Serial Measures of the Cortisol Awakening Response during Treatment for Depression in an Inpatient Setting

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
Institute of Medical Science
University of Toronto

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Abstract

Goal:
To determine whether the Cortisol Awakening Response (CAR) associates with treatment response and course in hospital for inpatients with major depressive disorder (MDD).

Methods:
The CAR was measured at admission and discharge in patients completing a four-week inpatient program for MDD. Self-report questionnaires were used to assess changes in depression, anxiety, and perceived stress.

Results:
Over the four week hospital stay measures of CAR reactivity (Delta, AUCi) decreased, but there was no significant correlation between the change in CAR reactivity and change in clinical symptoms. Cross-sectional measurements of the CAR reactivity at both admission and discharge were strongly correlated with the drop in depression scores in hospital. Furthermore, poor treatment responders had a significantly lower CAR reactivity at both admission and discharge than did good responders.

Conclusion:
Individuals with higher CAR reactivity at admission and discharge had the greatest reduction in depression over the course of treatment.
Acknowledgments

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<th>Definition</th>
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<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic releasing hormone</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<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>
</tr>
<tr>
<td>BAI</td>
<td>Beck Anxiety Inventory</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>BP</td>
<td>bipolar disorder</td>
</tr>
<tr>
<td>BPI</td>
<td>bipolar 1</td>
</tr>
<tr>
<td>BPII</td>
<td>bipolar 2</td>
</tr>
<tr>
<td>CAMH</td>
<td>centre for addiction and mental health</td>
</tr>
<tr>
<td>CAR</td>
<td>cortisol awakening response</td>
</tr>
<tr>
<td>CBG</td>
<td>corticosteroid-binding globulin</td>
</tr>
<tr>
<td>CMDD</td>
<td>chronic major depressive disorder</td>
</tr>
<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
</tr>
<tr>
<td>CRF</td>
<td>corticotropin releasing hormone</td>
</tr>
<tr>
<td>Dex</td>
<td>dexamethasone</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>diagnostic statistical Manual of Mental Disorders, Fourth Edition</td>
</tr>
<tr>
<td>DST</td>
<td>dexamethasone suppression test</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic pituitary adrenal</td>
</tr>
<tr>
<td>MDD</td>
<td>major depressive disorder</td>
</tr>
<tr>
<td>MDE</td>
<td>major depressive episode</td>
</tr>
<tr>
<td>MINI</td>
<td>Mini International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>PSS</td>
<td>Perceived Stress Scale</td>
</tr>
<tr>
<td>PVN</td>
<td>paraventricular nucleus</td>
</tr>
<tr>
<td>QIDS</td>
<td>Quick Inventory of Depressive Symptoms</td>
</tr>
<tr>
<td>RANOVA</td>
<td>repeated measures analysis of variance</td>
</tr>
<tr>
<td>SCID</td>
<td>structure clinical interview for DSM-IV</td>
</tr>
<tr>
<td>TSST</td>
<td>Trier Social Stress Test</td>
</tr>
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Chapter 1
Introduction

1 Review of the Literature

1.1 Overview of Major Depressive Disorder (MDD)

1.1.1 Definition, Epidemiology, and Economic Cost

In lay terms, “depression” describes feeling sad, blue or unhappy. It is a common experience in the face of negative life events such as perceived stress, failure, or loss. For the majority of people these feelings last only a short time, and have no long term effect on well-being. However, for a significant number of individuals, these symptoms become chronic and interfere with day-to-day functioning. At this point, these feelings are classified as a disease that affects multiple aspects of mental and physical well-being (Firestone & Marshall, 2003).

To meet the clinical definition of MDD, as outlined in the DSM-IV, an individual must have depressed mood and/or loss of interest or pleasure in life activities for at least 2 weeks, and at least five of the following symptoms that cause clinically significant impairment in social, work, or other important areas of functioning almost every day:

- Depressed mood most of the day.
- Diminished interest or pleasure in all or most activities.
- Significant unintentional weight loss or gain (increased/decreased appetite)
- Insomnia or hypersomnia
- Agitation or psychomotor retardation noticed by others (fidgety, restless have trouble sitting still/talking or moving more slowly)
- Fatigue or loss of energy
- Feelings of worthlessness or excessive guilt
- Diminished ability to think or concentrate, or indecisiveness
- Recurrent thoughts of death or feelings that one would be better off dead (DSM IV, 1994)

An MDD diagnosis is typically made by a family doctor, psychiatrist or psychologist based on subjective complaints and characteristic changes in mental status including dysphoric affect, highly pessimistic thinking that is disproportionate to actual circumstances, suicidal thinking, and psychomotor changes, either slowing or agitation. In research settings, MDD is often ascertained using structured clinical interviews based on the DSM criteria outlined above. Examples include the Structured Clinical Interview for DSM disorders (SCID)(First, Gibbon, Spitzer, & Williams, 1996), and the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). Alternatively, depression status can be measured by an inventory of depressive symptoms. The Quick Inventory of depressive symptoms (Q.I.D.S.) is one such tool used both clinically and in research to establish depression severity (Rush et al., 2003).

The DSM outlines various other subtypes of mood disorders. The current edition, DSM IV, characterizes other mood disorders under the following subtypes:

- Dysthymic disorder (mild depression, most days for at least 2 years)
- Bipolar episode; bipolar disorder (a combination of depressive and manic episodes)
- Substance-induced mood disorders (mood disturbances caused by the physiological changes due to substance abuse)
- Mood disorders due to a general medical condition (e.g. hypothyroidism, Parkinson’s disorder)
- Adjustment disorder with depressed mood (brief depression most likely triggered by overwhelming stress)
- Other psychiatric conditions in which depression can be a primary symptom (DSM IV, 1994)

MDD affects a large proportion of the population. Large epidemiological studies in the USA estimate the life-time prevalence rate for MDD to be 16.2% (Ronald C Kessler et al., 2003). MDD also has a significant economic burden. The most recent analysis in the USA reported that MDD costs $33 Billion in lost productivity, $12 Billion in treatment costs and $8 Billion in increased mortality each year (Greenberg PE, Kessler RC, 1996).

In their 2004 update to the original Global Burden of Disease (GBD) study, the WHO reports that unipolar depressive disorders are the leading cause of burden of disease (DALYS) in middle and high-income countries. In low-income countries, unipolar depressive disorders are the 8th leading cause of burden of disease (The World Health Organization, 2008). By 2020 the WHO projects that unipolar depression will be the leading cause of disability (WHO, 2001) and by 2030 the leading cause of burden of disease worldwide (WHO, 2008). It is evident that the effects of depression transcend far beyond the local/national level, and that it is truly a global issue. There is a pressing need to improve our understanding of what causes MDD and to optimize its prevention and treatment.
1.1.2 The Causes of MDD:

It is generally accepted that MDD is a complex disorder caused by the interaction of various biological and environmental factors including:

- Alcohol or drug abuse
- Medical conditions including underactive thyroid, cancer, or long-term pain
- Medications such as steroids or B-blockers
- Sleep problems
- Stressful life events, such as:
  - Interpersonal losses
  - Childhood abuse or neglect
  - Job loss
  - Social isolation (common in the elderly) (Encyclopedia, 2012)

A large body of literature supports the role of genetic and other biological vulnerabilities in the development of MDD. It can be argued that this line of thinking goes back as far as Hippocrates, who attributed melancholia to an overabundance of black bile. In recent decades, adoption studies were first used to study familial risk in MDD, and showed that MDD was up to 7 times more likely in biological relatives of depressed probands than in biological relatives of control cases (McGuffin, Katz, & Rutherford, 1991; McGuffin, Katz, Watkins, & Rutherford, 1996; Wender et al., 1986). Subsequent work has used linkage analysis, genetic association studies and genome-wide scans to determine the genetic vulnerabilities to depression. Taken as a whole, this body of work suggests that many genes of small effect, interacting with one another and with a variety of environmental adversities, contribute to the etiology and maintenance of
MDD (see reviews by Jia, Kao, Kuo, & Zhao, 2011; Macaluso & Preskorn, 2012; Sarnyai et al., 2011; Schneider & Prvulovic, 2012). MDD is a heterogeneous disorder, with different combinations of genetic and environmental factors likely to contribute to any one case.

While it is well established that there is a strong biological component to MDD, much more work is needed to fully characterize the specific mechanisms involved. Finding biological markers that could predict MDD onset, response to treatment and/or early relapse in a given individual is a particularly high priority for translational work.

1.1.3 Pathophysiology

There is increasing evidence that MDD may be associated with particular changes to brain structure and physiology. One recent area of work has focused on hippocampal changes in MDD, given the important role of this brain area in stress regulation, memory and cognition (Campbell, S., MacQueen, 2004; McKinnon, M., Yucel, K., Nazarov, A., MacQueen, 2009; Videbech, P., Ravnkilde, 2004). These studies have demonstrated that reduced hippocampal volume is a common neurological feature of MDD, especially in cases that are recurrent and/or chronic. These findings are further supported by studies showing reduced glucocorticoid receptors, which are highly localized in the hippocampus, in the brains of patients with MDD (Medina et al., 2013). A recent meta-analysis has shown that reduced hippocampal volume is associated with first-onset depression (Cole, Costafreda, McGuffin, & Fu, 2011) suggesting that it is a risk factor for de novo cases of MDD, and not just a consequence of chronic and recurrent episodes.
A large body of work has looked at stress system changes in MDD, particularly as it relates to the HPA-axis. As shown in figure 1, the HPA response to stress starts at the periventricular hypothalamus, where corticotropin-releasing hormone (CRH) is secreted into the hypothalamo-pituitary portal circulation. This triggers the release of adrenocorticotropic hormone (ACTH), which then stimulates the release of cortisol from the adrenal cortices. Cortisol, the most potent glucocorticoid, is found either in its free form (4%) or bound to proteins such as albumin and corticosteroid binding globulin (96%).

Cortisol secretion has a strong circadian rhythm. Healthy subjects have higher levels of cortisol in the morning, with a mean peak increase of 50-60% in the first 45 minutes of awakening. This process is referred to as the cortisol awakening response (CAR). The diurnal pattern of cortisol release then shows a gradual decrease throughout the day, with a minor blip upward in the early evening, ending with a nadir around midnight (Weitzman et al., 1971).
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/wwai/imsd/alcohol/Vanessa/vwhpa.htc, accessed May 27, 2013)

The CAR is an adaptive physiological response to awakening, which is a discrete and distinct component of the circadian rhythm (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). The circadian rhythm of the entire HPA-axis is largely controlled by the suprachiasmatic nucleus (SCN) via inputs in the periventricular nucleus of the hypothalamus (Buijs, van Eden, Goncharuk, & Kalsbeek, 2003; Dickmeis, 2009; Kalsbeek et al., 2006). The regulation of the CAR starts much before awakening with a gradual increase of cortisol towards the end of the sleep cycle, during non-REM when the hippocampus is suppressed (Buijs et al., 2003; Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007). It is suspected that this gradual increase in cortisol pre-awakening is controlled by the SCN and possibly the hippocampus. The hippocampus has been implicated based on prior research demonstrating that a normal CAR requires functional
integrity of the hippocampus (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004; Marita Pruessner, Pruessner, Hellhammer, Bruce Pike, & Lupien, 2007). Furthermore, anatomical and functional pathways linking the hippocampus with the SCN have been discovered (Krout, Kawano, Mettenleiter, & Loewy, 2002; Pace-Schott & Hobson, 2002; Stranahan, Lee, & Mattson, 2008).

