EXPLORING THE PATHOPHYSIOLOGY OF CHRONIC DEPRESSION: THE INTERPLAY BETWEEN DEPRESSION, CORTISOL RESPONSES, AND PERSONALITY

By

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ABSTRACT

Chronic major depressive disorder (CMDD) is a common and debilitating illness. Its pathophysiology needs further elucidation, before more effective targeted treatments can be developed for this condition. To gain a better understanding of the psychobiology of CMDD, three interconnected studies were conducted that examined the interplay between chronic depression, cortisol responses, and personality.

Study 1 examined cortisol responses to the Trier Social Stress Test (TSST) in CMDD participants (n=29) as compared to healthy controls (n=28). It was hypothesized that cortisol responses would be greater in the CMDD population. Results indicated that females with CMDD had increased cortisol output compared to female controls, a pattern consistent with the hypothesis. However, males with CMDD had decreased cortisol responses compared to male controls. These results suggest that cortisol responses to social stress are altered in those with CMDD; however, females and males experience fundamentally different changes.
Study 2 examined moderating effects of personality on cortisol responses to the TSST in those with CMDD (n=51) as compared to healthy controls (n=57). It was hypothesized that higher neuroticism and/or lower extraversion would be associated with increased cortisol responses in CMDD participants. As hypothesized, lower extraversion was associated with increased cortisol reactivity in those with CMDD but not in healthy controls. However, no association was found between neuroticism and cortisol responses. These findings could support the notion that lower extraversion is a vulnerability marker for chronic depression and thus a possible target for treatment.

Study 3, evaluated the cortisol awakening response (CAR) in CMDD participants (n=27) compared to healthy controls (n=30). It was hypothesized that such awakening responses would be more pronounced in the depressed population compared to controls. Contrary to expectation, no differences were found between the groups. However, lower extraversion was associated with a lower CAR in both CMDD and healthy controls, a finding that was not anticipated a priori.

These interconnected studies suggest that examining relationships between depression, cortisol responses, and personality, can assist with identifying distinct psychobiological profiles in those with chronic depression. It is proposed that this strategy will improve the likelihood of developing more targeted treatments for this population.
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<td>ACTH</td>
<td>adrenocorticotropic releasing hormone</td>
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<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>AUCg</td>
<td>area under the curve with respect to ground</td>
</tr>
<tr>
<td>AUCi</td>
<td>area under the curve with respect to increase</td>
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<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CAMH</td>
<td>Centre for Addiction and Mental Health</td>
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<td>CAR</td>
<td>cortisol awakening response</td>
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<td>CBASP</td>
<td>Cognitive Behavioral Analysis System of Psychotherapy</td>
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<td>CBG</td>
<td>corticosteroid-binding globulin</td>
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<td>CBT</td>
<td>cognitive behavioural therapy</td>
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<td>CMDD</td>
<td>chronic major depressive disorder</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>GR</td>
<td>glucocorticoid receptor</td>
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<td>CRF</td>
<td>corticotropin-releasing factor</td>
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<td>CRH</td>
<td>corticotropin-releasing hormone</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>DD</td>
<td>double depression</td>
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<td>Dex</td>
<td>dexamethasone</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision</td>
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<td>DST</td>
<td>dexamethasone suppression test</td>
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<td>ECT</td>
<td>electroconvulsive therapy</td>
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<td>FFM</td>
<td>Five-Factor Model</td>
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<td>HDRS</td>
<td>Hamilton Depression Rating Scale</td>
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<td>HPA</td>
<td>hypothalamic pituitary adrenal</td>
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<td>IPT</td>
<td>interpersonal therapy</td>
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<td>MDD</td>
<td>major depressive disorder</td>
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<td>MDE</td>
<td>major depressive episode</td>
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<td>MAOI</td>
<td>monoamine oxidase inhibitor</td>
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<td>MR</td>
<td>mineralocorticoid receptor</td>
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<td>NEO PI-R</td>
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<td>NIMH</td>
<td>National Institute for Mental Health</td>
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<td>NOS</td>
<td>not otherwise specified</td>
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<td>PTSD</td>
<td>post-traumatic stress disorder</td>
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<td>PVN</td>
<td>paraventricular nucleus</td>
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<td>RANOVA</td>
<td>repeated measures analysis of variance</td>
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<td>RIA</td>
<td>radioimmunoassay</td>
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<td>SCID-I/P</td>
<td>Structure Clinical Interview for DSM-IV-TR Axis I Disorders</td>
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<td>SSRIs</td>
<td>selective serotonin reuptake inhibitors</td>
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<td>SNRIs</td>
<td>serotonin-norepinephrine reuptake inhibitors</td>
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<td>STAR*D</td>
<td>Sequenced Treatment Alternatives to Relieve Depression</td>
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TCA: tricyclic antidepressant
TMS: transcranial magnetic stimulation
TSST: Trier Social Stress Test
CHAPTER 1

INTRODUCTION
1.1. Overview of Major Depressive Disorder (MDD)

1.1.1. Definition, Epidemiology, and Economic Costs

Depression affects more than 121 million people worldwide, and is among the leading causes of disability in the world (www.who.int). Strikingly, the World Health Organization predicts that by the year 2030, depression will become the leading cause of ill health globally, overtaking even heart disease (Lepine and Briley, 2011).

The diagnosis of depression, as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000), requires that a person experience two weeks of clinically significant dysphoria or anhedonia and five of the following nine symptoms:

1) depressed mood most of the day
2) marked diminished interest or pleasure
3) significant changes in appetite (either increased or decreased)
4) insomnia or hypersomnia
5) psychomotor agitation or retardation
6) fatigue
7) feelings of worthlessness or excessive guilt
8) diminished concentration
9) suicidal ideation
These symptoms result in functional impairment that cannot be explained by another psychiatric or medical illness.

About 20 percent of the population will develop depression at some point in their lives (Lewinsohn et al., 1991, Kessler et al., 1994). Twice as many women as men suffer from depression (Kessler et al., 2003). There are enormous economic costs associated with depressive illness. Greenberg et al. (2003) estimated the economic costs of depression to be $81.1 billion in 2000. The World Health Organization Global Burden of Disease Study ranked depression as the single most burdensome illness in terms of total disability-adjusted life years among middle-aged people (Murray and Lopez, 1996).

The economic costs associated with depressive illness are due to a combination of factors including its associated high lifetime prevalence, early age of onset (median age of onset is in the mid-twenties), high chronicity, and high functional impairment. The Collaborative Depression Study, a large naturalistic study evaluating the course of depression, revealed that the rate of depressive recurrence is 25 to 40 percent in the first two years but increases to 60 percent at five years (Lavori et al., 1994). Angst (1992) found that 75 percent of individuals who had a history of depression had a recurrence in a ten-year follow up study. About 20 percent of those who develop depression will have a chronic course of the illness (Keller and Hanks, 1994, Gilmer et al., 2005).

There is high rate of mortality associated with depression. The estimated lifetime risk of suicide has varied across studies, but has been found to be as high as 15 percent in more severely depressed patients. Studies suggest that more men commit suicide than women (CDC, 2007), but more women attempt suicide than men (Brockington, 2001).
The current project will focus on chronic depression, the subgroup of depressed individuals with the highest rates of morbidity and mortality.

1.1.2. Pathophysiology

**Biological Factors**

A number of biological mechanisms have been associated with depressive illness (for review, see Thase, 2009). Twin studies suggest that genes account for about 40 to 50 percent of the risk of depression in the population (reviewed in Sullivan et al., 2000). However, the genetic underpinnings of depression remain largely unknown. One landmark study by Caspi and colleagues (2003) reported that individuals with one or two copies of the short allele of the serotonin transporter gene promoter polymorphism are more susceptible to developing depression after major life events. It is likely that a number of genes interact to increase an individual’s susceptibility to depression (Moldin et al., 1991). Furthermore, the genetic effect on depression risk is probably also related to specific gene-environment interactions (Kendler et al., 2004).

Altered monoamine function (in particular serotonin and norepinephrine), has been one of the most significant neurobiological findings in depression research. It is believed that the therapeutic efficacy of many of the current antidepressant medications is via their effects on serotonin and norepinephrine neurons (Duman et al., 1997, Nemeroff, 1998). There is also a well-established link between the stress hormone cortisol and depressive illness. This biological pathway is the primary focus of this project, and will be discussed in depth in this thesis. Other biological abnormalities found to characterize depression
include REM sleep dysfunction (Thase, 2006) and EEG rhythm abnormalities (Davidson, 1998). Brain imaging has led to important insights into our understanding of the pathophysiology of depression. Structural imaging studies have found smaller volumes in some brain areas including the hippocampus (Sheline et al., 1996, Sheline, 2000, Bremner et al., 2000). Functional imaging studies have suggested different patterns of brain activation in depressive illness. The amygdala, prefrontal cortex, and anterior cingulate gyrus play significant roles in causing emotional dysregulation that characterizes depressive illness (Drevets et al., 1997, Strakowski et al., 1999, Pizzagalli et al., 2003).

**Psychological and Social Factors**

Aaron Beck’s (1976) cognitive theory of depression proposed that negative cognitive schemas were a psychological mechanism underlying depression. In general, individuals who are depressed have more negative views of themselves, the future, and the world around them (Ingram et al., 1998). There is evidence that negative cognitions are not just a state of depression, but also promote vulnerability to future depression. In a longitudinal study by Alloy et al. (2006), non-depressed individuals who had higher dysfunctional attitudes and negative attribution styles were more likely to experience depression. Negative cognitions also increase the risk of relapse in depression. In a study by Segal et al. (2006), remitted depressed individuals with greater levels of dysfunctional cognitions in response to a sad mood provocation were more likely to have a recurrence. One of the most effective treatments for depression is cognitive behavioural therapy, a
treatment that focuses on correcting negative cognitive appraisals that influence one’s behaviour and emotions (Beck, 1976).

The social environment also plays a key role in understanding mechanisms underlying depression. Early adverse experiences, such as early parental loss, are associated with an increased risk for developing depression (Cerel et al., 2006). Childhood abuse is also a risk factor for depression, and may promote the risk of depression through changes in the HPA axis (Heim et al., 2000). As well, inadequate parenting (e.g., neglectful, harsh, and inconsistent) can lead to insecure attachment relationships which can promote an elevated risk for depressive illness (Cummings and Cicchetti, 1990).

Major life stressors are associated with more severe forms of depression (Monroe et al., 2001). Low socioeconomic status has been associated with an increased risk of developing depression and is also associated with a more persistent depressive illness (Lorant et al., 2003).

In summary, there are a number of biological, psychological, and social pathways leading to depressive illness. One common thread appears to be stress, as mechanisms underlying depression are linked to stressful life events or are related to how an individual responds and/or copes with stress. In the current study, examination of the interplay between depression, cortisol responses (to social stress), and personality is in keeping with the biopsychosocial model of depression.
1.1.3. Treatment

Given the complexity of the etiological pathways for depression, not surprisingly, there are many avenues of treatment that are currently being used, including biological and psychosocial remedies.

Biological therapies

Over the past several decades there has been a dramatic increase in the number of antidepressant medications available for use. More than two dozen pharmacological agents are available to treat depression (Mignon and Stahl, 2010). The most commonly used drugs are selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) which have a more favourable side effect profile than older antidepressants, such as tricyclic antidepressants (TCAs) and monamine oxidase inhibitors (MAOIs). All commonly used antidepressants work on enhancing monoaminergic function and are similar in efficacy. Currently, there are no biological markers to aid in the choice of antidepressant medication. Side effect profile, family history of antidepressant response, cost, and physician preferences are factors that may be taken into account when choosing a specific antidepressant agent.

In randomized controlled studies, about 60 percent of individuals will respond to an antidepressant agent as compared to placebo (Klein et al., 1980, Gartlehner et al., 2011). Antidepressant medication response is often delayed by two or more weeks, with continuing improvement experienced over subsequent weeks (Posternak and Zimmerman, 2005). In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, the largest naturalistic treatment study of depression to date, many
patients did not achieve full remission until eight to fourteen weeks of active medication treatment (Trivedi et al., 2006).

Several brain stimulation therapies are now used in the treatment of depression. Electroconvulsive therapy (ECT) is a well-established and highly effective treatment (Husain et al., 2004, Petrides et al., 2011), but is generally reserved for more treatment-resistant cases. ECT is a procedure in which an electrical current is passed through the brain to induce a brief seizure. The mechanism of action of ECT remains unknown. Other more novel brain stimulation treatments for depression include repetitive transcranial magnetic stimulation (TMS), vagal nerve stimulation, and deep brain stimulation (Cusin and Dougherty, 2012).

**Psychotherapy**

Research has demonstrated efficacy of several forms of therapy for acute depression including individual, group, and marital therapy (Dobson, 1989, Leff et al., 2000, Huntley et al., 2012). Within these forms of therapy, many psychotherapeutic approaches can be taken. The two most empirically studied approaches are cognitive behavioural therapy (CBT) and interpersonal therapy (IPT). Randomized controlled trials have found that these are effective treatments for mild to moderate forms of depression (Dobson, 1989, Weissman, 1994, Parikh et al., 2009). In CBT, the therapist addresses negative cognitions and maladaptive thinking that promote and/or maintain depressive illness. IPT focuses on assisting patients with coping strategies to improve feelings, thoughts, and behaviours that occur in difficult interpersonal relationships. In mild to moderate depression, there is evidence that psychotherapy can be as effective as medication in
some individuals, although a combination of the two is generally accepted as the gold standard.

In summary, both medication and psychotherapy are effective treatment approaches for depressive illness. However, up to one third of patients will not respond to treatment and will have a chronic course of illness. At present, it is unknown what mechanism underlies lack of response and chronicity of depressive illness. One of the primary goals of the current project was to improve the understanding of the pathophysiology of CMDD.
1.2. Chronic Major Depressive Disorder (CMDD)

1.2.1. Definition and Epidemiology

The presence of chronic forms of depression has been noted for many decades (Kraepelin, 1921, Robins and Guze, 1970), although specific research in this population did not occur until the 1970’s and 1980’s (Weissman and Klerman, 1977, Akiskal et al., 1980, Keller and Shapiro, 1982). It was not until the DSM–III-R (American Psychiatric Association, 1987) that chronic depression was distinguished as a category. In the current version of this text, the DSM-IV-TR, (American Psychiatric Association, 2000), CMDD is defined as a major depressive episode (MDE) that persists for a minimum of two years. In the general population, it is estimated that the lifetime prevalence of chronic depression is over 6 percent (Kessler et al., 1994). It occurs in up to 20 percent of depressed individuals (Keller and Hanks, 1994, Gilmer et al., 2005), but constitutes the most common presentation of major depressive disorders (MDDs) in many specialized clinical settings including at the Centre for Addiction and Mental Health (CAMH) in Toronto, Canada.

1.2.2. Clinical Features and Morbidity

While some aspects of CMDD naturally overlap with those of acute depression, several clinical features distinguish these two groups. Compared to acute depression, CMDD is associated with greater comorbidity, particularly with anxiety, substance abuse, and personality disorders (Keller et al., 1998, Russell et al., 2003). In a large multicentre treatment study of chronic depression, almost a quarter of subjects had a lifetime history
of an anxiety disorder and over a third reported a lifetime history of a substance abuse or dependence disorder (Keller et al., 1998). Furthermore, over half of the patients in this study were diagnosed with a personality disorder, with avoidant (25.3 percent) and obsessive-compulsive (18.1 percent) personalities being the most frequently diagnosed. It should be noted that these high rates of comorbidity occurred despite strict exclusion criteria, and therefore underestimate the actual rates of comorbidity in naturalistic chronically depressed populations. There is controversy regarding the high rates of comorbidity, with many believing that the comorbidity observed in depression is merely an artifact of the current DSM diagnostic system which uses a categorical approach to define distinct disorders. Whether comorbidities between illnesses, such as depression and anxiety, are variants of a single disorder or represent qualitatively different disorders still remains to be determined.

In addition to seeing high rates of personality disorders among those suffering from CMDD, more recent studies focusing on dimensional measures of personality have found higher levels of neuroticism and lower levels of extraversion in CMDD individuals compared to those with acute depression (Wiersma et al., 2011, Klein et al., 2011). The association between CMDD, cortisol responses, and both neuroticism and extraversion forms an integral part of this thesis (see Chapter 4). Other features differentiating CMDD from acute depression include increased rates of attempted suicide (Gilmer et al., 2005), higher rates of childhood adversity, (Brown and Moran, 1994, Zlotnick et al., 1997, Riso et al., 2002), and more of the “atypical” symptoms of depression (e.g., over-eating, hypersomnia, leaden paralysis, and rejection sensitivity) (Horwath et al., 1992, Stewart et al., 1993, Matza et al., 2003).
Treatment response also differs in those with CMDD and acute depression in that CMDD is associated with poorer treatment outcomes with conventional antidepressant use (Kocsis et al., 1988, Akiskal et al., 1997, Riso et al., 2002, McGrath et al., 2006). Moreover, those with CMDD exhibit lower placebo response rates in comparison to those with acute depression (Khan et al., 1991, Brown et al., 1992) which is suggestive of a greater severity of illness.

The first large investigation of treatment response in CMDD was a double blind, randomized, multicentre study that evaluated treatment response to sertraline versus imipramine in 635 outpatients with chronic major depression and/or double depression (major depressive episode superimposed on dysthymic disorder) (Keller et al., 1998). Key findings included gender differences in both the response and tolerability of SSRIs versus tricyclic antidepressants. Premenopausal females had a significantly greater response to sertraline and were less likely to withdraw from sertraline than imipramine in comparison to males. In contrast, males on imipramine responded better and were less likely to drop out of the study than those on sertraline. There were no differences in treatment response between postmenopausal females and older males. When interpreting these findings, the authors suggested that the serotonergic potency of sertraline might be an important factor in the treatment of chronic depression in young females. Other studies, but not all, have also found gender differences in treatment response among those with chronic forms of depression. A study of subjects with atypical depression and associated panic attacks, a group known to have high rates of chronicity, found that females had a greater response to MAOIs, whereas males had a greater response to TCAs (Davidson and Pelton, 1986). In a study by Haykal and Akiskal (1999), females with
dysthymia had a greater response to fluoxetine compared to males. Taken as a whole, it is clear that it is important to consider sex differences in the study of CMDD. Whether differences in treatment response reflect differences in the underlying biology of chronic depression in males versus females is an intriguing question.

A specialized form of psychotherapy, Cognitive Behavioral Analysis System of Psychotherapy (CBASP), was developed to treat chronic forms of depression. This type of therapy aims to assist patients with interpersonal, cognitive-emotional, and maturational deficits that stem from early maltreatment by focusing on the therapeutic relationship. In a study by McCullough et al. (2000), CBASP in combination with antidepressant treatment was more effective than medication or therapy alone in chronic depression. In this same study population, CBASP was found to be particularly effective with or without additional medication in the subgroup of chronic depressives with early childhood adversity (Nemeroff et al., 2003). However, subsequent studies have questioned whether CBASP augmentation is more effective than antidepressant treatment alone and whether CBASP is more effective than other psychotherapies (Kocsis et al., 2009, Schramm et al., 2011).

Individual and Societal Costs

CMDD has a major impact on psychosocial functioning and is associated with significant individual and societal costs (Miller et al., 1998, Howland, 1993, Riso et al., 2002). CMDD is associated with significant impairments in numerous psychosocial domains including relationships, family functioning, and work performance (Miller et al., 2004). Greenberg et al. (2003) estimated the economic cost of depression in 2000 in the
United States to be $81.1 billion. As would be expected, chronic forms of depression are associated with significantly greater economic costs than acute depression (Greenberg et al., 2004). Several factors that contribute to this finding include greater impairments in work performance, increased health care utilization, and more frequent hospitalizations. In a study by Keller et al. (1998), individuals with chronic depression had a rate of unemployment of 20.6 percent which was considerably higher than the national average of 5 to 6 percent at the time of their study. Among the patients that were employed, about a third had been in a job that was below their educational level and training. In the recent STAR*D study, those with chronic depression had less education, lower income, higher rates of unemployment, and a higher burden of medical illness than non-chronically depressed individuals (Gilmer et al., 2005).

Whether psychosocial impairments associated with chronic depression are antecedents to or the result of the illness is a difficult question. Impairments in one or more psychosocial domains could be a risk factor for depression or could perpetuate the illness. Furthermore, as depressive illness does impact overall functioning, it would seem logical that the longer the depressive episode, the greater the impact on psychosocial functioning. It has been speculated that psychosocial impairments are, in fact, both antecedents to and the result of chronic depressive illness, although few studies have examined this directly (Miller et al., 2004).

1.2.3. CMDD versus Other Forms of Chronic Depression

There has been much debate over what constitutes chronic depression, with several studies attempting to subcategorize this illness. As stated above, CMDD is defined as a
MDE that has continued for at least two years. Dysthymic disorder has been identified as a milder form of chronic depression lasting two years. Double depression has been defined as a major depressive episode (MDE) superimposed on dysthymic disorder. MDE, recurrent, without full inter-episode recovery, is yet another putative subtype.

