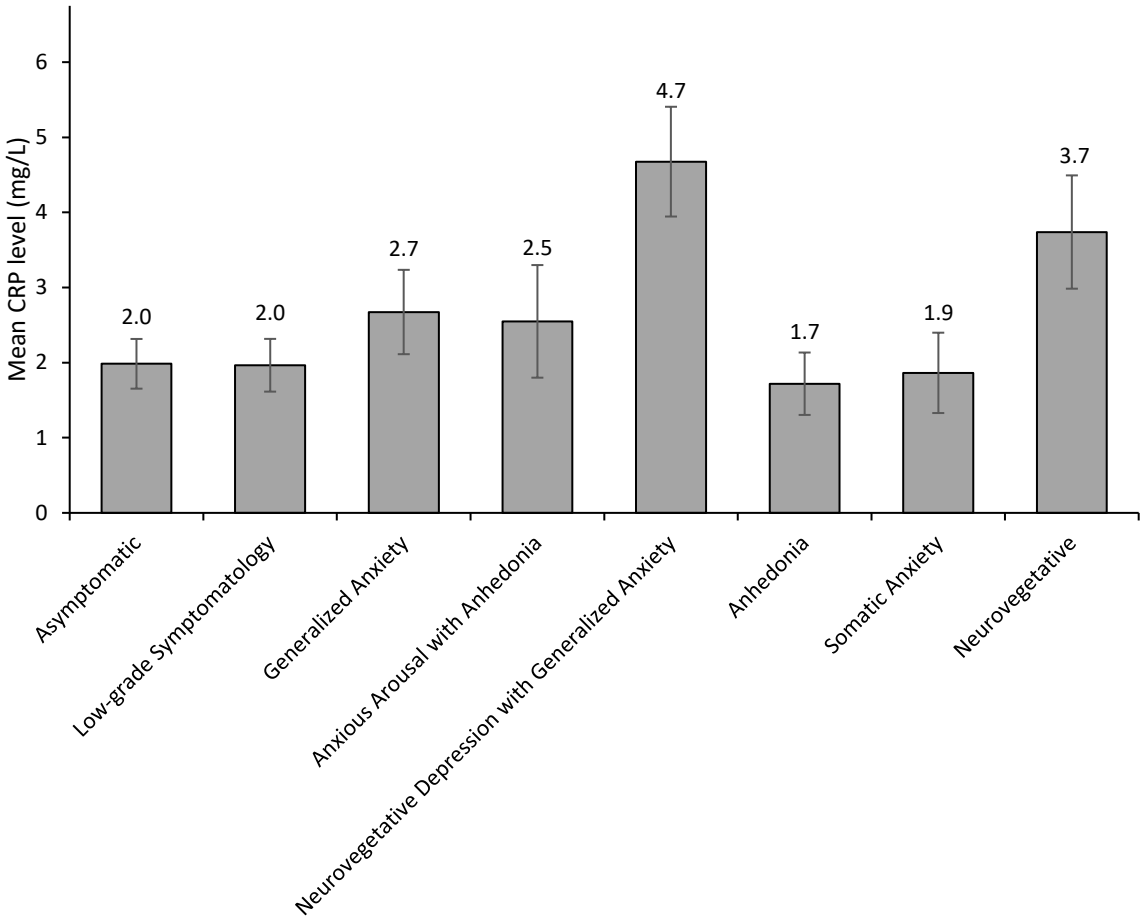
Basal Levels of Cortisol by Symptom Cluster (M ± SE)



Clusters also significantly differed according to CRP levels, $F_{(7,252)} = 3.30$, $p = .002$, $\eta^2 = .08$. As shown in Figure 11, CRP levels were highest among the *neurovegetative depression with generalized anxiety* cluster. Post hoc comparisons using Tukey test indicated that CRP levels for the *neurovegetative depression with generalized anxiety* cluster were significantly higher than the *asymptomatic* ($p = .005$), *low-grade symptomatology* ($p = .005$), *anhedonia* ($p = .036$) and *somatic anxiety* ($p = .021$) clusters, but did not differ from the *generalized an*xiety ($p = .162$), *anxious arousal with anhedonia* ($p = .223$), and *neurovegetative* clusters ($p = .961$). Notably, the *neurovegetative* subtype had the second highest levels of CRP.

Figure 11

Basal Levels of CRP by Symptom Cluster (M ± SE)



A number of potentially confounding factors in relation to peripheral biomarkers were considered. In this regard, BMI was weakly positively related to CRP, $r = .19$, $p = .038$, and negatively to cortisol, $r = -.18$, $p = .044$, whereas time of day of study (i.e., 12pm versus 2pm session) was not related to CRP, $p = .392$, or cortisol, $p = .132$. To be sure, CRP, $p = .002$, and cortisol, $p = .002$, effects by clusters remained significant when controlling for Body Mass Index (BMI).

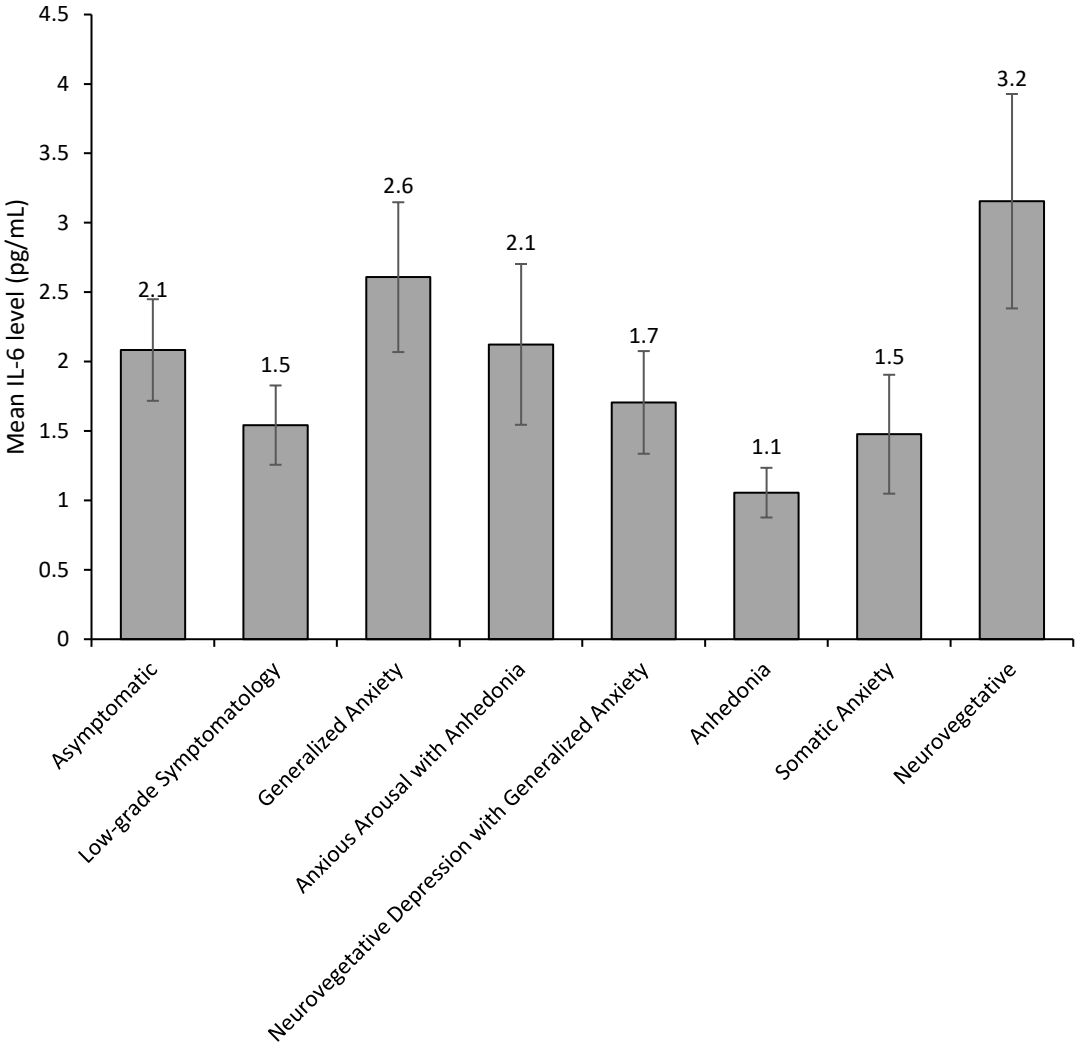
Oral contraceptive (OC) users have higher levels of plasma cortisol, $F_{(1,256)} = 205.69$, $p < .001$, $\eta^2 = .45$; and CRP, $F_{(1,94)} = 27.58$, $p < .001$, $\eta^2 = .10$; and also displayed higher mood states including; depression, $F_{(1,531)} = 16.35$, $p < .001$, $\eta^2 = .03$; and anxiety, $F_{(1,531)} = 15.83$, $p < .001$, $\eta^2 = .03$. OC users also differed by cluster, X^2 (7, N = 533) = 29.45, $p < .001$. As OC users differed significantly across clusters in mood scores, similar to gender, it was considered not appropriate to control for OC in our analyses[2].

Clusters did not significantly differ based on IL-6 levels, although this effect approached significance, $F_{(7,251)} = 1.65$, $p = .123$, $\eta^2 = .04$. As shown in Figure 12, basal levels of the pro-inflammatory cytokine IL-6 were highest among the *neurovegetative* cluster and lowest among the *anhedonia* cluster.

[2] To be sure, if OC were controlled for in biomarker analyses, cortisol becomes non-significant while CRP continues to significantly differ among clusters.

Figure 12

Basal Levels of IL-6 by Symptom Cluster (M ± SE)



Discussion

Depression, Anxiety and Suicidal Behaviours

In the current study, thirty-five percent of undergraduate students reported having a current mental health condition. This is consistent with literature indicating that between one in five to one in three university students meet the diagnostic criteria for a DSM-IV disorder in the past 12 months (Auerbach et al., 2016; Auerbach et al., 2018). Students were also twice as likely to report having a comorbid condition of anxiety and depression (30.7%) as opposed to reporting depression alone (16.9%). Moreover, suicidal thoughts and behaviours were prevalent, with half of students reporting having experienced suicidal thoughts at some point in their lifetime (51%); 11.9% reporting a lifetime suicide attempt; 25.4% reporting suicidal thoughts in the past year and 2.6% having attempted suicide in the past 12 months. These data emphasize the high comorbidity that exists among mental health disorders, in which depression co-occurs with anxiety more than 50% of the time (Kessler et al., 2008), and often exists together with suicidal thoughts and behaviours (Conner et al., 2019; Navaneelan, 2012). These findings are of concern given that comorbid disorders can indicate greater symptom severity and reduced treatment efficacy, but also bring to question the consideration and treatment of these disorders as separate independent diagnostic entities (Anisman et al., 2008; Dold et al., 2017; Fava et al., 2008; Fried & Nesse, 2015b; Hirschfeld, 2001; Kraus et al., 2019; Saveanu et al., 2014).

Due to not only the high comorbidity, but also the heterogeneity of these disorders (Fried, 2017; Grisanzio et al., 2018), focusing on symptomatologies across diagnoses and their corresponding biological markers might be more profitable to inform personalized

treatments (Ahmed et al., 2018; Anisman, 2014; Audet et al., 2013; Fried, 2017; Insel & Cuthbert, 2015). Indeed, recent novel approaches have taken a transdiagnostic perspective identifying symptom profiles that cut across mental health disorders (e.g., MDD, panic disorder and PTSD; Grisanzio et al., 2018). Such findings provide a basis for a more personalized approach to mental health, as classifying depression as a single illness may be counterproductive.

Symptom Heterogeneity

In the current study, we identified eight distinct transdiagnostic symptom clusters of depression and anxiety that significantly differed from one another on the basis of symptom dimensions, trauma and mood scales, and by peripheral biomarkers. Among our sample comprising both healthy participants and those experiencing mental health symptoms, two normative mood subtypes arose: *asymptomatic* and *low-grade symptomatology* clusters, represented by very low scores on all symptom dimensions and evidence of mild symptoms, respectively. Four additional clusters represented a single dimension, including: *generalized anxiety*, characterized by fearful and worrying symptoms of anxiety (e.g., a general sense of worry in the absence of physiological symptoms of anxiety); *somatic anxiety*, characterized by only physiological symptoms of anxiety (e.g., feeling faint, difficulty breathing); *anhedonia*, described mostly by a loss of pleasure in the absence of other symptoms (representing mood-related symptoms of depression), and *neurovegetative depression*, characterized mainly by somatic features of depression, such as, weight-gain, increased eating and fatigue and the absence of mood-related symptoms. Our analyses identified two additional clusters that were characterized by comorbid symptom profiles including; *anxious arousal with anhedonia*, a combination

of both somatic and generalized anxiety with anhedonia or a loss of pleasure, and; *neurovegetative depression with generalized anxiety*, comprising participants who displayed both neurovegetative symptoms of depression and a general sense of worry/anxiety.