The CAR is also regulated in a similar manner post-awakening. Studies have indicated that the levels of ACTH and cortisol immediately after awakening are positively correlated, suggesting the CAR is regulated by ACTH (Wilhelm et al., 2007). The SCN also helps regulate the CAR post awakening. The SCN is activated by the availability of light such that awakening in total dark is associated with a blunted CAR (Scheer & Buijs, 1999). Other brain regions and pathways may also work together prior to and post awakening to illicit the characteristic increase in cortisol, and a blunted or elevated CAR may be attributable to dysfunction in these pathways. The ultimate measurement of the CAR may also be influenced by lack of availability of free cortisol. The CAR measures the amount of free cortisol that has left the blood stream and entered saliva. As previously mentioned 96% of cortisol is bound to the glucocorticoid binding globulin. Once bound, cortisol is transported to nearly every cell in the body to bind to glucocorticoid receptors (GR) and/or mineralcorticoid receptors (MR) located in the cytosol. Areas of the brain such as the prefrontal cortex, the hippocampus, amygdala, and the hypothalamus have a wide distribution of these receptors (Gold & Chrousos, 2002). Acutely, activation of these receptors inhibits the HPA-axis. Having either too much or too little of these receptors may also contribute to an alteration in the CAR.
1.2 The HPA-axis and MDD

Research on the relation between stress and depression has been ongoing since the late 1950s. While early research in this area showed that many individuals with MDD have an overactive HPA axis (Board, Wadeson, & Persky, 1957), it was not until the seminal work of Carroll in the late 1970s that this work became popularized. Early studies found total and free cortisol to be elevated in urine (Carroll, Curtis, Davies, Mendels, & Sugerman, 1976), plasma (Carroll, Curtis, & Mendels, 1976; Gibbons & McHugh, 1962) and cerebrospinal fluid (Carroll, Curtis, & Mendels, 1976). Elevated cerebrospinal fluid corticotrophin releasing factor (CRF) has also been reported (Nemeroff et al., 1984). Pharmacologically-induced challenges to test HPA axis functioning have also shown impaired functioning of feedback mechanisms and general over activity of the axis as evidenced by non-suppression in response to the dexamethasone suppression test (Carroll et al., 1981), increased cortisol responses to ACTH (Amsterdam, Winokur, Abelman, Lucki, & Rickels, 1983), and blunted ACTH responses to CRH (Gold et al., 1984; Holsboer, Von Bardeleben, Gerken, Stalla, & Müller, 1984). Studies of diurnal cortisol secretion patterns have reported both a blunted and elevated cortisol awakening response or CAR (Huber, Issa, Schik, & Wolf, 2006) and an earlier and greater cortisol nadir (Halbreich, Asnis, Shindledecker, Zumoff, & Nathan, 1985; Jarrett, Coble, & Kupfer, 1983), in subgroups of MDD patients.

Several studies have shown changes in the glandular structures that comprise the HPA axis in MDD patients. Findings include an increased number of CRH and arginine vasopressin neurons in the paraventricular nucleus (Purba, Hoogendijk, Hofman, & Swaab, 1996; Raadsheer, Hoogendijk, Stam, Tilders, & Swaab, 1994), and adrenal gland enlargement in patients with MDD (Nemeroff et al., 1992).
While the early literature on HPA-axis changes in MDD supported a model of HPA-overactivity in this disorder, subsequent work suggests that these changes may be limited to the more acute and severe cases of depression presenting with melancholic features such as insomnia and weight loss (classic depression). However, many MDD patients present with more chronic and/or reversed vegetative features such as hypersomnia and weight gain (atypical depression). Studies of the HPA axis in atypical depression have found increased ACTH in response to CRH (O’Keane, Dinan, Scott, & Corcoran, 2005), normal responses to the DEX/CRH (Watson et al., 2002), hyper-suppression of cortisol in response to the low-dose DST (Levitan, Vaccarino, Brown, & Kennedy, 2002), and no difference in the CAR in atypical compared to melancholic depression (S. A. Vreeburg et al., 2009). In another study of premenopausal women with MDD, melancholic patients had hypercortisolemia, while atypical patients did not (Young, Carlson, & Brown, 2001). Taken as a whole, these studies point to a normal or even underactive HPA-axis in atypical depression. A recent review argues that there are different mechanisms underlying alterations in the HPA axis for atypical depression as compared to melancholic depression, and that in some cases patients switch from one pattern to another as depression becomes more chronic (O’Keane, Frodl, & Dinan, 2012).

Several other authors have suggested that after long periods of chronic stress and recurrent MDD episodes, the HPA axis becomes down regulated, less-responsive and secretes less cortisol (Heim et al. 2005, Fries et al. 2005). In support of this, chronic depression in geriatric populations is often associated with a hypo- rather than hyper-cortisolemic state (Bremmer et al. 2007). A study by Chopra et al. (2009) showed that sex differences may also play a role in this regard, with chronically depressed males showing blunted HPA activity and females exaggerated HPA reactivity in response to a social stressor (Chopra et al., 2009). It may
therefore be important to consider chronicity and sex differences when studying the stress system in MDD.

Few studies have examined HPA-function in bipolar disorder. Preliminary evidence suggests that bipolar patients may exhibit hyper-cortisolemia in both the manic and depressive phases of their illness (Daban, Vieta, Mackin, & Young, 2005; Manenschijn et al., 2012). Inter-generational studies have found that children of bipolar parents show the same changes in diurnal cortisol as do children of unipolar MDD parents (Ellenbogen, Santo, Linnen, Walker, & Hodgins, 2010).

Several pre-clinical models linking early life stress, HPA axis changes and MDD have been proposed. For example, decreased maternal licking and grooming in rat pups, a model of maternal adversity, causes significantly higher HPA axis activity (Liu et al., 1997), higher levels of CRH mRNA, and lower levels of gluco-corticoïd receptors in the brain during adulthood (Liu et al., 1997). Studies in rodent and non-human primates have shown that early separation of pups from their mothers elicits HPA axis changes similar to those demonstrated in depressed adult individuals (Sanchez, 2001). Primates classified as ‘dexamethasone-resistant’, a phenomenon common in humans with MDD, have significantly fewer glucocorticoid receptor binding sites in the hippocampus (Brooke, de Haas-Johnson, Kaplan, Manuck, & Sapolsky, 1994), while prolonged hypersecretion of cortisol in monkeys has been associated with hippocampal damage (Sapolsky, Uno, Rebert, & Finch, 1990). Furthermore, a study on the chronic mild stress (CMS) model of depression in rats, showed rats with anhedonic like symptoms to have an activation of the HPA-axis after 4 weeks of CMS exposure (Christiansen, Bouzinova, Palme, & Wiborg, 2012).
In sum, prior research has shown several associations between altered HPA-axis activity and MDD pathology. Hypersecretion of cortisol and major depression have overlapping brain circuitries and mediators (Gold & Chrousos, 1999.; Gold, Goodwin, & Chrousos, 1988; Gold, Kling, et al., 1988). Furthermore, both are characterized by a diminution of cognitive and affective flexibility, alterations in arousal and alterations in neuroendocrine and autonomic functions (Gold & Chrousos, 1999). Some studies suggest that sustained hyperactivity of the HPA-axis has negative effects on brain structures critical to MDD. These various findings have led many to theorize that stress hormones can precipitate the onset of major depression and its subsequent course and severity (Nemeroff, 1984; Frank et al., 1990; R C Kessler et al., 1994). If so, HPA-axis activity continues to offer significant promise as a vulnerability marker for MDD.
1.3 Can HPA-changes Predict Treatment Response and/or Relapse in MDD

Given the extensive research linking HPA-axis changes with MDD as outlined above, another line of research has examined whether one or more measures of HPA-function can predict treatment outcome and/or relapse vulnerability in heterogeneous MDD populations. Of all patients treated for MDD, only 30% fully respond to initial treatments while 70% require augmentation or a change in their primary anti-depressant, thereby lengthening the time until recovery (M Fava & Davidson, 1996; Maurizio Fava et al., 2006; Rush et al., 2006; Trivedi, Fava, et al., 2006; Trivedi, Rush, et al., 2006). While current guidelines suggest continuing treatment with an antidepressant for at least 4-6 weeks before augmentation or switching, it would be of great clinical utility to identify subgroups of patients who are less likely to respond to standard treatment at the outset (Davidson, 2010). This might inform more aggressive treatments for these patients earlier in their course while helping allocate limited clinical resources to those in greatest need.

Initial work on the HPA-axis and treatment outcome showed that continued DST non-suppression at the time of hospital discharge was predictive of a more difficult clinical course going forward (Greden et al., 1983; Holsboer, Liebl, & Hofschuster, 1982; Nemeroff & Evans, 1984; Ribeiro, Tandon, Grunhaus, & Greden, 1993). Though these findings garnered much excitement, the DST lacked the specificity needed for widespread implementation. The next HPA measure to be implemented in this way was the DEX/CRH test, which provides a more holistic assessment of overall HPA-axis activity. Some studies did show that normalization of HPA function as assessed using serial measures of the DEX/CRH was associated with a greater
reduction in symptoms, a greater chance of remission, and best predicted early response (Deuschle et al., 1997; Holsboer, von Bardeleben, Wiedemann, Müller, & Stalla, 1987; Holsboer-Trachsler, Stohler, & Hatzinger, 1991; Zobel et al., 2001). However, negative studies using the DEX/CRH have also been reported. Schuele et al. (2001) showed that changes in the DEX/CRH in the first week of treatment were not predictive of clinical response at 6 weeks (Schüle, Baghai, Zwanzger, & Rupprecht, 2001). Nickel et al. (2003) found that DEX/CRH assessments at admission, 3 weeks and 6 weeks had no predictive value of response at 6 weeks (Nickel et al., 2003). Given these inconsistencies across studies and the relative complexity of the DEX/CRH at a practical level, as with the DST, it has not found its way into routine clinical practice.
1.4 The Cortisol Awakening Response

1.4.1 Introduction

The cortisol awakening response or CAR refers to the increase in cortisol output that occurs within the first 30-60 minutes of awakening. It is usually followed by a gradual decline in cortisol levels throughout the rest of the day. Studies have indicated that the CAR is observable in approximately 77% of the population (Wüst et al., 2000). In research settings, sampling for the CAR involves taking 3 saliva samples for cortisol levels at time 0 (awakening), time +30 minutes and time +60 minutes after awakening.

Figure 2: The Cortisol Awakening Response. Cortisol levels spike at about 30-45 minutes post-awakening. The rise is termed the CAR which can be measures by having individuals collect salivary cortisol levels at awakening, 30 min., and 60 min. post awakening
The two main aspects of the CAR that are of interest to researchers are cortisol reactivity and overall cortisol output. The CAR reactivity is defined by either the CAR Delta or the AUCi. The CAR Delta simply subtracts the T0 cortisol level from the T30 cortisol level. The AUCi is an integrated measure of the area under the cortisol level X time curve, and also subtracts out the effects of the T0 cortisol measurement (Jens C Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The AUCg is a measure of total cortisol output and does not correct for baseline differences, however does provide a measure of total cortisol levels/exposure over a specified period of time (Jens C Pruessner et al., 2003).

In general, the CAR has been shown to have high levels of intra-individual stability across multiple samples i.e. the CAR on any given morning is generally consistent (J C Pruessner et al., 1997). It is suggested, however, that length of sleep and the transition from work days to weekend days can have a measurable effect on the CAR (Aubry et al., 2010). Where possible, it is thus helpful to control for length of sleep and day of sampling in research of this type. There are several other factors that can affect the CAR. Metabolic conditions, heavy substance abuse and cortisol medications can affect the HPA axis and are widely excluded from research of this type (Carroll et al., 1981). Worry and rumination the night before can cause an elevated CAR (Zoccola, Dickerson, & Yim, 2011), and a large body of literature shows the CAR to be dysregulated in psychiatric conditions, such as MDD. The next section of the thesis will specifically review the literature on the CAR as it relates to depressed mood in both non-clinical and clinical populations.
1.4.2 The CAR and Depressed Mood in Nonclinical Populations: Studies based on AUCi and CAR Delta

Several studies have looked at the possible association between CAR reactivity, as assessed using AUCi or CAR Delta, and either current mild depressive symptoms or vulnerability to depressive symptoms in non-clinical populations. Pruessner et al (2003) found that in a sample of 40 healthy young men, higher levels of depressive symptoms in the subclinical range were associated with increased aggregated AUC (M. Pruessner, 2003). Furthermore, both cortisol levels and depressive symptoms were positively correlated with perceived stress. This finding was of great interest to the field as it showed an initial link between depressive symptoms and an altered CAR profile. This study also provided cautious optimism for the use of the CAR as an economical and practical alternative to other HPA-axis measures available such as the DEX-CRH test.