Determining whether these diagnostic distinctions are meaningful for chronic forms of depression was the focus of two large out-patient studies by McCullough et al. (2000, 2003). In these studies, various forms of chronic depression including CMDD, double depression, recurrent depression without inter-episode recovery, and CMDD superimposed on antecedent dysthymic disorder were compared for symptomatology, clinical characteristics, family history, natural course, and treatment outcome. Interestingly, few differences between the chronically depressed groups were found, echoing results of previous studies in this area (McCullough et al., 1994, Donaldson et al., 1997). The authors concluded that the similarities between various forms of chronic depression outweighed the differences, thereby supporting a broad category of chronic depression rather than distinguishing between its multiple forms. If so, the findings from this project, which focuses on CMDD, are likely to be generalized to other forms of chronic depression.

1.2.4. Etiology and Pathophysiology

To date, there have been few prospective studies examining the antecedents of chronic depression, as most of the information known about chronic depression has been based on cross-sectional studies.
The most comprehensive review of the etiology and pathophysiology of chronic depression was by Riso et al. (2002). The goal of this review was to improve preventative strategies and management of chronic forms of depression through a greater understanding of their underlying causes. Riso et al. examined several areas including developmental factors, personality, psychosocial stressors, comorbid disorders, biological factors, and cognitive variables. Of all factors studied, childhood adversity, personality features associated with increased stress reactivity (e.g., high neuroticism), and chronic environmental stress were suggested as possible determinants of chronic depression. Although several important findings emerged from this study, three are most relevant to this thesis, namely the following: 1) there was some evidence that the hypothalamic-pituitary-adrenal (HPA) axis and immunological function are altered in dysthymia; 2) neuroticism was strongly associated with chronic depression in both cross-sectional and prospective research; and 3) there was a limited understanding of the pathophysiology of CMDD because of the dearth of research in this area. Despite this observation, only a small number of biological studies on CMDD have been undertaken since the Riso et al. report. As will be discussed below, some of these studies suggest that HPA activity may be different in CMDD as compared to acute forms of depression. These various findings provide a rationale to examine the interplay between CMDD, cortisol responses, and personality in this thesis.
1.3. Hypothalamic-Pituitary-Adrenal (HPA) Function and Depression

1.3.1. Introduction

This section provides an overview of cortisol responses and the HPA axis, and its association with various forms of depression.

**Definition of Stress**

There are a number of definitions of stress and the term “stress” can have different meanings for different people depending on the context. One of the pioneers in the field of stress, Hans Selye, sometimes referred to as the “father of stress,” defined stress in a generic fashion: a non-specific response of the body to any demand (Selye, 1956). This sparked controversy, as later researchers determined that characteristics of a stressor (namely cognitive, psychological, and biological variants), were shown to impact an individual’s unique response to stress. Others define stress as a behavioural response to the perception of threat that results in anxiety and tension. Sociologists might define stress as the result of social disequilibrium. To engineers, stress is simply the effect of an external force on a material, causing strain. Biologists might explain stress in purely neuroendocrinological terms, as any stimulus that provokes the release of adrenocorticotropic releasing hormone (ACTH) and adrenal glucocorticoids. Finally, many believe that there is no one overarching definition of stress that is sufficiently comprehensive in meaning, though most would agree that it is a universally recognized
condition that causes problems in human life deserving consideration from many vantage points. This project focuses on the psychoneuroendocrinology of stress.

**The HPA Axis**

The HPA axis is an integral part of the neuroendocrine system that controls one’s physiological reactions to stress. This axis also regulates many body processes including digestion, the immune system, energy storage and expenditure, and mood. Focusing on stress reactivity, the HPA axis mediates cortisol release as follows: when an individual experiences stress, either biological or psychological, the HPA axis is activated. Stress triggers the release of corticotropin-releasing hormone (CRH) from the hypothalamus and extra-hypothalamic regions. CRH is synthesized in the hypothalamus, primarily in the parvo cellular neurons of the paraventricular nucleus (PVN). CRH-containing neurons within the hypothalamus project to the median eminence which is the bridge between the hypothalamus and the anterior pituitary. CRH then acts on specific receptors on the pituitary to stimulate the release of ACTH which, in turn, stimulates the adrenal gland to release cortisol (see Figure 1 below).
Figure 1: The Hypothalamic-Pituitary-Adrenal Axis. Stress results in the release of CRH from the hypothalamus. This triggers the release of ACTH from the pituitary which then stimulates the release of cortisol from the adrenal gland. Cortisol has negative feedback effects at the level of the pituitary and hypothalamus (image online, available at www.montana.edu/wwwai/imsd/alcohol/Vanessa/vwhpa.htm, accessed November 12, 2012).
As shown above, the HPA axis is regulated by a cortisol-mediated negative feedback mechanism which controls its activity during the course of the day and during periods of stress. Cortisol acts on mineralocorticoid (MR) and glucocorticoid (GR) receptors. The MR is a high affinity receptor abundant in limbic areas of the brain. The GR differs from the MR in that it is a low affinity receptor which is widespread in the brain and is distributed throughout the body. There is a particularly high concentration of GRs in the hippocampus and hypothalamus. The GR is generally believed to be the central agent involved in the regulation of cortisol and is particularly important in the regulation of the response to stress when levels of glucocorticoids are high, such as in acute depression. However, more recent work has also lent support to the role of MRs in the response to stress. In fact, it may be that the synergy of both receptors may be important in mediating glucocorticoid negative feedback (Pariante and Lightman, 2008).

**Measurement of Cortisol**

Cortisol represents the most commonly measured stress hormone, and levels can be obtained through salivary or plasma sampling. About 90 to 95 percent of cortisol is bound to cortisosteroid-binding globulin (CBG) and other carrier molecules (e.g., albumin). Only free cortisol (unbound) penetrates cell membranes and activates MR and GR receptors and it is therefore believed to be the biological active form of cortisol. Salivary cortisol is a measure of unbound cortisol, whereas plasma measurements include both unbound and bound cortisol. Despite this difference, studies have shown a high correlation between plasma and salivary cortisol levels (Kirschbaum and Hellhammer, 1989). In the current study, salivary samples were used to measure cortisol, both because
saliva provides a measure of free (or biologically active) cortisol, and because it is less invasive to collect study participants’ saliva than to obtain their plasma samples.

1.3.2. The HPA Axis and Depression

Initial studies of HPA dysfunction in acute depression were published in the 1960s. A landmark study by Carroll and his colleagues (1976) reported non-suppression to the dexamethasone suppression test (DST) in about 50 percent of depressed individuals. In addition, elevated cortisol levels were observed in individuals with depression. These findings were subsequently replicated (reviewed by Carroll, 1982) and suggested dysregulation of cortisol feedback mechanisms in depression resulting in higher levels of cortisol secretion.

Later studies suggested that the abnormalities in the HPA axis seen in depression likely stem from central brain regions and are related to hypersecretion of CRH (Nemeroff et al., 1984, Nemeroff, 1988). Supporting this theory were the following findings: 1) the cerebrospinal fluid (CSF) of depressed individuals had higher levels of corticotropin releasing factor (CRF) peptide; 2) ACTH release was blunted in depressed individuals following exogenous systemic administration of CRF (Gold et al., 1984, Holsboer et al., 1984a, Holsboer et al., 1984b); 3) in post-mortem studies, CRF receptor-binding sites were significantly decreased in suicide victims suggesting that elevated levels of CRF resulted in downregulation of CRF receptors (Nemeroff, 1988, Merali et al., 2004); and 4) central administration of CRF and over-expression of CRF in transgenic mice led to symptoms consistent with a depressive syndrome (Britton et al.,
Overall, the above findings point to an overactive stress system in depression.

Implications of HPA Activity in Individuals with Depression

The link between an overactive stress system and depressive illness has significant medical implications, as prolonged exposure to glucocorticoids has been linked to various adverse medical outcomes. Past studies have suggested an association between long-term exposure to glucocorticoids and atrophy of the hippocampus, which could result in impaired memory function and a reduced ability of the body to appropriately respond to stress (MacQueen and Frodl, 2011). Higher glucocorticoid levels also contribute to an increased allostatic load, thus promoting risk to various illness processes including cardiovascular disease (Seeman et al., 2001).

The finding of altered HPA activity in depression also presents an opportunity to create novel antidepressants that target this biological pathway. There has been significant research and funding geared towards developing such medications that downregulate the HPA axis, such as CRH antagonists (Zoumakis et al., 2006). In fact, there is evidence that one CRH antagonist in particular, mifepristone, is effective in treating psychotic forms of depression (Flores et al., 2006, Blasey et al., 2011).

Challenges in the Field

Despite all of the positive implications noted above, there are several challenges that remain. First, whether HPA dysfunction represents a state effect of depression, is evident prior to depression, or is a later consequence of ongoing depressive illness continues to
be a point of controversy (Pariante and Lightman, 2008). Second, although there was initial promise that the DST may be a biological marker of depression, only about half of those with depression show this abnormality. It is possible that the DST should be reserved for certain subtypes of depression, for example melancholic or psychotic forms, which are more likely to show this abnormality (Stetler and Miller, 2011). This raises important questions about whether other measures of HPA activity (for example the CAR and cortisol responses to the TSST as used in this study) could be useful biological markers for other subtypes of depression (e.g., chronic depression).

1.3.3. The HPA Axis and Chronic Depression

The few studies conducted in this area suggest that HPA function may be distinct in individuals with CMDD as compared to those with acute MDD. Watson et al. (2002) were one of the first to conduct a study that specifically focused on HPA function in CMDD. In this study, twenty-nine individuals with CMDD and twenty-eight matched controls were administered both the DST and the Dex/CRH (the combined dexamethasone/CRH) challenge. Results revealed no significant differences in either the DST or Dex/CRH responses in those with CMDD as compared to the control group. In addition, illness characteristics and demographic variables did not predict baseline levels or response to challenge tests in CMDD. The majority of those with CMDD in this study were on psychotropic medications, although the medication regime did not predict cortisol response. Overall, this study did not show alterations in HPA activity in those with CMDD as compared to healthy controls, which contrasts previous studies of acute depression that point to heightened cortisol reactivity and decreased cortisol-mediated
feedback. In an effort to understand the unexpected negative findings, the authors speculated on the following possibilities: 1) normalization of the HPA axis in CMDD may be explained by chronic antidepressant use; 2) HPA patterns of activity change over time during the course of a depressive illness; and 3) normal HPA function in depression could be a vulnerability marker that predicts chronicity.

Other studies support the notion that HPA function may be different in chronic versus acute forms of depression. For example, chronic depression was associated with low basal cortisol levels in a geriatric population (Oldehinkel et al., 2001), a finding that is opposite to what is seen in acute depression. In another study, O’Keane et al., (2005) found increased ACTH responses to the CRH challenge test in CMDD, in contrast to studies on acute depression that have generally reported decreased ACTH responses to this challenge. Taken as a whole, studies on chronic depression point to either blunted or normal HPA activity in marked contrast to findings in acute melancholic depression (Nemeroff et al., 1984). Whether these findings reflect basic etiological differences in HPA activity in chronic and acute depression or possibly a normalization of the HPA axis in the transition from acute to chronic depression is unknown as of yet.
1.4. Social Stress Paradigms as a Method to Examine Cortisol Responses in Depression

1.4.1. Overview

Social stress paradigms may improve our understanding of stress reactivity and cortisol output by tapping into higher order emotional and cognitive processes that input into the HPA system. Thus, social stressors may provide a more naturalistic assessment of how individuals react to day-to-day stressors. Understanding the influence of cognitive processes on HPA function may be particularly important in chronic depression, as negative cognitive appraisals have been linked to both the etiology and maintenance of depressive episodes (Scher et al., 2005). Furthermore, animal studies suggest that physiological challenges may not elicit the same responses from limbic areas of the brain as do psychological stressors (Herman and Cullinan, 1997).

Hans Selye (1956), a pioneer in demonstrating critical links between social stress, physiology, and health, hypothesized that the characteristics of external stressors, either physical or psychological, were of little importance in determining the physiological stress response. However, recent reviews suggest that specific characteristics of a given stressor can, in fact, influence cortisol output. In a key paper, Dickerson and Kemeny (2004) reviewed 208 social stress studies and concluded that social stressors induce a significant cortisol stress response with an overall effect size of about .31. The authors also found that the effect size was greater with certain defined stressors, such as public speaking and cognitive tasks. Based on their review, Dickerson and Kemeny further concluded *that stressors encompassing both social evaluation and uncontrollability*
induced the greatest cortisol stress response. The TSST (Kirschbaum et al., 1993) includes both of these elements, and many consider it to be the gold standard of social stress paradigms.

1.4.2. The Trier Social Stress Test (TSST)

The TSST is the primary method used in this project to examine cortisol responses in CMDD. This paradigm includes a five-minute public speaking task followed by a five-minute mathematical challenge done in front of a live evaluating committee. As shown below in Figure 2, there are various props, such as a video camera and microphone, to further add to the stressful nature of this paradigm. TSST studies have been conducted in both healthy and clinical populations.
Figure 2: Trier Social Stress Test. This paradigm consists of a five-minute public speaking task followed by a five-minute mathematical challenge done in front of a live evaluating committee. After a preparatory phase, participants are led into a room that mirrors a standard interview room with various props including a video camera, microphone, and stand, and an “expert committee” of three people dressed in lab coats sitting behind a table. Participants are told they have ten minutes to prepare a five-minute speech for the expert committee as if they are at a job interview, after which the committee would assign them a second task, which will also be five minutes in duration. The participants prepare their speeches in a separate room. After participants complete the public speaking task, they complete a difficult mathematical problem that consists of serially subtracting 13 from 1022. Once the stress challenge is completed, there is a recovery phase in a separate room.
A number of demographic, psychosocial, and clinical factors contribute to individual differences in cortisol responses to the TSST. As reviewed by Kudielka and colleagues (2009), one of the most important variables to consider is sex differences. In healthy populations, males have more robust cortisol responses to the TSST than do females. While reasons for this are unknown, the type of social stressor may be one important factor accounting for sex differences. For example, a study by Stroud and colleagues (2002) found that males have a more robust cortisol response to achievement stressors, whereas females have a greater cortisol response to interpersonal stressors. This difference in response may reflect unique social roles of males and females.

Given the importance of sex differences in cortisol responses to social stress, this variable will be controlled for in the statistical analyses in Studies 1 and 2. Other demographic variables that may be relevant to cortisol responses to social stress include age, body mass index, and smoking status, all of which will also be examined in this project.

Psychosocial factors including personality and childhood adversity have also been associated with altered cortisol responses to the TSST. Whether or not personality dimensions of extraversion and neuroticism are associated with cortisol responses in CMDD is a major focus on this project and will be reviewed in detail in Chapter 4.

Biological factors, such as medication (e.g., steroid and psychotropic medications), and genetic factors have been found to contribute to individual differences in cortisol responses to social stress. Twin studies and candidate gene studies have found that polymorphisms in HPA axis genes (e.g., glucocorticoid) influence salivary responses to acute challenges including TSST responses (reviewed by Wust et al., 2004).
The TSST has been studied in those suffering from various clinical conditions including acute depression, anxiety disorders, post-traumatic stress disorder (PTSD), fibromyalgia, pain disorders, and asthma. While results of past studies have been somewhat mixed, researchers have found that differences in cortisol responses between clinical populations and healthy controls are more evident when a system is challenged, such as during the TSST. The studies examining TSST responses in acute depression are discussed below. This project will be the first to examine cortisol responses to the TSST in CMDD.

In summary, a number of demographic, clinical, and biological factors have been associated with cortisol responses to social stress. This presents a somewhat challenging situation, as it is not possible to account for countless potential covariates in a given study. However, careful defining of exclusion criteria, controlling for sex differences, and conducting social challenge tests in the afternoon have been suggested as possible approaches to improve validity of TSST studies (Kuldieka et al., 2009) and constitutes the approach used in this project. For completeness, Chapter 6 is dedicated to evaluating whether other pertinent variables that are not the primary focus of the project (e.g., childhood adversity, SSRI medication use, PTSD, and depressive subtype), are associated with cortisol responses in CMDD.
1.4.3. Studies using the TSST in Depressed Populations

More recent work has begun to examine social stress reactivity in clinically depressed populations (Burke et al., 2005), including several studies using the TSST. Young et al., (2000) compared TSST responses in ten acutely depressed individuals and ten healthy controls. Depressed individuals were untreated and had no history of childhood abuse, trauma, or PTSD. Results indicated that despite higher baseline cortisol levels in the depressed group, the cortisol stress response was similar in depressed individuals and healthy controls. However, those who were depressed did have a blunted B-endorphin (derived from the same precursor as ACTH) response compared to healthy controls. Although small, this study provided some preliminary evidence that social stress reactivity may be altered in acute forms of depression.

In a follow up study by this same group, Young et al., (2004) examined the influence of comorbid anxiety on TSST responses in depression. Results indicated an increased ACTH response to the TSST in depressed individuals as compared to healthy controls; however, this exaggerated ACTH response was exclusively in those who had both depression and an anxiety disorder. The authors concluded that increased ACTH reactivity following social stress may be a unique characteristic of the group suffering from comorbid depression and anxiety disorders.

While these preliminary studies by Young et al., provided modest evidence for altered social stress reactivity in acute depression, their findings should be interpreted in light of the particular TSST methodology used. Participants in the studies by Young et al. (2000, 2004) were informed at the time of recruitment that they would be performing a public speaking task. This differs from the original protocol where participants were not
made aware of the nature of the task until the day of the stressor (Kirschbaum et al., 1993). This difference in methodology likely decreased the novelty and unpredictability of the stressor, thus limiting the intensity of the cortisol stress response.

Another study using the TSST in an acutely depressed population was published by Heim et al., (2000b) and evaluated the role of childhood abuse on cortisol and ACTH responses in depressed and healthy populations. Overall, the study suggested that childhood abuse, and to a greater extent the combination of childhood abuse and depression, were significant determinants of increased cortisol reactivity to a social stressor in females. Therefore, the study by Heim et al., along with those by Young et al., support the theory that social stress responses are altered in acutely depressed populations. This work also suggests that in many cases, cortisol output in response to social stressors may be driven by comorbidities and/or adversities, such as anxiety and/or childhood abuse. The high prevalence of these factors in CMDD suggests that cortisol responses to social stress may be altered in this population.
1.5. Personality, Cortisol Responses, and Depression

While the cortisol responses to social stress may be impacted by several demographic, clinical, and biological factors, this project will focus on personality for two main reasons. First, personality pathology has been strongly linked with chronic depression. Second, the same personality factors that associate with chronic depression have been linked to stress reactivity and HPA activity. Thus, the second major goal of this project is to examine whether personality factors (specifically neuroticism and extraversion) have moderating effects on cortisol responses in CMDD.

The following sections will provide an overview of: 1) personality pathology in depressive illness; 2) associations between neuroticism and extraversion, and depressive illness; and 3) associations between neuroticism and extraversion and HPA activity.

1.5.1. Personality Pathology and Depressive Illness

Introduction

Associations between personality pathology and depressive illness have long been recognized. Kraepelin (1921) observed that similar personality profiles could be identified in individuals with affective disorder and in their relatives. Sneider (1958) also described certain personality characteristics in individuals with affective disorders. Akiskal proposed that mood and personality combined to result in “subaffective personalities” (Akiskal et al., 2005). This concept of subaffective personality disorders suggests that personality pathology and mood disorders exist on a spectrum. For example,
depressive personality disorder (a subaffective personality disorder currently in the appendix of DSM-IV-TR), may be the early onset, persistent trait-like variant of depressive disorders.

**Depression and Personality Disorders**

Personality disorders were introduced as part of the multi-axial diagnostic system in DSM-III (APA, 1980). The DSM-IV-TR categorizes ten different personality disorders in three overarching clusters. Cluster A disorders characterize odd and eccentric personalities (e.g., schizotypal, schizoid, and paranoid). Cluster B personality disorders describe traits such as impulsivity, emotional dysregulation, and aggression (e.g., borderline, histrionic, and narcissistic). Cluster C personality disorders are typified by obsessive, avoidant, and dependent traits.

There is significant comorbidity between personality disorders and depressive illness (Bagby *et al*., 2008). The rates of personality disorders in depressed populations have varied, but several studies suggested rates over 50 percent in both inpatient and outpatient settings (Charney *et al*., 1981, Shea *et al*., 1990, Fava *et al*., 2002). In general, cluster C personality disorders appear to be the most common in depressed outpatients (Shea *et al*., 1987) and Cluster B personality disorders being the most common amongst depressed inpatients (Black *et al*., 1999). *Higher rates of personality disorders have been reported in more chronic forms of depression* (Keller *et al*., 1998, Russell *et al*., 2003).

