The Effects of Cortisol on Emotion

by

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Dedication

To: My parents Dennis and Kay Sudheimer and also Dr. John I. Johnson for their guidance and for encouraging, then nourishing, then tolerating, and finally enduring my curiosity.

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Table of Contents

Dedication ..... **ii**

Acknowledgements ..... **iii**

List of Figures ..... **viii**

List of Tables ..... **xi**

List of Abbreviations ..... **xii**

Chapter 1: Introduction **1**

Physiology of Cortisol 1

Cortisol and Stress 4

Depression Neurophysiology, and Hypercortisolemia 5

Emotion and Hypercortisolemia 7

Neurophysiology of Emotion and Cortisol 8

Purpose of this Dissertation 9

Chapter 2: Cortisol Administration and the Hemodynamic Response **14**

Introduction 14

Methods 15

Subjects 15

Cortisol Administration 16

MRI image acquisition 16

fMRI Tasks 17
fMRI Analysis....18
Measures of the HRF 19
Results 19
Discussion 21
Limitations....23
Chapter 3: The Effect of Cortisol Administration on the Neural Correlates of Emotion 24
Introduction 24
Methods....27
Subjects....27
Cortisol Administration....28
fMRI Task....28
Measurement of Emotional States 29
Measurement of Cortisol 30
MRI Image Acquisition 30
Ratings of Emotion induced by Stimuli Presented During Scanning....31
fMRI Analysis....31
Results 33
Experimental Blinding....33
Circulating Cortisol Levels Elevated by Cortisol Administration....34
Cortisol Produces a Trend Level Increase in Sad Mood....36
Cortisol Increases Arousal Ratings of Sad Stimuli 36
Emotional Stimuli Robustly Activate Subcortical and Cortical Brain Regions....37

Cortisol Modulates Brain Activity Elicited by Emotional Stimuli....39

Discussion....54

Limitations....57

Chapter 4: The Effect of Cortisol Administration on the Time Course of Early Detection and Identification of Emotional Facial Expressions....60

Introduction....60

Methods....62

Subjects....62

Cortisol administration and Measurement:....63

Measurement of Emotional States....63

Equipment....64

Stimuli....64

Design....65

Analysis....68

Results....69

Experimental Blinding....69

Cortisol Administration does not affect Overall Emotional State....69

Circulating Cortisol Levels Elevated by Cortisol Administration....70

Type of Emotional Facial Expression Affects Detection Accuracy and Sensitivity But Cortisol Administration Does Not.....70

Type of Emotional Facial Expression Affects Identification Accuracy and Sensitivity But Cortisol Administration Does Not.....74

Discussion....80

Effects of Cortisol on Detection and Identification 80

Happy Faces Detected and Identified Better Than Sad or Neutral Faces. 81

Learning across sessions.....82

Limitations.....84

Chapter 5: Conclusion 87

Limitations.....89

Significance & Future Directions 92

Bibliography 95

List of Figures

Figure 1 Estimates of the time course of the visual cortex (Brodmann areas 17/18) HRF in subjects administered Placebo, a single dose of 100mg of hydrocortisone, or a extended dose of 25mg/day of hydrocortisone. 20

Figure 2 Average time to achieve peak BOLD response in the visual cortex in response to a 1 second visual stimulation for subjects administered placebo, a single dose of 100mg of hydrocortisone, or a split dose of 25mg/day of hydrocortisone/day over 5 days.....21

Figure 3 Average magnitude of the peak BOLD response in the visual cortex in response to a 1 second visual stimulation for subjects administered placebo, a single dose of 100mg of hydrocortisone, or a split dose of 25mg/day of hydrocortisone/day over 5 days.....21

Figure 4 Circulating cortisol concentrations as measured in saliva. The single dose group was administered 100mg oral hydrocortisone at time 0. The extended dose group was administered 25mg hydrocortisone/day over 5 days, ending 6 hours prior to time 0.....35

Figure 5 Average circulating cortisol during fMRI scanning was robustly elevated in the single dose group. 36

Figure 6 Arousal ratings in reaction to viewing sad stimuli were significantly elevated in the extended dose group but not the single dose group.....37

Figure 7 Brain Activity Changes Resulting from the Viewing of Happy (Row A), Neutral (Row B), and Sad (Row C) stimuli. All emotion condition activations/deactivations are relative to fixation cross for the placebo group only.....39

Figure 8 Subgenual cingulate suppression by a single dose (A) and an extended dose (B) of cortisol relative

to the placebo group. 40

Figure 9 Cortisol induced subgenual cingulate activity suppression is observed only during the sadness condition. 41

Figure 10 Sadness induced subgenual cingulate activity in the single dose group is negatively correlated with circulating cortisol levels at the time of scanning. 41

Figure 11 Cortisol induced VMPFC activity suppression is observed only during the sadness condition. The single dose produces only trend level suppression, whereas, the extended dose produces significant suppression. 42

Figure 12 Amygdala activity in response to emotional stimuli is not significantly altered by either the single dose or the extended dose of cortisol. 43

Figure 13 Parahippocampal/perimamygdala activity is increased in the single dose group during sadness (A) and the extended dose group during sadness (B), neutrality (C), and happiness (D). 44

Figure 14 Extended dose induced suppression of SLEA activity during sadness. 44

Figure 15 Superior colliculus and thalamus activity is suppressed by a single dose of cortisol. 45

Figure 16 Presentation time course of noise (Row A) and signal (Row B) trials in the face detection task. Each target face was preceded by a blank screen with a 300-500ms duration and then followed by a 100ms perceptual mask. 67

Figure 17 Presentation time course of identification trials. Each target face was preceded by a blank screen with a 300-500ms duration and then followed by a 100ms perceptual mask. 68

Figure 18 Cortisol administration does not significantly alter any of the 11 PANAS mood subscales. 69

Figure 19 Circulating cortisol levels are significantly elevated 2 hours after cortisol administration. 70