Adam et al (2010) examined whether the CAR was able to predict a clinical diagnosis of MDD after a one year follow up period in 230 adolescents. Indeed, Adam et al. found that a higher admission CAR delta was associated with a significantly increased risk of having MDD at follow-up, even after removing individuals with a prior diagnosis of MDD or anxiety (Adam et al., 2010). The authors concluded that an elevated CAR delta is a significant prospective risk factor for the development of MDD in young adults, and that this heightened CAR may play a role in the etiology of major depressive disorder.

A 2011 study by van Santen et al. examined whether the CAR was associated with psychological traits associated with vulnerability to depression in 381 controls without a personal or parental history of MDD or anxiety disorders. They found that hopelessness reactivity, a trait
associated with depression and suicide, was consistently associated with a higher CAR AUCi (van Santen et al., 2011). The authors concluded that hopelessness reactivity may represent a predisposing vulnerability for the development of a depressive or anxiety disorder, mediated in part by high HPA-axis reactivity.

When considered together, the three studies summarized above provide convincing evidence that higher than average CAR reactivity is associated with an increased risk of both current depressive symptoms, and a greater general vulnerability to depression, in non-clinical populations. These studies are also generally consistent with well-established models of depression that focus on sensitization and over-activity of the HPA-axis. However, as often occurs in biological studies of depression, several studies with the opposite pattern of results have also been reported.

Kuehner et al. (2007) examined whether cognitive vulnerability to depression was associated with the CAR in 42 healthy university students. They found that a decreased CAR delta was associated with self-focused rumination and with less improvement of sad mood after a negative mood induction followed by active distraction; both of these reflect cognitive patterns known to promote depression (Kuehner, Holzhauer, & Huffziger, 2007). Dedovic et al (2010) examined whether abnormalities in the CAR are present in subclinically depressed young adults and/or young adults designated high-risk for MDD, relative to controls. Results indicated that both the subclinical depressed and high-risk groups failed to show a significant increase in cortisol levels after awakening, and as a result had a lower mean CAR AUCi than did controls (Dedovic et al., 2010). A recent study examined the CAR in a sample of 55 Mexican adults, 18-35 years of age, who as a group had relatively high levels of lifetime trauma. This study examined whether the CAR could predict subclinical depressive symptomology after factoring
out the effects of early trauma. Results indicated that an attenuation of the CAR delta was the best predictor of greater subclinical depression symptomatology in this group (Mangold, Marino, & Javors, 2011).

In sum, studies linking CAR reactivity and depressive vulnerability in non-clinical populations have demonstrated both exaggerated and blunted CAR reactivity in mildly symptomatic and at-risk individuals. While at first glance this might seem highly problematic, it is highly consistent with prior HPA-findings in heterogeneous MDD populations whereby both hyper- and hypo-cortisolemia have been found. In MDD, this basic difference in HPA activity has been associated with the acuity of symptoms and presence of melancholic vs. atypical vegetative symptoms. Establishing the moderating factors that account for the CAR differences reported above in non-clinical populations is an important question for future work, but cannot be ascertained based on the extant literature.

1.4.3 The CAR and Depressed Mood in Nonclinical Populations: Studies based on AUCg (i.e. total cortisol output)

While almost all studies linking the CAR to depression in non-clinical populations have been based on CAR reactivity measures including AUCi and CAR Delta, a large study by Vreeburg et al. (2010) did report data for both AUCi and total cortisol output as assessed using the CAR AUCg. They found that healthy children at-risk for depression based on a parental history of MDD had a significantly higher AUCg, but no difference in AUCi, than did children with no parental history of depression (S. a Vreeburg et al., 2010). This finding is reminiscent of
the first set of studies summarized above in suggesting a link between higher cortisol levels and depressive vulnerability.

1.4.4 Summary of the CAR in Non-clinical Population

Taken as a whole, studies linking the CAR to depressive symptoms or vulnerability in non-clinical populations are somewhat difficult to interpret, given both the lack of consistency across studies and the lack of moderating factors to explain these differences. To shed more light on this issue, the next section summarizes studies looking at CAR changes in clinically depressed individuals.

1.4.5 Studies of the CAR in MDD Patients

One of the first studies in this area of research compared the CAR in depressed inpatients admitted for psychotherapy vs. a comparison group of other psychiatric inpatients (Huber et al, 2006). Results indicated that depressed inpatients exhibited an attenuated CAR delta compared to the other psychiatric inpatients considered together. A large cohort study by Vreeburg et al. (2009) showed that the CAR AUCi was significantly higher in remitted depressed patients, but not in currently depressed patients, than in normal controls. On the other hand, both currently depressed and remitted depressed patients had a significantly higher mean AUCg than did controls (S. A. Vreeburg et al., 2009). Aubry et al (2010) also found the CAR AUCi to be significantly higher in remitted depressed patients than in controls (Aubry et al., 2010).

One possible explanation for these various findings is that the combination of increased CAR reactivity, and moderately elevated total cortisol output, is a trait characteristic of MDD patients observable only in the remitted state. In the state of depression, there may be enough of an increase in baseline cortisol levels to establish a ceiling effect, reflected by the combination of a high AUCg but low AUCi i.e. if the first cortisol sample at awakening is at or near a maximum; there is no room for cortisol levels to increase at later time points. This interpretation would explain the combination of findings listed above.

A follow up study by Vreeburg et al (2013) showed that a lower CAR AUCi at baseline was associated with a less favorable 2 year outcome in patients with depression and anxiety disorders, which adds another element to this line of work, and might reflect a different phenomenon than observed in the cross sectional data e.g. longer term exhaustion of the HPA-axis as suggested by these very authors.

Another important finding comes from Wardenaar et al (2011). In a study of over 1000 participants with a lifetime history of depression or anxiety, Wardenaar et al found nonlinear inverted U-shaped associations between General Distress, Anhedonic Depression, and Anxious Arousal on the one hand and both the AUCg and AUCi of the CAR on the other. Thus, both high and low severity levels of these dimensional factors were associated with a lower CAR. This non-linear, inverted U distribution is reminiscent of prior work linking stress levels and performance, and may account for some of the discrepancies reviewed in the literature above.
1.5 Summary of Literature Review

Major depressive disorder is a highly disabling illness that affects millions of people worldwide. The effects of MDD are felt at a personal, familial and societal level. In some cases, MDD is effectively treated with ever more advancing pharmaceutical and psychological therapies. However, a significant subgroup of patients experiences incomplete treatment responses, chronicity and/or recurrent episodes. This often leads to repeated hospital admissions, which puts a further strain on the patient’s quality of life, health, and hospital resources. It is of utmost importance to improve our understanding of the factors contributing to sub-optimal treatment responses and to develop predictors of relapse that could help guide clinicians in identifying those at greatest risk.

While a large body of research has studied predictors of treatment response and relapse in MDD, more work is needed to translate these findings into clinical practice. The hypothalamic-pituitary adrenal (HPA) -axis continues to offer significant potential in this regard, given the importance of stress in the onset and maintenance of MDD, and the ability to measure HPA functioning in a minimally invasive, cost effective manner. In an attempt to use HPA-axis measures to inform MDD treatments, the field started with the DST. This test, however promising, lacked specificity. The combined DEX/CRH test has better sensitivity/specificity while providing a more holistic assessment of HPA functioning. However, though the DEX/CRH has been shown to be a strong predictor of treatment response as well as relapse, the relative impracticality of this test has limited its implementation in clinical settings.

The cortisol awakening response (CAR) is a relatively novel measurement of the HPA axis which is easy to administer, inexpensive and does not require significant resources or time.
While some studies have linked particular CAR profiles with an increased risk of depression, few studies have examined the effect of treatment on the CAR over time and/or the ability of the CAR to predict treatment response. With respect to the former, Vreeburg et al. (2009) showed that both current and remitted MDD was associated with a higher CAR vs. controls, particularly as it relates to AUCg. The notion that the CAR is more of a trait than a state marker was further supported by the findings of Gex-Fabry et al. (2011) who showed that the AUCi did not change following participation in mindfulness-based cognitive therapy, despite the clinical benefits of this treatment. With respect to the predictive ability of the CAR, a follow-up study by Vreeburg et al. 2013 did show that a lower AUCi and AUCg was able to predict an unfavorable course trajectory over a 2 year follow-up period in over 800 study participants. In sum, research to date suggests that the CAR may have utility as a predictor of longitudinal course in MDD patients and those at risk of MDD.

To further investigate the potential utility of the CAR in a busy inpatient setting, and to improve our understanding of individual differences in treatment response and course of illness in complex MDD patients, the current study measured the CAR at both admission and discharge in consecutive patients attending a structured four-week inpatient program (the Alternate Inpatient Milieu or AIM program) at the Centre for Addiction and Mental Health. No studies to date have examined changes in the CAR during an inpatient hospital stay. The next section summarizes the Specific Aims and Hypotheses related to this work.
Chapter 2
Rational and Study Questions

2       AIMs and Hypotheses

2.1     Rationale for Current Study

While the understanding and treatment of MDD has improved significantly in recent decades, MDD continues to follow a chronic and/or relapsing course in many cases. Preliminary evidence suggests that one or more measures of HPA-axis activity can help predict depression onset, treatment outcome and/or relapse over time. However, much more research is needed to improve the translational value of this work in busy clinical settings.

The cortisol awakening response (CAR) is a naturalistic measure of the HPA axis which is inexpensive, non-invasive and relatively easy to administer in large numbers of patients. While these practical advantages are noteworthy, more work is needed to assess whether the CAR can predict treatment response and/or course of illness in MDD patients. To date, alterations in the CAR have been shown to predict both mild depressive symptoms (Mangold et al., 2011)(van Santen et al., 2011)(M. Pruessner, 2003) and de novo cases of syndromal MDD (Adam et al., 2010). Having an elevated CAR AUCg may also be a trait marker in patients with established MDD (S. A. Vreeburg et al., 2009). A recent study has shown that a blunted cortisol awakening response predicts a poor outcome over 2 years in depressive outpatients (Vreeburg et al., 2013). While these studies suggest that the CAR may have utility in out-patient settings, no studies to date have looked at the potential utility of the CAR in an inpatient setting.
2.2 General AIM

The general goal of the current study was to determine whether the CAR might help us understand individual differences in treatment response and course in hospital for inpatients attending a 4 week inpatient program, the Alternate Inpatient Milieu (AIM). The three main study questions, related to the 4 week AIM stay, were as follows:

2.3 Question 1:

*Is there a significant change in the clinical measures relevant to depression (QIDS, BAI, PSS) and/or the CAR over the course of an AIM admission?*

2.3.1 Hypothesis 1:

Given the strong focus of the AIM program on stress reduction and behavioral activation, all three clinical measures and the CAR variables (Delta, AUCl, AUcg) will show changes during an AIM admission.
2.4  Question 2:

If so, does the change in the CAR correlate with the change in clinical measures?

2.4.1  Hypothesis 2:

Changes in the CAR over the four week hospital stay are associated with the change in depressive symptoms and/or anxiety symptoms and/or perceived stress.

2.5  Question 3:

Do cross-sectional assessments of the CAR at admission and discharge shed further light on these associations?