Certain personality disorders, such as depressive personality disorder (in appendix of DSM-IV-TR), have been found to be predictive of chronic depression. Depressive personality disorder includes characteristics such as gloominess, feelings of inadequacy,
self-blame, brooding, and pessimism. Kwon et al. (2000) found that young women with depressive personality disorder had an increased risk of developing dysthymic disorder over the course of a three-year follow up study. Depressive personality disorder has also been associated with poor treatment outcome in depression (Laptook et al., 2006, Ryder et al., 2010). Klein et al. (1999) reported that in family studies of MDD, those with chronic forms of depression had higher rates of depressive personality than their first degree relatives.

**Dimensional Models of Depression**

Recent research has suggested that there are a number of challenges when studying associations between categorical DSM-IV personality disorders and depressive illness including: 1) low inter-rater reliability in the diagnosis of personality disorders in depressed patients; 2) difficulty in differentiating personality traits from personality disorders in depressed individuals; and 3) the fact that there can be overlap in the diagnostic criteria between certain personality disorders and Axis I disorders (e.g., depressive personality disorder and dysthymic disorder). Based on the above limitations, dimensional models of personality have been proposed as a better approach to understanding personality pathology in depressive illness (Bagby et al., 2008) and will be the approach used in this project. It is noteworthy, that adding dimensional measures of personality is one of the major changes proposed for DSM-5.
The Five-Factor Model (FFM) of Personality

There are several dimensional models of personality that have been studied in psychiatric populations including Costa and McCrae’s (1992) Five-Factor Model (FFM) of personality, Cloninger’s Seven-Factor Psychobiological Model of Temperament and Character (Cloninger et al., 1993), and Livesley’s eighteen-factor model of personality pathology (Livesley and Jackson, 2002). The FFM represents one of the most commonly used dimensional models and was therefore the chosen model in this current project.

In the FFM, personality is organized hierarchically, with five major dimensions, namely neuroticism, extraversion, openness to experience, conscientiousness, and agreeableness. Each of these dimensions is made up of six narrower traits or facets. The dimensions of personality initially emerged through factor analyses of adjectives used in different languages in non-psychiatric populations. This type of inquiry, referred to as the lexical-semantic hypothesis, theorizes that the most salient personality characteristics are present in language.

Costa and McCrae’s five-factor personality inventory (NEO PI-R) that measures the above five personality dimensions was initially developed in non-clinical populations. However, studies have demonstrated that extremes in personality traits are related to psychopathology. A meta-analysis has demonstrated that there are meaningful correlations between personality traits of the FFM of personality and DSM-IV personality disorders (Saulsman and Page, 2004).

Neuroticism and extraversion are the two most studied personality dimensions in depression and will be the focus of this project.
1.5.2. Neuroticism, Extraversion, and Depression: Literature Review

Neuroticism and extraversion are both heritable and stable traits that describe a variety of moods and behaviours (Clark and Watson, 1991, Kirk et al., 2000). Neuroticism describes an individual’s sensitivity to experience negative emotions (Tellegen, 1985, Clark et al., 1994). These negative moods can include fear, anxiety, sadness, guilt, and self-dissatisfaction (Watson and Clark, 1984). Furthermore, neuroticism is also associated with negative cognitions (Clark et al., 1990), negative appraisals of others (Gara et al., 1993), and overall work, social, and life dissatisfaction. Extraversion on the other hand, includes characteristics such as positive emotionality, sociability, talkativeness, and dominance. Other characteristics associated with extraversion include high energy, friendliness, and assertiveness (Clark and Watson, 1991, Clark et al., 1994, Viken et al., 1994).

The relationship between neuroticism and/or extraversion and depressive illness has been studied over many decades, with several reviews having been published on this topic (Clark et al., 1994, Enns and Cox, 1997, Bagby et al., 2008, Klein et al., 2011) suggesting that:

1) There is a robust association between higher levels of neuroticism and/or lower levels of extraversion and depression (Clark et al., 1994, Enns and Cox, 1997, Clark and Watson, 1999, Kotov et al., 2010, Klein et al., 2011);

2) Higher neuroticism and/or lower extraversion have been associated with a chronic course of depressive illness, which is of significance for this project.
(Weissman et al., 1978, Hirschfeld et al., 1986, Duggan et al., 1990, Enns and Cox, 1997, Kotov et al., 2010, Wiersma et al., 2011);

3) In general, higher neuroticism and lower extraversion are associated with a poor prognosis in depression (Clark et al., 1994, Enns and Cox, 1997, Ormel et al., 2001, Quilty et al., 2008, Klein et al., 2011); and

4) Valid measures of neuroticism and extraversion personality “traits” can be obtained in depressed individuals. While it is true that being depressed can result in elevated neuroticism, and to a lesser extent, lower extraversion scores, prospective studies have shown that these personality traits remain extant even in the recovered state (Morey et al., 2010, Klein et al., 2011).

Recent research has also tried to evaluate whether personality dimensions, such as neuroticism and/or extraversion, can help guide treatment of depressive disorders. In a study by Bagby et al. (2006), individuals with higher neuroticism were found to preferentially respond to medications over psychotherapy. While these results may initially appear counterintuitive, the authors hypothesized that higher levels of neuroticism may make it challenging for individuals to adequately use psychotherapeutic approaches. In another paper, Quilty et al. (2008) found that depressed individuals who responded to both psychotherapy and medications had personality characteristics that included lower neuroticism and higher extraversion. In summary, these studies point to the usefulness of understanding personality pathology when evaluating treatment responses in depressed populations.
Whether there is a biological link between specific personality traits and depression is an intriguing question. As reviewed by Foster and MacQueen (2008), several of the neurobiological mechanisms believed to be associated with depressive illness (neuroendocrine, molecular, genetic, and neuroanatomical) have also been linked with personality traits that confer risk to depression (e.g., neuroticism). Foster and MacQueen highlighted that more integrative research is needed to better understand the complex relationship between personality, depression, and biological markers. This is the approach that will be used in this thesis. One of the major areas of study in this project and the focus of Study 2 is whether or not neuroticism and/or extraversion moderate cortisol responses to social stress in chronic depression.

In summary, there is an extensive body of work examining associations between neuroticism and extraversion and depressive illness. Neuroticism and extraversion have been linked to the onset of depression, chronicity of depressive illness, and treatment outcomes. At this point, an understanding of the biological links between personality dimensions and depressive illness is still limited, although biological pathways such as the HPA axis (as will be discussed in the next section) are promising avenues for future research.

1.5.3. Neuroticism, Extraversion, and HPA Function: Literature Review

In healthy populations, there has been some support for the association between neuroticism and/or extraversion and HPA function, although study results have varied. Higher neuroticism has been associated with various measures of increased HPA activity including higher cortisol awakening response (CAR) (Portella et al., 2005) and higher
daily cortisol levels (Nater et al., 2010). Higher neuroticism has also been associated with increased cortisol reactivity following the Dex/CRH test (Zobel et al., 2004) and naloxone administration (Mangold and Wand, 2006). However, some studies have linked higher neuroticism and/or lower extraversion with decreased HPA activity (Mc Cleery and Goodwin, 2001, LeBlanc and Ducharme, 2005, Phillips et al., 2005, Oswald et al., 2006), while others have found no relationship between neuroticism and/or extraversion and altered HPA function (Kirschbaum et al., 1992a, Schommer et al., 1999).

Only one study conducted in a healthy population specifically evaluated the role of personality dimensions (including neuroticism and extraversion) in cortisol responses to the TSST (Oswald et al., 2006). In this study, sixty-eight healthy adults completed the Revised NEO Personality Assessment and underwent the TSST. Cortisol responses to the TSST were influenced by personality dimensions in a sex-specific manner. Blunted cortisol responses were associated with higher neuroticism in females and with lower extraversion in males. Overall, this study supports sex-specific associations between neuroticism and extraversion and low cortisol reactivity. Specifically, it suggests that personality traits associated with greater psychopathology are associated with blunted social stress reactivity.

In summary, studies to date provide preliminary evidence that neuroticism and extraversion are associated with HPA activity, including cortisol responses to social stress. As individuals with CMDD have higher levels of neuroticism and lower levels of extraversion compared to healthy and acute MDD populations, it is possible that these personality dimensions may strongly contribute to individual differences in cortisol responses in CMDD.
1.6. The Cortisol Awakening Response (CAR)

*It has been suggested that one function of the CAR is to mobilize energy at awakening to prepare an individual for the demands of the day.*


1.6.1. Introduction

The cortisol awakening response is a novel measure of HPA activity which has generated increasing interest among those studying depression. While a number of studies have found altered CAR responses in acute forms of depression, there have been no published studies of the CAR in chronic depression. *Therefore, another major goal of this project was to evaluate the CAR in CMDD for the first time.* Advantages of the CAR test include its ease of administration and potential utility in longitudinal work in both clinical and research settings.

The following sections provide a summary of the CAR and a review of studies examining it in various depressed populations.

1.6.2. Overview of the CAR

Cortisol has a twenty-four-hour circadian rhythm with the highest levels occurring after awakening and lowest levels occurring between approximately 0200 to 0300 hrs. (see Figure 3 below).
Figure 3: Diurnal Cortisol Rhythm. Cortisol is released in a 24hr rhythm with the highest levels occurring just after awakening and the lowest levels between 0200 to 0300 hrs. (image online, available at www.wardelab.com/20_3.html, accessed November 12, 2012).

As shown in Figure 3, cortisol levels rise in the early hours before awakening. The CAR describes the spike in cortisol that occurs during the first thirty to forty-five minutes after awakening, during which time levels can increase about 50 to 100 percent (see Figure 4 below) (Pruessner et al., 1995, Pruessner et al., 1997, Wust et al., 2000b, Wilhelm et al., 2007).
Figure 4: The Cortisol Awakening Response. Cortisol levels spike at about 30 to 45 min. post-awakening. This rise is termed the CAR which can be measured by having individuals collect salivary cortisol levels at awakening, 30 min. and 60 min. post-awakening (image online, available at www.salimetrics.com/spit-report/archives/june-2010, accessed November 12, 2012).

The CAR can be detected in about 75 percent of individuals, is reasonably stable over time (r = .63 for AUC on day 1 and 2) (Wüst et al., 2000), and has moderate heritability (Wüst et al., 2000, Clow et al., 2004). These characteristics suggest that the CAR might be a useful trait marker of HPA function.

The exact mechanism underlying the CAR is unknown. While the CAR amplitude is partly determined by circadian factors, it has been found to be distinct from the circadian rise in cortisol in the morning hours (Wilhelm et al., 2007). Psychological processes may also be important in determining the CAR. For example, social stress, loneliness, and
work overload have all been associated with an altered CAR (Wust et al., 2000a, Kudielka and Kirschbaum, 2003, Schlotz et al., 2004).

1.6.3. The CAR and Depression

Several studies suggest that the CAR is altered in depressive illness. One of the largest such studies examined the CAR in actively depressed individuals (n=701), remitted depressed individuals (n=579), and controls (n=308) (Vreeburg et al., 2009). In this study, individuals with either active or remitted depression had higher total cortisol output (AUCg) and cortisol reactivity (AUCi) at awakening compared to healthy controls. There was no significant difference in the CARs between active and remitted depressed individuals. These findings are consistent with other studies that have also reported higher CARs in depression (Bhagwagar et al., 2005), in remitted depression (Bhagwagar et al., 2003, Aubry et al., 2010), in individuals with greater depressive symptomatology (Pruessner et al., 2003b), in unmedicated individuals with a history of depression, (Bhagwager et al., 2003), and in asymptomatic individuals with a familial risk of depression (Mannie et al., 2007, Vreeburg et al., 2010). In summary, studies have found that both the overall cortisol secretion (AUCg) and cortisol reactivity (AUCi) are greater in depressed than in healthy populations, although some conflicting results have also been reported (Huber et al., 2006). Given that CAR abnormalities have been found in both remitted and currently depressed individuals, it would appear that the CAR may represent a trait rather than a state marker of depression. However, as mentioned previously, there is evidence to suggest that the biology of CMDD, particularly as it
pertains to the HPA axis, may be different than that of acute depression. Study 3 will be the first to examine the CAR in those with CMDD versus healthy controls.
CHAPTER 2

RATIONALE AND STUDY DESIGN
2.1. Rationale and Study Design

This project was borne out of a need to improve the understanding and management of CMDD, a common and highly disabling illness. At present, clinicians have limited guidance in determining which medications and/or therapy are best suited for individual patients with this condition. In order to improve its management and develop more targeted treatments for CMDD, a greater understanding of its pathophysiology is needed.

As reviewed in Chapter 1, prior research has demonstrated that a multitude of biopsychosocial factors contribute to the etiology of CMDD. **Cortisol was chosen as the primary biological measure** based on an established link between this hormone and depressive illness. The TSST and the CAR were the two research methods chosen to examine salivary cortisol responses. From a social perspective, it is a well-observed finding that individuals with chronic forms of depression are highly sensitive to interpersonal stressors. This supported the use of a social stress paradigm, that being the TSST, as the main method to examine cortisol responses. This paradigm would capture cortisol fluctuations that occur in the context of higher order cognitive and emotional processes. Finally, from a psychological standpoint, literature suggests that the effect of social stressors on cortisol responses may be moderated by factors such as personality (e.g., neuroticism and extraversion). Therefore, whether or not neuroticism and/or extraversion moderated cortisol responses in CMDD was also evaluated.

In Study 1, discussed in Chapter 3, cortisol responses to social stress were compared in CMDD versus healthy controls. Given the links between higher levels of neuroticism and lower levels of extraversion and both chronic depressive illness and cortisol
responses, the moderating effects of these personality dimensions on cortisol responses in CMDD were evaluated in Study 2 as described in Chapter 4. There has been increasing interest in the CAR given the associations found between this measure of cortisol and depressive illness, although no studies have been reported specifically on CMDD. One advantage of the CAR test is that it is relatively easy to administer and it would be a simple and cost-effective test to use longitudinally in a clinical or research setting. In Study 3, described in Chapter 5, the CAR was examined for the first time in CMDD.

Given the number of factors that could impact cortisol responses to social stress (for review, see Kudielka et al., 2009) it is difficult to control for every potential covariate. Several key covariates including demographic variables (e.g., sex, age, and BMI) and clinical variables (e.g., anxiety comorbidity and psychiatric medication use) were examined in this project. For completeness and to help guide future research in this area, Chapter 6 explores how other pertinent clinical and social factors impact cortisol responses to social stress in those with CMDD. Out of the many variables found to be associated with cortisol responses, four stood out as having particular relevance to CMDD: childhood abuse, SSRI medication use, PTSD comorbidity, and depression subtype (atypical versus melancholic). The associations amongst these four variables and cortisol responses in CMDD participants were examined.
2.2. Conceptual Model for Thesis

The Conceptual Model of this thesis, illustrated below in Figure 5, proposes a tripartite relationship between chronic depression, cortisol responses, and the personality dimensions of neuroticism and extraversion. It also highlights the major study questions that have been derived from this model. In this tripartite model, the various relationships between the key variables were depicted as bidirectional in nature for two main reasons. First, it acknowledges that the true causal relationships between these variables are unknown due to the fact that most studies to date have been cross-sectional in nature. Second, the bidirectionality further addresses the possibility that CMDD is both caused by and causal of altered cortisol responses and/or personality. If so, this might explain why CMDD tends to worsen and persist over time. In other words, the state of having CMDD might further strengthen the very processes that caused it in the first place.
Figure 5: Conceptual Model for Thesis: the interplay between CMDD, cortisol responses, and personality. The current project first examines associations between cortisol responses and CMDD. The second major focus of the study is to determine whether personality dimensions of neuroticism and extraversion moderate this association. The arrows are depicted as bidirectional, as most studies to date have been cross-sectional. Therefore, conclusions about causality are limited.
2.3. Study Aims and Major Hypotheses

This project is comprised of three inter-related investigations as outlined below:

**Study 1:** This study examined cortisol responses to the TSST in those with CMDD compared to healthy controls.

*Hypothesis:* Given that individuals with chronic forms of depression are highly sensitive to interpersonal stressors, it was hypothesized that CMDD participants would have greater cortisol stress responses to the TSST than would healthy controls.

**Study 2:** This study examined whether higher levels of neuroticism and/or lower levels of extraversion moderated the association between cortisol responses and CMDD.

*Hypothesis:* Higher levels of neuroticism and/or lower levels of extraversion are often associated with greater interpersonal stress sensitivity. It was therefore hypothesized that these personality dimensions would be associated with increased cortisol responses to the TSST in individuals with CMDD as compared to healthy controls.

**Study 3:** This study examined the CAR in those with CMDD compared to healthy controls.

*Hypothesis:* Based on prior findings that the CAR is increased in acute and remitted depression, it was hypothesized that the CAR would be elevated in CMDD compared to healthy controls.
2.4. Study Implications

Leading national and international institutions, such as the National Institute of Mental Health (NIMH), have highlighted the need to conduct naturalistic studies on individuals with complex psychiatric disorders. In the recent, large, and NIMH-funded Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, treatment response was lower in a naturalistic, depressed sample, as compared to prior reports in narrowly-defined and less heterogeneous depressed populations (Gaynes et al., 2009). In the STAR*D study, comorbidity in depressive disorders was the norm and the authors emphasized the need to study “real life” patients. In particular, it was noted that there is a dearth of knowledge on chronic and treatment-resistant forms of depression (Gaynes et al., 2009). This project begins to tackle CMDD from a biological standpoint which is aligned with the needs highlighted by the STAR*D study and the NIMH. If the hypotheses of this project prove correct, it would validate the notion that biological research can be conducted in complex populations, such as those with CMDD, and can stimulate future work of this kind.

While there has been a well-established link between cortisol and depressive illness, it is unknown whether cortisol patterns observed in more acute depressed populations are also evident in CMDD populations. It is also unclear how cortisol patterns in depression differ based on the research method used, namely the TSST and the CAR, and whether one method is more reliable or informative than the other. The findings of this project will help shed light on these key questions.

At present, there is no reliable method by which clinicians can choose one treatment strategy over another in CMDD patients or predict how patients will respond to any given
treatment. The “one size fits all” approach to depression treatment involves trial and error until an appropriate treatment is found. A major goal of this project is to determine whether novel psychobiological markers in CMDD can be identified. It is hoped that these profiles could, in turn, lead to more targeted management strategies for this highly disabling illness.
CHAPTER 3

STUDY 1: EVALUATING CORTISOL RESPONSES TO A SOCIAL CHALLENGE IN CMDD

This study has been published by:

3.1. Introduction and Hypothesis

CMDD is a debilitating illness, affecting about 20 percent of depressed populations. Surprisingly few studies have examined its pathophysiology, although this is an essential step to developing targeted treatments for this population. It has been well established in the literature that associations exist between acute depression and increased HPA activity. Whether these same alterations are present in CMDD is unknown. As cortisol is the most commonly studied stress hormone within this axis, cortisol responses was chosen as the biological marker in the study of CMDD in this project.

Individuals with chronic forms of depression are highly sensitive to interpersonal stressors. Based on this clinical feature, the TSST, a social stress paradigm, was chosen to study cortisol responses. This paradigm captures cortisol fluctuations that occur in the context of higher order cognitive and emotional processes.

Hypothesis: Given that individuals with chronic forms of depression are highly sensitive to interpersonal stressors, it was hypothesized that CMDD participants would have greater cortisol stress responses to the TSST compared to healthy controls.
3.2. Methods

a) Participants

CMDD:

The CMDD group was recruited via posters and through the mood disorders clinic at CAMH. For clinic patients, only those who had signed global research consent were contacted regarding the study. All participants met criteria for CMDD (DSM-IV-TR), defined as a MDE without remission for a minimum of two years. Each participant also scored 18 or higher on the 29-item version of the Hamilton Depression Rating Scale (HDRS-29) (Williams et al., 1988).¹ This version of the HDRS was used to capture the atypical symptoms of depression, including hypersomnia and hyperphagia, which are common in the CMDD population (Horwath et al., 1992, Stewart et al., 1993).

Exclusion criteria for all participants included significant conditions likely to confound cortisol levels including: ischemic heart disease; heart arrhythmias; current steroid treatment; acute substance abuse; pregnancy; bipolar disorder; acute suicidal ideation; and a history of psychotic symptoms.

Menstrual phase was documented for all participants and was defined as menstrual phase 0 to 5, follicular phase 5 to 14, and luteal phase 15 to menses. Smoking history was also documented.