Figure 20 Detection accuracy curve for happy and sad facial expressions. 71

Figure 21 Accuracy of detecting happy facial expression across sessions for the placebo and cortisol groups. 72

Figure 22 Change in accuracy of detecting happy facial expression across sessions for the placebo and cortisol groups. 73
Figure 23 Accuracy of detecting sad facial expression across sessions for the placebo and cortisol groups.73
Figure 24 Change in accuracy of detecting sad facial expression across sessions for the placebo and cortisol groups. 74
Figure 25 Identification accuracy curve for happy, neutral and sad facial expressions.....75
Figure 26 Change in detection and identification accuracy across sessions for each type of emotional facial expression. 76
Figure 27 Accuracy of identifying happy facial expressions across sessions for the placebo and cortisol groups. 77
Figure 28 Change in accuracy of identifying happy facial expressions across sessions for the placebo and cortisol groups. 77
Figure 29 Accuracy of identifying neutral facial expressions across sessions for the placebo and cortisol groups. 78
Figure 30 Change in accuracy of identifying neutral facial expressions across sessions for the placebo and cortisol groups. 78
Figure 31 Accuracy of identifying sad facial expressions across sessions for the placebo and cortisol groups. 79
Figure 32 Change in accuracy of identifying sad facial expressions across sessions for the placebo and cortisol groups. 79

List of Tables

Table 1 Confusion matrix table indicates that subjects were poor at distinguishing between taking hydrocortisone and taking a placebo. Columns indicate the group assignment. Rows indicate the group the subject thought they were in. Cells indicate the rate of correct identification of group (diagonals) or misidentification of group (off diagonals). 34

Table 2 Table of significant group differences in brain activity while viewing sad stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of p< 0.005, uncorrected; Extend threshold k=5 voxels.....46

Table 3 Table of significant group differences in brain activity while viewing happy stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of p< 0.005, uncorrected; Extend threshold k=5 voxels.....48

Table 4 Table of significant group differences in brain activity while viewing neutral stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of p< 0.005, uncorrected; Extend threshold k=5 voxels.....51

Table 5 The confusion matrix of emotional facial expression. Columns indicate the emotion presented. Rows indicate the response. Cells indicate the rate of correct identification (diagonals) or misidentification (off diagonals).....80

List of Abbreviations

3AFC	Three Alternative Forced Choice
ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
BA	Brodmann Area
BOLD	Blood Oxygen Level Dependant
CBG	Corticosteroid Binding Globulin
cm	Centimeter
CRH	Corticotropin Releasing Hormone
CRT	Cathode Ray Tube
d’	D prime
dL	Deciliter
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
GB	Gigabyte
GHz	Gigahertz
GLM	General Linear Model
GR	Glucocorticoid Receptor
HPA	Hypothalamic-Pituitary-Adrenal
HRF	Hemodynamic Response Function
Hz	Hertz

IAPS	International Affective Picture System
K	Cluster
µg	Microgragms
mg	Milligrams
mm	Millimeter
MINI	Mini International Neuropsychiatric Interview
MNI	Montreal Neurological Institute
MR	Mineralcorticoid Receptor
mRNA	Messanger Ribonucleic acid
ms	Millisecond
PANAS	Positive And Negative Affect Schedule
RAM	Random Access Memory
SLEA	Sublenticular Extended Amygdala
SPGR	Spoiled Gradient Echo Pulse Sequence
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences
TE	Echo Time
TR	Repetition Time
VMPFC	Ventral Medial Prefrontal Cortex

Chapter 1: Introduction

There is long standing evidence linking depression and the hormone cortisol. A large amount of previous research has focused on establishing measures of cortisol secretion and cortisol regulation as biomarkers of depression. However, comparatively little work has been to investigate if cortisol is a contributing factor to the development or maintenance of depression. In this body of work we outline several studies designed to elucidate the physiological effects of cortisol administration on brain and behavioral processes relevant to depression.

Physiology of Cortisol

Cortisol is a steroid hormone, often secreted in response to stress (Selye, 1971). It is classified as a corticosteroid, due to its biosyntheses in the adrenal cortex. It is also classified as a glucocorticoid, due to early observations of its functions in glucose regulation and metabolism. Cortisol is produced in vivo from cholesterol and is secreted principally from the adrenal cortex. Endogenous cortisol has many known physiological effects including anti-inflammatory, energy regulatory, glucose regulatory, and immunosuppressive effects. The synthetic equivalent of endogenous cortisol is known as hydrocortisone, which is produced commercially from various plant sterols, and marketed in creams and as oral capsules to treat common inflammatory autoimmune

conditions. Other glucocorticoids such as prednisone and dexamethasone are also used for these purposes, with increased potency relative to cortisol. Cortisol is the major output hormone of the hypothalamic-pituitary-adrenal (HPA) axis in humans, whereas in rodents a closely related hormone called corticosterone serves this purpose. Cortisol secretion also maintains a regulated circadian rhythm. Activation of the HPA axis beyond the normal circadian rhythm and the subsequent release of cortisol are often associated with stress (Selye, 1985). However, cortisol secretion is not inextricably coupled with the subjective feelings of stress, and subjective feelings of stress do not always translate into high cortisol levels (Curtis et al., 1978). Thus, high levels of cortisol do not directly cause subjective feelings of stress.

Cortisol has two known types of principal intracellular receptors, the glucocorticoid receptor (GR) and the mineralcorticoid receptor (MR) which are distributed widely in the brain and periphery (Watzka et al., 2000b; Rashid and Lewis, 2005) and at least one additional cell membrane bound receptor (Orchinik et al., 1991; Borski, 2000). Activation of these receptors can produce a wide variety of physiological and psychological effects through gene transcription changes, mediated by glucocorticoid response elements, within hours of exposure to cortisol (Karst et al., 2002). Glucocorticoid hormones can also produce effects within seconds through membrane mediated mechanisms and through other unknown mechanisms that can affect neurotransmission and even complex behaviours. (Orchinik et al., 1991; ffrench-Mullen, 1995; Rose, 2000; Mikics et al., 2005; de Kloet et al., 2008a) (for a review see (Dallman, 2005)) .