2.5.1  Hypothesis 3:

Single cross sectional measurements of the CAR at admission and/or at discharge are associated with the change in depressive symptoms and/or anxiety and/or perceived stress.
2.6 The Research Plan:

To accomplish these goals, 3 major sets of analyses will be done. The first set of analyses looks at the change in the 3 clinical measures (QIDS, BAI, PSS) and the 3 variables of the CAR (CAR Delta, AUCi, and AUCg) over the four week hospital stay. The second examines the relationship between various change scores of the CAR and the change of depressive symptoms and/or anxiety symptoms and/or perceived stress from admission to discharge. The third examines whether one or more cross-sectional measures of the CAR in hospital were predictive of course of illness from admission to discharge. The next section outlines the methodology for the study.
Chapter 3
Methods and Study Design

3 Methods

3.1 Study Design and Subject Recruitment

This project is a longitudinal observational study of inpatients admitted to the Alternate Inpatient Milieu unit at CAMH (the AIM unit) who met full DSM-IV criteria for a current major depressive episode as assessed using the MINI (Sheehan et al., 1998). AIM is a unique 28-day inpatient program providing a home-like setting while emphasizing patient empowerment, goal setting, coping, social skills, and stress management. AIM patients take part in a variety group therapy sessions that start every week day at 10 am. Patients then have lunch and take part in afternoon group sessions. While on AIM patients are also followed by a primary psychiatrist that follows the progress of the patient and helps with medication optimization. The AIM unit has a staff of qualified psychiatrists, nurses, occupational therapists, social workers and recreational activity therapists.

To be admitted to AIM, patients are either transferred from other inpatient units at CAMH or recommended for the program by primary care physicians. Upon admission, staff psychiatrists identified patients suitable to take part in this research, and contacted the study coordinator (BJ) where appropriate. Potential participants were then approached by the study coordinator who implemented the informed consent protocol as approved by the CAMH research ethics board. Most patients recruited had a primary diagnosis of unipolar Major Depressive
Disorder, though a few had a primary diagnosis of bipolar-II disorder, depressed phase. All consenting patients were screened for inclusion and exclusion criteria to determine their suitability as follows:

Inclusion Criteria:

- 18-55 years of age
- Meeting full DSM-IV-TR criteria for a current major depressive episode, as the primary diagnosis based on the MINI module A
- Capable of consenting
- Absence of serious medical illness

3.1.1 Exclusion Criteria

- Meeting lifetime criteria for a primary psychotic disorder or dementia
- Organic brain syndrome or mental retardation
- Any current significant history of substance abuse/dependence (within the past 6 months)
- Acute risk of suicidality
- Psychotic symptoms
- Current diagnosis of Anorexia Nervosa and/or Bulimia Nervosa
- Major medical illnesses (asthma, heart disease, Chron’s, Rheumatoid arthritis, Diabetes, Hepatitis C, etc.)

- Pregnant or lactating women

- Medications likely to influence cortisol measures such as antibiotics and steroid medications

Exclusion factors such as serious medical illness, recent substance abuse, being pregnant or lactating, taking steroid medications, and having a diagnosis of Anorexia Nervosa and/or Bulimia Nervosa have known interactions with the HPA axis. Other exclusion criteria were based on clinical or ethical considerations. These inclusion and exclusion criteria are standard for clinical HPA-axis research.

3.1.2 Demographics and Clinical History

The current study is a part of a larger study looking at the overall effectiveness of AIM. For this thesis the cortisol measurements were specifically added in order to answer the questions posed above. While a significant amount of clinical and demographic data was collected for the larger study, only the most relevant data for the current hypotheses will be included in the thesis. While many variables are potential co-variates for the analyses described below, only a few were considered based on the limited sample size available at this time (discussed in more detail below).
3.1.3 Clinical Assessments

3.1.3.1 Week 1 (Admission)

Mini International Neuropsychiatric Interview (MINI)

The MINI was developed jointly in the United States and Europe in order that it covers both DSM-IV and ICD-10 psychiatric disorders. It has been validated based on the Standard Clinical Interview for the DSM (SCID), the gold standard for psychiatric assessment (Sheehan et al., 1998). The key advantage of the MINI is that it requires far less time to administer than the SCID, which reduces patient burden. Module A of the MINI covers the diagnosis of a current MDE, the essential inclusion criterion for this study. Co-morbid disorders as assessed by the other modules of the MINI were not a basis for exclusion, unless they were the primary reason for hospitalization and focus of treatment. The MINI assessment was completed within 2 days of admission to AIM.

Quick Inventory of Depressive Symptomology (QIDS)

The QIDS is a commonly used tool to measure changes in depressive symptoms over time. The main advantage of the QIDS is that it is a self-report requiring less time and resources to administer than does the Hamilton Depression Rating scale (HDRS). The QIDS has 16 questions which address the core symptoms of MDD including depressed mood, loss of interest, changes in appetite, insomnia/hypersomnia, agitation/retardation, fatigue or loss of energy, feelings of worthlessness or guilt, diminished ability to think or being indecisive or current thoughts of death or suicide. The QIDS is a well validated measure for depression research (Rush et al., 2003)
Beck Anxiety Inventory (BAI)

The BAI is a 21 question self-report measure used to assess subjective, somatic, or panic-related symptoms of anxiety. It is widely used in research and validated by several studies (Beck, Epstein, Brown, & Steer, 1988).

Perceived Stress Scale (PSS)

This is a validated 14-item scale that measures cognitive and affective responses to stress. The PSS was included in the current protocol based on its prior associations with various measures of HPA-activity (S. Cohen, Kamarck, & Mermelstein, 1983).
3.2 Cortisol Assessments

3.2.1 Saliva Collection

Participants were asked to collect 2 consecutive days of morning cortisol samples. For consistency these collections were completed on the same days each week. Saliva was collected using salivette tubes manufactured by Sarstedt Germany. Participants were given 3 tubes marked T-0, T-30 and T-60 the night before. Participants were asked to awake at their normal time, though this is highly controlled in an inpatient setting to ensure maximal participation in the morning treatment programs. They were asked to provide saliva samples immediately upon waking, and 30 and 60 minutes after awakening. Participants recorded what time they went to bed, when they awoke and when they performed each sample, in a journal that was provided. The research coordinator came to the AIM unit at 12 P.M. to obtain the completed samples from that morning and to provide new tubes for the following day. The second day of sampling was identical to the first. Samples were stored in a -74°C freezer on site at CAMH. During the 28-day program participants were asked to provide samples at admission and discharge.

3.2.2 Assays

Assays were performed at St. Joseph’s Healthcare Centre in Hamilton Ontario by the primary author of the thesis (BJ), under the direct supervision of a highly experienced lab technician (MC). Samples were transported on dry ice from the Centre for Addiction and Mental Health to St. Joseph’s Healthcare Centre in Hamilton and subsequently stored at -80°C. All samples were assayed using salimetrics expanded range high sensitivity salivary cortisol EIA kits
from lot number 1302507. The salimetric’s kit follows a standard protocol for all ELISAs and had a sensitivity of <0.087 nmol/L. All standards, controls, and unknowns were performed in duplicate. Upon completion all samples were transported back on dry ice to CAMH and remain in -80°C for future analysis. The intra and inter – variability among all assays was less than 10% for both. All cortisol values are reported in nmol/L.
4 Descriptive Results:

4.1 Patient Recruitment:

Figure 3 below summarizes the patient flow through the various study phases. As shown, a total of 51 participants completed informed consent, and 40 were enrolled after completing the screening protocol. Eleven other individuals did not meet entry criteria and were excluded at this point. Of the 40 participants enrolled, 26 completed the full study protocol. Most drop-outs occurred during the first week, due mostly to an inability to complete morning awakening samples. Another 3 subjects dropped out later during their hospital stay. Two participants left the inpatient unit before planned discharge and were excluded on this basis.
Figure 3: Patient Flow of Study. Figure 3 describes the flow of patients that took part in the AIM study. It outlines who many patients were recruited, how many completed consent, how many were eligible, and how many fully completed the study.
4.2 Patient Demographics

Clinical and demographic data were collected while patients were on the unit. This data can be seen in table 1.

**Table 1:** Demographic and Clinical Data for the Current Sample (means +/- S.D for continuous measures)

<table>
<thead>
<tr>
<th>Item</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>37.4 +/- 12.5</td>
</tr>
<tr>
<td>Length of Current Depressive Episode</td>
<td>4.1 +/- 1.2 years</td>
</tr>
<tr>
<td>Mean BMI</td>
<td>28.4 +/- 1.1</td>
</tr>
<tr>
<td>Smokers</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Male</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (65%)</td>
</tr>
<tr>
<td>MDD</td>
<td>23 (88%)</td>
</tr>
<tr>
<td>Bipolar II</td>
<td>3 (12%)</td>
</tr>
</tbody>
</table>
4.3 Calculation of CAR Delta, AUCi, and AUCg

To calculate CAR Delta, AUCi and AUCg we used the equations outlined in Pruessner et al. (2003) which are considered a standard in the field (Jens C Pruessner et al., 2003). As these equations assume regular time intervals between samples, participants were asked to record the specific clock times at which they completed each sample. A review of these sampling times revealed excellent overall compliance with the study protocol, with only a few significant deviations. One participant was excluded from the AUC calculations as they systemically took the 3rd sample 30 minutes later than the required time. This participant was included for the CAR Delta calculations. As delays exceeding 15 minutes have been shown to affect the CAR (Okun et al. 2010), two individual samples for two other participants were also excluded. One participant had compromised discharge samples and was thus excluded from all CAR analyses.

4.4 Normality of the Data

As cortisol data often require statistical transformation due to outliers and non-normal distributions, we first examined whether the key study variables met criteria for normality using the Explore function of SPSS-16 software. Normality was established based on the Kolmogorov-Smirnov (K-S) test using an a priori cut-off of p > .01 i.e. variables that had a distribution with a K-S statistic at p > .01 were considered normally distributed. Results indicated that all key variables used for subsequent analyses did in fact follow a normal distribution and did not contain extreme outliers. Importantly, the key Cortisol Awakening Response (CAR) measures including the Delta, AUCg and AUCi at both admission and discharge from hospital met criteria for normality.
4.5 Correlation of CAR on Day 1 and Day 2

The CAR was measured on 2 consecutive days while in hospital. To determine if the day 1 measurements were correlated with day 2 measurements we performed a series of Pearson correlations based on each individual time point (t0, t30, and t60) across the two days. Day 1 and Day 2 samples at admission were significantly correlated (See table 2 below), as were the corresponding measures at discharge (see table 3 below). Based on these findings, we used the mean values across day 1 and day 2 for all subsequent analyses including calculation of the AUC measures.

Table 2. Correlations between Day 1 and Day 2 Samples at Admission

<table>
<thead>
<tr>
<th>Cortisol Measurements at admission</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 and Day 2 t-0 correlation</td>
<td>r=0.38, p=0.09</td>
</tr>
<tr>
<td>Day 1 and Day 2 t-30 correlation</td>
<td>r=0.43, p=0.04</td>
</tr>
<tr>
<td>Day 1 and Day 2 t-60 correlation</td>
<td>r=0.63, p&lt;0.00</td>
</tr>
</tbody>
</table>
**Table 3:** Correlations between Day 1 and Day 2 Samples at Discharge

<table>
<thead>
<tr>
<th></th>
<th>Day 1 and Day 2 t-0 correlation</th>
<th>Day 1 and Day 2 t-30 correlation</th>
<th>Day 1 and Day 2 t-60 correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r=0.56, \ p=0.01 )</td>
<td>( r=0.59, \ p&lt;0.01 )</td>
<td>( r=0.38, \ p=0.11 )</td>
</tr>
</tbody>
</table>

4.6 Correlation of CAR Variables at Admission and Discharge

Pearson correlations were used to determine if the three CAR variables correlated with one another; this was done separately for the admission and discharge values. At admission we found all 3 CAR variables to be highly correlated with one other (see table 4).

**Table 4:** Correlation among CAR Variables at Admission

<table>
<thead>
<tr>
<th>Admission</th>
<th>CAR Delta</th>
<th>AUCi</th>
<th>AUCg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR Delta</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCi</td>
<td>( r=0.95, \ p&lt;0.01 )</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AUCg</td>
<td>( r=0.58, \ p&lt;0.01 )</td>
<td>( r=0.53, \ p=0.01 )</td>
<td>1</td>
</tr>
</tbody>
</table>
Completing the same analysis at discharge, we found that only the CAR Delta and AUCi remained highly correlated (see table 5).