¹ Similar to Watson, et al. (2002), it was concluded a priori that a drug-free study would be desirable but impractical in this population. However, subjects were required to be on stable doses of psychotropic medications for a minimum of one month before study entry to limit the effect of acute medication changes on the study results.
Controls:

The control group was recruited via posters and newspaper advertisements. Absence of psychiatric history was confirmed based on the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P) (First et al., 1995). Only controls with a score of 7 or less on the HDRS-29 were included in the study. The study received ethics approval from the institutional research ethics board. All potential study participants were screened using a brief, semi-structured phone interview to assess inclusion and exclusion criteria. Eligible participants then met with a research assistant and provided written informed consent.

b) Procedures

Clinical Assessment (Visit 1)

Participants were administered the Structured Clinical Interview for DSM-IV (First et al., 1995) and the HDRS-29 (Williams et al., 1988) by a trained research assistant.

The TSST (Visit 2)

A script of the protocol was followed as detailed on the following website: http://www.macses.ucsf.edu/Research/Allostatic/notebook/challenge.html. Trained undergraduate student volunteers, research assistants, and physicians conducted various components of the social stress paradigm.

After a preparatory phase, participants were led into a room that mirrored a standard interview room with various props including a video camera, microphone, and stand, and an “expert committee” of three people dressed in lab coats sitting behind a table.
Participants were told they had ten minutes to prepare a five-minute speech for the expert committee as if they were at a job interview, after which the committee would assign them a second task, which would also be five minutes in duration. The participants prepared their speeches in a separate room. After participants completed the public speaking task, they completed a difficult mathematical problem during a five-minute period that consisted of serially subtracting 13 from 1022. Protocols to deal with silences or errors during these tasks were established a priori and followed the scripts on the website noted above. Once the stress challenge was completed, there was a recovery phase in a separate room. Salivary cortisol samples were taken pre-stressor (−35 min. and −20 min.) just prior to entering the room to complete the public speaking and mathematical challenge (0 min.), and post-stressor (+20 min., +40 min., +60 min., and +80 min.). All participants were administered the TSST between 1400–1830 hours to control for circadian effects on cortisol secretion. Salivary samples were obtained by having participants lightly chew on cotton wool salivettes (Sarstedt, Montreal, Quebec). Cortisol levels were determined in duplicate in saliva by RIA using radioimmunoassay kits (ICN Biomedical Inc., Costa Mesa, CA.). The intra- and extra-assay variability was less than 10 percent. Saliva samples were stored until analysis at a minimum of −20°C.

Subjective appraisals of the TSST

To determine whether those with CMDD and healthy controls differentially appraised the TSST, participants completed a questionnaire post-challenge that evaluated how stressful they found the TSST. This questionnaire included the following six questions: 1) How stressful was the experiment overall? 2) How stressful was the public-
speaking task? 3) How stressful was the mathematical problem? 4) Did you feel especially anxious or tense in front of the committee? 5) Did you experience physical signs of stress (e.g., sweating, trembling, voice cracking, increased heart rate) in front of the committee? 6) How stressed do you think you appeared in front of the committee? The questions were assessed using a five-point Likert scale and a total score was calculated for each participant.

c) Statistical Analyses

Repeated measures analysis of variance (RANOVA) was conducted using the SAS System v. 9.1.3 to assess changes in cortisol over time during the social stressor, using both condition and sex as between group measures. Sex was included as a between group measure as this variable may be important in determining cortisol stress reactivity (for a review see Kajantie and Phillips, 2006) and may influence the presentation and treatment response of chronic depression (Kornstein et al., 2000). Other standard measures of the cortisol stress response including area under the curve (AUC) using the trapezoid method and peak percentage change in cortisol [(maximum post-stressor cortisol measure - T0 cortisol)/ T0 cortisol] *100 were also computed.

Pearson correlations were used to evaluate whether demographic variables, such as age and body mass index (BMI), were correlated with the cortisol measures. In addition, unpaired t tests were used to examine the potential effects of smoking status, medication status, menstrual phase, PTSD diagnosis, or other anxiety disorder comorbidity on these measures.
3.3. Results

Sample Characteristics

Twenty-six CMDD participants (twelve females and fourteen males) and twenty-eight controls (fourteen females and fourteen males) completed the study. The CMDD participants were statistically older than controls (43.3 +/- 5.7 years versus 39.4 +/- 6.0 years respectively; \( t (52) = -2.48, p = .016 \)). There was no difference in mean BMI between those with CMDD and controls (27.3 +/- 5.4 kg/m\(^2\) versus 25.0 +/- 4.4 kg/m\(^2\), \( t (45) = -1.64, p = .11 \)), but there were significantly more smokers in the CMDD group than in the control group (11/26 versus 3/26 respectively; \( \chi^2 = 7.01, df =1, p=.008 \)).

Eighteen out of twenty-six CMDD participants were on one or more psychotropic medication(s) including sixteen on antidepressants, one on a mood stabilizer (valproic acid), four on benzodiazepines, and two on antipsychotics. Thirteen out of twenty-six had a comorbid anxiety disorder, including six with PTSD, five with social phobia, one with specific phobia, and one with anxiety disorder not otherwise specified (NOS). Nine had a lifetime history of substance abuse and five had a lifetime history of an eating disorder (either anorexia nervosa or bulimia nervosa).

Cortisol Responses to the TSST

The analysis of cortisol levels revealed a significant time by sex interaction (\( F=3.03; df=4, 202; p=.02 \)) and a significant sex by condition (control versus CMDD) interaction (\( F=5.01; df=1, 52; p=.03 \)) (see Figure 6 below).
Figure 6: Mean (± SEM) Cortisol Responses to the TSST in CMDD Subjects vs. Healthy Controls Grouped by Condition and Sex

Males

Females
Summaries of the results for AUC and peak percentage change in cortisol are graphed in Figure 7. Females with CMDD had a significantly greater mean AUC (2.5 +/- 0.8 µg/dl) than controls (1.7 +/- 1.0 µg/dl, t (24) = -2.35, p = .027), but did not differ with respect to mean peak percentage change in cortisol (54.4 +/- 88.2 versus 63.0 +/- 107.8 µg/dl respectively, t (24) = 0.22, p = .83). A different pattern emerged in males, with no difference in mean AUC (CMDD = 2.1 +/- 0.6 versus controls = 2.2 +/- 0.9 µg/dl, t (25) = .52, p = .61), but with a significant difference in mean peak percentage change (CMDD versus controls: 48.6 +/- 66.4 versus 123.2 +/- 106.5 µg/dl; t (26) = 2.2, p = .035).

To examine whether the group differences in post-challenge cortisol levels might also be reflected in pre-challenge cortisol levels, possible group differences in the two pre-challenge cortisol samples taken at -35 min. and -20 min. before the TSST were evaluated. An initial analysis revealed that these two measures were highly correlated (r = .85, n = 54, p < .001). Based on these results, a single pre-challenge cortisol level was calculated based on the mean of the -35 min. and -20 min. samples. A univariate ANOVA was then calculated using this pre-challenge cortisol level as the dependent measure and both condition and sex as between-group variables. Results of this ANOVA revealed no significant interaction or main effects of diagnosis or sex on pre-challenge cortisol levels.

The exploratory examination of how relevant demographic and clinical characteristics of those with CMDD were associated with the primary cortisol measures is summarized in Table 1.
Figure 7: Comparison of Sex Differences in AUC and Peak Percentage Change in Cortisol in CMDD Subjects vs. Healthy Controls

Mean (±SEM) for AUC and Peak Percentage Change in Cortisol in CMDD vs. respective Controls. * p < .05.
Table 1: Association between Demographic and Clinical Variables to Cortisol Measures in CMDD Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Peak% Change</th>
<th>AUC</th>
<th>Pre-challenge cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r&lt;sup&gt;a&lt;/sup&gt;</td>
<td>r&lt;sup&gt;a&lt;/sup&gt;</td>
<td>r&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>43.3 (5.7)</td>
<td>.06</td>
<td>-.10</td>
<td>-.21</td>
</tr>
<tr>
<td>Body Mass Index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>27.3 (5.4)</td>
<td>.01</td>
<td>.04</td>
<td>.11</td>
</tr>
<tr>
<td>Age of onset of depression (year)</td>
<td>16.1 (10.0)</td>
<td>.24</td>
<td>-.42*</td>
<td>-.38</td>
</tr>
<tr>
<td>Current Duration (yrs.)</td>
<td>19.7 (12.0)</td>
<td>-.10</td>
<td>.02</td>
<td>-.08</td>
</tr>
<tr>
<td>HDRS-29</td>
<td>36.3 (9.3)</td>
<td>.11</td>
<td>.09</td>
<td>.15</td>
</tr>
<tr>
<td>HDRS-17</td>
<td>22.2 (5.1)</td>
<td>-.06</td>
<td>.03</td>
<td>.05</td>
</tr>
<tr>
<td>PTSD or Other Anxiety Disorder (Yes/No)</td>
<td>13/13</td>
<td>.08</td>
<td>.13</td>
<td>.50</td>
</tr>
<tr>
<td>On Psychotropic Medication (Yes/No)</td>
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<td>.58</td>
<td>1.0</td>
<td>-.07</td>
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<tr>
<td>Smoking Status (Yes/No)</td>
<td>11/26</td>
<td>.54</td>
<td>-1.0</td>
<td>-.34</td>
</tr>
</tbody>
</table>

* p < .05,  
<sup>a</sup> r= correlation coefficient,  
<sup>b</sup> df = 24
As shown above in Table 1, the only significant association was a negative correlation between age of onset of depression and AUC post-challenge, that being, those CMDD participants reporting an earlier age of onset had greater cortisol AUC. When these analyses were repeated in the two sexes considered separately, a negative correlation was found between age of onset and mean pre-challenge cortisol levels in females with CMDD, but not in males \( (r = - .66, p = .019) \). No other correlations reached statistical significance.

Importantly, there was no difference in AUC, peak percentage change in cortisol, or mean pre-challenge cortisol based on the presence or absence of psychotropic medications, a PTSD diagnosis, or other anxiety disorder diagnosis. In healthy controls, age and BMI were not significantly associated with AUC, peak percentage change, or mean pre-challenge cortisol measures. Across all study participants, and within the two diagnostic groups and sexes considered separately, those who were smokers \( (n = 14) \) did not differ significantly from non-smokers \( (n = 40) \) on these measures. Amongst the females, AUC, peak percentage change and mean pre-challenge cortisol levels did not differ based on menstrual phase.

**Subjective Appraisals of the TSST**

A univariate ANOVA using the total score from the subjective appraisal questionnaire as the dependent measure and both condition and sex as grouping variables revealed no significant interaction or main effects of sex. However, there was a trend for those with CMDD to appraise the TSST as more stressful than healthy controls \( \text{mean CMDD} = 22.5 \ (4.8), \text{mean control} = 19.9 \ (5.0); F=3.78, \text{df 1, 46, } p=.058 \). Further
analysis revealed no significant correlation between subjective appraisal of the TSST and key cortisol outcome measures in the entire sample and in each group and sex considered separately.

3.4. Discussion

Based on the clinical observation that CMDD is often associated with marked sensitivity to interpersonal stress, the working hypothesis for this study is that those with CMDD would exhibit greater cortisol responses to the TSST than would healthy controls. The overall pattern of results suggests that females with CMDD do, in fact, secrete more cortisol in response to the TSST than do female controls, pointing to sensitization at one or more levels of the (social) stress system. A novel and unexpected finding was that in males, CMDD was associated with significantly decreased peak percentage change in cortisol in response to the TSST. None of these results were readily attributable to basic demographic and clinical variables or differences in subjective experiences during the social challenge. Taken as a whole, these findings suggest that the nature of CMDD, at least as it relates to social stress responsivity, might be fundamentally different in the two sexes.

To date, few studies have evaluated HPA axis activity in chronically depressed populations. One study reported that the cortisol stress response to the DST and Dex/CRH challenge in CMDD participants was comparable to that of controls (Watson et al., 2002), however, sex was not included here as a grouping variable. The discrepancy in the results between the Watson et al. study and the current project may also reflect
methodological differences in evaluating the HPA axis, namely Dex/CRH versus TSST, and/or the different populations studied. Of note is the fact that those who partook in this project had an earlier onset of depression and a greater severity of depressive illness than did those assessed by Watson et al. As the current study found that age of onset of depression was associated with AUC in CMDD subjects as a whole and with mean pre-challenge cortisol levels in females, this clinical factor may be an important consideration in future cortisol studies in this population. Furthermore, it should not be assumed that HPA changes that stem from pharmacological and psychosocial challenges engage the same processes. In fact, it has been reported that amongst females with early life traumatic experiences, challenges involving CRH administration versus the TSST provoked very different outcomes (Nemeroff, 2004). It might similarly be the case that pharmacological and psychosocial challenges might have different effects amongst chronically depressed individuals.

Sex differences in HPA axis function have previously been reported in mixed samples of acute and chronically depressed individuals. Young and Ribeiro (2006) reported increased CRH drive in depressed females, but decreased CRH drive in depressed males relative to controls using a twenty-four-hour metyrapone challenge, a finding paralleling the current results. In addition, Young et al. (2007) found sex differences in ACTH pulsatility following metyrapone blockade in MDD. Furthermore, a treatment study of CMDD by Kornstein et al., (2000) found that females had a significantly greater response to sertraline compared to imipramine whereas the reverse pattern was seen in males. If one considers these studies together with the current results, one can reasonably hypothesize that there may be sex-specific interactions between
cortisol stress reactivity and treatment response in CMDD that merit future investigation.

With respect to the mechanisms underlying the current findings, given the nature of the TSST protocol, both higher order cognitive-emotional processes and differences in HPA function ought to be examined. Specifically, it is known that interpersonal rejection sensitivity and social anxiety are particularly common in females with chronic forms of depression (Matza et al., 2003, Novick et al., 2005) and that this elevated reactivity could account for increased cortisol responses to the TSST. However, this explanation does not account for the blunted response seen in males following the TSST. While the reason for this is as yet unknown, previous literature suggests that early life adversity, common in this population, may differentially influence HPA axis function in males and females. For example, in a study by Lingas and Matthews (2001), maternal nutrient restriction in late gestation in guinea pigs resulted in sex-specific, long-term effects on HPA function. Interestingly, restricted nutrient intake during pregnancy resulted in reduced basal pituitary-adrenal activity in male offspring, but increased basal pituitary-adrenal function in female offspring, findings that are similar to the current results. In another study, lower birth weight was associated with salivary cortisol stress responses to social stress in eight-to ten-year-old males but not females (Jones et al., 2006). Further work needs to be done to examine how early adversity variables may differentially impact HPA function in females and males with CMDD.

There were a number of limitations of the current study. For example, those with CMDD on psychotropic medications were included in the current project for ethical and pragmatic reasons, although ultimately, medication status was not associated with cortisol stress reactivity and could not account for the group by sex interaction. One explanation
for the lack of effect of medication status on cortisol measures, as suggested by Watson et al. (2002), is that over time, antidepressant use may have less of an impact on HPA function. However, the current findings should be interpreted cautiously, as the role of chronic psychotropic medication use on HPA activity in CMDD requires further study. Another potential limitation of the current study is its modest sample size even though it is consistent with other similar studies. As well, in the present study, only cortisol stress reactivity to a social stressor was examined. Other hormonal measures, such as ACTH or other indices of HPA activity (e.g., diurnal cortisol, Dex/CRH), might have provided a greater depth to the findings indicating the need for further research.

Despite the study limitations, though, this is the first time cortisol responses to a social stressor in the CMDD population has been studied. Whether current results reflect a fundamental difference in the pathophysiology of CMDD in females and males is also a matter in need of further research.

**ADDENDUM:** There were additional questions relating to the above manuscript that arose after its publication. An Appendix (pg 154) was added to respond to these questions in lieu of altering the content of the published manuscript. One specific question that is addressed is whether females with CMDD have greater cortisol responses compared to female controls or are the differences driven primarily by baseline differences in cortisol levels. As detailed in Appendix 1, differences in AUCg between females with CMDD and female controls need to be considered in light of baseline differences. Therefore, it can be more accurately concluded that females with CMDD had greater cortisol output during the TSST vs. having a heightened or sensitized response to this challenge.
CHAPTER 4

STUDY 2: DOES NEUROTICISM AND/OR EXTRAVERSION MODERATE CORTISOL RESPONSES IN CMDD?
4.1. Introduction and Hypothesis

In an attempt to understand the cortisol response patterns in CMDD, Study 1, described above, demonstrated the presence of sex differences to social stress in those with CMDD compared to healthy controls. This finding helps address question 1 of the Conceptual Model (see Figure 5, page 49). Yet, there are obviously many other factors that could contribute to individual differences beyond sex. Hence, in an effort to further narrow in on the psychobiology of the CMDD population, Study 2 focused on the personality dimensions of neuroticism and extraversion. Neuroticism and extraversion were the dimensions selected for two reasons, those being that they are strongly associated with CMDD and that there has been evidence linking them to cortisol responses. It was postulated that higher levels of neuroticism and/or lower levels of extraversion would moderate associations between cortisol responses and CMDD (see question 2 of the Conceptual Model). If this is the case, identifying distinct psychobiological profiles of CMDD based on cortisol responses and personality dimensions could lead to the development of more targeted management strategies for this difficult-to-treat population.

**Hypothesis:** Higher levels of neuroticism and lower levels of extraversion are often associated with greater interpersonal stress sensitivity. It was therefore hypothesized that these personality dimensions would be associated with increased cortisol responses to the TSST in individuals with CMDD as compared to healthy controls.
4.2. Methods

The methods, including recruitment, inclusion and exclusion criteria, and the procedure for the TSST have been detailed above for Study 1. Study 2 included both Study 1’s participants as well as additional participants recruited to increase the statistical power of this study. On visit 1, participants were administered the SCID-I/P and the HDRS-29 by a trained research assistant. Participants also completed the Revised NEO Personality Inventory (NEO PI-R) (Costa and McCrae, 1992). On visit 2, participants completed the TSST.

Revised NEO Personality Inventory (NEO PI-R)

Participants were administered the NEO PI-R which generates data based on the Five Factor Model (FFM) of personality (Costa and McCrae, 1992). The FFM evaluates individuals on personality domains of neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness. The NEO PI-R consists of 240 self-report items answered on a five-point Likert scale, with separate scales for each of the five domains. Each personality dimension consists of six correlated facets or subscales. A factor analytic study revealed that the same five factors and the thirty facets captured in community samples are also represented in psychiatric samples (Bagby et al., 1999). Stability estimates for the domains and facets are substantial in both community (Costa and McCrae, 1992) and psychiatric samples (Trull and Goodwin, 1993, Santor et al., 1997, Costa et al., 2005).
Statistical Analyses

Cortisol responses during the TSST were measured using the following: 1) area under the curve ground (AUCg) (using the trapezoid method), in order to measure of total cortisol output; and 2) area under the curve increase (AUCi), in order to measure stress reactivity (Pruessner et al., 2003a). Using the formulas described in Pruessner et al., 2003a, the following was used to calculate: a) AUCg = ((Time (T) 20min. + T0min.) / 2) + ((T40min. + T20min.) / 2) + ((T60min. + T40min.) / 2) + ((T80min. + T60min.) / 2), and b) AUCi = AUCg - 4 * T0min. An illustrative comparison of AUCg and AUCi is provided in Appendix 1 pg. 155. For ease and economy, the units for AUCg and AUCi have been summarized in units of concentration (µg/dl) throughout this thesis vs. concentration x time (i.e. µg/dl x min.). Cortisol measures that were not normally distributed were log transformed.

Separate hierarchical linear regressions were used to assess associations between neuroticism and extraversion and key outcome measures. In both instances, depression severity (using HDRS scores) and sex were entered as covariates in the first step of the regression. These factors were controlled for, given that severity of depression can influence personality measures (Kendler et al., 1993), and sex differences in cortisol responses have been found in both healthy and CMDD populations (Kajantie and Phillips, 2006, Chopra et al., 2009). At step 2, respective personality scores (neuroticism or extraversion), group classification (CMDD participants versus healthy controls), and the respective personality by group interaction were entered as predictor variables. Regressions were run separately for the two dependent measures, AUCg and AUCi.
4.3. Results

Participant Characteristics

Fifty-one CMDD individuals (twenty-nine females and twenty-two males) and fifty-seven healthy controls (twenty-nine females and twenty-eight males) completed the study. CMDD participants were older than the healthy controls (41.6 ±1.9 yrs. versus 36.9 ± 7.7 yrs., respectively). As expected, the CMDD participants had significantly higher mean neuroticism scores (70.1 ± 11.0 versus 46.5 ± 10.1, t = 11.6, df=106, p < .0001) and significantly lower extraversion scores (38.0 ± 11.2 versus 51.8 ± 9.5, t = -6.9, df = 106, p < .0001) than did the healthy controls.

For CMDD participants, the mean HDRS-29 and HDRS-17 scores were 33.7 ± 8.9 and 20.6 ± 4.6, respectively, suggesting a moderate severity of depression. As described in Study 1 (Chopra et al., 2009), there was a high degree of psychiatric comorbidity, particularly anxiety disorders, in the CMDD participants which is consistent with prior literature (Kessler et al., 2003). A large proportion of participants (36/51) were on one or more psychotropic medication(s) with the majority being on SSRIs or venlafaxine (30/36).