Females were most highly represented within the clusters that had the highest depressive and anxiety profiles (i.e., *anxious arousal with anhedonia* and *neurovegetative depression with generalized anxiety* clusters), whereas, within the more 'normative' mood clusters (i.e., *asymptomatic* and *low-grade symptomatology* clusters), there was the highest proportion of males. These results were anticipated based on the large body of existing literature reporting that young females are more likely to experience depression and anxiety compared to males (Acciai & Hardy, 2017; Auerbach et al., 2018; Kessler et al., 2005; Leach et al., 2008). In line with the above findings, females were most highly represented within the two comorbid clusters. This is consistent with literature indicating that comorbid diagnoses of depression and anxiety are significantly more common among females compared to men (Asher et al., 2017; McLean et al., 2011; Sramek et al., 2016). In addition, females are more likely to have a younger age of onset of depressive symptoms, a lower quality of life with diagnoses, and generally present symptomatologies that are more severe, prolonged and recurrent compared to males (Kornstein et al., 2000; Sramek et al., 2016). As gender differences are not often considered during the treatment of depression (Herzog et al., 2019), better understanding the characteristics of transdiagnostic subtypes to inform targeted interventions could be a useful mechanism in addressing these gender gaps (Herzog et al., 2019; Riecher-Rössler, 2017).

Comorbid clusters emerged to be an important subset of our sample, as they were characterized by higher mood (i.e., depression and anxiety), psychosocial scores (i.e., early life adversities), elevated levels of biomarkers (i.e., CRP and cortisol), and suicidal behaviours. Specifically, 78.2% of participants in the *anxious arousal with anhedonia* cluster and 82.5% of participants in the *neurovegetative depression with generalized anxiety* cluster reported lifetime experiences of suicide ideation, which is substantially higher than the average of our sample, and of those reported in literature (Liu et al., 2019). These data are in-line with evidence indicating higher complications with comorbid mood and anxiety conditions (Dold et al., 2017; Fava et al., 2008; Hirschfeld, 2001; Kraus et al., 2019), and might suggest that comorbidities have a role to play in suicide ideation, again highlighting the need to better understand transdiagnostic subtypes.

Our findings also demonstrate that anxiety and/or depression can present very differently according to the individual. Specifically, certain individuals displayed only the somatic or physical symptoms of anxiety, while others displayed anxiety in the form of excessive and constant worrying in the absence of physical symptoms. Similarly, depressive symptom clusters were mainly classified by either mood-related symptoms, such as anhedonia, or more physical, neurovegetative symptoms of depression. Based on these data, together with data of clinical populations who have confirmed diagnoses (Drysdale et al., 2017; Grisanzio et al., 2018), distinctly different subtypes exist within anxiety and depressive disorder/diagnoses. Unfortunately, current clinical practices often treat individuals with a diagnosis of MDD similarly, for example, with an SSRI or SNRI, regardless of the specific symptoms present, due to the lack of biological evidence

surrounding potential symptom subtypes (Herzog et al., 2018). Clearly, a better understanding of subtypes, and exploring their corresponding biological etiologies, could potentially inform more targeted treatment (Drysdale et al., 2017; Grisanzio et al., 2018).

Biomarker Profiles by Symptomatology

In the present study, both the inflammatory factor, CRP, and HPA biomarker, cortisol, meaningfully mapped onto and differentiated symptom clusters. The inflammatory cytokine, IL-6, also tended to differ according to symptom clusters, but only approached significance. Importantly, the clusters with the highest total anxiety and depression scores were not the clusters that had the most extreme biomarker profiles. In fact, independent of the symptom clusters, biomarkers were only weakly correlated to anxiety and depression total scores, measured by the BAI and BDI respectively. Previous studies have observed biomarkers (e.g., cortisol and inflammatory factors) to be related to overall or total anxiety and depression scores (Dowlati et al., 2010; Howren et al., 2009; Jeenger et al., 2017; Jia et al., 2019; Miller & Raison, 2016; Wang et al., 2017), while others have not found these associations (Dowlati et al., 2010; Howren et al., 2009; Maydych, 2019). Taken together, our findings suggest that it is specific symptoms that are more tightly linked to peripheral biomarkers rather than higher overall symptom scores.

Inflammation

Levels of circulating CRP were highest among the *neurovegetative depression with generalized anxiety* cluster and second highest among participants in the *neurovegetative* cluster. These effects remained significant when controlling for BMI, time of day of study session and oral contraceptive use. While only approaching

significance, IL-6, our pro-inflammatory cytokine marker, tended to be highest among the *neurovegetative* cluster. These comparisons suggest that elevated levels of inflammatory markers, namely CRP, appear to be related specifically to the neurovegetative features of depression and that elevated inflammation could be present in the absence of high mood-related depressive symptoms. These finding are in-line with emerging evidence indicating that the association between inflammatory markers and depression is likely to be symptom specific, and tied to neurovegetative features of depression, which are characterized mainly by changes in appetite and fatigue (Chu et al., 2019; Gialluisi et al., 2020; Jokela et al., 2016; Majd et al., 2019; Miller & Raison, 2016). In fact, others have found that associations between inflammation and other depressive symptoms, such as more cognitive and emotional symptoms (e.g., anhedonia), were no longer significant after controlling for neurovegetative features (Jokela et al., 2016; Majd et al., 2019). Similarly, after treatment with interferon (IFN)-α (i.e., a cytokine that stimulates the release of pro-inflammatory cytokines), all participants developed neurovegetative symptoms of depression within the first two weeks, whereas mood-related symptoms developed later in treatment, and only among 30-50% of participants (Capuron & Miller, 2004). As neurovegetative symptoms appear to be more resistant to traditional antidepressant treatments, and peripheral CRP levels have been found to be elevated among those with treatment-resistant depression, these findings could provide potential targets for alternative treatments related to inflammatory pathways (Chamberlain et al., 2019).

Cortisol

Basal peripheral cortisol levels were also highest among the *neurovegetative depression with generalized anxiety* cluster*,* in contrast, the *neurovegetative* cluster alone without generalized anxiety displayed very low cortisol levels that were similar to those in the *asymptomatic* cluster. These findings suggest that it might not be the neurovegetative features relating to increased cortisol levels. In fact, when considering the cluster with the next highest cortisol levels, it is the *anxious arousal with anhedonia* cluster. When we consider what these clusters have in common, while their depressive features are vastly different, these clusters display the highest generalized anxiety symptoms compared to the other clusters, such as worry, panic, and nervousness. Elevated salivary and basal blood cortisol levels have both been associated with symptoms of generalized anxiety in previous literature (Mantella et al., 2008; Wang et al., 2017), but have not been linked to neurovegetative features (Juruena et al., 2018). Elevated levels of cortisol among those with generalized anxiety disorders have been demonstrated to be dependent on the severity of symptoms expressed (Mantella et al., 2008). As comorbid conditions often increase the severity of illness, it is interesting that the *neurovegetative depression with generalized anxiety* and the *anxious arousal with anhedonia* clusters are the two groups representing comorbidities across anxiety and depressive symptoms. These two clusters also have the highest rates of individuals with self-reported depression and anxiety disorders. Thus, the high rates of cortisol in these groups could be due to the comorbidities themselves, which represent greater complexity, but also the greater severity of symptoms that comes along with comorbid disorders.

The cortisol finding remained significant when controlling for BMI and time of day of study; however, did not remain significant when controlling for the use of OCs. As OC use was related to both higher plasma cortisol levels and mood scores in this sample, it is important to consider how they might impact the current findings. The *neurovegetative depression with generalized anxiety* cluster had the highest proportion of OC users compared any other cluster. Females taking OCs can display higher levels of depression and elevated basal levels of plasma cortisol (Hertel et al., 2017; McQuaid et al., 2016; Skovlund et al., 2016), and the association between cortisol and depressive symptoms are suggested to be dependent on OC use (Hertel et al., 2017). This may explain why cortisol findings were no longer significant once controlling for OC use. Therefore, the high proportion of OC users in the *neurovegetative depression with generalized anxiety* could be driving this cortisol effect.

Early Life Adversity

The present study sought to identify whether early life adversity was associated with any specific set of symptoms. We found that total trauma, emotional abuse and sexual abuse scores were highest among the two comorbid clusters, *anxious arousal with anhedonia* and *neurovegetative depression with generalized anxiety*. As mentioned earlier, these clusters also had the highest overall depression and anxiety scores, in addition to the highest rates of suicidal behaviours. Previous studies have demonstrated a dose-response relationship regarding frequency and types of early life adversity and the presence of corresponding psychopathologies (Hovens et al., 2010). Specifically, trauma histories have been shown to present a higher risk of developing comorbid depression and anxiety disorders, compared to anxiety and depression on their own (Hovens et al.,

2010; Van Nierop et al., 2015). Thus, together, these findings suggest that early life adversity in the form of childhood trauma might be a predictor of comorbidities and of more severe symptomatologies.

Considering the strong existing link between inflammation and early life adversity (Danese et al., 2008; Munjiza et al., 2018), we had predicted that individuals falling within the clusters associated with elevated levels of inflammatory markers would also have the highest trauma scores, however, we did not find these results. While there are studies showing the link between inflammation and early life adversity (Bertone-Johnson et al., 2012; Hartwell et al., 2013; Munjiza et al., 2018; Moreira et al., 2018), there are also a handful of those that have not demonstrated such associations (Carpenter et al., 2012; Gouin et al., 2012). These discrepancies could be related to many methodological factors (Carpenter et al., 2012). In this regard, the present study was conducted on a sample of university students that comprised both healthy students and those struggling with their mental health. In some cases, the association between inflammatory factors and early life adversity is only seen in depressed patients with trauma histories compared to non-depressed patients with trauma histories (Lu et al., 2013). Thus, it is possible that their experiences of trauma and symptom severity was not as pronounced as in some clinical samples, particularly those in inpatient hospitals who often experience complex and severe mental illness together with extensive trauma histories (Munjiza et al., 2018).

Limitations

There are several limitations associated with the current study. The first being the generalizability of the sample. This study was based on self-selection for course credit in an undergraduate psychology course and had many more females compared to males,

which is in-line with the representation of females to males in psychology undergraduate programs. Thus, results from this study are not necessarily generalizable to other populations, including clinically depressed individuals. Despite these limitations, depression is more common among females (Herzog et al., 2019; Nolen-Hoeksema, 2001), and mental health concerns are very prevalent among university students (Auerbach et al., 2018), highlighting the importance of these data. The current study was based on self-report, which can lead to bias based on memory and current affective state. Moreover, this study was conducted to be as inclusive as possible, such that a wide range of symptoms and experiences would be considered. While this was viewed as important and was intentional to reflect the complexities of mental health and comorbidities that exist, it also serves as a potential limitation. In this regard, some participants were receiving treatment in the form of various therapies and/or psychopharmacological treatment at the time of this study, which may have normalized symptoms of depression and anxiety. The most common treatment was SSRI's, which can normalize levels of pro-inflammatory cytokines, and may be partially responsible for our inability to reach significant findings with our pro-inflammatory cytokine biomarker, IL-6 (Hannestad et al., 2011; O. Köhler et al., 2014). Despite this possibility, we did examine specific treatments participants were receiving for mental health disorders, and biomarkers were not significantly related. Future directions could conduct this study in a more controlled design with treatment naive populations to determine if the current findings replicate.

Conclusion

Taken together, transdiagnostic clusters of anxiety and depressive symptomatologies can be developed and are marked by specific psychosocial and

biological factors including trauma experiences, suicidal behaviours, neuroendocrine and inflammatory markers. Unfortunately, the majority of studies to date focus on overall depression and anxiety scores or diagnostic categories, neglecting to consider differences in the specific symptoms, or combination of symptoms being expressed (Majd et al., 2019). This has likely limited our ability to understand underlying biological mechanisms, owing in part to the limited efficacy in pharmacological treatments developed to date (Ahmed et al., 2018). This study serves as an important first step towards gaining a better understanding of the distinct biological and psychosocial features associated with various transdiagnostic symptoms of depression and anxiety. Such approaches could be used to help identify individuals that may benefit most from novel treatment methods, such as anti-inflammatory treatments, based on their presenting symptomatologies. In sum, the current findings can help contribute to a necessary shift towards a personalized medicine approach and away from diagnostic boundaries in treating and researching complex mental health disorders.