Table 5: Correlation among CAR Variables at Discharge

<table>
<thead>
<tr>
<th>Discharge</th>
<th>CAR Delta</th>
<th>AUCi</th>
<th>AUCg</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR Delta</td>
<td>1</td>
<td>r=0.95, p&lt;0.01</td>
<td>r=0.22, p=0.31</td>
</tr>
<tr>
<td>AUCi</td>
<td>r=0.95, p&lt;0.01</td>
<td>1</td>
<td>r=0.04, p=0.85</td>
</tr>
<tr>
<td>AUCg</td>
<td>r=0.22, p=0.31</td>
<td>r=0.04, p=0.85</td>
<td>1</td>
</tr>
</tbody>
</table>

In a separate set of analyses we examined whether the CAR measures at admission correlated with their corresponding measures at discharge. Results indicated that each admission CAR variable was highly correlated with its discharge value (see table 6). This suggests that for the most part, patients tended to maintain their rank order for each CAR value.
**Table 6:** Correlation of CAR variables at Admission and Discharge

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission CAR Delta vs. Discharge CAR Delta</td>
<td>$r=0.63$, $p&lt;0.01$</td>
</tr>
<tr>
<td>Admission AUCi vs. Discharge AUCi</td>
<td>$r=0.65$, $p&lt;0.01$</td>
</tr>
<tr>
<td>Admission AUCg vs. Discharge AUCg</td>
<td>$r=0.63$, $p&lt;0.01$</td>
</tr>
</tbody>
</table>

### 4.7 Exploration of Potential Covariates

While the small sample size (and thus limited statistical power) available for the current analyses limited our ability to include potential covariates, we did examine whether age, gender, smoking status, time of awakening, and body mass index (BMI) were associated with the CAR. We performed Pearson correlations to study the strength of the associations between CAR measurements at admission and discharge and both age and BMI. Unpaired t-tests were used to compare CAR measurements across the two sexes and whether or not participants were smokers.

Results indicated that none of these potential covariates was significantly associated with either AUCi or AUCg at admission or discharge. In light of this, we did not include these demographic variables as covariates for AUCi or AUCg in subsequent analyses.

Discharge CAR Delta was significantly correlated with age ($r=0.46; p=0.02$). All subsequent analyses based on the CAR Delta at discharge thus included age as a covariate.
4.8 Over all Statistical Approach

All analyses were done using SPSS-16 software. Analyses that examined the change in a single variable over two time points across all study subjects used paired t-tests. Analyses that examined the simple univariate association between two distinct variables used Pearson correlation. Where individual study subjects were assigned to a specific study group (e.g. good vs. poor responders), Analysis of Variance was used to compare means between groups. In general, means are reported +/- standard errors.

For all analyses, a p value of 0.05 or less was considered significant. Given that the results of this preliminary analysis will help inform future research on the AIM unit, and to minimize type 2 errors, we also wanted to identify Pearson correlations with a large effect size independent of p value. Based on Cohen et al. (1988), this would correspond to an r value of 0.371 or higher i.e. we considered Pearson correlations with an r value of greater than 0.371 to reflect a large and notable effect size independent of its p-value.
Chapter 5
Study Question Results

5 Examination of the CAR during the four Week AIM Program

The following summarizes the main study questions, hypotheses, statistical methods and results for this study:

Main Study Questions

**Question 1:**

*Is there a significant change in clinical measures relevant to depression (QIDS, BAI, PSS) and/or the CAR over the course of an AIM admission?*

**Hypothesis 1:**

*Given the strong focus of the AIM program on stress reduction and behavioral activation, all three clinical measures and the CAR variables (Delta, AUCi, AUCg) will show changes during an AIM admission.*
**Question 2:**

*If so, does the change in the CAR correlate with the change in clinical measures?*

**Hypothesis 2:**

*Changes in the CAR over the four week hospital stay will be associated with the change in depressive symptoms and/or anxiety symptoms and/or perceived stress.*

**Question 3:**

*Do cross-sectional assessments of the CAR at admission and discharge shed further light on these associations?*

**Hypothesis 3:**

*Single cross sectional measurements of the CAR at admission and/or at discharge will be associated with the change in depressive symptoms and/or anxiety and/or perceived stress.*

The following sections will consider each of the 3 hypotheses stated above in sequence including the statistical approach used, major results, and a specific discussion in each case.
5.1 Hypothesis 1:

*Given the strong focus of the AIM program on stress reduction and behavioral activation, all clinical measures and the CAR Delta, AUCg and AUCi will show changes during an AIM admission.*

5.1.1 Statistical approach

A series of paired t-tests were used to assess the percent change in clinical measures (QIDS, BAI, PSS) and the absolute change in the CAR (CAR Delta, AUCi and AUCg) from admission to discharge (weeks 1 and 4). When performing these calculations, we used absolute rather than percent changes for the CAR measures as several patients had very low CAR values at admission which can greatly exaggerate statistics based on percentage change.

5.1.2 Results:

5.1.2.1 Percent Change in Depressive Symptoms as Measured using the QIDS:

As shown in figure 4, as expected, there was a notable decrease in the QIDS over the course of the inpatient stay.
Figure 4: Reduction in QIDS from Admission to Discharge. Figure 4 shows that there was a significant decrease in QIDS from admission to discharge (p<0.01)

The mean QIDS scores at admission and discharge were 16.9 (+- 1.1) and 12.7 (+-1.3) respectively (paired t=5.64, df= 25, p < .01).

5.1.2.2 Percent Change in Anxiety symptoms as measured by the BAI

As shown in figure 5 below there was a decrease in anxiety symptoms as assessed using the BAI over the course of the inpatient stay.
**Figure 5: Reduction of Anxiety from Admission to Discharge.** Figure 5 shows that there was a significant decrease of anxiety symptoms from admission to discharge (p<0.01).

The mean BAI scores at admission and discharge were 30.8 (+1.9) and 23.4 (+2.3) respectively (paired t=4.14, df= 22, p < 0.01).
5.1.2.3 Percent Change Perceived Stress Scale as measured by the PSS

As shown in figure 6 below there was a decrease in perceived stress as assessed using the PSS over the course of the inpatient stay.

**Figure 6: Reduction of Perceived Stress from Admission to Discharge.** Figure 6 shows that there was a significant decrease of anxiety symptoms from admission to discharge (p<0.01).

The mean PSS scores at admission and discharge were 38.2 (+-1.2) and 33.1 (+-1.3) respectively (paired t=4.96, df= 24, p<0.01).
5.1.2.4 Changes in the Cortisol Awakening Response from Admission to Discharge

Figure 7 below plots the individual mean cortisol measures taken at awakening (t 0) and +30 and +60 minutes post-awakening, at both admission and discharge.

**Figure 7:** The Cortisol Awakening Response at Admission and Discharge among all Patients. Figure 7 shows the individual measure of the CAR at both admission and discharge in all patients. There was a trend towards significant change in the t=30 measure.
When we compared admission and discharge values of the T0, +30 and +60 time points, each considered separately, paired t-tests revealed that the +30 measures changed at a trend level (p=0.09) over time, with the admission week +30 values being higher than the discharge week +30 values as shown (see also table 7). Neither the t0 nor the +60 measures changed significantly from admission to discharge.

**Table 7:** Paired t-tests to compare Means of the 3 Time Point of the CAR at Admission vs. Discharge

<table>
<thead>
<tr>
<th>Time</th>
<th>T</th>
<th>Df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0 Admission vs. Discharge</td>
<td>-0.47</td>
<td>24</td>
<td>0.64</td>
</tr>
<tr>
<td>Time +30 Admission vs. Discharge</td>
<td>1.78</td>
<td>24</td>
<td>0.09</td>
</tr>
<tr>
<td>Time +60 Admission vs. Discharge</td>
<td>-0.22</td>
<td>23</td>
<td>0.83</td>
</tr>
</tbody>
</table>

When the admission data was next considered separately, we found the difference between time 0 and time+30, as well as the difference between time +30 and time+60, to be highly significant (see table 8), consistent with a robust CAR at 30 minutes followed by a rapid return to baseline by 60 minutes.
Table 8: Paired T-tests examining the Change in Sequential Cortisol Values of the CAR at Admission

<table>
<thead>
<tr>
<th>Admission Paired T-tests</th>
<th>T</th>
<th>Df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0 – time +30</td>
<td>-3.71</td>
<td>24</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Time +30 – time+60</td>
<td>2.34</td>
<td>23</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

The same analyses performed on the discharge data revealed only a statistical trend for an increase from time 0 to +30 minutes, and no significant difference from T+30 to T+60, further demonstrating the relative blunting of the CAR at discharge.

Table 9: Paired T-tests examining the Change in Sequential Cortisol Values of the CAR at Discharge

<table>
<thead>
<tr>
<th>Discharge Paired T-tests</th>
<th>T</th>
<th>Df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 0 – time +30</td>
<td>-2.01</td>
<td>24</td>
<td>0.06</td>
</tr>
<tr>
<td>Time +30 – time+60</td>
<td>0.80</td>
<td>23</td>
<td>0.43</td>
</tr>
</tbody>
</table>

The following sections will now address changes in the integrated measures of the CAR as defined by CAR delta, AUCi and AUCg, from admission to discharge.
5.1.2.5 Absolute Change in the CAR Delta (T+30 – T0) from Admission to Discharge

As shown in figure 8 below there was a significant decrease in the CAR delta over the course of the inpatient stay.

![Figure 8: CAR Delta over the 4 week Inpatient Stay](image)

**Figure 8: CAR Delta over the 4 week Inpatient Stay.** Figure 8 shows that from admission to discharge there was a significant change in the CAR delta variable (p=0.02)

The mean CAR Delta values at admission and discharge were 3.20 (+- 0.90) and 1.46 (+- 0.70) respectively (paired t=2.50 df=24 p=0.02).
5.1.2.6 Absolute Change in AUCi from Admission to Discharge

As shown in figure 9 below there was a trend towards a significant decrease in the AUCi over the course of the inpatient stay.

![Graph of AUCi over the 4 week Inpatient Stay](image)

**Figure 9: AUCi over the 4 week Inpatient Stay.** Figure 9 shows that from admission to discharge there was a trend towards significant change in the AUCi variable (p=0.07)

The mean AUCi values at admission and discharge were 4.1 (± 1.2) and 2.1 (±1.2) respectively (paired t=1.88 df=23 p=0.07).
5.1.2.7 Absolute Change in AUCg from Admission to Discharge

As shown in figure 10 below there was a nominal decrease in AUCg over the course of the inpatient stay.

![AUCg over the 4 week Inpatient Stay](image)

**Figure 10: AUCg over the 4 week Inpatient Stay.** Figure 10 shows that from admission to discharge there was no change in the AUCg among all participants.

The mean AUCg at admission was 24.0 (+- 1.7) and at discharge it was 22.6 (+-1.6). This decrease was not significant.

5.1.3 Summary of Hypothesis 1 Results

In sum, over the course of their four week stay on the AIM unit, study subjects exhibited a statistically significant decrease in depression scores, anxiety and perceived stress. Over the
same four weeks there was a statistically significant decrease in CAR Delta and a trend for a
decrease in the CAR AUCi. Of note, both the CAR Delta and AUCi measure the reactivity of the
HPA-axis upon awakening. No significant decrease in AUCg from admission to discharge was
found. Taken as a whole, this pattern of results suggests that across all subjects, there was a
decrease in the reactivity of the HPA-axis at awakening from admission to discharge, but no
change in total cortisol output at awakening over this same period.