Associations between Neuroticism and Cortisol Responses during the TSST in Participants with CMDD and in Healthy Controls

As shown below in Table 2, a) and b), no significant associations between neuroticism and cortisol responses (either AUCg or AUCi) were found in either the CMDD or control group.
Table 2: Results of Hierarchical Linear Regressions Evaluating Associations between Neuroticism and Extraversion and Cortisol Responses in CMDD Subjects vs. Healthy Controls

a) Neuroticism and AUCi

<table>
<thead>
<tr>
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<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.15</td>
<td>-1.55</td>
<td>ns</td>
</tr>
<tr>
<td>HDRS-17</td>
<td>-0.22</td>
<td>-0.69</td>
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<tr>
<td>Neuroticism x Group</td>
<td>-0.37</td>
<td>-0.99</td>
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</tr>
<tr>
<td>Neuroticism</td>
<td>0.26</td>
<td>0.56</td>
<td>ns</td>
</tr>
<tr>
<td>Group</td>
<td>0.32</td>
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</table>

b) Neuroticism and AUCg

<table>
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<tr>
<td>Neuroticism x Group</td>
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<tr>
<td>Neuroticism</td>
<td>0.40</td>
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<tr>
<td>Group</td>
<td>0.51</td>
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Table 2: Results of Hierarchical Linear Regressions Evaluating Associations between Neuroticism and Extraversion and Cortisol Responses in CMDD Subjects vs. Healthy Controls (cont’d.)

c) Extraversion and AUCi

<table>
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<tr>
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<tr>
<td>HDRS-17</td>
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<td>.091</td>
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<td>Extraversion x Group</td>
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<tr>
<td>Extraversion</td>
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<td>-3.02</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Group</td>
<td>-1.38</td>
<td>-2.58</td>
<td>&lt;.05</td>
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</table>

d) Extraversion and AUCg

<table>
<thead>
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<th>t</th>
<th>p</th>
</tr>
</thead>
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<td>HDRS-17</td>
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<td>Extraversion x Group</td>
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</tr>
<tr>
<td>Extraversion</td>
<td>-0.30</td>
<td>-0.81</td>
<td>ns</td>
</tr>
<tr>
<td>Group</td>
<td>-0.78</td>
<td>-1.40</td>
<td>ns</td>
</tr>
</tbody>
</table>
**Associations between Extraversion and Cortisol Responses during TSST in CMDD**

**Participants and Healthy Controls**

For extraversion, the overall model significantly predicted AUCi ($F=2.90$, $df = 5, 102$, $p < .05$). Results revealed a significant association, shown above in Table 2 c), between the extraversion by group interaction and cortisol reactivity as measured by AUCi. Further analyses revealed that, as is shown in Figure 8 below, in those with CMDD but not in healthy controls, a negative correlation was found such that lower extraversion scores were associated with increased AUCi ($r = 0.34$, $p< .05$ versus $r=-.12$, $p = ns$, respectively). When the regression was repeated for AUCg, a measure of total cortisol output, no significant associations were found in either group, as shown in Table 2d) above.
**Figure 8:** Association between Extraversion and Cortisol Reactivity in CMDD Subjects and Healthy Controls

1. CMDD

![Graph showing the association between Extraversion and Cortisol Reactivity in CMDD subjects.](image)

AUCi
μg/dl (log transformed)

\[ r^2 = .12 \]
\[ p < .05 \]

2. Healthy Controls

![Graph showing the association between Extraversion and Cortisol Reactivity in Healthy Controls.](image)

AUCi
μg/dl (log transformed)

\[ r^2 = .01 \]
\[ p = \text{ns} \]
**Subjective Appraisals of the TSST**

Based on the cortisol findings, it was further examined whether participants with higher levels of neuroticism and/or lower levels of extraversion appraised the TSST differently, and if so, whether differences in appraisal were associated with cortisol stress responses. Each participant completed a post-challenge questionnaire that evaluated how stressful they found the TSST. The details of the questionnaire are described above in Section 3.2.

In the CMDD group only, lower extraversion was associated with increased subjective appraisals of stress during the TSST ($r (46) = -.31, p < .05$) (see Figure 9 below). There was no association between subjective appraisals of the TSST and neuroticism in either the CMDD or healthy control participants. Of note is the finding that subjective appraisals of the social challenge were not associated with cortisol responses, as measured by AUCg and AUCi, in either the CMDD participants or the healthy controls.

In summary, in CMDD participants, lower levels of extraversion were associated with both increased subjective stress and increased cortisol reactivity during the TSST, suggestive of both high psychological and physiological reactivity. However, these two types of reactivity were not correlated with one another, meaning that increased subjective appraisal of stress did not predict increased cortisol reactivity. A similar disconnect between subjective distress and cortisol reactivity has been reported in prior research (Dickerson and Kemeny, 2004).
Figure 9: Association between Extraversion and Subjective Stress Ratings of the TSST in CMDD Subjects and Healthy Controls

1. CMDD

Subjective Stress Ratings of TSST

Extraversion

$r^2 = .10$
$p < .05$

2. Healthy Controls

Subjective Stress Ratings of TSST

Extraversion

$r^2 = .03$
$p = ns$
Pre-Challenge Cortisol Levels

To explore the possible role of anticipatory stress on the current results, associations between neuroticism and extraversion and pre-TSST cortisol levels were examined next. As the two pre-challenge cortisol levels (-35min. and -20min.) were significantly correlated, $r (106) = .77, p < .001$), a single mean pre-challenge cortisol measure was computed. Results indicated no significant association between mean pre-challenge cortisol measures and neuroticism or extraversion in the entire sample as a whole and in the CMDD and healthy control populations individually. This suggests that the relationship between low extraversion and increased cortisol reactivity in CMDD participants was independent of pre-challenge cortisol measures.

Evaluation of Other Potential Covariates

As a final step, several potential covariates were examined. Age, BMI, and smoking status (yes/no) were not associated with cortisol reactivity. There was a trend for CMDD subjects who were on psychotropic medications to have lower total cortisol output (AUCg) ($0.67 \pm 0.42 \, \mu g/dl$) as compared to CMDD subjects who were not taking psychotropic medications ($0.91 \pm 0.28 \, \mu g/dl$, $t = 0.71$, df =49, $p = .06$). However, psychotropic medication use (yes/no) was not associated with cortisol reactivity (AUCi) ($0.81\mu g/dl \pm .25$ versus $0.87 \pm 0.30, \mu g/dl$ $t =2.0$, df =49, $p = ns$, respectively). When psychotropic medication use (yes/no) was controlled for in the regression model, the association between the extraversion by group interaction and AUCi remained significant, further suggesting that the results were not related to the effects of psychotropic medications.
4.4. Discussion

Study 2 is the first study to evaluate the association between dimensional personality traits and cortisol responses to the TSST in chronically depressed individuals. The main finding was that lower levels of extraversion were associated with increased cortisol responses during the social stressor in CMDD participants but not in healthy controls. Furthermore, CMDD participants with low levels of extraversion appraised the TSST as more stressful, although appraisal ratings were not correlated with cortisol reactivity. Interestingly, neuroticism was not associated with cortisol responses or subjective stress ratings in either group. These findings could not be attributed to clinical or demographic variables, medication status, or pre-challenge cortisol levels.

The current findings add to the growing literature highlighting the important relationship between personality factors and chronic depressive illness. In a recent study by Wiersma et al. (2011), multiple psychological characteristics including personality, cognitive reactivity, and external locus of control were evaluated in 690 non-chronic and 312 chronically depressed individuals. Chronicity of depression was associated with neuroticism, hopelessness, risk aversion, rumination, and external locus of control, as well as with lower levels of extraversion, agreeableness, and conscientiousness. However, multivariate analyses revealed that only low extraversion, rumination, and external locus of control remained significantly associated with chronicity when controlling for other psychological factors. In combination with Study 2’s results, this suggests that lower levels of extraversion may have unique relevance in CMDD that is distinct from other psychological factors including neuroticism.
The association between lower extraversion and increased cortisol reactivity in CMDD participants is intriguing and presents two possible interpretations of this finding. First, an increased cortisol response could suggest a pathological process. This interpretation is consistent with a study by Heim et al. (2000b) which reported increased cortisol reactivity to the TSST in women with depression and a history of childhood abuse. However, it is important to note that robust cortisol responses have also been found to be normal or adaptive in certain populations such as healthy males (Kirschbaum et al., 1992b), while blunted responses have been associated with psychopathology (Heim et al., 2000a). At this time, until normal parameters for cortisol responses to social stress can be defined, determining whether an increased cortisol response is pathological or adaptive remains a key challenge for the field.

Nevertheless, from a clinical standpoint it appears plausible that the association between lower extraversion and increased cortisol responses to social stress represents an alteration in HPA activity in those with CMDD. If future work supports this hypothesis this could have important theoretical and clinical implications for this difficult-to-treat population. Recent research has highlighted the need to identify distinct intermediate phenotypes within heterogeneous depressed populations that can provide more tangible targets for both neurobiological research and tailored treatment approaches (Klein et al., 2011). Could the current findings suggest that those with CMDD who have low levels of extraversion may be in need of treatments that can moderate cortisol reactivity to social stress? It is already known that increased social connections, development of relationships, and engagement in activities are proven psychological treatment strategies for CMDD (Keller et al., 2000, Bhagwagar et al., 2003, Schramm et al., 2008). However,
it would be valuable to determine whether CMDD patients who have lower extraversion scores have the greatest benefit from this treatment approach, both symptomatically and in terms of cortisol reactivity. Alternatively stated, does treatment that targets lower levels of extraversion improve outcomes for this subgroup of CMDD individuals and is this effect mediated through cortisol reactivity?

There are several limitations that merit consideration. To begin with, the study is cross-sectional. Therefore, the degree to which the personality scores represent longstanding personality traits versus those influenced by the state of depression cannot be determined. However, previous studies have suggested that meaningful measures of personality can be conducted in the depressed state (Trull and Goodwin, 1993; Santor et al., 1997, Costa et al., 2005). Furthermore, studies have found that extraversion, in particular, is a personality dimension that is less affected by the state of depression than are other personality measures, such as neuroticism (Morey et al., 2010, Klein et al., 2011). Other limitations of this study include the modest sample size and the fact that most CMDD participants were on psychotropic medications. Importantly though, controlling for medication status did not influence the primary findings of the study. As well, despite the fact that clinical and demographic variables were non-significant predictors of the cortisol stress response, it is possible that potential covariates were not sufficiently accounted for.

In summary, Study 2 suggests that lower levels of extraversion are associated with increased cortisol reactivity to a social challenge in CMDD participants but not in healthy controls. The negative effects of long-term cortisol exposure on mood regulation and
brain pathology indicate the possible role of extraversion as a clinical target in CMDD treatment and merits further research.
CHAPTER 5

STUDY 3: THE CORTISOL AWAKENING RESPONSE (CAR) IN CMDD
5.1. Introduction and Hypotheses

The cortisol awakening response (CAR) is a unique measure of HPA activity describing the sharp rise in cortisol during the first thirty to forty-five minutes after awakening. Interestingly, a number of studies have found a higher CAR in acute and remitted depression, although this has yet to be studied in the CMDD population. It was particularly appealing to investigate the CAR due its ease of measurement relative to the TSST. If proven to be a useful biological marker in CMDD, it would be a simple and cost-effective test to administer longitudinally in a clinical or research setting. The goal of Study 3, the final study of this project, was to evaluate the CAR in those with CMDD.

Hypotheses:

1) Based on prior findings that the CAR is increased in acute and remitted depression, it was hypothesized that **the CAR would be elevated in CMDD as compared to healthy controls.**

2) Given the association between lower levels of extraversion and higher cortisol response to the TSST in those with CMDD (as seen in Study 2), it was expected that **lower levels of extraversion would also predict a higher CAR in those with CMDD as compared to healthy controls.**

3) Given the lack of association between higher levels of neuroticism and cortisol responses to the TSST in both CMDD and healthy participants (as seen in Study 2), it
was similarly expected that no association would exist between neuroticism and the CAR.

### 5.2. Methods

A subgroup of study participants comprising of twenty-seven CMDD participants and thirty healthy controls were administered both the CAR test and the TSST. Each participant completed the TSST (Kirschbaum et al., 1993) during a weekday between 1400 and 1800 hrs. The TSST methodology is described above in Section 3.2. Two consecutive days of awakening salivary samples were collected by participants at home to determine the CAR. In most cases, the CAR tests and TSST were completed between 24hrs and one week apart. For logistical reasons, five participants had the CAR and TSST completed more than a week apart, with the maximum length between the two tests being thirty-five days. Salivary samples were obtained by having participants lightly chew on cotton wool salivettes (Sarstedt, Montreal, Quebec). Cortisol levels were determined in saliva by RIA using radioimmunoassay kits (ICN Biomedical Inc., Costa Mesa, CA.). The intra- and extra-assay variability was less than 10 percent. Saliva samples were stored until analysis at –20°C. Smoking history was documented for each participant. Menstrual phase was also documented for all female participants and defined as menstrual phase 0 to 5, follicular phase 5 to 14, and luteal phase 15 to menses.

#### CAR Collection

Each participant collected salivary samples at home upon awakening, as well as at thirty and sixty minutes post-awakening on each of two consecutive days. Participants
were given verbal and written instructions regarding proper collection of cortisol samples and were to refrain from eating or brushing their teeth until after the three morning salivary samples were collected. Samples were to be refrigerated up until they were returned to the study coordinator, at which time they were stored at a minimum of −20°C until analyses were done.

**Statistical Analyses**

Repeated measures analysis of variance (RANOVA) was used to assess changes in cortisol over time during the first hour of awakening (awakening, +30 min., and + 60 min.) using both condition and sex as between group measures. Other standard measures of the CAR were examined, including the AUCg and AUCi (Pruessner et al., 2003). Cortisol measures that were not normally distributed were log transformed.

Four separate step-wise linear regressions were used to examine associations between extraversion and neuroticism and either AUCg or AUCi. In each model, depression severity (HDRS scores) and sex were entered in the first step of the regression. At step 2, respective personality scores (extraversion or neuroticism), group classification (CMDD participants versus healthy controls), and the respective personality by group interaction were entered as predictor variables.
5.3. Results

Sample Characteristics

Twenty-seven CMDD participants (nineteen females and eight males) and thirty controls (fifteen females and fifteen males) completed the study. The CMDD participants were older than the control group at a trend level (40.0 +/- 9.8 years versus 35.3 +/- 8.9 years respectively; t (55) = 1.9, p = .07). There was no difference in mean BMI between CMDD participants and controls (27.4 +/- 5.5 kg/m² versus 25.5 +/- 4.7 kg/m², respectively; t (49) = 1.3, p = 0.2).

Twenty out of the twenty-seven CMDD participants were on one or more psychotropic medications including seventeen on antidepressants, four on benzodiazepines, and one on an antipsychotic. Eighteen out of the twenty-seven CMDD participants had one or more comorbid anxiety disorder(s), including ten with generalized anxiety disorder, six with PTSD, seven with social phobia, and one with specific phobia.

CAR Analyses

Prior to completing the full analyses of CAR data, correlations between awakening cortisol measures on Day 1 and Day 2 were examined. The awakening, 30 min., and 60 min. post-awakening cortisol measures were significantly correlated across Day 1 and Day 2 (at awakening: r (55)=.55, p< .0001; 30 min. post awakening: r (55) =.68, p< .0001; 60 min. post awakening: r (54) =.60, p <.0001). Similar findings were found when correlations were repeated in depressed and healthy participants considered separately. Based on these results, mean values were determined for each participant using the data.
from both days, as shown in Table 3 below, and these values were used for computation of the AUCg and AUCi for each participant.

### Table 3: Mean Values for Awakening, 30 min., and 60 min. Post-Awakening Cortisol Measures in CMDD Subjects and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Awakening µg/dl</th>
<th>30 min. Post-awakening µg/dl</th>
<th>60 min. Post-awakening µg/dl</th>
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<tr>
<td>CMDD</td>
<td>.79 ± .33</td>
<td>.91 ± .42</td>
<td>.84 ± .41</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>.65 ± .36</td>
<td>.85 ± .37</td>
<td>.76 ± .33</td>
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</table>

**Examining the CAR in CMDD Participants Compared to Healthy Controls**

RANOVA revealed a significant change in cortisol during the first hour post-awakening ($F=8.5$, df 2, 51, $p = .001$). As shown in Figure 10 below, cortisol levels increased from awakening to the 30 min. time point in both groups and then declined toward the 60 min. time point. However, there was *no significant time by group* ($F=.34$; df=2, 51; $p=.71$), time by sex ($F=1.0$; df=2, 51; $p=.37$), or time by sex by group interaction ($F=.005$; df= 2, 51; $p=.995$). The main effects of group and sex were also not significant.
Group comparisons of cortisol AUCg and AUCi at awakening are illustrated in Figure 11 below. There was no significant difference in AUCg in individuals with CMDD (1.7 +/- 0.7 µg/dl) versus healthy controls (1.6 +/- 0.6 µg/dl, t (55) = .87, p= .39). There was also no difference in AUCi in CMDD versus healthy controls (.15 +/- .39 versus .27 +/- .46 µg/dl respectively, t (55) = -1.1, p= .29).
**Figure 11:** Mean (± SEM) AUCg and AUCi Cortisol at Awakening in CMDD Subjects vs. Healthy Controls

**Conclusion 1:**

*There were no group differences in the CAR in those with CMDD compared to healthy controls.*
Associations between Extraversion and Neuroticism and the CAR in CMDD Participants Compared to Healthy Controls

As would be expected, the CMDD participants had significantly lower extraversion scores (37.8 ± 8.9 versus 50.0 ± 10.5, t=-4.6, df=53, p<.000) and significantly higher mean neuroticism scores (68.7 ± 9.8 versus 49.4 ± 11.4, t=6.7, df=53, p<.000) than did the healthy controls. As noted previously, separate, stepwise linear regression analyses were conducted to determine whether extraversion and/or neuroticism were associated with the CAR. In each model, Step 1 included sex and HDRS scores, while Step 2 included personality (extraversion or neuroticism), group classification (CMDD participants versus healthy controls), and the respective personality by group interaction.

Interestingly, extraversion was significantly associated with AUCi (β = .286, t 2.2, p = .034), while none of the other variables (sex, HDRS, or extraversion by group) contributed significantly to the model. As is shown in Figure 12 below, there was a significant association between lower levels of extraversion and cortisol reactivity at awakening (AUCi) in both groups, but in the opposite direction of what was expected. Specifically, lower levels of extraversion were associated with lower cortisol reactivity upon awakening. There was no association between lower extraversion and AUCg.
Figure 12: Association between Extraversion and AUCi Cortisol at Awakening in CMDD Subjects and Healthy Controls

CMDD and Controls: $r^2 = .08$, $p < .05$
Consistent with Hypothesis #3, step-wise linear regressions revealed no significant association between neuroticism or the neuroticism by group interaction and the CAR (AUCg and AUCi). Figure 13, shown below, illustrates the lack of association between neuroticism and AUCi in both those with CMDD and healthy controls.

**Figure 13: Association between Neuroticism and AUCi Cortisol at Awakening in CMDD Subjects and Healthy Controls**

\[ r^2 = .038, p = .16 \]
Conclusion 2:

Unexpectedly, lower levels of extraversion were associated with decreased CARs (AUCi) in both the CMDD and control groups. As hypothesized, there was no significant association between neuroticism and the CAR in either those with CMDD or healthy controls.

5.4. Discussion

Study 3 examined group differences in the CAR in CMDD participants compared to healthy controls and the potential role of personality (e.g., extraversion and neuroticism) in shaping the CAR.

Contrary to Hypothesis #1, no significant difference was found in the CAR in participants with CMDD compared to healthy controls. This was an unexpected finding in light of literature that links elevated CARs to acute depression (Bhagwager et al., 2005, Vreeburg et al., 2009, 2010). In another study of CMDD, Watson et al. (2002) reported no difference in cortisol responses following either the DST or the Dex/CRH challenge in those with CMDD compared to healthy controls. Taking these various findings into consideration, it is concluded that basic physiological measures of the HPA axis are not elevated in CMDD, which contrasts most work in acute depression. However, sex differences in cortisol responses are evident in this population during a social stress challenge (Chopra et al., 2009 (Study 1)). This highlights the unique value of using social stress paradigms to assess cortisol responses in psychiatric populations. More specifically, the TSST may be better able to capture higher order central nervous system
(CNS) processes that impact the HPA axis. It is well established that negative cognitions and emotions are fundamental to the etiology and maintenance of depressive episodes.