References

Acciai, F., & Hardy, M. (2017). Depression in later life: A closer look at the gender gap. *Social Science Research*. https://doi.org/10.1016/j.ssresearch.2017.08.003

Afifi, T. O., MacMillan, H. L., Boyle, M., Taillieu, T., Cheung, K., & Sareen, J. (2014). Child abuse and mental disorders in Canada. *CMAJ*. https://doi.org/10.1503/cmaj.131792

Ahmed, A. T., Frye, M. A., Rush, A. J., Biernacka, J. M., Craighead, W. E., McDonald, W. M., Bobo, W. V., Riva-Posse, P., Tye, S. J., Mayberg, H. S., Flavin, D. H., Skime, M. K., Jenkins, G. D., Wang, L., Krishnan, R. R., Weinshilboum, R. M., Kaddurah-Daouk, R., & Dunlop, B. W. (2018). Mapping depression rating scale phenotypes onto research domain criteria (RDoC) to inform biological research in mood disorders. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2018.05.005

American Psychiatric Association. (2013a). DSM-5 diagnostic classification. In *Diagnostic and Statistical Manual of Mental Disorders*. https://doi.org/10.1176/appi.books.9780890425596.x00diagnosticclassification

American Psychiatric Association. (2013b). DSM 5. In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.books.9780890425596.744053

Anisman, H. (2014). An introduction to stress & health. In *An Introduction to Stress & Health*. https://doi.org/10.4135/9781473920781

Anisman, H., Gibb, J., & Hayley, S. (2008). Influence of continuous infusion of interleukin-1β on depression-related processes in mice: Corticosterone, circulating cytokines, brain monoamines, and cytokine mRNA expression.

Psychopharmacology. https://doi.org/10.1007/s00213-008-1166-z

Anisman, H., & Merali, Z. (1999). Understanding stress: Characteristics and caveats. *Alcohol Research and Health*.

Arnow, B. A., Blasey, C., Williams, L. M., Palmer, D. M., Rekshan, W., Schatzberg, A. F., Etkin, A., Kulkarni, J., Luther, J. F., & Rush, A. J. (2015). Depression subtypes in predicting antidepressant response: A report from the iSPOT-D trial. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2015.14020181

Asher, M., Asnaani, A., & Aderka, I. M. (2017). Gender differences in social anxiety disorder: A review. In *Clinical Psychology Review*. https://doi.org/10.1016/j.cpr.2017.05.004

Audet, M. C. Jacobson-Pick, S. McQuaid, R. Anisman, H. (2013). An inflammatory perspective of stress and human depressive disorder. In H. Kusnecov, A. Anisman (Ed.), *The Wiley-Blackwell Handbook of Psychoneuroimmunology* (pp. 469–487). John Wiley & Sons, Ltd.

Audet, M. C., McQuaid, R. J., Merali, Z., & Anisman, H. (2014). Cytokine variations and mood disorders: Influence of social stressors and social support. In *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2014.00416

Auerbach, R. P., Alonso, J., Axinn, W. G., Cuijpers, P., Ebert, D. D., Green, J. G., Hwang, I., Kessler, R. C., Liu, H., Mortier, P., Nock, M. K., Pinder-Amaker, S., Sampson, N. A., Aguilar-Gaxiola, S., Al-Hamzawi, A., Andrade, L. H., Benjet, C., Caldas-De-Almeida, J. M., Demyttenaere, K., … Bruffaerts, R. (2016). Mental disorders among college students in the World Health Organization World Mental Health Surveys. In *Psychological Medicine*.

https://doi.org/10.1017/S0033291716001665

Auerbach, R. P., Mortier, P., Bruffaerts, R., Alonso, J., Benjet, C., Cuijpers, P., Demyttenaere, K., Ebert, D. D., Green, J. G., Hasking, P., Murray, E., Nock, M. K., Pinder-Amaker, S., Sampson, N. A., Stein, D. J., Vilagut, G., Zaslavsky, A. M., & Kessler, R. C. (2018). WHO world mental health surveys international college student project: Prevalence and distribution of mental disorders. *Journal of Abnormal Psychology*. https://doi.org/10.1037/abn0000362

Baumeister, D., Lightman, S. L., & Pariante, C. M. (2014). The interface of stress and the HPA axis in behavioural phenotypes of mental illness. *Current Topics in Behavioral Neurosciences*. https://doi.org/10.1007/7854_2014_304

Beck, A. T., & Steer, R. A. (1990). Manual for the Beck Anxiety Inventory. In *Behaviour research and therapy*.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.1961.01710120031004

Benton, S. A., Robertson, J. M., Tseng, W. C., Newton, F. B., & Benton, S. L. (2003). Changes in counseling center client problems across 13 years. *Professional Psychology: Research and Practice*. https://doi.org/10.1037/0735-7028.34.1.66

Bertone-Johnson, E. R., Whitcomb, B. W., Missmer, S. A., Karlson, E. W., & Rich-Edwards, J. W. (2012). Inflammation and early-life abuse in women. *American Journal of Preventive Medicine*, *43*(6), 611–620. https://doi.org/10.1016/j.amepre.2012.08.014

Brådvik, L. (2018). Suicide risk and mental disorders. In *International Journal of*

Environmental Research and Public Health. https://doi.org/10.3390/ijerph15092028

Bremner, J. D., Bolus, R., & Mayer, E. A. (2007). Psychometric properties of the early trauma inventory-self report. *Journal of Nervous and Mental Disease*. https://doi.org/10.1097/01.nmd.0000243824.84651.6c

Bücker, J., Fries, G. R., Kapczinski, F., Post, R. M., Yatham, L. N., Vianna, P., Bogo Chies, J. A., Gama, C. S., Magalhães, P. V., Aguiar, B. W., Pfaffenseller, B., & Kauer-Sant'Anna, M. (2015). Brain-derived neurotrophic factor and inflammatory markers in school-aged children with early trauma. *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/acps.12358

Bylsma, L. M., Taylor-Clift, A., & Rottenberg, J. (2011). Emotional reactivity to daily events in major and minor depression. *Journal of Abnormal Psychology*. https://doi.org/10.1037/a0021662

Capuron, L., Gumnick, J. F., Musselman, D. L., Lawson, D. H., Reemsnyder, A., Nemeroff, C. B., & Miller, A. H. (2002). Neurobehavioral effects of interferon-α in cancer patients: Phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology*. https://doi.org/10.1016/S0893-133X(01)00407-9

Capuron, L., & Miller, A. H. (2004). Cytokines and psychopathology: Lessons from interferon-α. In *Biological Psychiatry*. https://doi.org/10.1016/j.biopsych.2004.02.009

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., & Price, L. H. (2012). C-reactive protein, early life stress, and wellbeing in healthy adults. *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2012.01892.x

Cavanagh, J. T. O., Carson, A. J., Sharpe, M., & Lawrie, S. M. (2003). Psychological autopsy studies of suicide: A systematic review. In *Psychological Medicine*. https://doi.org/10.1017/S0033291702006943

Chamberlain, S. R., Cavanagh, J., De Boer, P., Mondelli, V., Jones, D. N. C., Drevets, W. C., Cowen, P. J., Harrison, N. A., Pointon, L., Pariante, C. M., & Bullmore, E. T. (2019). Treatment-resistant depression and peripheral C-reactive protein. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.2018.66

Chapman, D. P., Whitfield, C. L., Felitti, V. J., Dube, S. R., Edwards, V. J., & Anda, R. F. (2004). Adverse childhood experiences and the risk of depressive disorders in adulthood. *Journal of Affective Disorders*, *82*(2), 217–225. https://doi.org/10.1016/j.jad.2003.12.013

Chen, M. H., Li, C. T., Lin, W. C., Hong, C. J., Tu, P. C., Bai, Y. M., Cheng, C. M., & Su, T. P. (2018). Rapid inflammation modulation and antidepressant efficacy of a low-dose ketamine infusion in treatment-resistant depression: A randomized, double-blind control study. *Psychiatry Research*. https://doi.org/10.1016/j.psychres.2018.08.078

Chrousos, G. P. (2009). Stress and disorders of the stress system. In *Nature Reviews Endocrinology*. https://doi.org/10.1038/nrendo.2009.106

Chu, A. L., Stochl, J., Lewis, G., Zammit, S., Jones, P. B., & Khandaker, G. M. (2019). Longitudinal association between inflammatory markers and specific symptoms of depression in a prospective birth cohort. *Brain, Behavior, and Immunity*. https://doi.org/https://doi.org/10.1016/j.bbi.2018.11.007

Claes, S. (2009). Glucocorticoid receptor polymorphisms in major depression. *Annals of*

the New York Academy of Sciences. https://doi.org/10.1111/j.1749-6632.2009.05012.x

Cohen, S., Murphy, M. L. M., & Prather, A. A. (2019). Ten surprising facts about stressful life events and disease risk. *Annual Review of Psychology*. https://doi.org/10.1146/annurev-psych-010418-102857

Collins, F. S., & Varmus, H. (2015). A new initiative on precision medicine. *New England Journal of Medicine*. https://doi.org/10.1056/NEJMp1500523

Conner, K. R., Bridge, J. A., Davidson, D. J., Pilcher, C., & Brent, D. A. (2019). Meta-analysis of mood and substance use disorders in proximal risk for suicide deaths. *Suicide and Life-Threatening Behavior*. https://doi.org/10.1111/sltb.12422

Craggs, S. (2012). *One-third of McMaster students battle depression: survey*.

Dale, E., Bang-Andersen, B., & Sánchez, C. (2015). Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. In *Biochemical Pharmacology*. https://doi.org/10.1016/j.bcp.2015.03.011

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

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. In *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2297

Dhabhar, F. S., Burke, H. M., Epel, E. S., Mellon, S. H., Rosser, R., Reus, V. I., &

Wolkowitz, O. M. (2009). Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2009.05.010

Dold, M., Bartova, L., Souery, D., Mendlewicz, J., Serretti, A., Porcelli, S., Zohar, J., Montgomery, S., & Kasper, S. (2017). Clinical characteristics and treatment outcomes of patients with major depressive disorder and comorbid anxiety disorders - results from a European multicenter study. *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2017.02.020

Douglas, K. R., Chan, G., Gelernter, J., Arias, A. J., Anton, R. F., Weiss, R. D., Brady, K., Poling, J., Farrer, L., & Kranzler, H. R. (2010). Adverse childhood events as risk factors for substance dependence: Partial mediation by mood and anxiety disorders. *Addictive Behaviors*. https://doi.org/10.1016/j.addbeh.2009.07.004

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

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., Fetcho, R. N., Zebley, B., Oathes, D. J., Etkin, A., Schatzberg, A. F., Sudheimer, K., Keller, J., Mayberg, H. S., Gunning, F. M., Alexopoulos, G. S., Fox, M. D., Pascual-Leone, A., Voss, H. U., … Liston, C. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nature Medicine*. https://doi.org/10.1038/nm.4246

Dube, S. R., Anda, R. F., Felitti, V. J., Chapman, D. P., Williamson, D. F., & Giles, W. H. (2001). Childhood abuse, household dysfunction, and the risk of attempted

suicide throughout the life span: Findings from the adverse childhood experiences study. *Journal of the American Medical Association*, *286*(24), 3089–3096. https://doi.org/10.1001/jama.286.24.3089

Eisenberg, D., Golberstein, E., & Hunt, J. B. (2009). Mental health and academic success in college. *B.E. Journal of Economic Analysis and Policy*. https://doi.org/10.2202/1935-1682.2191

Espejo, E. P., Hammen, C. L., Connolly, N. P., Brennan, P. A., Najman, J. M., & Bor, W. (2006). Stress sensitization and adolescent depressive severity as a function of childhood adversity: A link to anxiety disorders. *Journal of Abnormal Child Psychology*. https://doi.org/10.1007/s10802-006-9090-3

Fava, M., Rush, A. J., Alpert, J. E., Balasubramani, G. K., Wisniewski, S. R., Carmin, C. N., Biggs, M. M., Zisook, S., Leuchter, A., Howland, R., Warden, D., & Trivedi, M. H. (2008). Difference in treatment outcome in outpatients with anxious versus nonanxious depression: A STAR*D report. *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2007.06111868

Field, A. (2005). Discovering statistics using SPSS (2nd ed.). In *Discovering statistics using SPSS (2nd ed.).*

Fried, E. (2017). Moving forward: how depression heterogeneity hinders progress in treatment and research. *Expert Review of Neurotherapeutics*. https://doi.org/10.1080/14737175.2017.1307737

Fried, E. I., & Nesse, R. M. (2015a). Depression is not a consistent syndrome: An investigation of unique symptom patterns in the STAR∗D study. *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2014.10.010

Fried, E. I., & Nesse, R. M. (2015b). Depression sum-scores don't add up: Why analyzing specific depression symptoms is essential. *BMC Medicine*. https://doi.org/10.1186/s12916-015-0325-4

Frodl, T., Carballedo, A., Hughes, M. M., Saleh, K., Fagan, A., Skokauskas, N., McLoughlin, D. M., Meaney, J., O'Keane, V., & Connor, T. J. (2012). Reduced expression of glucocorticoid-inducible genes GILZ and SGK-1: High IL-6 levels are associated with reduced hippocampal volumes in major depressive disorder. *Translational Psychiatry*. https://doi.org/10.1038/tp.2012.14

Gałecki, P., Mossakowska-Wójcik, J., & Talarowska, M. (2018). The anti-inflammatory mechanism of antidepressants – SSRIs, SNRIs. In *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. https://doi.org/10.1016/j.pnpbp.2017.03.016

Gallagher, R. P. (2014). National survey of college counseling. *American College Counseling Association (ACCA)*.