5.1.4 Specific Discussion of Hypothesis 1:

This study was the first to look at serial measures of the CAR in an inpatient population
being treated for Major Depressive Disorder. The results suggest that over the four week AIM
hospital stay, across all study subjects, there was a decrease in the reactive component of the
CAR as measured by the CAR Delta and AUCi, but no change in total cortisol output as
measured by the AUCg. As expected, all three clinical measures decreased significantly from
admission to discharge. While there is no comparative CAR data available from other inpatient
studies of MDD, and no outpatient studies looking at serial CAR measures in acutely depressed
patients undergoing treatment, Gex-Fabry et al (2012) examined serial CAR measures over an 8
week period in 56 remitted outpatients with MDD. These individuals were being treated with
either Mindfulness-Based Cognitive Therapy (MBCT) or treatment as usual (TAU), with a
clinical goal of preventing relapse. In their study, no effect of time, treatment group, or group X
time interaction was found for the CAR measures, including AUCi. This contrasts the current
study which found a significant drop in the CAR Delta, and a trend for a decrease in AUCi
measures, over the four week hospital stay. While both the remitted and outpatient status of the
Gex-Fabry sample makes it difficult to compare results across studies, it is of note that the AIM
unit also emphasizes stress reduction techniques including MBCT. It may be that stress reduction with MBCT has no effect on the CAR response in euthymic individuals, but can help lower the CAR in individuals who present with acute symptoms. However, it may also be the case that the drop in AUCi observed in the current study reflects non-specific aspects of inpatient treatment. This is discussed further in the next section.

In interpreting the decrease in CAR reactivity from admission to discharge it is notable that the T +30 measure of the CAR, and not baseline differences, accounted for the significant differences found i.e. only the +30 measure dropped meaningfully from admission to discharge (at a trend level), while the t0 and t60 measures changed little over time. This indicates that the relatively blunted CAR Delta and AUCi’s at discharge were not simply attributable to high T0 cortisol values and a ceiling effect related to this.

It is of note that only measures of CAR reactivity (CAR Delta and AUCi) tended to decrease in hospital while total cortisol output (AUCg) did not, underlining the importance of considering CAR reactivity distinct from total output (Pruessner et al, 2002). Interestingly, the three CAR variables were highly correlated at admission, while at discharge the AUCg was no longer correlated with the CAR Delta and the AUCi. This further suggests that the reactive measures of the CAR were differentially affected by the inpatient program than was the CAR AUCg. It would appear that CAR reactivity may have unique relevance in monitoring the course of MDD treatment vs. other cortisol measures.

The next section will examine whether the observed decrease in the reactive measures of the CAR while in hospital were associated with the concomitant decreases in depressive symptoms, anxiety and/or perceived stress.
5.2 Hypothesis 2:

Changes in the CAR over the four week hospital stay will be associated with the change in depressive symptoms and/or anxiety and/or perceived stress.

5.2.1 Statistical Approach

Pearson correlations were used to test the strength of the associations between the change in the CAR Delta, AUCi and AUCg from admission to discharge and the percent change in the clinical variables (QIDS, BAI and PSS) over this same time period. When using the CAR delta at discharge, partial correlations were used with age as a covariate. When performing these calculations, we used absolute rather than percent changes for the CAR measures as several patients had very low CAR values at admission which can greatly exaggerate statistics based on percentage change.

5.2.2 Results:

5.2.2.1 Correlations between the Absolute Change in the CAR Delta and the Percent Change in Clinical Symptoms from Admission to Discharge

As shown in table 10 below, the absolute change in the CAR Delta over the four week hospital stay was not significantly correlated with the percent change in depression symptoms, anxiety symptoms, or perceived stress over this same time period.
Table 10: Univariate Correlations between CAR Delta Change Scores and Percent Decrease in Clinical Symptoms over the Four Week Hospital Stay

<table>
<thead>
<tr>
<th>Net Decrease in CAR Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent Decrease in QIDS</strong></td>
</tr>
<tr>
<td><strong>Percent Decrease in BAI</strong></td>
</tr>
<tr>
<td><strong>Percent Decrease in PSS</strong></td>
</tr>
</tbody>
</table>

5.2.2.2 Correlations between the Absolute Change in the AUCi and the Percent Change in Clinical Symptoms from Admission to Discharge

As shown in table 11 below, the absolute change in the CAR AUCi from admission to discharge was not correlated with the percent change in depression symptoms, anxiety symptoms, or perceived stress.
Table 11: Univariate Correlations between AUCi Change Scores and Percent Decrease in Clinical Symptoms over the Four Week Hospital Stay

<table>
<thead>
<tr>
<th>Net Decrease in AUCi</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Decrease in QIDS</td>
<td>r=-0.03, p=0.90</td>
</tr>
<tr>
<td>Percent Decrease in BAI</td>
<td>r=0.19, p=0.39</td>
</tr>
<tr>
<td>Percent Decrease in PSS</td>
<td>r=0.28, p=0.19</td>
</tr>
</tbody>
</table>

5.2.2.3 Correlation between the Absolute Change in AUCg and the Percent Change in Clinical Symptoms from Admission to Discharge

As shown in table 12 below, the absolute change in AUCg from admission to discharge was not significantly correlated with the percent changes in depression symptoms, anxiety symptoms, or perceived stress.
Table 12: Univariate Correlations between Change Scores of the AUCg and Percent Decrease of Clinical Symptoms

<table>
<thead>
<tr>
<th>Percent Decrease in QIDS</th>
<th>r=0.15, p=0.47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Decrease in BAI</td>
<td>r=0.22, p=0.33</td>
</tr>
<tr>
<td>Percent Decrease in PSS</td>
<td>r=0.27, p=0.20</td>
</tr>
</tbody>
</table>

5.2.3 Summary of Hypothesis 2 Results

In sum, while the results summarized in section 5.2 above demonstrated significant decreases in the CAR Delta, AUCi and all three clinical measures from admission to discharge, the CAR change scores did not correlate significantly with the clinical measures change scores.
5.2.4 Specific Discussion of Hypothesis 2:

The second study question assessed whether the changes in clinical measures over the four week hospital stay were significantly correlated with a change in one or more of the CAR measures under investigation. If so, this would provide a relatively clear explanation for why the CAR may have changed during the AIM program. Interestingly, despite finding a drop in CAR reactivity from admission to discharge (and a drop in clinical measures as expected), the changes in CAR Delta and AUCi in hospital were not associated with the concomitant changes in the various clinical measures. These results suggest that the reduction in the CAR delta and AUCi in hospital may have been mediated by one or more factors unrelated to the state of depression per se.

One possible contributor to the overall decrease in the CAR in hospital may have been the introduction of new medications that can moderate HPA-axis function independently of actual clinical efficacy. Indeed, a recent study of over 1500 individuals with MDD demonstrated that different classes of anti-depressant medications might have differential effects on various HPA measures including the CAR (Manthey et al., 2011). Some patients are administered anti-psychotic medications for the first time on the AIM unit, which might in theory influence the CAR, although preliminary research has been negative in this regard (Mondelli et al., 2010). Examining the possible effects of medication changes on the CAR is a longer term goal for this line of research, however was beyond the scope of the current project based on the wide range of treatment changes performed in hospital and thus the very large sample sizes needed for this type of work.

Another possible contributor to the decrease in cortisol reactivity while on AIM is a change in sleep-wake schedules. The influence of sleep duration and wake-up time on the CAR
has recently been studied in a mixed sample of remitted depressed patients and controls (Aubry et al., 2010). Results indicated that longer sleep duration and later awakening time were both associated with a decrease in the CAR AUCg in these individuals, although these same sleep measures had no effect on the reactive component of the CAR as assessed using CAR Delta. As the current findings found decreases in CAR reactivity but not AUCg over the four week hospital stay, the opposite pattern to that described by Aubry et al, it is somewhat less likely that similar sleep changes accounted for the current changes in CAR reactivity, although this will be testable empirically once the sample size is increased. One major advantage of an inpatient setting for work of this type is the ability to measure and control sleep-wake times relative to outpatient settings, making this an interesting focus for future AIM work.

While no correlation between the change in the CAR reactivity measures and the change in clinical measures from admission to discharge was found, a potential association between single cross-sectional measurements of the CAR and the clinical change scores was also examined. This is discussed in the following section.
5.3 Hypothesis 3:

Single cross sectional measurements of the CAR at admission and/or discharge will be associated with the change in depressive symptoms and/or anxiety and/or perceived stress.

5.3.1 Statistical Approach

Pearson correlations were used to examine the association between single cross-sectional measurements of the CAR Delta, AUCi and AUCg at either admission or discharge with the percent change in QIDS, BAI and PSS over the course of treatment. When looking at the discharge CAR delta, partial correlations were used with age as a co-variate.

5.3.2 Results

5.3.2.1 Correlations between Cross-sectional Measurements of the CAR Delta and the Percent Change in Clinical Symptoms from Admission to Discharge

As shown in table 13 below, both the admission and discharge CAR deltas were significantly correlated with the percent decrease in QIDS from admission to discharge. Figures 11 and 12 below plot these correlations at admission and discharge. This suggests that patients who experienced the greatest decrease in depressive symptoms while on the AIM unit also entered hospital and left the program with greater reactivity of the CAR than did their more
treatment-resistant peers. This finding will be further analyzed and discussed in section 5.4 below.

There was no significant correlation between any of the cross-sectional measurements of the CAR Delta with the percent change in BAI and PSS.

**Table 13:** Univariate Correlations between Individual Measures of CAR Delta and Percent Decrease in Clinical Data from Admission to Discharge

<table>
<thead>
<tr>
<th></th>
<th>Admission CAR Delta</th>
<th>Discharge CAR Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Decrease in QIDS</td>
<td>r=0.40, p=0.05</td>
<td>r=0.55, p&lt;0.01</td>
</tr>
<tr>
<td>Percent Decrease in BAI</td>
<td>r=0.27, p=0.22</td>
<td>r=0.19, p=0.37</td>
</tr>
<tr>
<td>Percent Decrease in PSS</td>
<td>r=0.04, p=0.83</td>
<td>r=-0.26, p=0.21</td>
</tr>
</tbody>
</table>
Figure 11: Admission CAR Delta vs. Percent Reduction in QIDS (Admission to Discharge).
Figure 11 plots the admission CAR Delta vs. the percent reduction in QIDS from admission to discharge. It shown that the is a positive linear correlation among a higher admission CAR Delta and a great percent reduction in QIDS ($R^2=0.16$). All x-values were transformed by adding +5 so that all values were positive.

*all data were transformed by adding 5 in order to make all x axis values positive
Figure 12: Discharge CAR Delta vs. Percent Reduction in QIDS (Admission to Discharge). Figure 12 plots the discharge CAR Delta vs. the percent reduction in QIDS from admission to discharge. It shown that ther is a positive linear correlation among a higher discharge CAR Delta and a great percent reduction in QIDS ($R^2=0.31$).

*all data were transformed by adding 5 in order to make all x axis values positive

5.3.2.2 Correlations between Cross-sectional Measurements of the AUCi and the Percent Change in Clinical Symptoms from Admission to Discharge

As shown in table 14 below, the percent decrease in QIDS from admission to discharge was significantly correlated with both admission and discharge AUCi. Figures 13 and 14 plots these correlations. This pattern of results replicates the findings summarized immediately above
for CAR Delta. There was no significant correlation between the percent change in BAI and PSS with any of the cross-sectional measurements of the AUCi.

Table 14. Univariate Correlations between Individual Measures of AUCi and Percent Decrease in Clinical Data from Admission to Discharge

<table>
<thead>
<tr>
<th></th>
<th>Admission AUCi</th>
<th>Discharge AUCi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Decrease in QIDS</td>
<td>r=0.48, p=0.02</td>
<td>r=0.54, p=0.01</td>
</tr>
<tr>
<td>Percent Decrease in BAI</td>
<td>r=0.32, p=0.15</td>
<td>r=0.19, p=0.40</td>
</tr>
<tr>
<td>Percent Decrease in PSS</td>
<td>r=0.05, p=0.83</td>
<td>r=0.19, p=0.37</td>
</tr>
</tbody>
</table>
Figure 13: Admission AUCi vs. Percent Reduction in QIDS (Admission to Discharge). Figure 13 plots the admission CAR Delta vs. the percent reduction in QIDS from admission to discharge. It shown that there is a positive linear correlation among a higher admission AUCi and a greater percent reduction in QIDS ($R^2=0.23$).