The amount of circulating cortisol that is free to exert effects on receptors in the brain or periphery is tightly regulated. Two known isoforms of the enzyme 11-beta-hydroxysteroid dehydrogenase serve to interconvert cortisol and its much less active form cortisone (for review see (Tomlinson and Stewart, 2001)). In the blood the vast majority of cortisol is bound and inactivated by a protein called corticosteroid binding globulin (CBG/transcortin) (Westphal, 1983). Cortisol release is also regulated at multiple levels by redundant feedback inhibition within the HPA axis. When the HPA axis is activated brain mediated signals prompt the release of corticotropin releasing hormone (CRH) at the level of the hypothalamus. CRH then prompts the release of a second hormone known as adrenocorticotropic hormone (ACTH) at the level of the pituitary gland. ACTH is released into the general circulation and serves to prompt the release of cortisol from the adrenal cortex. When cortisol is released into circulation, it serves to inhibit its own release by providing inhibitory signals at the level of the pituitary, hypothalamus, and hippocampus. These inhibitory signals are achived extremely rapidly at the level of the hypothalamus (paraventricular nucleus) and hippocampus (cornu ammonis 1 cells) through non-genomic mechanisms that affects glutamate neurotransmission and subsequent miniature excitatory postsynaptic currents. Intermediated and slower delayed negative feedback mechanisms also occur on the level of the hypothalamus, pituitary, and hippocampus acting predominantly via cytosolic GR, which affect the structure and function of neurons in these and other subcortical and cortical brain regions (for reviews see (Jacobson, 2005; de Kloet et al., 2008b)).

Failure to regulate cortisol production can result in serious pathological conditions. Addison’s disease, which is characterized by underproduction of cortisol brought on by

insufficient production of cortisol in the adrenal cortex, results in chronic and worsening fatigue over time, muscle weakness and loss of weight and appetite. Cushing's syndrome, characterized by overproduction of cortisol (hypercortisolemia), is often caused by pituitary or adrenal tumors and results in severe fatigue, muscle weakness, high blood pressure, high blood glucose, upper body obesity and, notably, increases in anxiety and depression (Haskett, 1985; Nieman and Ilias, 2005).

Cortisol and Stress

The release of glucocorticoids in humans and animals is triggered by a wide variety of stimuli that are often conceptualized as stressful or threatening to the integrity or homeostasis of an organism. These triggers include physical stressors such as bodily injury and also psychological stressors which may predict such injury. For example, invasive surgical procedures result in massive increases in circulating glucocorticoid levels (Naito et al., 1992). Less invasive procedures such as electric shocks (Bassett et al., 1973) and exposure to extreme cold (Edelson and Robertson, 1986) also produce a significant glucocorticoid response. Exposure to physically threatening stimuli which could predict injury can also elicit a similarly robust glucocorticoid response. For example, in animals, exposure to the smell of predators can causes robust increases in glucocorticoid responses, even when the predator inflicts no physical harm (for a review see (Apfelbach et al., 2005)). Similarly, physical restraint in animals can also produce a large glucocorticoid response (Keim and Sigg, 1976) without producing any physical harm. In human studies, potentially physically threatening situations that do not result in physical harm also generate a robust increase in cortisol secretion. For example,

novice skydivers performing their first jump show marked increases in cortisol secretion (Chatterton et al., 1997).

In humans recent evidence suggests that psychosocial stress or threats to the social self (social value, esteem, status, worth etc.) can generate a robust glucocorticoid response (Dickerson and Kemeny, 2004). Laboratory studies employing the Trier social stress test (TSST) (Kirschbaum et al., 1993), which involves subjects delivering a speech and performing mental arithmetic in front of an audience, demonstrate a robust and reliable increase in cortisol secretion and seem to support psychosocial stress theories. In addition, a meta-analysis of studies attempting to induce stress via cognitive tasks, public speaking tasks, noise exposure and emotion induction suggests that cortisol is most reactive to social evaluative/psychosocial stress. It also suggests that amongst psychosocial stressors the cortisol response is the greatest when subjects are in experimental contexts that involve dimensions of social evaluation threat and uncontrollability (Dickerson and Kemeny, 2004).

Depression Neurophysiology, and Hypercortisolemia

Depression is a psychiatric disorder characterized by symptoms that include low mood, decreased interest in pleasurable activities, changes in appetite or weight, insomnia/hypersomnia, psychomotor agitation/retardation, fatigue, inappropriate guilt, difficulties with cognition, and thoughts of suicide (American Psychiatric Association., 2000). Some investigations have reported that over 17% of the population will develop major depression in their lifetime (Blazer et al., 1994) and the World Health Organization

estimates that depression is the second leading cause of potential years of life lost to disability for both men and women between the ages of 15 and 44, making the global burden of depression immense.

Hypercortisolemia has been observed in large portions of depressed patients (Carroll et al., 1976; Young et al., 2001). Hypercortisolemia is accompanied by increases in the size of the adrenal cortex (Rubin et al., 1995). This hypercortisolemia is thought to arise from decreased sensitivity to inhibitory feedback (Kolebinov et al., 1975; Carroll, 1980; Greden et al., 1980; Carroll, 1982a, b; Holsboer et al., 1982; Carroll, 1984; Zobel et al., 2001). Amelioration of the hypercortisolemic state in depression is associated with recovery from depression, resistance to relapse (Greden et al., 1980; Holsboer et al., 1982; Zobel et al., 1999; Zobel et al., 2001) and normalization of the adrenal gland size(Rubin et al., 1995). However, it is currently unknown if this hypercortisolemia is a consequence of depression, or if cortisol may be actively contributing to the maintenance or etiology of depressive symptomatology through its effects on the brain.