Gialluisi, A., Di Castelnuovo, A., Bracone, F., De Curtis, A., Cerletti, C., Donati, M. B., de Gaetano, G., & Iacoviello, L. (2020). Associations between systemic inflammation and somatic depressive symptoms: Findings from the Moli-sani study. *Depression and Anxiety*. https://doi.org/10.1002/da.23070

Gibb, J., Audet, M. C., Hayley, S., & Anisman, H. (2009). Neurochemical and behavioral responses to inflammatory immune stressors. *Frontiers in Bioscience - Scholar*.

Gold, P. W., & Chrousos, G. P. (2013). Melancholic and atypical subtypes of depression represent distinct pathophysiological entities: CRH, neural circuits, and the diathesis for anxiety and depression. In *Molecular Psychiatry*.

https://doi.org/10.1038/mp.2013.5

Gold, P. W., Goodwin, F. K., & Chrousos, G. P. (1988). Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress. *New England Journal of Medicine*.

Gold, P. W., Machado-Vieira, R., & Pavlatou, M. G. (2015). Clinical and biochemical manifestations of depression: Relation to the neurobiology of stress. In *Neural Plasticity*. https://doi.org/10.1155/2015/581976

Goldsmith, D. R., Rapaport, M. H., & Miller, B. J. (2016). A meta-analysis of blood cytokine network alterations in psychiatric patients: Comparisons between schizophrenia, bipolar disorder and depression. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2016.3

Gouin, J. P., Glaser, R., Malarkey, W. B., Beversdorf, D., & Kiecolt-Glaser, J. K. (2012). Childhood abuse and inflammatory responses to daily stressors. *Annals of Behavioral Medicine*. https://doi.org/10.1007/s12160-012-9386-1

Grisanzio, K. A., Goldstein-Piekarski, A. N., Wang, M. Y., Ahmed, A. P. R., Samara, Z., & Williams, L. M. (2018). Transdiagnostic symptom clusters and associations with brain, behavior, and daily function in mood, anxiety, and trauma disorders. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2017.3951

Haapakoski, R., Mathieu, J., Ebmeier, K. P., Alenius, H., & Kivimäki, M. (2015). Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2015.06.001

Hair, J. F., Black, W. C., Babin, B. J., & Anderson, R. E. (2010). Multivariate data

analysis. In *Vectors*. https://doi.org/10.1016/j.ijpharm.2011.02.019

Hammen, C. (2016). Depression and stressful environments: identifying gaps in conceptualization and measurement. *Anxiety, Stress and Coping*. https://doi.org/10.1080/10615806.2015.1134788

Hannestad, J., Dellagioia, N., & Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: A meta-analysis. *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2011.132

Harald, B., & Gordon, P. (2012). Meta-review of depressive subtyping models. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2011.07.015

Hartwell, K. J., Moran-Santa Maria, M. M., Twal, W. O., Shaftman, S., DeSantis, S. M., McRae-Clark, A. L., & Brady, K. T. (2013). Association of elevated cytokines with childhood adversity in a sample of healthy adults. *Journal of Psychiatric Research*, *47*(5), 604–610. https://doi.org/10.1016/j.jpsychires.2013.01.008

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., Miller, A. H., & Nemeroff, C. B. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual abuse. *The Journal of American Medical Association*. https://doi.org/10.1001/jama.2013.285447.

Hertel, J., König, J., Homuth, G., Van Der Auwera, S., Wittfeld, K., Pietzner, M., Kacprowski, T., Pfeiffer, L., Kretschmer, A., Waldenberger, M., Kastenmüller, G., Artati, A., Suhre, K., Adamski, J., Langner, S., Völker, U., Völzke, H., Nauck, M., Friedrich, N., & Grabe, H. J. (2017). Evidence for stress-like alterations in the HPA-axis in women taking oral contraceptives. *Scientific Reports*. https://doi.org/10.1038/s41598-017-13927-7

Herzog, D. P., Beckmann, H., Lieb, K., Ryu, S., & Müller, M. B. (2018). Understanding and predicting antidepressant response: Using animal models to move toward precision psychiatry. *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2018.00512

Herzog, D. P., Wegener, G., Lieb, K., Müller, M. B., & Treccani, G. (2019). Decoding the mechanism of action of rapid-acting antidepressant treatment strategies: Does gender matter? In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms20040949

Hirschfeld, R. M. A. (2001). The comorbidity of major depression and anxiety disorders: Recognition and management in primary care. *Primary Care Companion to the Journal of Clinical Psychiatry*.

Hoban, M., & Leino, E. V. (2006). American College Health Association National College Health Assessment (ACHA-NCHA) Spring 2005 reference group data report (abridged). *Journal of American College Health*. https://doi.org/10.3200/JACH.55.1.5-16

Hostinar, C. E., Nusslock, R., & Miller, G. E. (2018). Future directions in the study of early-life stress and physical and emotional health: Implications of the neuroimmune network hypothesis. *Journal of Clinical Child and Adolescent Psychology*. https://doi.org/10.1080/15374416.2016.1266647

Hovens, J. G. F. M., Wiersma, J. E., Giltay, E. J., Van Oppen, P., Spinhoven, P., Penninx, B. W. J. H., & Zitman, F. G. (2010). Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2009.01491.x

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with c-reactive protein, IL-1, and IL-6: A meta-analysis. *Psychosomatic Medicine*. https://doi.org/10.1097/PSY.0b013e3181907c1b

Hybels, C. F., Blazer, D. G., Landerman, L. R., & Steffens, D. C. (2011). Heterogeneity in symptom profiles among older adults diagnosed with major depression. *International Psychogeriatrics*. https://doi.org/10.1017/S1041610210002346

Insel, T. R., & Cuthbert, B. N. (2015). Brain disorders? Precisely. *Science*. https://doi.org/10.1126/science.aab2358

Irwin, M. R., & Cole, S. W. (2011). Reciprocal regulation of the neural and innate immune systems. In *Nature Reviews Immunology*. https://doi.org/10.1038/nri3042

Jeenger, J., Sharma, M., Mathur, D. M., & Amandeep. (2017). Associations of number and severity of depressive episodes with C-reactive protein and Interleukin-6. *Asian Journal of Psychiatry*. https://doi.org/10.1016/j.ajp.2017.02.016

Jia, Y., Liu, L., Sheng, C., Cheng, Z., Cui, L., Li, M., Zhao, Y., Shi, T., Yau, T. O., Li, F., & Chen, L. (2019). Increased serum levels of cortisol and inflammatory cytokines in people with depression. *Journal of Nervous and Mental Disease*. https://doi.org/10.1097/NMD.0000000000000957

Jokela, M., Virtanen, M., Batty, G. D., & Kivimäki, M. (2016). Inflammation and specific symptoms of depression. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2015.1977

Jones, P. J., Park, S. Y., & Lefevor, G. T. (2018). Contemporary college student anxiety: The role of academic distress, financial stress, and support. *Journal of College Counseling*. https://doi.org/10.1002/jocc.12107

Juruena, M. F., Bocharova, M., Agustini, B., & Young, A. H. (2018). Atypical depression and non-atypical depression: Is HPA axis function a biomarker? A systematic review. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2017.09.052

Karalunas, S. L., Fair, D., Musser, E. D., Aykes, K., Iyer, S. P., & Nigg, J. T. (2014). Subtyping attention-deficit/hyperactivity disorder using temperament dimensions. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2014.763

Karatekin, C. (2018). Adverse childhood experiences (ACEs), stress and mental health in college students. *Stress and Health*. https://doi.org/10.1002/smi.2761

Kendler, K. S. (2016). The phenomenology of major depression and the representativeness and nature of DSM criteria. In *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2016.15121509

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. In *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.62.6.593

Kessler, R. C., Gruber, M., Hettema, J. M., Hwang, I., Sampson, N., & Yonkers, K. A. (2008). Co-morbid major depression and generalized anxiety disorders in the National Comorbidity Survey follow-up. *Psychological Medicine*. https://doi.org/10.1017/S0033291707002012

Kessler, R. C., Nelson, C. B., Mcgonagle, K. A., Liu, J., Swartz, M., & Blazer, D. G. (1996). Comorbidity of DSM-III-R major depressive disorder in the general population: Results from the US National Comorbidity Survey. *British Journal of Psychiatry*.

Kessler, R. C., Wai, T. C., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. In *Archives of General Psychiatry*. https://doi.org/10.1001/archpsyc.62.6.617

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., & Jones, P. B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life a population-based longitudinal study. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2014.1332

Klein, D. N., & Riso, L. P. (1993). Psychiatric disorders: Problems of boundaries and comorbidity. In *Basic Issues in Psychopathology*.

Köhler, C. A., Freitas, T. H., Stubbs, B., Maes, M., Solmi, M., Veronese, N., de Andrade, N. Q., Morris, G., Fernandes, B. S., Brunoni, A. R., Herrmann, N., Raison, C. L., Miller, B. J., Lanctôt, K. L., & Carvalho, A. F. (2018). Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: Systematic review and meta-analysis. In *Molecular Neurobiology*. https://doi.org/10.1007/s12035-017-0632-1

Köhler, O., Benros, M. E., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., & Krogh, J. (2014). Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2014.1611

Kornstein, S. G., Schatzberg, A. F., Thase, M. E., Yonkers, K. A., McCullough, J. P., Keitner, G. I., Gelenberg, A. J., Ryan, C. E., Hess, A. L., Harrison, W., Davis, S. M., & Keller, M. B. (2000). Gender differences in chronic major and double depression.

Journal of Affective Disorders. https://doi.org/10.1016/S0165-0327(99)00158-5

Kraus, C., Kadriu, B., Lanzenberger, R., Zarate, C. A., & Kasper, S. (2019). Prognosis and improved outcomes in major depression: a review. In *Translational Psychiatry*. https://doi.org/10.1038/s41398-019-0460-3

Lamers, F., Vogelzangs, N., Merikangas, K. R., De Jonge, P., Beekman, A. T. F., & Penninx, B. W. J. H. (2013). Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. *Molecular Psychiatry*. https://doi.org/10.1038/mp.2012.144

Leach, L. S., Christensen, H., Mackinnon, A. J., Windsor, T. D., & Butterworth, P. (2008). Gender differences in depression and anxiety across the adult lifespan: The role of psychosocial mediators. *Social Psychiatry and Psychiatric Epidemiology*. https://doi.org/10.1007/s00127-008-0388-z

Lee, K., Kim, D., & Cho, Y. (2018). Exploratory factor analysis of the beck anxiety inventory and the beck depression Inventory-II in a psychiatric outpatient population. *Journal of Korean Medical Science*. https://doi.org/10.3346/jkms.2018.33.e128

Liu, C. H., Stevens, C., Wong, S. H. M., Yasui, M., & Chen, J. A. (2019). The prevalence and predictors of mental health diagnoses and suicide among U.S. college students: Implications for addressing disparities in service use. *Depression and Anxiety*. https://doi.org/10.1002/da.22830

Lovibond, S. H., & Lovibond, P. F. (1995). Manual for the Depression Anxiety Stress Scales. In *Psychology Foundation of Australia*. https://doi.org/DOI: 10.1016/0005-7967(94)00075-U

Lu, S., Peng, H., Wang, L., Vasish, S., Zhang, Y., Gao, W., Wu, W., Liao, M., Wang, M., Tang, H., Li, W., Li, W., Li, Z., Zhou, J., Zhang, Z., & Li, L. (2013). Elevated specific peripheral cytokines found in major depressive disorder patients with childhood trauma exposure: A cytokine antibody array analysis. *Comprehensive Psychiatry*, *54*(7), 953–961. https://doi.org/10.1016/j.comppsych.2013.03.026

Maes, M. (2008). The cytokine hypothesis of depression: Inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. In *Neuroendocrinology Letters*.