*all data were transformed by adding 5 in order to make all x axis values positive
**Figure 14:** Discharge AUCi vs. Percent Reduction in QIDS (Admission to Discharge). Figure 14 plots the discharge AUCi vs. the percent reduction in QIDS from admission to discharge. It shown that there is a positive linear correlation among a higher discharge AUCi and a greater percent reduction in QIDS ($R^2=0.29$).

*all data were transformed by adding 12 in order to make all x axis values positive*
5.3.2.3 Correlations between Cross-sectional Measurements of the AUCg and the Percent Change in Clinical Symptoms from Admission to Discharge

Cross sectional measurements of the AUCg during treatment were not significantly correlated with the percent drop in the QIDS, BAI or PSS from admission to discharge.

**Table 15:** Univariate Correlations between Individual Measures of AUCg and Percent Decrease in Clinical Data from Admission to Discharge

<table>
<thead>
<tr>
<th></th>
<th>Admission AUCg</th>
<th>Discharge AUCg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Decrease in QIDS</td>
<td>r=0.33, p=0.12</td>
<td>r=0.23, p=0.29</td>
</tr>
<tr>
<td>Percent Decrease in BAI</td>
<td>r=0.14, p=0.54</td>
<td>r=0.04, p=0.86</td>
</tr>
<tr>
<td>Percent Decrease in PSS</td>
<td>r=0.13, p=0.54</td>
<td>r=0.10, p=0.63</td>
</tr>
</tbody>
</table>
5.3.3 Specific Discussion of Hypothesis 3:

To put the results of question 3 into a larger context, the main findings from hypotheses 1-3 were summarized as follows:

During the course of the 4 week AIM admission, across all study subjects, the following were demonstrated:

- There was a significant decrease of all 3 clinical measures as assessed using the QIDS, BAI and PSS.
- There was a significant decrease in the CAR Delta, a trend towards a significant decrease in the AUCi, and no change in the AUCg.
- There was no correlation between absolute changes in the CAR measures and percent changes in clinical measures.
- Interestingly, while there was a trend for the CAR Delta and AUCi to decrease from admission to discharge, higher cross-sectional measurements of the CAR delta and AUCi were significantly correlated with a greater percent reduction in depressive symptoms.

Taken as a whole, these findings suggest that there is an overall effect of being on AIM which causes the CAR reactivity to decrease across all subjects, yet having lower CAR reactivity was also associated with less clinical improvement based on the QIDS. To help reconcile this apparent contradiction, a series of analyses was completed post hoc as described in the next section.
5.4 Post hoc Analyses Grouping Patients based on Treatment Response during AIM

5.4.1 Rationale

In combining the results for hypotheses 1, 2 and 3 above, it is somewhat difficult to reconcile the main positive findings related to the CAR Delta and AUCi. The first set of analyses demonstrated that the CAR Delta and AUCi both decreased from admission to discharge, suggesting that lowering the reactivity of the CAR may be a positive aspect of inpatient treatment. On the other hand, there was no significant association between the drop in these CAR measures and the drop in actual clinical measures while on the AIM unit. Furthermore, having a high CAR Delta or AUCi at admission or discharge was associated with a greater decrease in depressive symptoms as measured using the QIDS. These latter findings would suggest that higher reactivity of the CAR is a positive clinical sign.

To shed further light on this apparent discrepancy, it was decided to designate each patient as a good or poor responder to the AIM unit, and to perform repeated measures ANOVAs predicting the CAR using a 2 treatment response (good or poor) X 2 time (admission, discharge) design. Given the highly chronic nature of the current sample, and their low rate of achieving a full treatment response, we defined treatment response groups based on a median split of the QIDS change score i.e. patients who exhibited an above -the- median QIDS response (a 25% or greater reduction in QIDS scores) were considered good responders (n=12) while patients exhibiting a below-the median change in QIDS were designated poor responders (n=12).

The following section summarizes the results of these RANOVA models.
5.4.2 Comparing the Change in the CAR Delta and AUCi from Admission to Discharge in Good and Poor Responders to the AIM Program

5.4.2.1 Changes in the CAR Delta from Admission to Discharge in Good and Poor Responders

Figure 15 below illustrates the pattern of change of the CAR Delta in good and poor treatment responders over the four week AIM program.

**Figure 15: Change in CAR Delta from Admission to Discharge Split by Good vs. Poor Responders.** Figure 15 plots the admission and discharge CAR Delta split by good and poor responders (median split of 25% reduction in QIDS). RANOVA analysis shows that there was a significant effect of time and a significant group effect, but no group X time effect. This suggests that though both groups change over time there is no significant difference in the pattern of change.
A 2 (treatment response group) x 2 (time) repeated measures analysis of variance predicting the CAR Delta demonstrated that the tendency for good responders to have a higher CAR Delta was in fact a trait effect i.e. there was a significant main effect of time and a significant between-groups effect for the CAR Delta, but no group X time interaction. This suggests that 1. both good and poor responders experience a decrease in CAR delta while in hospital and 2. good responders have higher CAR deltas than do poor responders both before and after treatment on AIM (see also table 16).

**Table 16: Repeated Measures Analysis of CAR Delta from Admission to Discharge in Good and Poor Responders**

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>p</th>
<th>Partial Eta²</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>6.06</td>
<td>1.23</td>
<td>0.02</td>
<td>0.21</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>Response</strong></td>
<td>5.23</td>
<td>1</td>
<td>0.03</td>
<td>0.19</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>ResponsexTime</strong></td>
<td>0.09</td>
<td>1.23</td>
<td>0.77</td>
<td>0</td>
<td>0.06</td>
</tr>
</tbody>
</table>

5.4.2.2 Changes in AUCi from Admission to Discharge in Good and Poor Responders

Figure 15 below illustrates the pattern of change of the CAR AUCi in good and poor treatment responders over the four week AIM program.
Figure 16: Change in AUCi from Admission to Discharge Split by Good vs. Poor Responders. Figure 16 plots the admission and discharge AUCi split by good and poor responders (median split of 25% reduction in QIDS). RANOVA analysis shows that there was a trend effect of time and a significant group effect, but no group X time effect. This suggests that though both groups change over time there is no significant difference in the pattern of change.

A 2 (response group) x 2 (time) repeated measures of variance predicting the CAR AUCi at admission and discharge demonstrated a trend for a main effect of time, and a significant between-groups effect, but no group X time interaction. This suggests that 1. both good and poor responders experience some decrease in AUCi while in hospital and 2. good responders have higher AUCi’s than do poor responders both before and after treatment on AIM (see table 17).
Table 17: Repeated Measures Analysis of AUCi from Admission to Discharge in Good and Poor Responders

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>p</th>
<th>Partial Eta²</th>
<th>Power</th>
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<tr>
<td>Time</td>
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<td>1.22</td>
<td>0.08</td>
<td>0.13</td>
<td>0.42</td>
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<tr>
<td>Response</td>
<td>5.5</td>
<td>1</td>
<td><strong>0.03</strong></td>
<td>0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>ResponsexTime</td>
<td>0</td>
<td>1.22</td>
<td>1</td>
<td>0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

5.4.2.3 Changes in AUCg from Admission to Discharge in Good and Poor Responders

Figure 17 below illustrates the pattern of change of the CAR AUCg in good and poor treatment responders over the four week AIM program.
Figure 17: Change in AUCg from Admission to Discharge Split by Good vs. Poor Responders. Figure 17 plots the admission and discharge AUCg split by good and poor responders (median split of 25% reduction in QIDS). RANOVA analysis shows that there was no effect of time, group, and/or group X time.

As shown in table 18 below, when grouping patients based on good vs. poor responders, there was no significant effect of time, response group, or a group X time interaction in predicting AUCg.
Table 18. Repeated Measures Analysis of AUCg from Admission to Discharge Split by Good vs. Poor Responders

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>P</th>
<th>Partial Eta²</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
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<td>0.37</td>
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<td>Response</td>
<td>1.23</td>
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<td>0.19</td>
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<tr>
<td>ResponsexTime</td>
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<td>1.22</td>
<td>0.64</td>
<td>0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>

5.4.2.4 Summary

In sum, while the CAR Delta and AUCi decreased from admission to discharge across all subjects, as indicated by the main effect of time in these respective RANOVAS, there was no difference in the pattern of this decrease between good and poor responders. Good responders had a higher CAR Delta and AUCi than did poor responders at both admission and discharge. There were no statistically significant effects related to AUCg.

5.4.3 Summary of Analyses based on Treatment Response

- good responders on the AIM unit had a higher CAR Delta and AUCi at admission and discharge than did poor responders. There was no difference in AUCg between groups.
- Though the CAR Delta and AUCi changed significantly over time, there was no difference in the pattern of this change among good and poor responders.
5.4.4 Specific Discussion of Analyses done Post Hoc:

Looking at the analyses done post-hoc it is clear that study participants who did well on AIM (good responders based on a median split) had a more robust CAR Delta and AUCi than did poor responders. Furthermore, this difference was evident for both admission and discharge values. These data are generally consistent with a recent study by Vreeburg et al. (2013) showing that a blunted AUCi and AUCg in MDD patients was associated with a poor clinical outcome over the course of 2 years (S. A. Vreeburg et al., 2013). It would be reasonable to conclude that in both inpatients and outpatients with MDD, a blunted CAR is associated with poor short- and long-term outcomes. If replicated in a larger AIM sample, the current findings may have clinical application in identifying a simple and practical HPA-measure that can predict response to treatment on the ward.

Given that a higher CAR Delta and AUCi at admission were predictive of a better treatment response on the unit over the subsequent four weeks, it is interesting to speculate on whether a robust CAR contributes to differential treatment responses rather than simply being a result of successful treatment. Prior authors have suggested that having a robust CAR enables behavioral activation and prepares an individual for the demands of the day (Clow, Hucklebridge, & Thorn, 2010). If so, it is reasonable to speculate that individuals with a more robust CAR at admission were better able to benefit from the AIM program because of greater energetic arousal, cognition and executive function in the early part of the day. This takes on added relevance when it is considered that many of the psychological treatments on the AIM unit occur during the morning hours, favoring individuals who are alert and able to engage in treatment in the early part of the day. Conversely, individuals with a blunted CAR may experience morning fatigue, poor attention and concentration and thus less engagement in the
overall treatment process. Clinically, it is often the case that some individuals are more or less engaged in the treatment process due to individual differences in basic energy and arousal; whether the CAR contributes to these individual differences is an interesting and novel question for future work on the unit.
Chapter 6
Discussion

6 General Discussion

6.1 Summary of Main Study findings

6.1.1 Findings based on the Overall Sample:

- There was a significant decrease of all 3 clinical measures as assessed using the QIDS, BAI and PSS.
- There was a significant decrease in the CAR Delta, a trend towards a significant decrease in the AUCi, and no change in the AUCg.
- There was no correlation between absolute changes in the CAR measures and percent changes in the clinical measures.
- Interestingly, while there was a trend for the CAR Delta and AUCi to decrease from admission to discharge, higher cross-sectional measurements of the CAR delta and AUCi, both at admission and at discharge, were significantly correlated with a greater percent reduction in depressive symptoms.