Contrary to Hypothesis #2, lower levels of extraversion were associated with decreased cortisol reactivity to awakening in both those with CMDD and healthy controls, even after controlling for depression severity and sex. Higher cortisol reactivity upon awakening was expected based on findings in acutely depressed populations and the central dogma that higher cortisol levels are associated with psychopathology. However, there is a growing body of literature that links blunted cortisol responses, even upon awakening, with psychopathology such as PTSD, fibromyalgia, and chronic stress (Gaynes et al., 2009, Wessa et al., 2006, Chida and Steptoe, 2009). As one function of the CAR is believed to be the mobilization of energy to prepare an individual for the demands of the day (Clow et al., 2004, Pruessner et al. 1997, Kudielka and Wüst, 2010), a blunted CAR could suggest malfunction of this otherwise adaptive process.

Several limitations of this study merit consideration. To begin with the study had a modest sample size although it is consistent with several past studies in the field. A type II error cannot be ruled out, particularly if the differences between the two groups are subtle in nature. Another limitation was that data on waking time and sleep quality was incomplete. Although participants were provided with both verbal and written instructions, similar to prior studies of this kind, full compliance at home is never guaranteed. Any inconsistencies, however, are mitigated by the fact that there was consistency between awakening cortisol measures on Days 1 and 2. Electronic devices have been used in a few previous studies in an effort to track when participants removed the lid to access the cotton swab for cortisol sampling. However, these devices do not
assure the accuracy of the time of the first awakening sample, and indicate only that the subsequent samples were taken at the correct intervals.
CHAPTER 6

A CLOSER EXAMINATION OF CLINICAL AND SOCIAL FACTORS THAT MAY BE LINKED TO CORTISOL RESPONSES IN CMDD
6.1. Introduction

The first two studies of this project revealed that cortisol responses to social stress was different in those with CMDD as compared to healthy participants, and highlighted the important role of sex and personality (namely, lower extraversion) in moderating these effects. In both studies, several potential covariates including demographic variables (sex, age, and BMI) and key clinical variables (anxiety comorbidity and psychiatric medication use) were examined. It was found that none of these factors impacted the main study findings.

Given the number of factors that could impact cortisol responses to social stress (for review, see Kudielka et al., 2009) it was difficult to control for every potential covariate. However, to help guide future research in this area, this chapter explores how other pertinent clinical and social factors might impact cortisol responses to social stress in CMDD. Of the many variables found to be associated with cortisol responses in prior research, four stood out as having particular relevance to CMDD, those being childhood abuse, SSRI medication use, PTSD comorbidity, and depression subtype (atypical versus melancholic). The potential roles of these four variables in shaping cortisol responses in CMDD are described in the following sections.
6.2. Is a History of Childhood Abuse Associated with Cortisol Responses to Social Stress in CMDD?

A history of childhood abuse is a risk factor for depression and predisposes individuals to a chronic course of the illness (Brown and Moran, 1994). In addition, a history of childhood abuse has been associated with alterations in the HPA axis in both healthy and acutely depressed populations (Heim et al., 2000b, Newport et al., 2004). For example, Heim and colleagues (2000) compared responses to the TSST in women with and without a history of MDD and/or childhood abuse. They found that women with a history of childhood abuse exhibited increased pituitary and autonomic responses to the TSST compared to normal controls. This effect was particularly robust in women experiencing current symptoms of depression. This study suggests that acute depression and a history of trauma can have a synergistic effect in enhancing HPA reactivity and arousal mechanisms in MDD.

However, whether a history of childhood abuse is associated with cortisol responses in CMDD is unknown. As childhood abuse is highly prevalent in CMDD (Hovens et al., 2012), can individual differences in cortisol responses based on this variable be determined?

**Research question:**

Does a history of childhood abuse contribute to individual differences in cortisol responses in CMDD?

**Hypothesis:**

Childhood abuse measures **will** be associated with higher cortisol responses to the TSST.
**Method:**
Participants completed the Childhood Trauma Questionnaire (CTQ), a validated and reliable measure of childhood abuse (Bernstein *et al.*, 1994). The CTQ is a 28-item inventory that assesses self-reported experiences of abuse and neglect before age eighteen (Bernstein *et al.*, 1994). Items on the CTQ begin with the phrase: "When I was growing up," and are rated on a 5-point Likert scale. Individuals are determined to have none, low to moderate, moderate to severe, or severe to extreme levels of childhood abuse based on the cutoff scores summarized in Table 4 below.

**Table 4: Cutoff Scores for Childhood Abuse Subscales of the CTQ**

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>Low to Moderate</th>
<th>Moderate to Severe</th>
<th>Severe to Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual abuse</td>
<td>0–5</td>
<td>6,7</td>
<td>8–12</td>
<td>≥ 13</td>
</tr>
<tr>
<td>Physical abuse</td>
<td>0–7</td>
<td>8,9</td>
<td>10–12</td>
<td>≥ 13</td>
</tr>
<tr>
<td>Physical neglect</td>
<td>0–7</td>
<td>8,9</td>
<td>10–12</td>
<td>≥ 13</td>
</tr>
<tr>
<td>Emotional abuse</td>
<td>0–8</td>
<td>9–12</td>
<td>13–15</td>
<td>≥16</td>
</tr>
<tr>
<td>Emotional neglect</td>
<td>0–9</td>
<td>10–14</td>
<td>15–17</td>
<td>≥ 18</td>
</tr>
</tbody>
</table>
**Statistical Analyses:**

Pearson correlations were computed to examine associations between the mean CTQ scores and mean CTQ subscale scores and cortisol responses (AUCg and AUCi) during the TSST in CMDD.

**Results:**

a) **Childhood Abuse Scores in the CMDD Group**

In total, out of forty-eight participants who completed the CTQ, forty-three suffered at least mild to moderate abuse as a child. Furthermore, thirty-four of the participants suffered from abuse that was of moderate or greater severity. As summarized below in Table 5, the mean scores for CMDD participants indicated at least low to moderate degrees of childhood abuse on each of the subscales. CMDD participants scored the highest on the emotional abuse and emotional neglect scales, with moderate to severe levels of abuse on these subscales.
Table 5: Mean Childhood Abuse Scores in CMDD Participants

<table>
<thead>
<tr>
<th></th>
<th>CMDD (n=48) (mean ± SD)</th>
<th>Females (n=26) (mean ± SD)</th>
<th>Males (n=22) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CTQ</strong>(^{*})</td>
<td>60.7 ± 16.9 (n=45)</td>
<td>58.7 ± 16.6 (n=23)</td>
<td>62.8 ± 17.4 (n=22)</td>
</tr>
<tr>
<td>Sexual Abuse</td>
<td>7.8 ± 4.8 (n=46)</td>
<td>8.7 ± 5.6 (n=24)</td>
<td>6.8 ± 3.6 (n=22)</td>
</tr>
<tr>
<td>Physical Abuse</td>
<td>9.7 ± 5.1 (n=48)</td>
<td>8.9 ± 4.4 (n=26)</td>
<td>10.6 ± 5.7 (n=22)</td>
</tr>
<tr>
<td>Physical Neglect</td>
<td>8.5 ± 3.7 (n=48)</td>
<td>8.0 ± 3.3 (n=26)</td>
<td>9.2 ± 4.1 (n=22)</td>
</tr>
<tr>
<td>Emotional Abuse</td>
<td>12.8 ± 5.6 (48)</td>
<td>11.5 ± 4.9 (n=26)</td>
<td>14.2 ± 6.0 (n=22)</td>
</tr>
<tr>
<td>Emotional Neglect</td>
<td>14.8 ± 5.3 (n=47)</td>
<td>13.5 ± 4.9 (n=25)</td>
<td>16.2 ± 5.5 (n=22)</td>
</tr>
</tbody>
</table>

* 3 subjects (3/51) were missing complete CTQ data resulting in n=48. In addition, two participants were missing data for the sexual abuse subscales and one participant did not complete the emotional neglect subscale. One subject was not included in the analyses based on outlying scores.
b) Correlations between Childhood Abuse Scores and Cortisol Responses during the TSST

Table 6, shown below, summarizes correlations between the CTQ scores and cortisol responses to the TSST in CMDD subjects. There was no significant correlation between cortisol responses (AUCg and AUCi) and mean CTQ scores. There was also no correlation between cortisol responses and the mean CTQ subscales scores (e.g., sexual abuse, physical abuse, physical neglect, emotional abuse, and emotional neglect subscales). Further, there was no association between mean CTQ scores and cortisol responses (AUCg and AUCi) in female and male CMDD participants considered separately.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>AUCi (µg/dl)</th>
<th>AUCg (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10'</td>
<td>(47) 0.10</td>
<td>(48) 0.12</td>
</tr>
<tr>
<td>60'</td>
<td>(47) 0.14</td>
<td>(48) 0.19</td>
</tr>
<tr>
<td>1'</td>
<td>(47) 0.16</td>
<td>(48) 0.13</td>
</tr>
<tr>
<td>2'</td>
<td>(47) 0.18</td>
<td>(48) 0.20</td>
</tr>
<tr>
<td>3'</td>
<td>(47) 0.21</td>
<td>(48) 0.23</td>
</tr>
</tbody>
</table>

Table 6: Correlation of Cortisol Responses to the TSST with Mean CTQ scores and Mean CTQ subscale scores in CMDD.

* Differences in number (n) reflect missing data.
Discussion:

Unexpectedly, childhood abuse was not associated with cortisol responses to social stress. In interpreting this negative finding, it is noteworthy that 90 percent of CMDD participants had experienced at least a mild form of childhood abuse while 71 percent had experienced moderate to severe childhood abuse. It may be that these very high rates of childhood abuse in CMDD make it difficult to statistically determine the independent effects of this factor on cortisol responses. A study comparing cortisol responses in CMDD participants with and without childhood abuse may be needed; however, it would be a challenge to find a CMDD group without childhood abuse. Longitudinal studies, ideally beginning in childhood, would also assist in examining the interplay between childhood abuse, cortisol responses, and depressive illness.

6.3. Are the Cortisol Responses to Social Stress Different for those with CMDD who are on SSRIs from those who are Unmedicated?

The monoamine hypothesis of depression attributes depressive symptomatology to a lack of monoamines in various brain regions. SSRI medications are believed to exert their therapeutic effects by increasing levels and synaptic effects of monoamines, particularly serotonin.

Antidepressant effects may also arise from normalization of the HPA axis (Holsboer, 2000, Pariante and Miller, 2001, Barden et al., 2005). Studies have found that antidepressant medications enhance the expression and function of the glucocorticoid receptor (GR), a key regulator of the negative feedback of the HPA axis (Holsboer, 2000,
Pariante and Miller, 2001, Pariante et al., 2004). The precise mechanism underlying improved negative feedback is unknown. However, molecular mechanisms such as antidepressant-induced GR activation and translocation from the cytoplasm to the nucleus have been implicated (Funato et al., 2006, Pariante et al., 2012). This section specifically examined the association between SSRIs and cortisol responses, given that different antidepressant classes may have specific effects on GR receptor function and HPA activity (Funato et al., 2006).

**Research question:**

Do cortisol responses to the TSST differ in CMDD individuals treated with SSRIs from those not on psychotropic medications?

**Hypothesis:**

Given that SSRI treatment has been found to increase GR negative feedback, it was hypothesized that SSRI treatment will be associated with lower cortisol responses to the TSST in CMDD.
**Method:**

CMDD participants on psychotropic medications had to be on stable doses for at least one month before entering the study to limit the potential effects of dose changes on cortisol responses. T tests were computed to compare cortisol responses in CMDD participants receiving SSRI treatment (either alone or in combination with another psychotropic medication) with CMDD participants not on psychotropic medications.

**Results:**

a) **SSRIs and Psychotropic Medication Usage in CMDD Participants**

Table 7, below, summarizes psychotropic medication usage in CMDD participants. As shown in the table, thirty-six out of fifty-one CMDD participants were on one or more antidepressant medications. Eighteen participants were taking an SSRI either alone (12/18) or in combination with another psychotropic medication (three were on benzodiazepines, two were on Welbutrin, and one was on trazodone).
Table 7: Psychotropic Medication Usage in CMDD Subjects

<table>
<thead>
<tr>
<th>Medication Type</th>
<th>CMDD n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychotropic Medications (yes/total)</td>
<td>36/51</td>
</tr>
<tr>
<td>SSRIs (yes/total)</td>
<td>18/51</td>
</tr>
<tr>
<td>SNRIs (yes/total)</td>
<td>12/51</td>
</tr>
<tr>
<td>Bupropion (yes/total)</td>
<td>5/51</td>
</tr>
<tr>
<td>Atypical Antipsychotics (yes/total)</td>
<td>4/51</td>
</tr>
<tr>
<td>Benzodiazepines (yes/total)</td>
<td>7/51</td>
</tr>
<tr>
<td>Trazodone (yes/total)</td>
<td>1/51</td>
</tr>
</tbody>
</table>

b) SSRI Medication Usage (yes/no) and Cortisol Responses to the TSST

As reported previously in this project (see results of Study 1 and 2), there were no significant differences in cortisol responses to the TSST in CMDD participants on psychotropic medications compared to those not on medications.

The current analyses focused on whether SSRI medication use (alone or in combination with another psychotropic medication) was associated with cortisol responses. As summarized below in Table 8, CMDD participants on SSRIs had a significantly lower AUCg than those not on psychotropic medications. Table 9, below, illustrates that there was no significant difference in AUCi in SSRI-treated CMDD
participants compared to those not on psychotropic medications. The analyses were repeated excluding the six out of the eighteen participants on SSRIs who were taking other psychotropic medications. A similar pattern of results were found. CMDD participants who were only on SSRI medication had reduced AUCg cortisol compared to those not on psychotropic medications (.66 ± .31µg/dl versus .91 ± .28µg/dl, t= - 2.1, df=25, p<.05, respectively). There remained no association between AUCi cortisol and SSRI medication usage in CMDD participants who were on SSRIs only compared to those not on psychotropic medications.

As SNRI medications overlap in mechanism with SSRIs, a final analysis was conducted to determine whether cortisol responses differed in CMDD participants on either SSRIs or SNRIs (alone or in combination with other psychotropic medications) compared to those not on medications. A similar pattern emerged with CMDD participants on either SSRIs or SNRIs having a significantly lower AUCg cortisol compared to unmedicated CMDD participants (see Table 8). There was no significant difference in AUCi in CMDD individuals on SSRIs or SNRIs compared to those not on medications (see Table 9).
<table>
<thead>
<tr>
<th></th>
<th>SSRI or SNRI-treated (n=43)</th>
<th>Unmedicated (n=2.1)</th>
<th>67 ± 38 vs. 66 ± 32 (t=31, df=31, p&lt;.04)</th>
<th>AUCg (log transformed) mean ± SD (µg/dl)</th>
<th>Unmedicated-CMDD patients vs. SSRI/SNRI-treated-CMDD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>04'</td>
<td>2.1</td>
<td>1.9</td>
<td>1.9 ± 1.1</td>
<td>67 ± 38 vs. 66 ± 32 (t=31, df=31, p&lt;.04)</td>
<td>Unmedicated-CMDD patients vs. SSRI/SNRI-treated-CMDD patients</td>
</tr>
</tbody>
</table>

Table 8: Comparison of AUCg Cortisol during the TSST in CMDD Subjects Treated with SSRI/SNRI/S with those not on Psychotropic Medications.
<table>
<thead>
<tr>
<th>p</th>
<th>43</th>
<th>63</th>
<th>82 ± .24 vs. 87 ± .30</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>31</td>
<td>1.3</td>
<td>75 ± .25 vs. 87 ± .30</td>
</tr>
</tbody>
</table>

Table 9: Comparison of AUCi CORTisol during the TSST in CMDD Subjects Treated with SSRI/SNRIs with those not on Psychotropic Medications
**Discussion:**

As hypothesized, CMDD participants on SSRI medications had significantly lower AUCg cortisol during the TSST compared to those not on psychotropic medications. However, SSRI treatment in CMDD was not associated with AUCi cortisol.

It is believed that antidepressant-induced change in HPA activity is one mechanism through which therapeutic effects is mediated. In CMDD, SSRIIs/SNRIs are inducing the expected decrease in overall HPA activity as defined by AUCg cortisol. However, this change is not associated with treatment response. Could this point to a unique biology in CMDD as compared to more acute forms of depression? Might alternative treatment strategies be needed for individuals with CMDD (e.g., medication strategies that do not target GR)?

Based on the association between SSRI/SNRI medication usage and decreased AUCg cortisol during the TSST, a re-analysis of AUCg cortisol findings was conducted for Studies 1 and 2. In the subsample of Study 1, after controlling for SSRI/SNRI medication use (yes/no), the sex by condition interaction remained significant in predicting cortisol responses during the TSST. Furthermore, females with CMDD continued to have a greater AUCg cortisol during the TSST as compared to female controls. In Study 2, after controlling for SSRI/SNRI medication usage (yes/no), there remained no significant association between neuroticism and extraversion and AUCg cortisol in CMDD participants compared to healthy controls. Therefore, while SSRI/SNRI usage did significantly decrease AUCg in CMDD participants, it did not have a major impact on AUCg findings reported in Study 1 and 2 of this project.
6.4. Is PTSD Comorbidity Associated with Individual Differences in Cortisol Stress Responses to Social Stress in CMDD?

PTSD is a severe and disabling illness and a common comorbidity in CMDD. Individuals suffering from PTSD experience three distinct, but co-occurring symptom clusters. First, patients with PTSD have re-experiencing symptoms, such as flashbacks and nightmares of the traumatic event. The second feature of PTSD is severe avoidance of any reminders (persons, places, or things) of the traumatic event. The third feature is hyperarousal symptoms, such as anxiety and an increased startle response. In the DSM-IV-TR, these symptoms must be associated with functional impairment and continue for at least one month following the trauma to meet criteria for PTSD.

Research has revealed that the HPA profiles are different in PTSD than in depressed populations (for review, see Yehuda, 2009). In general, PTSD has been associated with lowered urinary and plasma cortisol as compared to the higher levels usually reported in depression. Furthermore, PTSD is associated with increased suppression to dexamethasone which also differs from findings in depression.

Research question:
Is PTSD comorbidity in CMDD associated with individual differences in cortisol responses to the TSST?
Hypothesis:
Given findings of decreased cortisol levels in PTSD, it was hypothesized that CMDD participants with comorbid PTSD will have a blunted cortisol stress response to the TSST.

Method:
PTSD was diagnosed using the SCID-I/P (First et al., 1995). A series of t tests were computed to determine differences in cortisol responses to the TSST in CMDD subjects with or without comorbid PTSD.

Results:
Eleven out of fifty CMDD participants had comorbid PTSD. T test revealed no significant difference in AUCg cortisol in CMDD participants with or without PTSD comorbidity (.83 ± .27µg/dl versus .72 ± .43µg/dl, t = -.81, df = 48, p = .42, respectively). Similarly, there was no difference in AUCi cortisol in CMDD participants with PTSD compared to those without PTSD (.85 ± .26µg/dl versus .77 ± .29µg/dl, t = 0.9, df = 48, p = .37, respectively).

Discussion:
Contrary to our hypothesis, PTSD comorbidity was not associated with blunted cortisol responses to the TSST in CMDD. In general, lower cortisol levels have been associated with PTSD, although less is known about cortisol responses to social stress in
these populations. In a review by Yehuda et al. (2009), it was noted that the presence of lower baseline cortisol levels in PTSD does not necessarily imply that these individuals will have blunted neuroendocrine responses to psychosocial challenges. In fact, in PTSD higher neuroendocrine responses to psychosocial challenges have been reported (Liberzon et al., 1999, Elzinga et al., 2003). Therefore, it may be that individuals with PTSD have blunted basal levels as compared to depressed populations but have similar cortisol responses to social stress. It may also be that cortisol responses differ in pure PTSD versus PTSD comorbid with chronic depressive illness, but this has not been explored to date.

6.5. Do Cortisol Responses to Social Stress Differ Between Atypical and Melancholic Forms of Depression?

The DSM-IV-TR defines two major depressive subtypes, namely atypical and melancholic depression. Atypical depression is characterized by rejection sensitivity, leaden paralyses, and reversed vegetative symptoms (e.g., overeating and oversleeping). In contrast, melancholic depression is typified by decreased sleep, diminished appetite, and a profound lack of interest and enjoyment in daily activities. Twenty-five to 30 percent of depressed patients present with melancholic depression and 15 to 30 percent present with atypical depression (Gold and Chrousos, 2002). However, in chronic forms of depression, there are much higher rates of atypical than melancholic depression (Stewart et al., 1993, Matza et al., 2003).
Research has demonstrated that cortisol responses and HPA activity differ in atypical and melancholic forms of depression. Most studies of melancholic depression support the hypothesis that HPA activity is increased (Lightman, 2008, Stetler and Miller, 2011, O'Keane et al., 2012). In atypical depression, a unique pattern of decreased HPA activity has been found. For example, O'Keane et al. (2005) found that chronically depressed individuals with atypical features had increased ACTH responses to the CRH challenge. Levitan et al. (2002), found that individuals with atypical depression had increased cortisol suppression to the low dose DST compared to healthy controls. The findings by O'Keene et al. and Levitan et al. are indicative of decreased HPA activity and are in contrast to prior work in melancholic depression (Stetler and Miller, 2011). The unique HPA profiles in melancholic and atypical depression are believed to be central in origin. Higher CRF levels centrally in melancholic depression result in higher cortisol release from the adrenal gland, whereas CRF deficiency in atypical depression leads to the opposite pattern (Gold and Chrousos, 2002).