Majd, M., Saunders, E., & Engeland, C. (2019). Inflammation and the dimensions of depression: A Review. *Frontiers in Neuroendocrinology*. https://doi.org/https://doi.org/10.1016/j.yfrne. 2019.100800

Mantella, R. C., Butters, M. A., Amico, J. A., Mazumdar, S., Rollman, B. L., Begley, A. E., Reynolds, C. F., & Lenze, E. J. (2008). Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2008.03.002

Maydych, V. (2019). The interplay between stress, inflammation, and emotional attention: Relevance for depression. In *Frontiers in Neuroscience*. https://doi.org/10.3389/fnins.2019.00384

Mc Elroy, S., & Hevey, D. (2014). Relationship between adverse early experiences, stressors, psychosocial resources and wellbeing. *Child Abuse and Neglect*. https://doi.org/10.1016/j.chiabu.2013.07.017

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness.

Journal of Psychiatric Research. https://doi.org/10.1016/j.jpsychires.2011.03.006

McQuaid, R. J., McInnis, O. A., Paric, A., Al-Yawer, F., Matheson, K., & Anisman, H. (2016). Relations between plasma oxytocin and cortisol: The stress buffering role of social support. *Neurobiology of Stress*. https://doi.org/10.1016/j.ynstr.2016.01.001

Miller, A. H., & Raison, C. L. (2015a). Are anti-inflammatory therapies viable treatments for psychiatric disorders? Where the rubber meets the road. In *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2015.22

Miller, A. H., & Raison, C. L. (2015b). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nature Reviews Immunology*, *16*, 22. https://doi.org/10.1038/nri.2015.5

Miller, A. H., & Raison, C. L. (2016). The role of inflammation in depression: From evolutionary imperative to modern treatment target. In *Nature Reviews Immunology*. https://doi.org/10.1038/nri.2015.5

Mokdad, A. H., Forouzanfar, M. H., Daoud, F., Mokdad, A. A., El Bcheraoui, C., Moradi-Lakeh, M., Kyu, H. H., Barber, R. M., Wagner, J., Cercy, K., Kravitz, H., Coggeshall, M., Chew, A., O'Rourke, K. F., Steiner, C., Tuffaha, M., Charara, R., Al-Ghamdi, E. A., Adi, Y., … Murray, C. J. L. (2016). Global burden of diseases, injuries, and risk factors for young people's health during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*. https://doi.org/10.1016/S0140-6736(16)00648-6

Moreira, F., Wiener, C. D., Jansen, K., Portela, L. V., Lara, D. R., Souza, L. D. de M., da Silva, R. A., & Oses, J. P. (2018). Childhood trauma and increased peripheral cytokines in young adults with major depressive: Population-based study. *Journal of*

Neuroimmunology, *319*(February), 112–116. https://doi.org/10.1016/j.jneuroim.2018.02.018

Munjiza, A., Kostic, M., Pesic, D., Gajic, M., Markovic, I., & Tosevski, D. L. (2018). Higher concentration of interleukin 6 - A possible link between major depressive disorder and childhood abuse. *Psychiatry Research*, *264*(March), 26–30. https://doi.org/10.1016/j.psychres.2018.03.072

Musselman, D. L., Lawson, D. H., Gumnick, J. F., Manatunga, A. K., Penna, S., Goodkin, R. S., Greiner, K., Nemeroff, C. B., & Miller, A. H. (2001). Paroxetine for the prevention of depression induced by high-dose interferon alfa. *New England Journal of Medicine*. https://doi.org/10.1056/NEJM200103293441303

Navaneelan, T. (2012). Health at a Glance. Suicide rates: An overview. *Statistics Canada*.

Nemeroff, C. B. (1996). The corticotropin-releasing factor (CRF) hypothesis of depression: New findings and new directions. *Molecular Psychiatry*.

Nolen-Hoeksema, S. (2001). Gender differences in depression. In *Current Directions in Psychological Science*. https://doi.org/10.1111/1467-8721.00142

O'Donovan, A., Hughes, B. M., Slavich, G. M., Lynch, L., Cronin, M. T., O'Farrelly, C., & Malone, K. M. (2010). Clinical anxiety, cortisol and interleukin-6: Evidence for specificity in emotion-biology relationships. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2010.03.003

Osimo, E. F., Baxter, L. J., Lewis, G., Jones, P. B., & Khandaker, G. M. (2019). Prevalence of low-grade inflammation in depression: A systematic review and meta-analysis of CRP levels. In *Psychological Medicine*.

https://doi.org/10.1017/S0033291719001454

Østergaard, S. D., Jensen, S. O. W., & Bech, P. (2011). The heterogeneity of the depressive syndrome: When numbers get serious. In *Acta Psychiatrica Scandinavica*. https://doi.org/10.1111/j.1600-0447.2011.01744.x

Ottawa Public Health. (2018). *Status of Mental Health in Ottawa*. 94. https://www.ottawapublichealth.ca/en/reports-research-and-statistics/resources/Documents/mental_health_report_2018_en.pdf

Owens, M. J., & Nemeroff, C. B. (1993). The role of corticotropin-releasing factor in the pathophysiology of affective and anxiety disorders: laboratory and clinical studies. In *Ciba Foundation symposium*.

Pace, T. W. W., Hu, F., & Miller, A. H. (2007). Cytokine-effects on glucocorticoid receptor function: Relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. In *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2006.08.009

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. In *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2008.06.006

Pariante, C. M., & Miller, A. H. (2001). Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. In *Biological Psychiatry*. https://doi.org/10.1016/S0006-3223(00)01088-X

Patten, S. B. (2017). Age of onset of mental disorders. In *Canadian Journal of Psychiatry*. https://doi.org/10.1177/0706743716685043

Poluzzi, E., Piccinni, C., Sangiorgi, E., Clo, M., Tarricone, I., Menchetti, M., & De Ponti,

F. (2013). Trend in SSRI-SNRI antidepressants prescription over a 6-year period and predictors of poor adherence. *European Journal of Clinical Pharmacology*. https://doi.org/10.1007/s00228-013-1567-8

Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: Inflammation and the pathogenesis of depression. In *Trends in Immunology*. https://doi.org/10.1016/j.it.2005.11.006

Regehr, C., Glancy, D., & Pitts, A. (2013). Interventions to reduce stress in university students: A review and meta-analysis. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2012.11.026

Riecher-Rössler, A. (2017). Sex and gender differences in mental disorders. In *The Lancet Psychiatry*. https://doi.org/10.1016/S2215-0366(16)30348-0

Sarstedt, M., & Mooi, E. (2019). Cluster Analysis. In: A Concise Guide to Market Research. In *Springer*. https://doi.org/10.1007/978-3-642-53965-7_9

Saveanu, R., Etkin, A., Duchemin, A. M., Goldstein-Piekarski, A., Gyurak, A., Debattista, C., Schatzberg, A. F., Sood, S., Day, C. V. A., Palmer, D. M., Rekshan, W. R., Gordon, E., Rush, A. J., & Williams, L. M. (2014). The International Study to Predict Optimized Treatment in Depression (iSPOT-D): Outcomes from the acute phase of antidepressant treatment. *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2014.12.018

Schilling, E. A., Aseltine, R. H., & Gore, S. (2008). The impact of cumulative childhood adversity on young adult mental health: Measures, models, and interpretations. *Social Science and Medicine*, *66*(5), 1140–1151. https://doi.org/10.1016/j.socscimed.2007.11.023

Serafini, G., Pompili, M., Elena Seretti, M., Stefani, H., Palermo, M., Coryell, W., & Girardi, P. (2013). The role of inflammatory cytokines in suicidal behavior: A systematic review. *European Neuropsychopharmacology*. https://doi.org/10.1016/j.euroneuro.2013.06.002

Silverman, M. N., Pearce, B. D., Biron, C. A., & Miller, A. H. (2005). Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. In *Viral Immunology*. https://doi.org/10.1089/vim.2005.18.41

Skovlund, C. W., Mørch, L. S., Kessing, L. V., & Lidegaard, O. (2016). Association of hormonal contraception with depression. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2016.2387

Slavich, G. M. (2016). Life stress and health: A review of conceptual issues and recent findings. *Teaching of Psychology*. https://doi.org/10.1177/0098628316662768

Slavich, G. M., & Auerbach, R. P. (2018). Stress and its sequelae: Depression, suicide, inflammation, and physical illness. In *APA handbook of psychopathology: Psychopathology: Understanding, assessing, and treating adult mental disorders (Vol. 1).* https://doi.org/10.1037/0000064-016

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: A social signal transduction theory of depression. *Psychological Bulletin*. https://doi.org/10.1037/a0035302

Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995). A scale for the assessment of hedonic tone. The Snaith-Hamilton Pleasure Scale. *British Journal of Psychiatry*. https://doi.org/10.1192/bjp.167.1.99

Sramek, J. J., Murphy, M. F., & Cutler, N. R. (2016). Sex differences in the

psychopharmacological treatment of depression. *Dialogues in Clinical Neuroscience*.

Statistics Canada. (2019). *Table 13-10-0394-01 Leading causes of death, total population, by age group*. CANSIM.

Valkanova, V., Ebmeier, K. P., & Allan, C. L. (2013). CRP, IL-6 and depression: A systematic review and meta-analysis of longitudinal studies. In *Journal of Affective Disorders*. https://doi.org/10.1016/j.jad.2013.06.004

Van Nierop, M., Viechtbauer, W., Gunther, N., Van Zelst, C., De Graaf, R., Ten Have, M., Van Dorsselaer, S., Bak, M., Van Winkel, R., Bruggeman, R., Wiersma, D., Cahn, W., Kahn, R. S., De Haan, L., Meijer, C. J., Myin-Germeys, I., & Van Os, J. (2015). Childhood trauma is associated with a specific admixture of affective, anxiety, and psychosis symptoms cutting across traditional diagnostic boundaries. *Psychological Medicine*. https://doi.org/10.1017/S0033291714002372

Wang, W., Feng, J., Ji, C., Mu, X., Ma, Q., Fan, Y., Chen, C., Gao, C., Ma, X., & Zhu, F. (2017). Increased methylation of glucocorticoid receptor gene promoter 1F in peripheral blood of patients with generalized anxiety disorder. *Journal of Psychiatric Research*. https://doi.org/10.1016/j.jpsychires.2017.01.019

West, D., & Maes, M. (1999). Major depression and activation of the inflammatory response system. In *Ceska a Slovenska Psychiatrie*. https://doi.org/10.1007/978-0-585-37970-8_2

WHO. (2008). The World Health Report 2008: Primary Health Care: Now More Than Ever. In *The World Health Report*. https://doi.org/10.12927/hcpol.2013.22778

World Federation For Mental Health (WFMH). (2012). Depression: A Global Crisis.

World Mental Health Day.

Appendices

Appendix A: Ethics Certificate

Office of Research Ethics 5110 Human Computer Interaction Bldg | 1125 Colonel By Drive | Ottawa, Ontario K1S 5B6 613-520-2600 Ext: 4085 ethics@carleton.ca

CERTIFICATION OF INSTITUTIONAL ETHICS CLEARANCE

The Carleton University Research Ethics Board-B (CUREB-B) has granted ethics clearance for the research project described below and research may now proceed. CUREB-B is constituted and operates in compliance with the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* (TCPS2).

Ethics Protocol Clearance ID: Project # 109133

Research Team: Robyn McQuaid (Primary Investigator)

Kelly Moreland (Co-Investigator) Sabina Franklyn (Co-Investigator (External)) Zachary Kaminsky (Other) Dr. Kimberly Matheson (Research Supervisor) Jennifer Kemp (Research Assistant) Dr. Hymie Anisman (Collaborator)

Project Title: Full Board: A Biological and Psychosocial Characterization of Mental Health Symptoms [Sabina Franklyn and Kelly Moreland]

Funding Source (If applicable): Effective: **July 31, 2018** Expires: **July 31, 2019**. **Please ensure the study clearance number is prominently placed in all recruitment**

and consent materials: CUREB-B Clearance # 109133.