6.1.2 Findings Comparing Good vs. Poor Responders:

- Good responders on the AIM unit had a higher CAR Delta and AUCi at admission and discharge than did poor responders. There was no difference in AUCg between groups.
• While the CAR Delta and AUCi decreased from admission to discharge across all subjects, there was no difference in the pattern of this change among good and poor responders.
6.2 General Discussion

The current study was the first to examine serial CAR measures in a group of MDD patients undergoing four weeks of treatment on an inpatient unit. The main study findings can be summarized as follows: 1. There was a general decrease in CAR reactivity from admission to discharge across the full study sample, independent of treatment outcome 2. This decrease in CAR reactivity was not simply attributable to changes in depressive symptoms, anxiety symptoms or perceived stress, as evidenced by the lack of correlation between CAR change scores and clinical change scores 3. Having more CAR reactivity at either admission or discharge was associated with a better clinical outcome. This raises the interesting question of whether individual differences in the CAR at baseline might contribute to differential treatment responses.

In trying to reconcile these various findings, one explanation is that the general decrease in CAR reactivity across all subjects has to do with non-specific effects of the AIM program, such as a change in sleep-wake schedules, that may or may not play a major role in treatment response overall. This would be consistent with the lack of relationship between clinical changes and CAR changes over the four weeks, and the lack of difference between good and poor responders with respect to CAR reactivity changes from admission to discharge. On the other hand, the enhanced CAR reactivity in good vs. poor responders, which is apparent at the time of admission, likely reflects a trait resiliency factor that differentiates these two groups over time. As alluded to above, it is not unreasonable to speculate that the existence of a robust CAR, in addition to being a long-term marker of resilience, may also contribute directly to improved treatment response through enhanced energetic arousal, cognition and executive function and overall engagement in the treatment program. If having a sluggish CAR prevents individuals
from benefiting fully from the AIM program, it might be important in future years to target the CAR as an initial treatment outcome unto itself i.e. the first step in treatment might be to optimize the reactivity of the CAR after which patients would be in a better position to benefit from other treatments.

6.2.1 The CAR in MDD: What is the Direction of Change?

One important question raised by prior authors is whether an exaggerated or blunted CAR is most characteristic of MDD pathology. Vreeburg et al (2009) compared the CAR in currently depressed individuals (N=701), those with remitted MDD (N=571), and normal controls (N=308). Results indicated that both the remitted and current MDD groups showed a significantly higher CAR than did controls, suggesting that an elevated CAR is a trait vulnerability marker in MDD (S. A. Vreeburg et al., 2009). A similar conclusion was made by Adam et al (2010) in a longitudinal study of older adolescents. Adam et al found that a higher baseline CAR was associated with a significantly increased risk of developing MDD, even when excluding individuals with baseline MDD. The authors concluded that an elevated CAR is a significant prospective risk factor for the development of MDD in young adults, providing some support for the possibility that a heightened CAR may play a role in the etiology of major depressive disorder (Adam et al., 2010). While these two large cohort studies suggest that a higher CAR is associated with greater MDD risk, it should be noted that in a subsequent follow-up study, Vreeburg et al (2013) found that among a cohort of adults with MDD, a blunted rather than exaggerated CAR was associated with an unfavorable longitudinal course over 2 years (S. A. Vreeburg et al., 2013). An association between a lower CAR and MDD in a group of inpatients undergoing psychotherapy has also been reported by Huber et al (2006) (Huber et al.,
The current finding that lower CAR reactivity at admission or discharge was associated with poor treatment response is reminiscent of the second Vreeburg study and Huber et al.’s findings. On the other hand, it contrasts several other studies demonstrating that a high CAR confers MDD risk over time. How might these various findings be reconciled?

One explanation is that MDD encompasses several different phenotypes that may be associated with different HPA-physiology. In support of this hypothesis, there is now substantial evidence that more acute, severe forms of MDD are likely to exhibit HPA over-activity, while less acute, more chronic forms of depression often entail a blunted or hypoactive HPA-axis (Chopra et al., 2009; Gold & Chrousos, 1999). A recent review has further suggested that the degree of HPA-axis hyperactivity differs substantially across MDD subtypes (Stetler & Miller, 2011). To understand these phenotypic differences, it is critical to consider stage of illness and the impact of chronic stress on MDD and the HPA-axis.

Several authors have suggested that after long periods of chronic stress and MDD episodes, the HPA axis becomes down regulated, less-responsive and secretes less cortisol (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Heim, Ehlert, & Hellhammer, 2000). In support of this, studies in geriatric depression have shown that hypocortisolism is associated with more chronic depression (Bremmer et al., 2007). Additionally, O’Keane and colleagues have proposed that with a more chronic atypical course of MDD there is a switch from a CRH regulated to an arginine vasopressin (AVP) regulated HPA axis (O’Keane et al., 2012). This switch may in part cause an alteration in the homeostasis of the HPA-axis. On the other hand, studies of unaffected children with a parental or genetic predisposition for MDD show them to have a higher CAR AUCi (S. a Vreeburg et al., 2010). Preclinical studies in animals have also reported that adverse events early in life are associated with an increased HPA-axis activity (Liu
et al., 1997). Another recent study showed that persistent depressive state in rats 3 months following chronic stress was associated with non-suppression in the DST (Mizoguchi, Shoji, Ikeda, Tanaka, & Tabira, 2008). A study in non-human primates showed that chronic exposure to glucocorticoids was associated with a neuronal damage of the hippocampus (Sapolsky et al., 1990). This is of importance as studies have shown that a functional hippocampus is necessary for a robust CAR (Buchanan et al., 2004). Other studies have indicated that exposure to chronic stress per se is associated with a blunted CAR (Meinschmidt & Heim, 2005; O’Connor et al., 2009). In sum, overactive HPA functioning including a higher CAR may indicate a trait-based vulnerability to MDD earlier in life, while a ‘scar’ effect as a result of high allostatic load on the HPA axis over time may lead to a more chronic form of MDD associated with a blunted CAR (Vreeburg et al., 2013).

Taking this into context when interpreting the results from the current study, a higher CAR might be considered a healthier pattern than a blunted response in patients with more chronic forms of MDD, which is the type of MDD experienced by most AIM patients. If so, this would explain why patients with greater CAR reactivity exhibited a favorable treatment response on the unit.

6.2.2 Prediction of Relapse:

The current study was the first to assess serial measurements of the CAR in MDD inpatients. Previous studies using the DST and DEX/CRH generally concluded that a normalization of the HPA axis predicted not only a successful treatment response but also a lower risk of relapse. Though there is not yet a consensus on what a ‘normal’ CAR is, our study has suggested that a higher CAR among MDD is associated with a better outcome while in hospital. This in the context of the argument previously put forward that a higher CAR may be a
more ‘normal’ and/or ‘healthier’ CAR in MDD patients, it could be hypothesized that patients that have a lower CAR may also be at a greater chance of poor clinical outcome post-discharge. Vreeburg et al. in fact showed that a higher AUCi and/or AUCg was associated with a better 2-year clinical outcome (S. A. Vreeburg et al., 2013). This hypothesis would be further supported by previous research which showed that a normalization (i.e. a healthier) of the HPA-axis following treatment for MDD predicted resilience post-discharge (Aubry et al., 2006; Schweitzer et al., 1987; Zobel et al., 2001.). Research needs to be done in order to determine if in an elevated CAR is a more healthy response in MDD patients and if it is associated with risk of relapse, specifically for inpatients.
6.3 Study Strengths

The current study is the first to examine serial CAR measures in MDD patients undergoing a four week inpatient stay. It is also one of the first studies to relate CAR measures to treatment response in currently depressed MDD patients. The fact that AUCi measures at admission were able to differentiate good vs poor responders in a prospective way is of great interest. If this is replicated as the sample increases, it would not be unreasonable to make the CAR a routine aspect of treatment planning on the AIM unit. For example, it might prove to be the case that MDD patients with a blunted CAR AUCi at admission will benefit from a different treatment approach than for patients with a more robust CAR. It might also be the case that in the former subgroup, increasing the AUCi could be a treatment goal unto itself. This would be particularly pertinent if the CAR does in fact play a role in behavioural activation, energetic arousal and cognition, all of which contribute to greater engagement in the program.
6.4 Study Limitations

The major limitation is that the current sample size is small, which greatly limits statistical power and our ability to use co-variates in the analyses. The small sample also increases the chance of statistical errors. While this is acknowledged, this study will help guide future research on AIM and was able to shed significant new light on the use of the CAR in MDD inpatients.

The current sample was quite heterogeneous, as is usually the case for naturalistic clinical samples, although most participants would be considered relatively chronic vs. the MDD population as a whole. Given this reality, the current findings may not translate well for more acute MDD cases. There was not a strong emphasis on subtyping MDD in the current analyses, which might prove to be a critical issue going forward, given evidence for different patterns of HPA-activity in atypical vs melancholic depression for example. The possible role of sleep differences in these depressive subtypes will also need to be considered.

Another limitation which is common to all studies using the CAR is that it is difficult to control exactly when patients take their samples. In order to control this we asked patients to record the exact clock time that they took their sample. Though we had a very high rate of compliance among participants, we cannot be certain that these times were accurately recorded. Knowing exactly when each patient fell asleep and awoke is also difficult to ascertain, though standardization of sleep and activity rhythms is a relative benefit when studying inpatients. In a similar vein, though patients were required to go to classes while on AIM, adherence to assigned homework as well as actual participation in classes could not be controlled for. Having said that, more consistent participation is also a relative benefit of inpatient vs. outpatient programs.
The fact that medication regimens were quite varied and might have contributed to variation in the CAR is a notable limitation, but will be controlled for as the sample increases. The fact that we observed significant differences in the CAR based on treatment outcome per se suggests that this may not be a major issue going forward.
6.5 Future directions

One longer term goal for this body of work is to follow patients longitudinally after discharge. Prediction of relapse based on CAR values while on AIM would be of great benefit in this regard. Complete follow up data through 4 months post-discharge is currently available for 20 patients. This data was left out of the current thesis as the sample size is too small to reliably assess the risk of relapse based on CAR values in hospital. However, a preliminary analysis is suggesting that patients who do well 4 months post-discharge leave hospital with a higher CAR AUCg than do other patients ($t=2.97, \ df= 18, \ p=.01$). This is well illustrated in figure 18 and table 19 below:

![Graph showing CAR split by Good and Poor Outcome at 4-Month Follow-up](image)

**Figure 18:** Discharge CAR split by Good and Poor Outcome at 4-Month Follow-up shows that when splitting patients based upon a QIDS>14 at 4 months follow, there was significantly greater CAR in the good outcome (QIDS<15) vs. the poor outcome group (QIDS>14).
Table 19 shows that the AUCg at discharge is significantly different in both groups

<table>
<thead>
<tr>
<th></th>
<th>AUCg</th>
<th>T</th>
<th>Df</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>1.53</td>
<td>18</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Discharge</td>
<td>2.97</td>
<td>18</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

These findings are reminiscent of the recent study by Vreeburg et al. (2013) showing that a blunted AUCg (and AUCi) predicted a poor clinical outcome over 2 years in MDD outpatients. As discussed previously, given the relatively chronic nature of the current sample, a blunted CAR may reflect a “burn-out” phenomenon in many cases. If so, it is entirely reasonable that this subgroup would have a difficult clinical course over time.

Other biological markers associated with the HPA could also be utilized in future work. Recent research has suggested that salivary alpha-amylase (sAA), a measure of the autonomic nervous system, may have particular value in this regard (Ali & Pruessner, 2012). This latter study showed that sAA, when combined with cortisol measurements, provides greater correlation with MDD than either sAA or cortisol considered alone (Ali & Pruessner, 2012).

Future studies should determine if it is only patients with chronic depression who exhibit a blunted CAR while on AIM. A study by Chopra et al. was able to find this association in males using the TSST (Chopra 2009). Future studies need to determine if the CAR is altered in CMDD vs MDD and if this has treatment implications for either group.
Yet another consideration for future work is the use of fMRI to visualize the awakening response. One might expect significant differences in brain activation patterns in individuals with a blunted vs robust CAR response.

Lastly there is growing body of literature showing that various coping strategies are associated with different patterns of the CAR. As coping strategies are widely used as a tool to treat MDD, it would be of great utility to determine which ones might optimize both the CAR and treatment outcome.
7 References