**Research question:**

Does the depressive subtype (namely, atypical and melancholic depression) contribute to individual differences in cortisol responses in CMDD?

**Hypothesis:**

As atypical depression has been associated with decreased HPA activity, it was hypothesized that among individuals with CMDD, those with atypical depression will have lower cortisol responses to the TSST than those with melancholic depression.
Method:

Atypical and melancholic depression were diagnosed using the SCID-I/P (First et al., 1995). A series of t tests were computed to determine differences in cortisol responses to the TSST in individuals with atypical depression as compared to melancholic depression.

Results:

a) Rates of Depressive Subtype in CMDD

Table 10 summarizes the frequencies of melancholic, atypical, and the neither subtype (that is, criteria for melancholic or atypical depression were not met) in CMDD. As expected, there were much higher rates of atypical depression than melancholic depression in CMDD, consistent with prior literature (Stewart et al., 1993, Matza et al., 2003).
### Table 10: Frequency of Depressive Subtypes in CMDD

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Depression</td>
<td>28</td>
<td>54.9</td>
</tr>
<tr>
<td>Melancholic Depression</td>
<td>9</td>
<td>17.6</td>
</tr>
<tr>
<td>Neither Subtype *</td>
<td>11</td>
<td>21.6</td>
</tr>
<tr>
<td>Missing data</td>
<td>3</td>
<td>5.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>51</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

* criteria for atypical or melancholic depression not met

b) Cortisol responses in Atypical, Melancholic, and Neither Subtypes in CMDD

Tables 11 and 12 summarize cortisol AUCg and AUCi during the TSST in the atypical, melancholic, and neither subtype groups in CMDD. As shown, there was no significant difference in cortisol AUCg and AUCi during the TSST among the three groups.
### Table 1: Comparison of AUCg Cortisol during the TSST in Atypical, Melancholic, and Neither Subtype in CMDD

<table>
<thead>
<tr>
<th>Subtype Comparison</th>
<th>AUCg (µg/dl)</th>
<th>Mean ± SD</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical vs. Neither Subtype</td>
<td>.80 ± .40 vs. .69 ± .55</td>
<td>.73 ± .36 vs. .69 ± .55</td>
<td>37</td>
<td>.03</td>
</tr>
<tr>
<td>Melancholic vs. Neither Subtype</td>
<td>.49</td>
<td>.23</td>
<td>35</td>
<td>.62</td>
</tr>
</tbody>
</table>

Note: AUCg values are log-transformed means ± SD.
<table>
<thead>
<tr>
<th></th>
<th>18</th>
<th>3.3</th>
<th>89.8 ± 28.8 vs. 34.3 ± 28.8</th>
<th>Melancholic vs. Neither Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>37</td>
<td>5.8</td>
<td>79.2 ± 28.2 vs. 85.7 ± 28.7</td>
<td>Atypical vs. Neither Subtype</td>
</tr>
<tr>
<td>56</td>
<td>25</td>
<td>5.2</td>
<td>79.2 ± 28.2 vs. 69.8 ± 24.2</td>
<td>Atypical vs. Melancholic</td>
</tr>
<tr>
<td>62</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>d</td>
<td>1</td>
<td>(log-transformed) mean ± SD</td>
<td>AUCI (μg/dl)</td>
</tr>
</tbody>
</table>

Table 12: Comparison of AUCI Cortisol during the TSST in Atypical, Melancholic, and Neither Subtype in CMD.
**Discussion:**

There were higher rates of atypical (28/51) versus melancholic depression (9/51) in the CMDD group, which is consistent with past literature. Contrary to the hypothesis, there was no significant difference in cortisol responses to the TSST between the two depressive subtypes. There was also no difference in cortisol response between CMDD participants with atypical and melancholic depression and those who did not meet criteria for either subtype.

If these preliminary findings are replicated, what are the potential explanations? One explanation (similar to that mentioned for PTSD above) is that differences in cortisol responses between these two depressive subtypes might be dependent on the methodology used. In other words, while differences in diurnal cortisol levels and cortisol responses to the DST have been demonstrated in atypical versus melancholic depression (Gold and Chrousos, 2002), these differences may not be relevant when using social stress paradigms. Alternatively, it may be that differences in cortisol responses in atypical versus melancholic depression are less pronounced in more chronic forms of depression.

In summary, depressive subtype was not associated with cortisol responses to social stress in CMDD. Given the high rates of atypical depression in CMDD and the prior association found between atypical depression and lowered HPA activity, it would be important to re-examine this negative finding in the CMDD population in larger studies.
6.6. Overall Summary

There are numerous variables that could potentially be associated with cortisol responses in any given population. Out of the many variables, four stood out as having particular relevance to CMDD. This chapter was an exploratory examination of how a history of childhood abuse, SSRI treatment, PTSD comorbidity, and depressive subtype (namely, atypical and melancholic depression) were associated with cortisol responses to social stress in CMDD. The goal was that findings from this examination could inform future work in CMDD.

Several important findings were revealed, some surprising. As expected, SSRI usage in CMDD was found to be associated with decreased AUCg cortisol. This suggests that although SSRIs are able to lower total cortisol output, this effect does not result in treatment response in CMDD, hence these individuals remain symptomatic. Future studies are needed to better understand whether therapeutic mechanisms of SSRIs differ in acute and chronic depression. A second finding was the extremely high rate of childhood abuse in CMDD, consistent with the literature. This finding in conjunction with the high rate of PTSD, points to trauma being a primary feature of CMDD. However, neither childhood abuse nor PTSD was associated with cortisol responses in the CMDD group. It may be that these very high rates of childhood abuse in CMDD make it difficult to statistically determine the independent effects of this factor on cortisol responses. Finally, contrary to expectations, there was no difference in cortisol responses to the TSST in atypical compared to melancholic depression.
In conclusion, the work described in this chapter added another dimension to the understanding of cortisol responses to social stress in CMDD. Based on these results, it is apparent that cortisol findings in the general depression literature are *not indicative* of cortisol findings in CMDD. Furthermore, cortisol responses using a social stress challenge appear to differ from prior findings using other measures of HPA function (e.g., diurnal and DST).

There was a general lack of association between the variables examined in this chapter and cortisol responses. Given the number of covariates examined in this chapter and in Studies 1 and 2, the main findings of this project appear to be highly significant. Taken as a whole, *sex differences and lower levels of extraversion appear to be two key drivers of cortisol responses to social stress in CMDD.*
CHAPTER 7

GENERAL DISCUSSION OF THESIS
7.1. Introduction

CMDD is a common and debilitating psychiatric illness, with a lifetime prevalence of over 6 percent (Howland, 1993, Kessler et al., 1994, Keller et al., 1998, Gilmer et al., 2005). Despite the significant morbidity and mortality associated with CMDD, few studies have examined its biological underpinnings, an essential step to developing targeted treatment regimens for those who suffer from this illness.

There is robust evidence linking stress and depressive illness. Stressful life events can precipitate depressive episodes (Hammen, 2005). Seventy percent of initial depressive episodes are preceded by a stressor (Monroe and Harkness, 2005). Alterations in stress physiology, specifically HPA activity, are well documented in depression. As cortisol is the most commonly studied stress hormone within this axis, cortisol responses was chosen as the biological marker in this study of CMDD.

Personality dimensions, such as higher levels of neuroticism and lower levels of extraversion, are associated with chronic depressive illness and have been linked to altered cortisol responses. It follows that these personality dimensions may then have moderating effects on the relationship between chronic depression and cortisol responses.

To gain a better understanding of the underlying psychobiology of CMDD, three studies were conducted that examined the interplay between chronic depression, cortisol responses, and the personality dimensions of neuroticism and extraversion. This interplay is illustrated below in Figure 14 in the Conceptual Model (this figure is the same as Figure 5 on page 49, but has been included again here for the benefit of the reader).
Figure 14: Conceptual Model for Thesis: the interplay between CMDD, cortisol responses, and personality

CHRONIC MAJOR DEPRESSIVE DISORDER  Question 1  CORTISOL RESPONSES

Question 2

HIGH NEUROTICISM
LOW EXTRAVERSION
**Summary of Major Findings:**

a) **Study 1:**

In Study 1, cortisol responses to the TSST were examined in CMDD participants compared to healthy controls. It was hypothesized that individuals with CMDD would have greater cortisol responses to the TSST. Overall, the results support the notion that an association exists between CMDD and cortisol responses, as represented by Question 1 of the Conceptual Model shown above. Consistent with the hypothesis, females with CMDD had greater cortisol output during the TSST as compared to female controls. However, males with CMDD had decreased cortisol stress responses compared to male controls. These results suggest that cortisol responses to social stress are altered in CMDD but with fundamentally different changes in each sex.

b) **Study 2:**

Many factors besides sex can contribute to individual differences in cortisol responses in CMDD. In an effort to further understand cortisol responses in CMDD, and to identify distinct cortisol response profiles in this heterogeneous population, focus was placed on the effects of the personality dimensions of neuroticism and extraversion. These dimensions were selected for two reasons, namely that they are strongly associated with CMDD and that there has been evidence linking them to cortisol responses. It was hypothesized that higher levels of neuroticism and/or lower levels of extraversion would contribute to greater cortisol responses to the TSST in those with CMDD compared to healthy controls.
Contrary to this hypothesis, no association between neuroticism and cortisol responses was found. However, as postulated, comparatively lower levels of extraversion were associated with increased cortisol reactivity in those with CMDD but were not so in healthy controls. These findings address Question 2 of the Conceptual Model shown above, suggesting that lower extraversion does moderate the relationship between CMDD and cortisol responses. These results also raise the possibility that lower extraversion is a unique vulnerability marker and potential treatment target in CMDD.

c) Study 3:

Study 3 examined the CAR in CMDD. Based on the fact that acute depression has been associated with an increased CAR, it was hypothesized that the CAR would be greater in those with CMDD than in healthy controls.

Contrary to this hypothesis, no significant difference was found in the CAR between CMDD participants and healthy controls. In light of Study 1 results demonstrating an association between cortisol responses to the TSST and CMDD, one might postulate that a social stress paradigm taps into the psychobiology of CMDD to a greater extent than do other measures of cortisol.

Given the association between lower levels of extraversion and higher cortisol responses to the TSST in CMDD participants found in Study 2, it was also hypothesized that lower levels of extraversion would predict a higher CAR in those with CMDD. Surprisingly, while extraversion did significantly associate with cortisol reactivity, the direction of this relationship was opposite to expectations. Specifically, lower extraversion predicted a lower CAR across both the CMDD population and healthy
controls. Going back to the Conceptual Model shown above, there was no unique relationship between CMDD and cortisol responses as measured by the CAR. Furthermore, although lower extraversion is associated with the CAR, it does not moderate the relationship between the CAR and CMDD, as the association is the same across CMDD and healthy control groups.

7.2. Relevance of Study Findings

The current findings may have relevance to future biological and treatment studies in CMDD as follows:

1. Biological studies, specifically those related to cortisol responses, can be conducted in CMDD

   The inherent complexity and heterogeneity of CMDD explain why few biological studies have been conducted to date. However, leading national and international institutions, such as the NIMH, have highlighted the need to conduct naturalistic studies on individuals with complex psychiatric disorders. In the recent, large, NIMH-funded STAR*D trial, treatment response was lower in a naturalistic, depressed sample compared to those reported in narrowly defined, less heterogeneous depressed populations (Gaynes et al., 2009). In the STAR*D study, comorbidity in depressive disorders was the norm and the authors emphasized the need to study “real life” patients. In particular, it was noted that there is a dearth of knowledge on chronic and treatment-resistant forms of depression (Gaynes et al., 2009).
In keeping with the needs stated above, this project begins to tackle this complex and heterogeneous population using a naturalistic sample. Despite the challenges inherent to this approach, sex differences were observed in cortisol responses in those with CMDD compared to healthy controls. Furthermore, strong links were found between extraversion and cortisol reactivity. **These findings offer strong initial support that biological research can be conducted in this population.** More specifically, the results support the use of cortisol responses in the context of a social stressor, as well as dimensional measures of personality, as one means to define biologically distinct profiles in CMDD.

2. **The choice of test to measure cortisol responses matters**

   While there may be several tests to measure cortisol responses, the results of this project suggest that using a social challenge may uncover differences in cortisol responses in CMDD that are not evident when using other tests. Study 1 revealed sex differences in cortisol responses to the TSST in those with CMDD compared to healthy controls. In contrast to this, a previous study by Watson *et al.* (2000) using pharmacological challenges (namely, the DST and Dex/CRH tests) did not find alterations in cortisol reactivity in those with CMDD compared to healthy controls. It would appear that the TSST taps into higher order emotional and cognitive pathways that may underlie the deficits in interpersonal functioning so characteristic of those suffering from CMDD. These same pathways may not be directly assessed by other measures of HPA function. *It can therefore be concluded that social stress reactivity is a valuable measure of HPA function, particularly where emotional and cognitive processes are believed to play a key role in the etiology and maintenance of the illness.*
While the TSST is highly useful in research settings, it is not practical for use in daily clinical practice. Furthermore, longitudinal studies may be problematic when using the TSST given the critical role of novelty in eliciting cortisol release. Previous studies have demonstrated that the cortisol responses to the TSST decreases with repeated administration (Kirschbaum et al., 1995, Wust et al., 2005). Given the limitations of the TSST, another goal of this project was to assess the utility and practicality of the CAR in CMDD with a view to use this measure in future longitudinal studies.

Though highly preliminary and based on a modest sample size, the results of this project, described in Study 3, suggest that assessing cortisol responses using the CAR can be informative given the significant association found between lower extraversion and a blunted CAR across all study participants. Whether the CAR can demonstrate unique vulnerabilities in the CMDD population will require further study of a larger population.

3. Both increased and blunted cortisol responses should be considered when studying the interplay between chronic depression, cortisol responses, and personality.

Although the central dogma in the literature is that depression is associated with higher levels of cortisol, findings of both blunted and increased cortisol responses were found in CMDD as compared to healthy controls in this project. Study 1 showed that while females with CMDD had greater cortisol output during the TSST, consistent with the current dogma, males with CMDD unexpectedly demonstrated blunted cortisol responses to the TSST. In Study 2, lower levels of extraversion in the CMDD population were associated with increased cortisol responses to the TSST. This suggests that the
relationship between cortisol responses and CMDD should include both heightened and blunted cortisol responses as potential biological markers.

The finding of blunted cortisol responses in males during the TSST in Study 1 is in keeping with a growing body of literature that has found associations between blunted cortisol responses and psychopathology (Heim et al., 2000a, Wessa et al., 2006, Chida and Steptoe, 2009). One theory is that cortisol responses changes from being sensitized to hypoactive in the transition from acute to chronic illness (Miller et al., 2007). Another possibility is that blunted cortisol responses are a vulnerability factor for the development of stress-related illnesses.

4. Identification of psychobiological markers in CMDD may assist in the development of targeted management strategies for this difficult-to-treat population

In examining the interplay of CMDD, cortisol responses, and personality, the following unique psychobiological profiles within a heterogeneous CMDD population were identified:

1) Males with CMDD had blunted cortisol reactivity to a social stressor.
2) Females with CMDD had greater cortisol output during the social stressor.
3) CMDD individuals with lower levels of extraversion had increased cortisol reactivity to a social stressor.
Defining novel and empirically defined psychobiological profiles raises the prospects of developing more targeted management strategies in this difficult-to-treat population. For example, Study 2 found that CMDD individuals with lower levels of extraversion had greater cortisol reactivity. It is known that increased social connections, development of relationships, and engagement in activities are proven psychological treatment strategies for CMDD (Keller et al., 2000, Browne et al., 2002, Schramm et al., 2008). Based on this knowledge, it would be valuable to progress to the next level and determine whether the CMDD individuals with lower extraversion scores derive greater benefit from specific treatment approaches. For example, does psychological treatment approaches geared at increasing aspects of extraversion (e.g., increased social connections and increased activity level) improve outcomes in CMDD individuals with lower levels of extraversion? Furthermore, could this beneficial effect be mediated through changes in cortisol responses?

7.3. Study Limitations

a) Cross-Sectional Study:

There are several limitations of this project that merit consideration. First, the cross-sectional nature of this study limits the ability to establish causal relationships. It is not known whether findings in this project (e.g., blunted cortisol responses in males with CMDD) are evident in these individuals prior to the onset of illness and represent a vulnerability factor for chronic depression. Second, it is plausible that cortisol responses changes from being sensitized to hypoactive in the transition from acute to chronic
depression. Ultimately, studies comparing acute and chronic depression directly are needed, as well as longitudinal studies examining how the relationships between personality (e.g., lower levels of extraversion), cortisol responses, and depression evolve over time.

b) **Psychotropic Medication Use:**

Another important limitation of this project was the inability to study a drug-free CMDD population. Antidepressant medications can influence HPA function and normalize the HPA changes found in acutely depressed individuals (Nickel *et al.*, 2003). Ideally, it would be preferable to study a drug-free population, but for practical and ethical reasons this was not possible. As well, consideration was given to the possibility that the actual process itself of discontinuing long-term medications in CMDD participants could have a major effect on cortisol responses that would confound study results. Furthermore, this study aimed to better understand the psychobiology of a naturalistic CMDD population, given the need for studies of this kind. Studying a drug-free CMDD population would not be as “naturalistic,” as most CMDD patients are treated with psychotropic medications.

In the total sample, CMDD participants on SSRI or SNRI medications had lower AUCg compared to those not on psychotropic medications. Nevertheless, there are reasons to conclude that medication use did not have a major impact on the main findings of this project. First, medication usage was controlled for in the statistical analyses. Second, medication usage, including SSRI usage, was not a significant predictor of cortisol AUCi (cortisol reactivity) to the TSST or to the CAR test. Nevertheless, the
mechanism by which psychotropic medications impact cortisol responses and how this relates to treatment response in CMDD and acute depression requires further elucidation.

c) **Comorbidity and Heterogeneity:**

As expected, there was significant heterogeneity and comorbidity in this project’s naturalistic study population, which is often mentioned as a reason to avoid biological studies in complex patients. CMDD, as compared to acute forms of depression, has high rates of comorbidity with other psychiatric illnesses, such as anxiety disorders (including PTSD), personality disorders, and substance use disorders. High rates of comorbidity lead to many methodological and research challenges. One of the known limitations of the DSM is that the diagnoses are not etiologically based, rather have been developed based on clusters of symptoms. Due to the overlap of these symptom clusters in psychiatric illness, multiple diagnoses become inevitable. This limitation raises two issues. First, it can be difficult to determine what the primary diagnosis in these complex individuals is. Second, it is probable that diagnoses with overlapping symptom profiles, as defined in the DSM, (e.g., MDD and generalized anxiety disorder) may, in fact, have similar biological underpinnings.

Another challenge is that the comorbid disorders in CMDD have also been independently associated with alterations in the HPA axis. For example, PTSD is associated with decreased baseline cortisol and hypersuppression to the TSST (Yehuda, 2009). This is contrary to findings of increased baseline cortisol levels and non-suppression to the TSST that is commonly reported in depressed populations. In this project, PTSD comorbidity was not associated with cortisol responses to the TSST. It is
possible that the sample was not large enough to independently assess the role of this comorbidity on HPA function in CMDD.

In summary, comorbid conditions are commonly found in CMDD and can present methodological challenges. However, as multiple comorbidities amongst chronically depressed patients are the norm rather than the exception, it may be reasonable to expect to see biological patterns in CMDD despite it being a complex population.

d) Generalizability:

This project examined cortisol responses in a highly complex population of CMDD. It should be noted that the majority of participants were recruited from the Centre for Addiction and Mental Health, a tertiary care centre in Toronto, Canada. It is possible that this population is more severe than chronically depressed patients treated in community settings. Also, information about treatment resistance was not obtained from participants in this study, although it is likely that many were treatment resistant given the nature of patients seen at this centre. Whether the results of this project generalize to community or treatment resistant populations needs further elucidation.

Understanding the generalizability of this project’s results would also be enhanced by examining cortisol responses in other depressed populations. This will be the approach used in the Alternative Inpatient Milieu (AIM) study (discussed in 7.4.2.) which will examine the cortisol awakening response in both unipolar and bipolar depression, as well as acute and chronic depression.
7.4. Future Directions

7.4.1. Overview

The goal of this project was to define novel psychobiological profiles within a heterogeneous CMDD population which could, in turn, lead to more targeted management for this highly disabling illness. It is likely that the mechanisms underlying CMDD and depression involve a complex interplay among a host of biopsychosocial factors, including altered cortisol responses and personality. Moving forward, how can the current understanding of mechanisms underlying CMDD be built upon to ultimately improve treatment for this disabling illness?