Restrictions:

This certification is subject to the following conditions:

Clearance is granted only for the research and purposes described in the application.

Any modification to the approved research must be submitted to CUREB-B via a Change to Protocol Form. All changes must be cleared prior to the continuance of the research.

3. An Annual Status Report for the renewal of ethics clearance must be submitted and cleared by the renewal date listed above. Failure to submit the Annual Status Report will result in the closure of the file. If funding is associated, funds will be frozen.

4. A closure request must be sent to CUREB-B when the research is complete or terminated.

5. Should any participant suffer adversely from their participation in the project you are required to report the matter to CUREB-B.

Failure to conduct the research in accordance with the principles of the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans 2ndedition* and the *Carleton University Policies and Procedures for the Ethical Conduct of Research* may result in the suspension or termination of the research project.

Upon reasonable request, it is the policy of CUREB, for cleared protocols, to release the name of the PI, the title of the project, and the date of clearance and any renewal(s).

Please contact the Research Compliance Coordinators, at ethics@carleton.ca, if you have any questions.

CLEARED BY: Date: July 31, 2018

Bernadette Campbell, PhD, Chair, CUREB-B Andy Adler, PhD, Vice-Chair, CUREB-B

Appendix B: Informed Consent

Study Title: A Biological and Psychosocial Characterization of Mental Health Symptoms

Study Personnel: Sabina Franklyn, Carleton University (Graduate Researcher), Maya Atlas, Carleton University (Research Assistant), Jennifer Kemp, Carleton University (Undergraduate Researcher), Emily Thaw, Carleton University (Undergraduate Researcher), Maria Anam, Carleton University (Undergraduate Researcher), Dr. Kim Matheson, Carleton University (Faculty Supervisor), Dr. Robyn McQuaid, The Royal's Institute of Mental Health Research (IMHR; Co-Supervisor), Dr. Hymie Anisman, Carleton University (Collaborator) and Dr. Zach Kaminsky, The Royal's IMHR (External Collaborator).

Purpose and task requirements: The purpose of this study is to assess how factors such as social resources (e.g. support from friends, and social connections) are used to cope with negative life events or stressors, and the impact of these events on mental health. We are also interested in examining how certain genetic and hormonal/immune factors relate to well-being and mental health symptoms.

In this session, we will be asking everyone to fill out a number of questionnaires regarding information related to your background (e.g., demographic, family and medical history), early life experiences (e.g., trauma, parental bonding), mood (e.g., have they recently been feeling sad or anxious), substance use, and the quality of your interpersonal relationships (e.g., social support, group identities). We are also asking all participants to provide a DNA sample through the simple act of spitting into a tube however, due to the relatively large volume of saliva required (~1 ml) this can take up to 5 minutes. We will use the saliva sample (that you will provide today) to determine the presence or absence of particular genes.

We are also interested in examining differences in stress hormones and immune system functioning among participants. To measure these hormones, we will be asking certain participants to provide a single blood sample at the end of this session. We will have everyone complete an eligibility screening questionnaire to assess whether you would be a good candidate to provide a blood sample, but also if this is something you are comfortable providing. If you are willing and eligible to provide a blood sample, you will go a separate room and a Registered Nurse/Phlebotomist will draw a blood sample (just as they do when you have a blood test at your doctor's office or clinic). A very small amount (approximately 8mL) will be taken. All instruments used for blood withdrawal will be sterile and one-time use only. The blood samples will be analyzed for levels of related hormones (cortisol), cytokines (CRP, IL-6, IL-10, TNF-α, etc.) and epigenetic factors (i.e. tags on your DNA that show how environmental experiences shape the way our genes express). Some of this work will be conducted at the Institute of Mental Health Research by Dr. Robyn McQuaid and Dr. Zachary Kaminsky.

By understanding the biological and psychosocial factors that lead to different mood outcomes, we will gain a better understanding of how mood changes develop. This

research will therefore help to develop improved approaches to both treat, and prevent mood disorders in the future.

Who can participate: University undergraduate students between the ages of 17-30 who have fluent English comprehension.

You will receive 2% course credit. This session will take 1 hour and 45 minutes.

Potential risks or causes of discomfort for participants

Some individuals might feel discomfort when asked to provide personal, sensitive information. Furthermore, responding to some of the questions in this study or thinking about various stressors or difficulties in your life can be anxiety provoking or make you unhappy. If this is the case, the Debriefing form at the end of the study contains contact information for people who are available to help. You can also choose to withdraw from the study at any point. Inserting the needle usually gives a small pin-prick (as it does when you get blood tests at the doctor's office or clinic). The blood draw is very quick, however, if at any time you wish to stop, please let us know and the nurse will take out the needle. We are looking to collect about a teaspoon of saliva. Some participants may therefore feel discomfort or embarrassment about spitting into a tube in the presence of the researcher, in which case he/she will be happy to leave the room to give you privacy. You will be asked to not drink, eat, smoke or chew gum for 30 minutes before providing this sample.

Anonymity and confidentiality

All information and samples collected from you for this study will be identified with a code number, as opposed to any personal identifiers such as your name or address. We ensure that all information you share with us, such as personal information (i.e. adverse life experiences) and information regarding illegal activities (i.e. substance use) will remain strictly confidential. We will maintain a separate record that matches your personal identification details with this code number. This is necessary in order for us to re-contact you in the future (with your express permission to do so), and so that we can respect your right to withdraw from the study at a later date (to be described below). However, this record that allows your identity to be linked to your information will kept in a separate and secured location, and will only be accessible to the primary researchers, in order to maintain anonymity and confidentiality of your information. All personal identifying information will be destroyed within 5 years of study completion. Furthermore, the saliva and blood samples will be stored in a secured storage area that is only accessible by the researcher and research assistants.

Genetic testing: Common questions and concerns

What is DNA?

DNA is a large molecule that contains information necessary for our bodies to build all the components needed for our development, growth and survival. This information is commonly referred to as the genetic code or the DNA sequence. Some rare diseases can be attributed entirely to simple errors in our DNA sequence. However, the majority of common diseases (including depression) are caused by a combination of many different genetic factors, together with environmental factors (how we grew up, life events, etc).

What will my DNA be used for?

If you compare any two people, their DNA will be about 99% identical. We are interested in the 1% of DNA that is different between people. Our current plan is to investigate these differences, focusing on just a small proportion of your genes (we are targeting less than 100 of the ~30,000 genes that humans have), which we anticipate may be involved in risk of either anxiety or depression. With your express permission, we will conduct future follow-up studies on your DNA, which will extend the analysis to substantially more genes – potentially all genes. These future studies will be limited to analyses that examine polymorphisms, mutations and epigenetic modifications to DNA molecule, and will not involve any other use or manipulation of your DNA sample. However, in no case will your samples be kept for more than 5 years from study completion, at which time the samples and the sample container will be incinerated. Thus, the data derived from the sample will be kept (stripped of any information that could identify you), but the sample or remnants of the sample, including DNA, will be destroyed (incinerated). At the end of this form, you have the option to opt-out of any such future uses of your DNA sample.

How long will my DNA be stored, and potentially used in research?

By providing a DNA sample and signing this form, you are indicating that you are willing for us to preserve and analyze your DNA sample for an extended period of time (5 years or less). During this period, use of the sample is guaranteed to be limited to studies that examine polymorphisms, mutations and epigenetic modifications to DNA molecule. As indicated above, your saliva and any DNA samples will be destroyed, by incineration, no later than 5 years after study completion.

Will I be told the results of my own genetic analysis?

No. Your DNA sample and genetic information will be identified by a code number, and not your name. This preserves confidentiality of this information. Returning your personal genetic information to you would require that confidentiality to be compromised, so will be avoided. Furthermore, as described above, genetic data collected in this study will not allow accurate prediction of whether or not you will develop any disease. Although in the future we hope to be able to use genetic materials to examine disease susceptibility, our research is in a preliminary phase and we are not currently able to provide this kind of information on an individual basis.

What if something unexpected and potentially dangerous is discovered in my DNA?

None of the DNA sites that we plan to analyze are currently known to be predictive of disease with any real accuracy. However, future advances in genetic research could allow disease predictions to be possible based on information from these, or other genetic sites. In exceptional circumstances, if genetic research reveals information about a serious or life-threatening condition that can be prevented or treated through intervention, then we have an obligation to inform you of this information, and potentially also inform your biological relatives who may share similar risk of disease. This would therefore represent a potential breach of confidentiality. In this instance, only information directly relating to disease diagnosis, and participant identity, would be shared. We wish to make clear, however, that it is not our intent to systematically go through the samples after our initial analyses, and so even if it was subsequently discovered that certain genes might carry relevant information for you, it is unlikely that we would actually spot any vulnerabilities you might have.

Can my DNA ever be used to identify me?

This is a complicated question to answer. Unless you have an identical twin (whose DNA will be identical to yours), your DNA is absolutely unique to you. It is this unique nature of genetic material that allows individuals to be identified based entirely on their DNA, through techniques such as DNA fingerprinting. It is therefore theoretically possible that in the future, your identity could be determined from simply analyzing your DNA sample. It is extremely unlikely, however, that you could be identified based on your DNA sample. In order to identify you based purely on your DNA sample, it would be necessary to compare your DNA sample that you provide today, with another DNA sample from you in a DNA database, which is linked to your identity. DNA databases do exist in countries including Canada, Australia, USA and UK, but are limited to samples from criminal offenders. Access to these databases is strictly limited to law enforcement agencies thus cannot be accessed by researchers. Access to DNA samples taken for this study will similarly be limited to the researchers, and will not be provided to any law enforcement agency unless we become legally obliged to do so (to our knowledge, this has never happened to any research group). Furthermore, these government DNA databases typically contain information about only 13 regions of human DNA, none of which are to be analyzed in the present study.

Right to withdraw from this study

Participation in this study is entirely voluntary. At any point during the study you have the right to not complete certain questions or to withdraw with no penalty whatsoever and will still receive course credit. Furthermore, if at a later date you wish to withdraw from the study, you can contact the principal investigators and we will destroy all of your records (questionnaire answers, responses from the interview, plus DNA sample) from this study. The only exceptions are where data has already been published, in this instance, unpublished data plus your DNA sample will be destroyed, or if 5 years have passed, in which case, all personal identifying information will have been destroyed and there will be no way to re-identify participants.

If you have any additional questions or concerns, please ask the researcher today, or

contact any of the graduate researchers or supervisors at a later date.

Sabina Franklyn, Department of Psychology, Carleton University
Email: SabinaFranklyn@cmail.carleton.ca

Dr. Robyn McQuaid, The Royal's Institute of Mental Health Research
Phone: 613 722-6521 ext. 6490, Email: Robyn.McQuaid@theroyal.ca

Dr. Kim Matheson, Department of Neuroscience, Carleton University
Phone: 613 520-2600 ext. 2652, Email: kim.matheson@carleton.ca

If you have any ethical concerns with the study, please contact Dr. Bernadette Campbell, Chair, Carleton University Research Ethics Board-B (by phone: 613-520-2600 ext. 4085 or by email: ethics@carleton.ca).

Signatures

Participant's Full Name: _____ Participant's Signature: _____

Researcher's Name: _____ Researcher's Signature: _____

Date_____

How can we use your DNA?:
While we would ideally obtain consent to use your DNA for any future research studies that are aimed at analyzing DNA sequence (Option 1 below), please indicate below how you would like your DNA sample to be treated in the future. There are no penalties for you associated with your selection.

☐ Option 1: I grant the researcher permission to use my DNA for any future research studies that involve analyses of polymorphisms, mutations and epigenetic modifications to the DNA molecule, within a 5 year period of study completion.

☐ Option 2: I grant the researcher permission to re-contact me to seek consent to use my DNA in future research studies, within a 5 year period of study completion.
Email:_____ Phone Number:_____

☐ Option 3: I grant use of my DNA, but this use will be strictly limited to the analysis of <100 genes, as described in the current research plan. This can only be done within a 5 year period of study completion.

This study has been cleared by the Carleton University Research Ethics Committee (CUREB-B Clearance #109133) and is funded by the Royal's Institute of Mental Health Research.

Appendix C: Blood Eligibility Screening Questionnaire

In order to examine certain hormones and immune factors for this study, we require a blood sample. We would like to collect a blood sample from any participants who meet our criteria on the below questionnaire and are comfortable doing so. This would be done by a registered nurse or registered phlebotomist (exactly as when one gives a sample of blood at a clinic/doctor's office). Please answer the following questions as honestly and accurately as possible by selecting the appropriate statement.