Several brain functional, volumetric, and histological abnormalities have been documented in depression (See (Drevets et al., 2008) for a current review). Among these the subgenual cingulate cortex is most notable in that it demonstrates reduced volume, cell counts and glucose metabolism in depression. However, once the reduce volume of the subgenual cingulate is accounted for activity within this region appears to increase in depression relative to healthy controls. Reductions in volume, cell counts, and markers of functionality (glucose metabolism or cerebral blood flow) have also been observed in the dorsal medial prefrontal cortex, pregenual anterior cingulate, vental

lateral prefrontal cortex, and parahippocampal cortex. Decreased volume in the hippocampus of depressed patients has also been observed in multiple studies and correlates with time spent untreated (Sheline et al., 1999; Sheline et al., 2003). However, there are not consistent observations of decreased hippocampal functional activity to accompany this reduced volume. In the amygdala the literature is mixed with respect to volume and functional activity, both increases and decreases in these measures have been observed. The ventral striatum is another region that has reduced grey matter volume and functional activity. Also notable amongst these findings is that the medial thalamus demonstrates decreased functional activity in depressed patients relative to healthy controls.

Emotion and Hypercortisolemia

Some evidence suggests that hypercortisolemia could be contributing to mood changes in depression. For example, patients with Cushing's disease often have high rates of co-morbid depression (up to 80%). Exogenous hydrocortisone administration in large doses can also directly induce severe mood dysregulation, including both depression and mania (Ling et al., 1981), with depression the more common outcome. Measures of endogenous cortisol levels in humans have been shown to correlate with depressed mood (Van Honk et al., 2003). Animal studies also support this notion by demonstrating that corticosterone administration increases depression-like behaviors (Kalynchuk et al., 2004), and anxiety in rats (Mitra and Sapolsky, 2008).

Neurophysiology of Emotion and Cortisol

Consistent findings across studies of the neurophysiology of emotion are rare due to heterogeneity of laboratory tasks designed to evoke emotion and the highly subjective nature of measurements of emotion induction magnitude. However, some patterns of brain activity seem to associate strongly with certain emotions. Fear is strongly associated with amygdala activity, sadness with subgenual cingulate activity and happiness with basal ganglia activity (Phan et al., 2002). These regions however do not respond exclusively to these emotions, and these emotions involve additional brain regions. The amygdala for example responds most robustly to fear (Aggleton, 1992) but it also responds to many other additional emotions (Fitzgerald et al., 2006). The subgenual cingulate, while less extensively studied than the amygdala, shows strong associations with negative mood and sadness. Critically, this subgenual cingulate responsiveness seems to overlap between sadness in healthy subjects and the pathological sadness central to depression (Mayberg et al., 1999). Moreover, early evidence from clinical trials of deep brain stimulation aiming to modulate subgenual cingulate activity for use in treatment resistant depression has demonstrated promising results (Lozano et al., 2008; McNeely et al., 2008).

Further evidence suggests that cortisol can influence emotion and the structure and physiology of neurons in brain regions thought to underlie emotional responses. Animal studies have demonstrated that corticosterone has direct modulatory effects on the physiological responsiveness of neurons in neural structures associated with emotion, such as the hippocampus (Birnstiel et al., 1995; De Kloet et al., 1998), amygdala (Karst et al., 2002; Mitra and Sapolsky, 2008) , and ventral tegmental area (Cho and Little, 1999).

High levels of corticosterone in animals also causes dendritic reorganization of the hippocampus (Woolley et al., 1990) and prefrontal cortex (Wellman, 2001). In humans, MR and GR are expressed in high levels in brain regions associated with emotional processing such as the hippocampus (Watzka et al., 2000a), amygdala (Sarrieau et al., 1986), and in both the frontal and temporal lobes (Watzka et al., 2000b). Endogenous cortisol levels in humans have been shown to correlate with activity in a variety of subcortical brain regions thought to process emotion, such as the amygdala (Drevets et al., 2002), insula and subgenual cingulate cortex (Liberzon et al., 2007). Furthermore, cortisol administration has been shown to enhance memory for selective types of emotional material (Buchanan and Lovallo, 2001), and is capable of blunting emotional responses to some stimuli (Reuter, 2002).

Purpose of this Dissertation

Despite long-standing evidence that cortisol is associated with depression, alters emotional behaviors, and affects brain regions involved in emotion, to date, little work has been done to explore what influence cortisol may be having on emotional processes relevant to depression. Understanding how cortisol may be altering emotional processes and the brain activity underlying those processes may be a critical step in understanding the root causes of emotional and brain activity changes that are observed in depression. Furthermore, identifying neurophysiological effects that explain the core symptoms of depression would be a critical step forward in the progression towards informed treatments, and could potentially speed development of novel pharmaceuticals.

The general approach used in this dissertation is to begin to uncover the effects of cortisol on emotion and brain activity associated with emotion by actively manipulating circulating cortisol levels. In contrast, the majority of previous studies have relied on correlating circulating endogenous cortisol levels with measures of emotion and brain activity. The approach that we use here decreases the inherent ambiguity in correlation studies, as it allows directionality of effects to be inferred. Are emotional changes driving changes in cortisol levels or are cortisol levels driving emotional changes? Since we are administering cortisol, the possibility of emotional changes driving up cortisol levels is all but eliminated, allowing inferences about the effects of cortisol on emotion.