In answer to this question, one must look at future research directions. *In the short-term (ongoing and to be completed within three years)*, the author is investigating how changes in the CAR over time may assist in understanding patterns of response to and relapse after a novel inpatient treatment program for depression. This study will be discussed in the next section. *In the medium term (three to five years)*, the author plans to engage in research that will move towards identification of more reliable biomarkers (cortisol and beyond) in CMDD and other depressive subtypes. As will be reviewed, using multiple HPA markers, evaluating both HPA and other biological measures in combination with one another, and incorporating imaging techniques are all approaches that may lead to the identification of a reliable biomarker in CMDD. In the *long term (five to ten years)*, the author plans to focus his research on personalized medicine by incorporating the information about patients’ psychobiological profiles in determining
specific treatment choices. An overview of what personalized medicine entails and what it could mean for the management of depression is discussed below in Section 7.4.4.

7.4.2. Future Research Direction (Short term): The CAR and the Prediction of Treatment Response and Relapse in Depressive Illness

One major challenge in treating inpatients with depression is the high rate of relapse post-discharge. As it stands, there is very little empirical research to guide individualized approaches to treatment and discharge planning. The general goal of this ongoing project is to determine whether psychobiological profiling can assist in this process. There is a evidence demonstrating that persistent dysregulation of the HPA axis after depression remits is associated with a higher risk of recurrence (Zobel et al., 2001, Lok et al., 2012). This ongoing project combines elements of these studies with the findings of this thesis work in order to accomplish this goal.

More specifically, this ongoing study investigates whether longitudinal changes in the CAR over a four-week hospital stay and post-discharge can predict response and relapse in depressed patients. The study will investigate whether patterns of the CAR predict relapse one and four months post-treatment. This study is being done at the Alternative Inpatient Milieu (AIM), a novel in-patient unit at CAMH for the treatment of severe and chronic mood disorders. Novel aspects of AIM include the development of coping and stress management skills and the promotion of patient autonomy within a “home-like,” in-patient environment. The treatment team is multidisciplinary, including psychiatrists, nurses, psychologists, occupational therapists, and social workers, in keeping with the complex nature of depressive illness and in an effort to target
biopsychosocial mechanisms inherent to depression. It is highly plausible that many improve while on the AIM unit through changes in stress reactivity. Based on the research findings described in this thesis, the examination of the role of depression chronicity, sex, and the personality dimension of extraversion in shaping CAR patterns will be an important component of the AIM study. One working hypothesis of the study is that those with CMDD with lower levels of extraversion will show a different pattern of CAR changes over the four-week hospital stay than will those with higher levels of extraversion. It also predicted that this psychobiological profile, defined by extraversion and the CAR over time, will be associated with patterns of relapse. Additional goals of the study are to determine whether psychosocial therapies that focus on aspects of extraversion (e.g., increased social connectedness and activity level) impact on pre- and post-therapy cortisol levels and whether this, in turn, impacts on remission and relapse rates.

7.4.3. Future Research Direction (Medium term): Identification of Biomarkers in Depressive Illness: HPA Axis and Beyond

“A biomarker is defined as a characteristic that can be objectively measured and evaluated as a measure of a normal biological process, pathogenic process or a pharmacological response to a therapeutic intervention.”

(Jain, 2009b)
A reliable biological marker could have several functions including: 1) being a sensitive and specific measure of a disease state; 2) being a target for drug development; 3) assisting in predicting response; 4) being an indicator of prognosis; and 5) reducing the financial and safety risks associated with clinical trials (Feuerstein and Chavez, 2009).

The identification of biomarkers is a major goal of both medical and psychiatric research. While several biomarkers have been identified for medical illnesses (e.g., cholesterol measures and blood pressure), no such markers have been identified for complex psychiatric disorders including depression. One of the most studied areas of psychiatric research, as it pertains to biomarkers, has focused on cortisol and the HPA axis. Despite significant research in this area, no single marker of HPA activity has been shown to have sufficient sensitivity and specificity to have widespread clinical utility. Going forward, how can research improve this state of affairs?

As will be discussed below, a profiling approach that uses multiple HPA measures in tandem and/or HPA measures in combination with other biological measures, while incorporating imaging studies to assess the higher order mechanisms tied to HPA function, may be needed to address this challenge.

a) Using Multiple HPA Measures may be Necessary to Identify a Reliable Biomarker of Depression

There are a number of methods to examine HPA activity including diurnal measures, pharmacological challenges, and social stress responses. As highlighted previously, each test is distinct and examines a different component of the HPA axis. For example, a
diurnal measure, such as the CAR, provides insights into basal cortisol secretion, whereas pharmacological challenges, such as the DST, provide a measure of glucocorticoid receptor activity, a critical component of HPA regulation. Social stressors, such as the TSST, help assess how higher level CNS processes impact cortisol responses. In reviewing this literature, one limitation of work to date has been the tendency to focus on only one component of the HPA axis at a time. *Assessing multiple measures would provide a comprehensive assessment of HPA activity.* This is the approach used when assessing biological markers in other fields of medicine. For example, when examining cholesterol levels, treatment is not based on a single cholesterol marker but is based on a number of markers as well as ratios between these markers. It might be necessary for future studies to go beyond a single measure of HPA axis activity and instead examine HPA profiles in depressed populations. Whether an HPA profile or a single HPA measure is more meaningful in depressed populations needs to be examined directly in future studies.

b) **Evaluating Both HPA and Other Biological Measures in Combination**

It was suggested above that a HPA profile that includes several measures of HPA function may be a more useful biological marker of depression than a single HPA measure. Similarly, it may be that multiple biological marker profiles of different biological systems could be even more informative in assisting with diagnosis and management in various depressed populations. Again, this is an approach that has been used in other fields of medicine. For example, multiple biological markers such as cholesterol levels, blood pressure, and blood glucose determine an individual’s
cardiovascular risk and, in turn, guide management of cardiovascular disease. Could the assessment of multiple biological systems in depression assist in a similar manner?

Schmidt et al. (2011) argued that a biomarker panel that aims to profile multiple biological markers (e.g., growth, immune and endocrine) may be needed to stratify depressed patients into more distinct subpopulations. Possibly, a panel of biomarkers would aid in subdividing a heterogeneous depressive illness that presents with a similar phenotype. This may, in turn, lead to more effective, etiologically based treatments for subgroups of patients with depression.

There are a number of potential biological markers that have been linked to depression. In reviewing the literature, growth factors and cytokine and inflammatory markers are measures other than cortisol that appear particularly promising and that will be strongly considered by the author in future work.

**Growth Factors:**

One growth factor that has received significant attention is the brain-derived neurotrophic factor (BDNF). This growth factor is involved in regulating synaptic plasticity in neuronal networks associated with depression (Pittenger and Duman, 2008). A number of clinical studies suggest that BDNF levels are lower in depressed patients and levels increase after antidepressant treatment (Gervasoni et al., 2005, Aydemir et al., 2006, Brunoni et al., 2008). These findings support the notion that serum BDNF levels could be a useful biomarker and may assist in our understanding of the pathophysiology and treatment response in depression. Other growth factors that may also have relevance
as biological markers include insulin-like growth factor-1 and vascular endothelial growth factor (Schmidt et al., 2011).

**Cytokines and Inflammatory Markers:**

There is a growing body of work suggesting alterations in cytokines and inflammatory markers in depressive illness (Miller et al., 2009). For example, higher levels of TNF-α and IL-6 have been found in depressed patients (Dowlati et al., 2010). As well, there is evidence that antidepressant treatment can normalize elevations in cytokines and that persistent elevation of cytokines may be associated with treatment resistance (Maes et al., 1997, Steptoe et al., 2007). Another inflammatory marker of interest in depression is high-sensitivity C-reactive protein, which has been positively associated with depressive illness (Howren et al., 2009).

There is a dynamic relationship between cytokines and inflammatory markers and the HPA axis (Zunszain et al., 2011). Therefore, understanding both inflammatory, cytokine, and HPA systems simultaneously in depression may be more useful than examining each system on its own.

c) **The Role of Imaging Studies in Identifying an HPA Axis Biomarker in Depression**

As reviewed by Dedovic et al. (1998), amygdala, hippocampal, and prefrontal regions of the brain play regulatory roles in response to social stressors in humans. In addition, research has shown that repeated and chronic stress can change brain structure, morphology, and function in these regions (Bremner et al., 2000, Ingram et al., 1998). Functional imaging studies done following social stress challenges, with a particular
focus on the hippocampal, amygdala, and prefrontal cortex regions, may provide new insights regarding the mechanism underlying altered cortisol stress responses in CMDD and how this critical circuitry might differ in CMDD and more acute forms of depression. Potentially, TSST responses in conjunction with imaging findings may lead to a more reliable biomarker of CMDD.

Studies incorporating both imaging studies and HPA activity are starting to be done. For example, Aihara et al. (2007) found that hypometabolism in the right medial prefrontal cortices discriminated Dex/CRH non-suppressors from suppressors in unmedicated MDD. In another study by Drevets et al. (2002), left amygdala metabolism was positively correlated with plasma cortisol levels in MDD and bipolar depressed groups.

An exciting developing field is imaging genetics which examines how genetic variations can influence brain structure and function in various illnesses such as depression. For example, gene variants of the serotonin transporter gene (LAL versus S or LG alleles) have been associated with enhanced amygdala activation to fearful faces in patients with depressive or anxiety disorders (Lau et al., 2009). Interestingly, this gene has also been linked with alterations in HPA activity (Miller et al., 2012). Taken as a whole, imaging genetics may provide an important added dimension in understanding the mechanisms (both genetic and brain function/circuitry) underlying altered cortisol responses to social stress in CMDD, and may prove to be a powerful technique for identifying biomarkers in depressed populations.
d) **Next Steps in Biomarker Research**

To date, a number of putative markers of depression have been identified, but as of yet, no one marker has been found to be a sensitive or reliable marker of depressive illness. It is likely that a number of biological processes are involved in depression and therefore, examining multiple biological systems may be a logical approach in future studies. Studies of this nature will involve a large investment of resources and infrastructure, though, and much larger samples of depressed patients will need to be studied. Even so, research along these lines is starting to be done. For example, Dunlop and colleagues (2012) are examining how multiple clinical, biological, genetic, and psychological factors moderate response to treatment in patients with MDD who have never received treatment before. Biological measures in this study include fMRI, immune markers, genetic markers, and the Dex/CRH test. This is an intriguing study and hopefully will provide insights into which biomarkers are most relevant in depressive illness. This work may also provide clues to treatment resistance and possible markers of chronicity in depression.

**7.4.4. Future Research Direction (Long term): Personalized Medicine**

*“Prescription of specific treatment and therapeutics best suited for an individual taking into consideration of both genetic and environmental factors that influence response to therapy.”* (Jain, 2009a)
Personalized medicine is one of the strategic objectives of the NIMH in the hope that more specific treatments will be developed for illnesses such as depression. However, at present, there is no reliable method for clinicians to choose one antidepressant over another and predict patient response to a given treatment. The “one size fits all” approach to depression treatment usually does not work and instead, there must be a great deal of trial and error until an appropriate treatment is found. The goal of personalized medicine is to choose and/or design the right treatment for the right patient based on the individual characteristics of the patient. Designing such an approach is particularly important in depression. By not having personalized treatment strategies for depression, a disservice is being done to patients given the lag time in treatment response, which can be up to six weeks, and the increased risk of treatment resistance the longer a patient is symptomatic. The current project was a step towards personalized medicine, by identifying unique psychobiological profiles in chronic depression. Over the long-term, the author plans to focus his research on personalized medicine by incorporating information about patients’ psychobiological profiles when determining specific treatment choices.

One area that appears particularly promising in personalized medicine is the field of pharmacogenomics (Lin et al., 2010), defined as the study of how variations in genes affect the response to medications. For example, almost all antidepressants are metabolized by cytochrome P450 enzymes (CYPs) in the liver. Studies suggest that polymorphisms in these enzymes can provide valuable information on medication dose and the emergence of its side effects (Luo et al., 2005, Yin et al., 2006). There is intriguing research examining how polymorphisms of genes encoding monoamines, the targets for current antidepressants, influence treatment response. Several studies have
found that polymorphisms of the serotonin transporter gene may assist in determining treatment response and the emergent of side effects (Lin et al., 2010).

So what could personalized medicine look like in depression treatment in the future? Ultimately, personalized medicine for depression would include a comprehensive biopsychosocial profile of the patient, one of which is pharmacogenomics. Physicians would be able to use information from this profile to determine more effective and targeted management strategies for patients with depression. Practically speaking, the steps towards instituting a personalized approach to treatment of CMDD may be as follows:

- Upon entering the clinic, patients would provide a blood sample and using a biomarker panel, several vulnerability markers would be immediately assessed (e.g., HPA axis, immune and inflammatory markers, and growth factors).
- An imaging study would be done to examine brain function and/or structure.
- A genetic panel would be completed that could assist with diagnosis and treatment decisions. For example, a genetic profile could assist with determining pharmacokinetic and pharmodynamic profiles of patients which would aid in determining the dose and choice of antidepressant.
- Comprehensive psychological (e.g., personality) and social assessments (e.g., childhood adversity) would be done.
- All of the information collected would be entered into a computer program (possibly using a hand-held device) which would provide the clinician with information that would augment the clinical interview.
• The clinician would then be able to use this comprehensive assessment to determine the most effective treatment strategy for the patient.

Although, personalized medicine may be in its infancy with respect to depressive treatment, it is the way of the future. It is anticipated that over the coming years, knowledge of a patient’s psychobiology will assist in providing a more effective and individualized approach to treatment.

7.5. Final Summary

This project was developed out of a need to improve the management of CMDD, a common and highly disabling illness. At present, clinicians are often at a loss when faced with these patients and have limited guidance in determining which medications and/or therapy would be best suited for them. In order to improve management and develop more targeted treatments for CMDD, a greater understanding of its underlying pathophysiology is needed.

The author’s graduate work has led to several important findings related to cortisol responses in a naturalistic and highly complex sample of CMDD individuals. Various unique psychobiological profiles were identified in chronic depression that merits further investigation. Study 1 demonstrated that cortisol responses are different in those with CMDD than in healthy controls, but only when sex differences are considered. Females with CMDD had greater cortisol output following the social challenge relative to healthy controls. In contrast, males with CMDD have blunted cortisol reactivity under the same
challenge compared to male controls. This suggests the existence of opposite stress response mechanisms in females and males. The cortisol-mediated over-arousal by females and under-arousal by males in the face of social stress could be reflective of sex-specific defence and coping mechanisms. This finding could present interesting possibilities for psychological therapies that cater to the unique defense style of each sex.

Studies 2 and 3 demonstrated that psychobiological profiles of CMDD based on the personality dimension of extraversion may also have great utility in future work. CMDD individuals with lower levels of extraversion secrete more cortisol when socially challenged than do those with higher levels of extraversion. These findings suggest that CMDD individuals with lower levels of extraversion may, in fact, represent a distinct phenotype. In addition, these findings suggest that those with CMDD who have low levels of extraversion may be in need of treatments that can moderate cortisol reactivity to social stress. It is already known that increased social connections, development of relationships, and engagement in activities (all of which are features of extraversion) are proven psychological treatment strategies for CMDD (Keller et al., 2000, Bhagwagar et al., 2003, Schramm et al., 2008). However, it would be valuable to determine whether CMDD patients who have lower extraversion scores have the greatest benefit from this treatment approach both symptomatically and in terms of cortisol reactivity. Alternatively stated, does a treatment targeting lower level of extraversion improve outcomes for CMDD individuals who score lower on this personality dimension, and is this effect mediated through cortisol reactivity?

The results of the project point to several interesting questions that may eventually lead to improved management of CMDD, including the following:
1) Should unique treatment approaches be considered for females and males with CMDD?

2) Are lower levels of extraversion a target for treatment or a predictor of relapse in CMDD?

3) Will alterations in cortisol responses be a quantifiable biological marker to measure response or risk of relapse, much like assessment scales used in clinical practice?

4) Should unique treatment approaches be considered for individuals with CMDD compared to those with acute depression?

The author is currently involved in a study that will start addressing some of these questions. The AIM study is an ongoing study that examines whether patterns of the CAR can assist in predicting response and relapse in depressive illness. Based on the research findings described in this project, examination of the role of depression chronicity, sex, and the personality dimension of extraversion on the CAR will be an important component of the AIM study.

Over the long term, the author plans to examine biomarkers above and beyond cortisol, for example immune, inflammatory, and growth factors. Imaging studies would also be an interesting future direction that would allow for the examination of brain circuitry and how this may be related to altered cortisol responses and treatment response in CMDD. It is anticipated that over the next several years, research of this kind will start identifying new targets for treatment that reflect a more personalized approach for managing individuals with CMDD.
APPENDIX 1

Additional Analyses and Interpretations of Study 1 Results

There were additional questions pertaining to Study 1 that arose after this study was published. Chapter 3 includes the published manuscript i.e. Chopra et al., 2009. An appendix was added to the thesis to address the following questions:

1. Do females with CMDD have greater cortisol responses to the TSST than female controls or are the differences mainly due to baseline differences in cortisol levels given that 1) pre-challenge levels are higher in female depressed as compared to female controls and 2) there were only differences in AUCg (a measure of total cortisol output) and no difference in peak percentage change in cortisol in females with CMDD compared to female controls.

2. Are the results of Study 1 replicated in the larger study sample that was recruited to examine moderating effects of personality on cortisol responses?

Response to Question 1:

In Study 1, it was found that females with CMDD had greater AUCg compared to females controls (page 61 first paragraph; figure 7, page 62). As illustrated below, AUCg is a measure of total cortisol output that includes both baseline (AUCB) levels and cortisol changes over time or cortisol reactivity (ACi).
Therefore, it is important to consider differences in baseline cortisol levels when interpreting AUCg findings. The degree to which higher AUCg cortisol following the TSST in female depressed as compared to female controls is determined by higher pre-challenge cortisol levels remains an open question. However, it should be noted that Time 0 cortisol levels (figure 6, pg 60) obtained just prior to the TSST were not significantly different between females with CMDD and female healthy controls. This suggests that the TSST also contributed to the differences found between these two groups. A more parsimonious interpretation of Study 1 findings is that females with CMDD had greater cortisol output vs. a heightened or sensitized cortisol response during this challenge.
Response to Question 2:

Additional participants were recruited for Study 2 to increase the statistical power so that moderating effects of personality on cortisol responses could be examined. Whether the finding of sex differences in cortisol responses in those with CMDD as compared to healthy controls in Study 1 was replicated in the larger sample was examined to ensure the robustness of Study 1 findings.

A linear mixed model was conducted using The SAS System v.9.1.3 in order to assess changes in cortisol associated with sex and group (CMDD vs. healthy controls) over the course of the TSST in the larger sample. As with the initial model in Study 1, all possible interactions between sex, condition, and time were included in the model. Model diagnostics found that cortisol values for two subjects were unusually high and had an undue influence on the estimation of model parameters. The cortisol value for one participant at 60 minutes was replaced with a missing value and another participant was excluded from the analysis altogether. Diagnostics for the model based on the reduced sample found that that the underlying model assumptions were reasonably well met. The results of this model are summarized in the following table:

<table>
<thead>
<tr>
<th>Predictor</th>
<th>F, df</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>F=14.84, df=4,418</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>F=1.55, df=1,105</td>
<td>p=0.2165</td>
</tr>
<tr>
<td>Condition</td>
<td>F=1.86, df=1,105</td>
<td>p=0.1754</td>
</tr>
<tr>
<td>Time*Gender</td>
<td>F=2.45, df=4,418</td>
<td>p=0.0457</td>
</tr>
<tr>
<td>Time*Condition</td>
<td>F=0.54, df=4,418</td>
<td>p=0.7053</td>
</tr>
<tr>
<td>Sex*Condition</td>
<td>F=2.76, df=1,105</td>
<td>p=0.0993</td>
</tr>
<tr>
<td>Time<em>Sex</em>Condition</td>
<td>F=1.99, df=4,418</td>
<td>p=0.0947</td>
</tr>
</tbody>
</table>
As shown in the above table, the results in the larger sample are consistent with those in Study 1. There remained a significant main effect of time and the interaction between sex and condition trends towards significance. It is important to note that in the larger sample, the sex distribution between male CMDD participants (n= 22) and male controls (n=28) was not evenly matched which might be one reason for the decreased level of significance in the larger sample.

Taken as a whole, the pattern of results in the larger sample were consistent with those in Study 1 which supports the finding that there are sex differences in cortisol responses in CMDD participants as compared to healthy controls.
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