1) Have you been diagnosed with any of the following conditions? (Please select all that apply)

   ____ Diabetes
   ____ Heart condition
   ____ Autoimmune disorder
   ____ None of the above

2) Are you currently being treated for any physical illness or injury?

   ____ Yes
   ____ No

3) If you answered "yes" to the previous question, please specify the illness or injury you are being treated for

   _____
   _____
   _____
   _____
   _____

4) Are you currently taking any of the following medications? (please check all that apply)

   ____ Anti-inflammatories
   ____ Anti-depressants
   ____ Anti-anxieties
   ____ Allergy medication
   ____ Other prescription drugs

5) If you answered "yes" to the previous question, please indicate the name of all medications you are currently taking

   _____
   _____
   _____
   _____

_____
_____
_____
_____

6) Have you given blood before?

____ Yes, I have donated blood at Canadian Blood Services/Red Cross
____ Yes, I have had blood tests (e.g., at a doctor's office)
____ No, I have never given blood before

7) Have you had any issues during blood tests at a clinic or doctor's office before?

____Yes
____ No

If yes please specify:

_____
_____
_____
_____
_____

8) When you think about needles and blood tests, how anxious/nervous do you feel?

____Not anxious at all
____ Slightly anxious/nervous
____ Moderately anxious/nervous
____ Extremely anxious or nervous

Appendix D: Blood Consent Form

Study Title: A Biological and Psychosocial Characterization of Mental Health Symptoms

In order to understand the biological basis of mental health symptoms, we require a blood sample, which allows us examine stress hormones, immune factors, and epigenetic factors that we cannot assess with the saliva sample you provided. Understanding how these biological factors are linked to mental health is very important to our research questions and to help inform the causes and treatments of mental health disorders in the future. However, providing a blood sample is completely optional.

Blood collection is done by a registered nurse or registered phlebotomist (exactly as when one gives a sample of blood at a clinic/doctor's office).

Your responses to the screening questionnaire met our criteria for providing a blood sample. Are you willing to provide a blood sample (will take roughly 5 minutes).

____ Yes
____ No

_____
Participant Name

_____ _____
Participant Signature Date

_____
Researcher Name

_____ _____
Researcher Signature Date

Appendix E: Measures

Background Information

The purpose of the following set of questions is to collect demographic information about various aspects of your life. Although some of the questions may seem unrelated to the present study (e.g. weight, height, religion, etc...) these factors may be important determinants of your health and well-being.

1. Gender: ___Female ___Male ___Transgender ___Gender non-Conforming ___Not listed (please specify) _____

2. Age: _____
3. What is your citizenship status?

_____ Canadian citizen
_____ Landed immigrant Since what year?_____ Country of origin _____
_____ Student visa Since what year?_____ Country of origin_____
_____ Temporary visa Since what year?_____ Country of origin _____
_____ Refugee Since what year?_____ Country of origin _____
_____ Permanent Resident Since what year?_____ Country of origin _____

Other (please specify): _____

4. What is your first language? _____

If your first language is not English, how long have you been **fluent** in reading, writing and comprehension of the English language?

5. What is your ethnic/racial background? *Please select the one that best applies to you.*
____Asian (e.g., Chinese, Japanese, Korean)
____South Asian (e.g., East Indian, Pakistani, Punjabi, Sri Lankan)
____South East Asian (e.g., Cambodian, Indonesian, Laotian)
____Arab/West Asian (e.g., Armenian, Egyptian, Iranian, Lebanese, Moroccan)
____Black (e.g., African, Haitian, Jamaican, Somali)
____Latin American/Hispanic
____Aboriginal

____White/Euro-Caucasian
____Other (please specify): _____

6. What is your religious affiliation? *Please select the one that best applies to you*

____None—Atheist (e.g., belief that there is NO God)
____None—Agnostic (e.g., belief that the existence of God cannot be known)
____Protestant (e.g., United, Anglican, Baptist, Presbyterian, Lutheran, Pentecostal,

7. What is your current living arrangement? *Please select the one that best applies to you.*
_____ Living alone in residence (at Carleton University)
_____ Living alone off-campus
_____ Living with friends in residence (at Carleton University)
_____ Living with friends off-campus
_____ Living with roommates in residence (at Carleton University)
_____ Living with roommates off-campus
_____ Living with parents
_____ Living with spouse/significant other
_____ Living with spouse/significant other and young children (13 years and younger)
_____ Living with spouse/significant other and older children (13 years and older)
_____ Living alone with young children (13 years and younger)
_____ Living alone with older children (13 years and older)
_____ Other (please specify) _____

8. What is your current relationship status? *Please select the one that best applies to you.*
_____ Single, and not seeing anyone
_____ Going out with someone
_____ In a serious dating relationship
_____ Have recently broken up. Please specify how many weeks ago you broke up_____ _____ Living with an intimate other
_____ Engaged
_____ Married
_____ Separated/Divorced. Please specify how many months ago you separated_____ _____ Widowed

9. Is your current (or most recent) partner: Male _____, Female _____, Other_____?

10. Have you had or do you currently have any health related (i.e., medical) illnesses or physical conditions? *Please select the one that best applies to you.* No, I don't

_____ Yes, I did but I no longer do _____ Yes, I do

If YES, please specify illness/condition you had/have
_____

If YES, please specify treatment received or currently receiving_____

11. Are you currently sick with a cold? No_____ Yes_____

Flu? No_____ Yes_____

12. Do you currently have a mental health condition (e.g. depression, anxiety, etc.)?

No_____

Yes_____

If YES, please specify disorder/condition _____

If YES, are you currently being treated for these symptoms?
No_____
Yes_____ (if yes please specify)_____

13. Have you ever in the past had a psychological disorder/condition (e.g. depression, anxiety, etc.) but no longer do?

NO, I haven't ____ YES, I have_____
If YES, please specify the disorder/condition you had_____

14. Have you ever had thoughts of suicide in your lifetime? No_____ Yes_____

15. Have you had thoughts of suicide in the past 12 months? No_____ Yes_____

16. Have you ever attempted suicide in your lifetime? No_____ Yes_____

17. Have you attempted suicide in the past 12 months? No_____ Yes_____

18. In your opinion, how would you describe your health?
_____Poor _____Fair _____Good _____Very good _____Excellent

19. Are you on any of the following medications (please check all that apply)?

_____ Anti-inflammatories (please specify)
_____
_____ Anti-depressants (please specify)
_____
_____ Anti-anxieties (please specify)
_____
_____ Allergy medication (please specify)

_____
_____ Other prescription drugs (please specify)
_____

20. Have you taken any of these medications in the past 24 hours? _____NO _____YES
If yes:

1. a) Which medication(s)? _____
2. b) What time did you take it/them? _____
3. c) How much did you take? _____

21. Are you taking any form of hormonal based contraception (birth control)? _____NO _____YES

If yes, which one?
____Standard birth control pill
____Evra Patch
____NuvaRing
____Depo-Provera Shot
____Mini (progestin-only) birth control pill
____Other: _____

22. Are you currently on your period? _____NO _____YES
When did your last period begin (how many days ago)? _____ And end (how many days ago)? _____

23. How often do you exercise/week? _____

24. Have you exercised in the last 24hrs? _____NO _____YES
What form of exercise was it? _____

25.Do you currently smoke? _____NO _____YES
If YES, how many/day? _____

26.Do you drink alcohol? _____NO _____YES

If YES,
How much alcohol do you drink on average? _____ drinks per day _____ drinks per week

27.Do you take or use any drugs? _____NO _____YES

If YES, which drugs have you used in the past month? (check as many as apply)
____Cannabis/hash; How many times in the past month? _____
____Ecstasy; How many times in the past month? _____

____Cocaine; How many times in the past month? _____
____Opioids (non-prescription); How many times in the past month? _____
____Other; Please specify _____; How many times in the past month?_____

28.What is your estimate of your family's gross income per year? *Please select the one that best applies to you.*

____ under $15,000
____ $15,000 - $29,999
____ $30,000 - $44,999
____ $45,000 - $59,999
____ $60,000 - $74,999
____ $75,000 - $89,999
____ $90,000 - $104,999
____ $105,000 or more
____ Not sure

29. What is your employment status (aside from being a student)?
_____ Employed Part-time
_____ Employed Full-time
_____ Unemployed
_____ Retired
_____ Other (please specify_____

30. What time did you wake up this morning? _____
31. When was the last time you ate today? _____
32. What did you have to eat and drink today?
_____

33. How many pets do you have?
_____ None
_____ One
_____ Two
_____ Three
_____ 4+

34. If you have pets, what kinds of pets do you own (check all that apply)? Cat ____ Dog _____
_____ Other (specify) : _____

35. Are you currently a primary (daily) caregiver of your pet(s)? _____Yes _____ No

Beck Anxiety Inventory (BAI)

Please rate how much you have been bothered by each of the following symptoms over the past week.

Not at All	*Mildly but it didn't bother me much*	*Moderately-it wasn't pleasant at time*	*Severely-it bothered me a lot*
0	1	2	3

1. Numbness or tingling	0	1	2	3
2. Feeling hot	0	1	2	3
3. Wobbliness in legs	0	1	2	3
4. Unable to relax	0	1	2	3
5. Fear of the worst happening	0	1	2	3
6. Dizzy or light-headed	0	1	2	3
7. Heart pounding or racing	0	1	2	3
8. Unsteady	0	1	2	3
9. Terrified	0	1	2	3
10. Nervous	0	1	2	3
11. Feelings of choking	0	1	2	3
12. Hands trembling	0	1	2	3
13. Shaky	0	1	2	3
14. Fear of losing control	0	1	2	3
15. Difficulty breathing	0	1	2	3
16. Fear of dying	0	1	2	3
17. Scared	0	1	2	3
18. Indigestion or discomfort in abdomen	0	1	2	3
19. Faint	0	1	2	3
20. Face flushed	0	1	2	3
21. Sweating (not due to heat)	0	1	2	3

Source: Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: psychometric properties. *Journal of consulting and clinical psychology*, *56*(6), 893.

Beck Depression Inventory (BDI)

On this questionnaire are groups of statements. Please read the entire group of statements in each category. Then pick out **ONE** statement in that group which best describes the way you feel. Check off the number beside the statement you have chosen.

1. ___ 0 = I do not feel sad
   ___ 1 = I feel sad or blue
   ___ 2a = I am blue or sad all of the time and I can't snap out of it
   ___ 2b = I am so sad or unhappy that it is very painful
   ___ 3 = I am so sad or unhappy that I can't stand it

2. ___ 0 = I am not particularly pessimistic or discouraged about the future
   ___ 1 = I feel discouraged about the future
   ___ 2a = I feel I have nothing to look forward to
   ___ 2b = I feel I won't every get over my troubles
   ___ 3 = I feel that the future is hopeless and things cannot improve

3. ___ 0 = I do not feel like a failure
   ___ 1 = I feel I have failed more than the average person
   ___ 2a = I feel I have accomplished very little that is worthwhile or that means anything
   ___ 2b = As I look back on my life, all I can see is a lot of failures
   ___ 3 = I feel I am a complete failure as a person

4. ___ 0 = I am not particularly dissatisfied
   ___ 1a = I feel bored most of the time
   ___ 1b = I don't enjoy things the way I used to
   ___ 2 = I don't get satisfaction out of anything anymore
   ___ 3 = I am dissatisfied with everything

5. ___ 0 = I don't feel particularly guilty
   ___ 1 = I feel bad or unworthy a good part of the time
   ___ 2a = I feel quite guilty
   ___ 2b = I feel bad or unworthy practically of the time now
   ___ 3 = I feel as though I am very bad or worthless

6. ___ 0 = I don't feel I am being punished
   ___ 1 = I have a feeling that something bad may happen to me
   ___ 2 = I feel I am being punished or will be punished
   ___ 3a = I feel I deserve to be punished
   ___ 3b = I want to be punished

7. ___ 0 = I don't feel disappointed in myself
   ___ 1a = I am disappointed in myself
   ___ 1b = I don't like myself
   ___ 2 = I am disgusted with myself
   ___ 3 = I hate myself

8. ___ 0 = I do not feel I am any worse than anybody else
   ___ 1 = I am very critical of myself for my weaknesses or mistakes
   ___ 2a = I blame myself for everything that goes wrong

___ 2b = I feel I have many bad faults

9. ___ 0 = I don't have thoughts of harming myself
___ 1 = I have thoughts of harming myself but I would not carry them out
___ 2a = I feel I would be better off dead
___ 2b = I have definite plans about committing suicide
___ 2c = I feel my family would be better off if I were dead
___ 3 = I would kill myself if I could