In chapter 2 we present a necessary prerequisite study to determine if cortisol alters brain hemodynamic responses, which are used to make inferences about emotion related brain activity. The advent of neuroimaging techniques in recent decades has provided a method of studying the neurophysiology of emotion in humans, opening realms of knowledge almost entirely inaccessible previously. However, successful implementation of these techniques requires obsequious recognition of the underlying assumptions and limitations of the technique being utilized. The incorporation of pharmacology in fMRI studies presents a potent example of this principle. The fMRI signal is particularly reliant on the dynamics of oxygenated and deoxygenated blood within the cerebral vasculature, which is closely correlated with local neural activity. Therefore any pharmacological agent that impacts the dynamics of the cerebral vasculature could lead to atypical fMRI signal from the underlying neural activity. This would then require adapted models of the fMRI signal to be used to infer functioning of

the underlying neural activity. Chapter 2 aims to address this issue directly by empirically deriving the shape of the hemodynamic response function derived from the visual cortex of subjects administered placebo or various dose regimens of exogenous cortisol. A novel characterization of shape changes in the hemodynamic response function would allow for individualized hemodynamic models to be built accounting for cortisol differences. Alternatively, verification of a typical hemodynamic response function would allow for the use of standard hemodynamic models to infer neural activity patterns.

Chapter 3 aims to determine if cortisol administration produces changes in subjective emotional experience, and the neural correlates of that experience. Furthermore, in this chapter we aim to be sensitive to subjective emotional changes and brain activity changes that are relevant to depression, such as cortisol induced increases in measures of sadness, decreases in measures of happiness, and changes in subgenual cingulate and amygdala activity during emotional processes. In Chapter 3 we are informed by the results of Chapter 2, which allow valid inferences to be made in Chapter 3 regarding the effects of cortisol on brain activity associated with emotion. In service of the aims of Chapter 3 we incorporate two different types of measures of subjective emotional experience. The first is a measurement of emotional states (see page 29) and the second measures the magnitude of the emotional reaction elicited by a stimulus (see page 31). We also utilize both happiness and sadness conditions within the fMRI scanning session to allow sensitivity to observe cortisol induced changes relevant to sadness or anhedonia symptoms in depression. Results from Chapter 3 provide several findings potentially relevant to the pathophysiology of depression and additional unexpected effects of

cortisol administration.

Chapter 4 seeks to test if cortisol administration produces changes in the perceptual processing of emotional stimuli. This study was informed by unexpected results in Chapter 3 (see Figure 15 on page 45), indicating that cortisol affects brain activity in regions traditionally associated with sensory processing of visual stimuli, regions such as the superior colliculus, thalamus, and the visual cortex. Each of these brain regions is also thought to be a critical node in the expeditious visual processing of emotional stimuli which drive amygdala based behavioral responses (LeDoux, 1994). In Chapter 4 we use these findings to generate a hypothesis that cortisol might be affecting early sensory processes in such a way as to constitute a perceptual bias. Such a perceptual bias could theoretically drive emotional changes such as those observed in Chapter 3. In order to test this hypothesis we assess the effects of cortisol on perception by utilizing two measures of emotional perception, a test of ability to detect emotional facial expression (see Figure 16 on page 68) and a test of ability to identify emotional facial expression (see Figure 17 on page 68). Both of these tests make use of a perceptual "masking" technique called backwards masking where a mask image is presented immediately after a target image in order to interfere with the subjective awareness of the target image. In the study outlined in Chapter 4 we use a swirl mask to interfere with the subjective awareness of emotional facial expressions. We hypothesized that cortisol administration would lead to greater accuracy and sensitivity to sad facial expressions and lower accuracy and sensitivity to happy facial expression under conditions that limit the subjective awareness of the faces. We characterize the majority of the psychophysical response curve associated with the detection of emotional facial expression and the curve

associated with identification of emotional facial expressions. We characterize the response curves of detection of happy and sad facial expressions, and the response curves of the identification of happy, sad and neutral facial expressions. The results of experiments in Chapter 4 suggest that cortisol does not affect aspects of the perception of emotional stimuli, such as detection and identification. These results can be used to shape inferences drawn in Chapter 3 regarding the nature of the effects of cortisol on emotion, suggesting that cortisol may affect emotional processing that occurs subsequent to an unmodified perceptual experience.

The experiments chronicled in Chapter 2, Chapter 3, and Chapter 4 fit together as an overall approach to characterize depression relevant emotional processes that are affected by cortisol administration. The central focus of this approach provides insight that could help to explain the emotional, neurophysiological, and perceptual consequences of endogenous hypercortisolemia, and how those consequences could theoretically manifest themselves in components of the symptom profiles of depressed patients.

Chapter 2: Cortisol Administration and the Hemodynamic Response

Introduction

Functional magnetic resonance imaging (fMRI) studies incorporating pharmacological agents are becoming increasingly common. Many pharmacological agents can affect components of the blood oxygen level dependant (BOLD). Drug effects on blood pressure, heart rate, or the oxygen extraction fraction could produce alterations in the shape of the BOLD signal. For example, commonly used drugs like caffeine work as a brain vasoconstrictor and, consequently, alter the BOLD signal collected in fMRI experiments (Mulderink et al., 2002). Use of any pharmacological agent that affects components of the BOLD signal could potentially make the results of fMRI experiments difficult to interpret, by changing the BOLD signal without affecting correlated neural activity. Determining that a pharmacological agent influences functional neural activity patterns requires that pharmacological effects on the BOLD signal are either accounted for or eliminated experimentally.

fMRI studies in Chapter 3 aim to use hydrocortisone (cortisol) administration to make inferences regarding how increased endogenous cortisol levels might influence functional activity patterns associated with emotion. Implicit in these studies is the assumption that exogenous hydrocortisone administration does not alter the shape of the BOLD response, the hemodynamic response function (HRF), in any significant way. However,

this assumption has not yet been addressed empirically and some evidence suggests that cortisol may alter systemic blood pressure and, therefore, could potentially alter the HRF (Sudhir et al., 1989; van den Berg et al., 1990; Mantero and Boscaro, 1992; Pirpiris et al., 1992; Tonolo et al., 1993; Dodt et al., 2000).

In the current study we divide subjects into a placebo group and two different dose regimens of hydrocortisone administration. We then use a canonical visual stimulation paradigm to empirically derive the shape of the visual cortex HRF.