10. ___ 0 = I don't cry anymore than usual
___ 1 = I cry more now than I used to
___ 2 = I cry all the time now. I can't stop it
___ 3 = I used to be able to cry but now I can't cry at all even though I want to

11. ___ 0 = I am no more irritable than usual
___ 1 = I am more irritable than usual
___ 2 = I am much more irritable than usual
___ 3 = I am irritable all the time

12. ___ 0 = I have not lost interest in other people
___ 1 = I am less interested in other people than I used to be
___ 2 = I have lost most of my interest in other people and I have little feeling for them
___ 3 = I have lost all my interest in other people and don't care about them at all

13. ___ 0 = I make decisions about as well as ever
___ 1 = I am less sure of myself now and try to put off making decisions
___ 2 = I can't make decisions anymore without help
___ 3 = I can't make decisions at all anymore

14. ___ 0 = I don't feel I look any worse than I used to
___ 1 = I am worried that I am looking old or unattractive
___ 2 = I feel that there are permanent changes in my appearance and they make me look unattractive
___ 3 = I feel that I am ugly or repulsive looking

15. ___ 0 = I can work about as well as before
___ 1a = It takes extra effort to get started at doing something
___ 1b = I don't work as well as I used to
___ 2 = I have to push myself very hard to do anything
___ 3 = I can't do any work at all

16. ___ 0 = I can sleep as well as usual
___ 1 = I wake up more tired in the morning than I used to
___ 2 = I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
___ 3 = I wake up early every day and can't get more than 5 hours sleep

17. ___ 0 = I don't get any more tired than usual
___ 1 = I get tired more easily than I used to
___ 2 = I get tired from doing anything
___ 3 = I get too tired to do anything

18a. ___ 0 = My appetite is no worse than usual
___ 1 = My appetite is not as good as it used to be
___ 2 = My appetite is much worse now
___ 3 = I have no appetite at all any more

18b. ___ 0 = I am not eating more than usual
___ 1 = I am eating a little more than usual
___ 2 = I am eating somewhat more than usual
___ 3 = I am eating a lot more than usual

18c. ___ 0 = I have had no change in food preferences lately
___ 1 = I have been craving more carbohydrates (starches or sweets lately)
___ 2 = I have had irresistible craving for sweets and starches lately

19a. ___ 0 = I haven't lost much weight, if any, lately
___ 1 = I have lost more than 5 pounds
___ 2 = I have lost more than 10 pounds
___ 3 = I have lost more than 15 pounds

19b. ___ 0 = I have not gained any weight lately
___ 1 = I have gained more than 5 pounds
___ 2 = I have gained more than 10 pounds
___ 3 = I have gained more than 15 pounds

20. ___ 0 = I am no more concerned about my health than usual
___ 1 = I am concerned about aches and pains or upset stomach or constipation or other
unpleasant feelings in my body
___ 2 = I am so concerned with how I feel or what I feel that it's hard to think of much else
___ 3 = I am completely absorbed in what I feel

21. ___ 0 = I have not noticed any recent change in my interest in sex
___ 1 = I am less interested in sex than I used to be
___ 2 = I am much less interested in sex now
___ 3 = I have lost interest in sex completely

22. ___ 0 = I have not had any increase in nightly sleep length lately
___ 1 = I have had at least 1-hour increase in sleep length
___ 2 = I have had at least 2-hour increase in sleep length
___ 3 = I have had at least 3-hour increase in sleep length

My average nightly sleep length in the past week is_____hours

23. ___ 0 = I am not feeling more fatigued than usual
___ 1 = I feel more fatigued than usual lately, but it does not interfere with my daily functioning
___ 2 = I feel more fatigued than usual lately, and it interferes somewhat with my daily functioning
___ 3 = I feel more fatigued than usual lately, and it significantly interferes with my daily functioning

24. ___ 0 = I have not had any mood swings or slumps lately
___ 1 = I have had some mood swings or slumps lately but very minor
___ 2 = I have had more mood swings or slumps than usual
___ 3 = I have had severe mood swings or slumps lately

25. ___0 = I have not been more sensitive in social situations lately
___ 1 = I have been feeling slightly more sensitive in social situations lately
___ 2 = I have been feeling much more sensitive in social situations lately
___ 3 = I have been feeling extremely sensitive in social situations lately

Source: Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory of measuring depression. *Archives of General Psychiatry, 4*, 561-571.
In addition we have added 7 items regarding sleep, eating and sensitivity that reflect a-typical depression, as our lab has done in the past.

Depression Anxiety and Stress Scale (DASS – 21)

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3

4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

Source: Lovibond. L. Manual for the Depression Anxiety and Stress Scales. Sydney, Australia; Psychology Foundation of Australia; 1995.

The Snaith-Hamilton Pleasure Scale

This scale was designed to measure your ability to experience pleasure *in the last few days*. It is important to read each statement carefully and indicate how much you agree or disagree with each statement.

Strongly Agree	*Agree*	*Disagree*	*Strongly Disagree*
1	2	3	4

1. I would enjoy my favourite television or radio programme 1 2 3 4
2. I would enjoy being with family or close friends 1 2 3 4
3. I would find pleasure in my hobbies and pastimes 1 2 3 4
4. I would be able to enjoy my favourite meal 1 2 3 4
5. I would enjoy a warm bath or refreshing shower 1 2 3 4
6. I would find pleasure in the scent of flowers or the smell 1 2 3 4 of a fresh sea breeze or freshly baked bread
7. I would enjoy seeing other people's smiling faces 1 2 3 4
8. I would enjoy looking smart when I have made an effort 1 2 3 4 with my appearance
9. I would enjoy reading a book, magazine or newspaper 1 2 3 4
10. I would enjoy a cup of tea or coffee or my favourite drink 1 2 3 4
11. I would find pleasure in small things, e.g. bright sunny day, 1 2 3 4 a telephone call from a friend
12. I would be able to enjoy a beautiful landscape or view 1 2 3 4
13. I would get pleasure from helping others 1 2 3 4
14. I would feel pleasure when I receive praise from other 1 2 3 4 people

Source: Snaith, R.P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., Trigwell, P., 1995. A scale for the assessment of hedonic tone the Snaith–Hamilton Pleasure Scale. *Br. J. Psychiatry* 167, 99–103.

Early Trauma Inventory Self Report-Short Form (ETISR-SF)

Part 1. General Traumas. Before the age of 18

1. Were you ever exposed to a life-threatening natural disaster?..... YES NO

2. Were you involved in a serious accident? ……..... YES NO

3. Did you ever suffer a serious personal injury or illness?..... YES NO

4. Did you ever experience the death or serious illness of a parent or a primary caretaker? ..... YES NO

5. Did you experience the divorce or separation of your parents?..... YES NO

6. Did you experience the death or serious injury of a sibling? ..... YES NO

7. Did you ever experience the death or serious injury of a friend?..... YES NO

8. Did you ever witness violence towards others, including family members? ..... YES NO

9. Did anyone in your family ever suffer from mental or psychiatric illness or have a a “breakdown”? ..... YES NO

10. Did your parents or primary caretaker have a problem with alcoholism or drug abuse? ..... YES NO

11. Did you ever see someone murdered? ..... YES NO

Part 2. Physical Punishment. Before the age of 18

1. Were you ever slapped in the face with an open hand? ..... YES NO

2. Were you ever burned with hot water, a cigarette or something else.....YES NO

3. Were you ever punched or kicked? ..... YES
NO

4. Were you ever hit with an object that was thrown at you? ..... YES
NO

5. Were you ever pushed or shoved? ..... YES
NO

Part 3. Emotional Abuse. Before the age of 18

1. Were you often put down or ridiculed? ..... YES
NO

2. Were you often ignored or made to feel that you didn't count? ..... YES
NO

3. Were you often told you were no good? ..... YES
NO

4. Most of the time were you treated in a cold, uncaring way or made to feel like you were not loved? ..... YES
NO

5. Did your parents or caretakers often fail to understand you or your needs..... YES
NO

Part 4. Sexual Events. Before the age of 18

1. Were you ever touched in an intimate or private part of your body (e.g. breast, thighs, genitals) in a way that surprised you or made you feel uncomfortable? ..... YES
NO

2. Did you ever experience someone rubbing their genitals against you?..... YES
NO

3. Were you ever forced or coerced to touch another person in an intimate or private part of their body? ..... YES
NO

4. Did anyone ever have genital sex with you against your will? ..... YES
NO

5. Were you ever forced or coerced to perform oral sex on someone against your will? ..... YES
NO

6. Were you ever forced or coerced to kiss someone in a sexual rather than an affectionate way? ..... YES
NO

If you responded "YES" for any of the above events, answer the following for the one that has had the greatest impact on your life. In answering consider how you felt at the time of the event.

1. Did you experience emotions of intense fear, horror or helplessness?..... YES NO
2. Did you feel out-of-your-body or as if you were in a dream? .....YES NO

Source; Bremner, J. D., Bolus, R., & Mayer, E. A. (2007). Psychometric properties of the early trauma inventory–self report. *The Journal of nervous and mental disease*, *195*(3), 211.

Appendix F: Debriefing

Study Title: A Biological and Psychosocial Characterization of Mental Health Symptoms

What are we trying to learn in this research?
In this study we are evaluating the effects of one's early life experiences (e.g., stressors, trauma) and the quality of our social relationships/connections with others on mental health outcomes (e.g., depression, anxiety symptoms and problematic substance use). Recently, there have been efforts made to better understand the association between our social connections and depressive and anxiety symptoms. We asked you to provide blood samples in order to assess how these variables influence cortisol, cytokines (proteins related to immune system functioning) and epigenetic factors (i.e. tags on your DNA that show how environmental experiences shape the way our genes express). These biological factors have been linked to depression, however, not all individuals who have depression exhibit these markers and it is thought that this might be due to the heterogeneity of those with depression and the high comorbidity with other disorders such as anxiety. Therefore, by linking different biological and psychosocial factors to distinct depressive and anxiety symptoms, we can gain a better understanding of theses disorders.

Moreover, not all individuals respond to stressful life events in the same way. Some people react strongly, whereas others do not. Research has focused on important genetic factors, which may underlie increased risk to develop depression and anxiety related symptoms. These genetic factors include small deviations of certain genes that control the functioning of neurochemical systems in the brain. The slight deviations are called polymorphisms, and are fairly common. We are assessing whether adverse life experiences affect people differently depending on the presence of different polymorphisms. You were asked to provide a saliva sample, genetic material (e.g., DNA) can be extracted from this saliva sample. Therefore we were interested in examining the relationships between your responses on some of the questionnaires (e.g., experiences of trauma, depressive and anxiety symptoms) with certain genes. These polymorphisms are different from the epigenetic ‘tags’ on your DNA, but both are important factors when understanding stress responses and mental health.

Why is this research important? By understanding the genetic, hormonal and psychosocial factors that lead to different mood outcomes, we will gain a better understanding of how mood changes develop. This research will help to develop improved approaches to both treat, and prevent mental health concerns in the future.

We hope that being a participant in this research has proven to be a worthwhile learning experience, and that you find some benefit in being a participant in this study. Thank you very much for your willingness to participate.

Where can I learn more?
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3721019/
https://www.ncbi.nlm.nih.gov/pubmed/28235397

https://www.ncbi.nlm.nih.gov/pubmed/29684053
What if I have questions later?

Please contact:

Sabina Franklyn, Department of Psychology, Carleton University
Email: SabinaFranklyn@cmail.carleton.ca

Dr. Robyn McQuaid, The Royal's Institute of Mental Health Research
Phone: 613 722-6521 ext. 6490, Email: Robyn.McQuaid@theroyal.ca

Dr. Kim Matheson, Department of Neuroscience, Carleton University
Phone: 613 520-2600 ext. 2652, Email: kim.matheson@carleton.ca

This ethics protocol for this project has been cleared by Carleton University Research Ethics Board-B (CUREB-B Clearance #109133).

If you have any ethical concerns with the study, please contact Dr. Bernadette Campbell, Chair, Carleton University Research Ethics Board-B (by phone: 613-520-2600 ext. 4085 or by email: ethics@carleton.ca).

Is there anything that I can do if I found this experiment to be emotionally draining?
Thank you very much for your participation in this study. If you have experienced any distress while completing these measures, please consult the resources below:

Carleton University Health and Counseling Services: 613-520-6674
Distress Centre Ottawa and Region: (613) 238-3311, Web Site: www.dcottawa.on.ca/.
Mental Health Crisis Line: within Ottawa (613) 722-6914, Web Site: http://www.crisisline.ca/

Thank you for participating in this research!