Methods

Subjects

Sixty healthy (30 male, 30 female), right-handed subjects, ages 18-30 years old, were recruited from the local population and signed a comprehensive written consent form, as approved by the local ethics committee. Exclusion criteria included a history of endocrine disorders, head injury, psychiatric or neurologic disorders, presence of an acute medical condition, medication use, recent major surgeries, a history of traumatic life events, current illicit drug use or dependence, smoking, and current exposure to excessive psychological stress. Subjects meeting mini international neuropsychiatric interview (MINI) criteria for axis-1 psychiatric disorders (Sheehan et al., 1998), or scoring more than 7 points on the Beck Depression inventory-II, were excluded from the study. All female subjects were free of hormone-based contraceptives and fMRI scanning was scheduled to coincide with the luteal phase of their menstrual cycle.

Subjects were matched by age, sex, weight, and ethnic/racial background and were

randomly assigned to one of three groups: placebo, single dose 100 mg hydrocortisone, extended dose 25 mg/day hydrocortisone over the course of five days. This formed a 2 x 3 (gender X dose) factorial design. Subjects and the experimenters were blind to group assignment until the completion of data collection for each subject.

Cortisol Administration

Cortisol was administered in a double blind fashion, either as a single oral dose of 100 mg hydrocortisone given two hours prior to fMRI scanning or as an extended dose, which was a split dose of 25 mg/day oral hydrocortisone given as a 3:2 dose ratio (15 mg at 8 a.m. and 10 mg at 8 p.m.), similar to previous memory studies (Newcomer et al., 1999). A placebo group acted as an experimental control for both the extended dose group and the single dose group. The single dose group and the placebo group were given placebos at 8 a.m. and 8 p.m., over the 5 day study, in the same fashion as the extended exposure regimen.

MRI image acquisition

MRI imaging was performed on a 3.0 Tesla GE Signa system using a standard radio frequency coil. A T1 weighted image was acquired for land marketing and positioning of subsequent scans. Functional scans were acquired using a T2* weighted, single shot, reverse-spiral pulse sequence (TR = 1000 ms,TE = 30 ms, flip angle = 90°, FOV = 22 cm, slice thickness = 3 mm, number of the slices = 20) to minimize susceptibility artifact (Yang et al., 2002).

Functional scanning was divided into 2 runs. At the beginning of each functional run, 8

functional volumes (8 seconds) were collected and subsequently discarded to allow for T1 equilibrium. High-resolution, T1 weighted, inversion recovery SPGR anatomical images were also collected for each subject (TR = 10.5 ms, TE= 3.4 ms, flip angle = 25°, FOV = 24 cm, slice thickness = 1.5 mm, number of slices = 106) to allow for normalization of functional volumes to standard anatomical space (MNI).

fMRI Tasks

Two separate tasks were used. The first task, a block design fMRI task, was used to localize visual cortex regions that respond maximally to visual stimulation for each subject. This block design was used because, by concatenating multiple events, block designs are relatively insensitive to perturbations in the shape of any single HRF.

The block design task used a 4 Hz counter-flashing checkerboard presentation in order to identify subject-specific maximally stimulated regions within the visual cortex. Six presentations, 20 seconds in duration, of the 4 Hz checkerboard patterns were interleaved with 6 presentations of fixation crosses, with the same 20 second duration (total time 4 minutes).

The second task was an event-related task that allowed us to estimate the shape of the HRF for each subject. This task also incorporated a 4 Hz counter-flashing checkerboard pattern. Subjects viewed 15 presentations of a flashing checkerboard with a 1 second duration. These presentations were separated by a fixation cross with random durations of 14-22 seconds (average 16 seconds).

fMRI Analysis

Functional volumes were slice-time corrected, realigned, co-registered within the native anatomy of each subject. Preprocessing and statistical analysis were performed using SPM version 5 (Wellcome Institute of Cognitive Neurology, London) and Matlab (www.mathworks.com Natick, MA.).

Functional imaging data from the block design task were analyzed using the SPM general linear model approach with a parameter associated with the experimental condition (flashing checkerboard on). Using the functional scans as input to the model, a parameter estimate was generated for each subject in the form of beta images. Contrast images were also generated for each subject by applying a linear contrast between the beta images and the implicit baseline (fixation cross).

In the analysis of the event-related task, estimates of the percent signal change at each time point along the HRF were derived using a deconvolution algorithm (Friston et al., 1998). This algorithm employs a linear regression approach to estimate the percent signal change during time points in which two HRF functions overlap. This allowed for an efficient event related experimental design, but required that events be jittered. Events were jittered in time by randomizing the duration of the fixation cross presentations that occurred between trials, as described above. A sphere with a 5mm radius was centered on the voxel with maximal statistical increase in BOLD signal between the fixation cross condition and the flashing checkerboard condition. Care was

taken to ensure that sphere locations were placed in visual cortex but laterally enough to avoid activation irregularities caused by midline veins.

Measures of the HRF

We used several measures of the HRF, derived from the deconvolution estimates, in order to determine if administration of cortisol impacted the fMRI signal. First, in order to determine if significant signal time course changes were occurring we employed a repeated measures ANOVA analysis. Second, we used a time to peak response as an indicator of temporal shifts in the shape of the HRF. We also measured peak percent signal change for group comparisons, in order to assess potential signal magnitude effects of cortisol administration. These analyses were used because the standard canonical HRF models, typically used in GLM approaches to fMRI data analysis, rely heavily on assumptions about the shape of the HRF. Thus any cortisol induced shifts in the overall shape of the HRF, or time to peak response, would impact the validity of inferences made using models that rely on the canonical HRF.

Results

The fMRI scans from 2 subjects in the placebo group, 3 subjects in the single dose, and 3 subjects in the extended dose group were corrupted. These subjects were excluded from the analysis.

Overall, visual stimuli evoked changes in visual cortex were observed with typical magnitudes of approximately 0.5% for all subject groups. The HRF shape was also

typical with a peak magnitude at 6 seconds and a subsequent undershoot for all subject groups (See Figure 1).

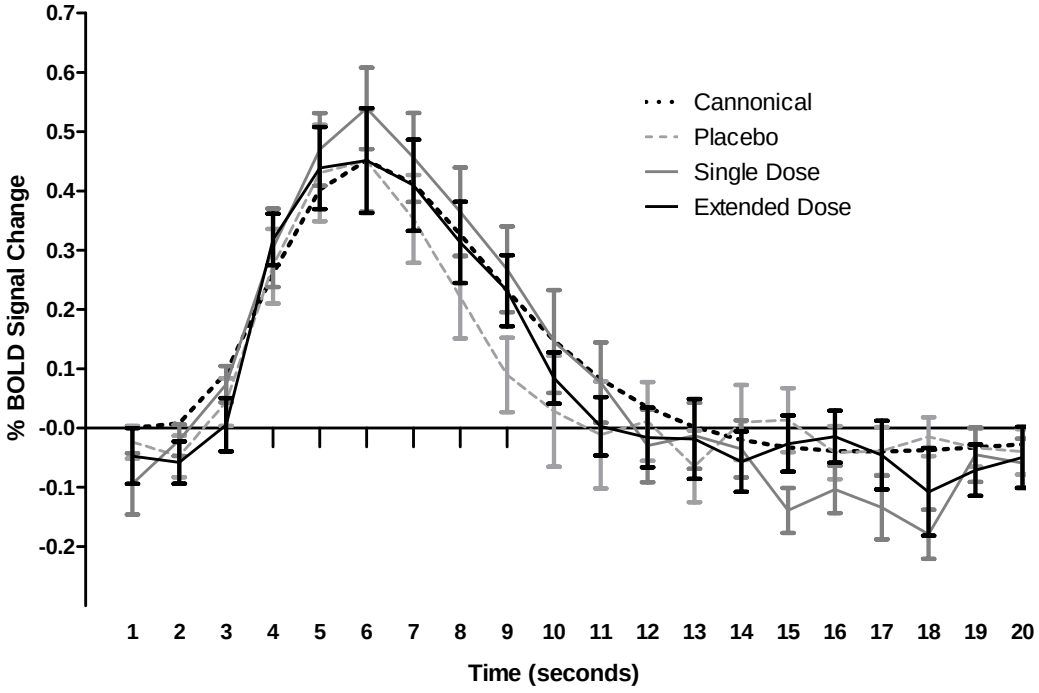

Figure 1 Estimates of the time course of the visual cortex (Brodmann areas 17/18) HRF in subjects administered Placebo, a single dose of 100mg of hydrocortisone, or a extended dose of 25mg/day of hydrocortisone.

Cortisol administration did not significantly affect the shape of the bold signal over time [Group x Time interaction $F(38,931) = 0.96$, $p = 0.542$] (See Figure 1). Cortisol also did not significantly impact the time to peak [Single Dose $T(33)=0.00$, $p = 1$; Extended Dose $T(33)=0.12$, $p = 0.909$] (See Figure 2) or the magnitude of the peak BOLD response [Single Dose $T(33)=1.19$, $p = 0.243$; Extended Dose $T(33)=0.124$, $p = 0.902$](See Figure 3).

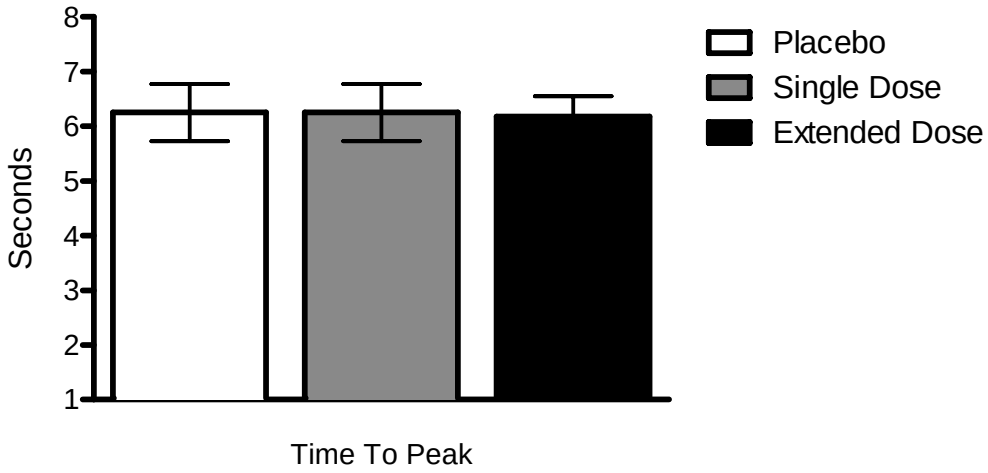

Figure 2 Average time to achieve peak BOLD response in the visual cortex in response to a 1 second visual stimulation for subjects administered placebo, a single dose of 100mg of hydrocortisone, or a split dose of 25mg/day of hydrocortisone/day over 5 days.

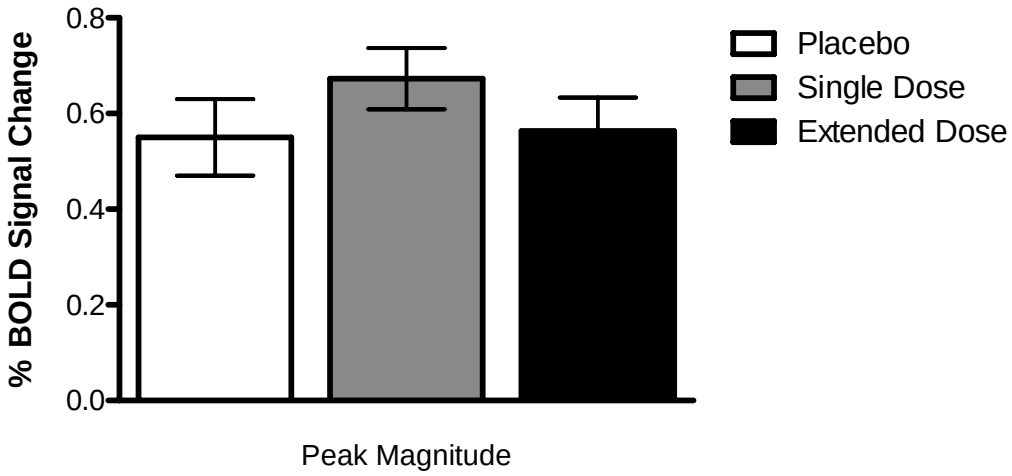

Figure 3 Average magnitude of the peak BOLD response in the visual cortex in response to a 1 second visual stimulation for subjects administered placebo, a single dose of 100mg of hydrocortisone, or a split dose of 25mg/day of hydrocortisone/day over 5 days.

Discussion

Studies of the effects of cortisol on systemic blood pressure are provide mixed and

contradictory results. In addition, glucocorticoid effects on brain vasculature are thought to be a possible mechanism of clinical efficacy in the reduction of intracranial pressure (Leenders et al., 1985; Behrens et al., 1998). However, studies of cerebral blood flow after treatment with glucocorticoids also yield contradictory results (Bastin et al., 2006). Nevertheless the possibility of these effects makes this empirical evaluation of the effects of cortisol on the HRF essential if valid inferences about the effects of cortisol on brain activity are to be drawn (See Chapter 3).

Measures of both shape and magnitude of the HRF were not significantly impacted by cortisol administration. The shape of the HRF is a critical assumption necessary in standard GLM approaches to fMRI imaging analysis, like those used in statistical parametric mapping software packages. These assumptions are most critical when employing event-related fMRI task designs in which single events are isolated in time. Block type fMRI task designs (like those used in Chapter 3), on the other hand, concatenate multiple events, and therefore the shape of the brain response to any single event overlaps with several others, reducing the importance of the model shape. However, the magnitude of the HRF response could also affect inferences by creating the illusion of larger or smaller brain activation changes in cortisol groups compared to the placebo group.

These findings support previous observations that suggest the HRF is generally resistant to pharmacological influences (Murphy et al., 2006). It also suggests that investigators using neuroimaging and hydrocortisone administration as tools may not necessarily need to restrict themselves to tasks that are suited to block designs.

Limitations

Caution should be used in the extrapolation of these findings. Studies of HRF variability across brain structures demonstrate many region specific patterns (Handwerker et al., 2004). Cortisol may yet be influencing regional hemodynamics outside the visual cortex in a significant way. Effects of cortisol on the HRF could potentially vary by dose as well. Here we investigate only 2 different dosing regimens.

Conservative approaches to integration of pharmacology in fMRI studies require the verification that underlying assumptions about HRF are not violated, if valid inferences about brain activity are to be made. Therefore, future studies may consider incorporating similar empirical validations of the shape of the HRF in brain regions of interest. However, when this type of comprehensive approach is infeasible or impractical, blocked experimental designs can serve to reduce the impact of atypical HRF shapes.

Chapter 3: The Effect of Cortisol Administration on the Neural Correlates of Emotion

Introduction

Activation of the HPA axis and the release of cortisol are often associated with stress (Selye, 1985). Extreme dysregulation of cortisol secretion by the HPA axis causes severe pathologies such as Addison's disease and Cushing's syndrome. These conditions result in symptoms which include changes in emotion and energy levels. Poor regulation of cortisol, overproduction of cortisol, and increased adrenal cortex volume are commonly observed in a large population of depressed patients (Carroll et al., 1976; Carroll, 1980; Rubin et al., 1995) , who also exhibit changes in energy levels and emotion. However, to what extent cortisol may contribute to the maintenance or etiology of aberrant emotional processing in depression is unknown.

Some evidence suggests that cortisol may play a critical role in the emotional pathologies of depression and in emotion processes in general. Evidence from post-mortem studies of depressed patients demonstrates decreased GR mRNA expression in several cortical and subcortical structures (Webster et al., 2002) suggesting possible adaptations to repeated activation or hyperactivation of the HPA axis and/or breakdown of regulatory mechanisms. Amelioration of the hypercortisolemic state is associated with recovery from depression and resistance to relapse (Greden et al., 1980; Holsboer et al., 1982;

Zobel et al., 1999; Zobel et al., 2001). Better understanding of the influence of cortisol on brain regions that process emotion, could elucidate the developmental timeline of pathological emotional processes at work in depressed patients with hypercortisolemia.

A large corpus of previous studies suggests that cortisol can affect memory and emotion (Newcomer et al., 1999; Buchanan and Lovallo, 2001; de Quervain et al., 2003). There is also evidence cortisol can influence the structure and physiology of neurons in brain regions thought to underlie emotional processes and behaviors. Animal studies have demonstrated that corticosterone increases anxiety (Mitra and Sapolsky, 2008) and depression-like behaviors (Kalynchuk et al., 2004), and has direct modulatory effects on the physiological responsiveness of neurons in neural structures associated with emotion, such as the hippocampus (De Kloet et al., 1998), amygdala (Karst et al., 2002; Mitra and Sapolsky, 2008) , and ventral tegmental area (Cho and Little, 1999). High levels of corticosterone in animals have also been shown to cause dendritic reorganization of the hippocampus (Woolley et al., 1990; Sapolsky, 2000) and PFC (Wellman, 2001).

In humans the primary receptors for cortisol, MR and GR, are expressed in high levels in brain regions associated with emotional processing such as the hippocampus (Watzka et al., 2000a), amygdala (Sarrieau et al., 1986), frontal and temporal lobes (Watzka et al., 2000b). Endogenous cortisol levels, in humans, have been shown to correlate with activity in a variety of subcortical brain regions (Liberzon et al., 2007). Furthermore, cortisol administration has been shown to enhance memory for selective types of emotional material (Buchanan and Lovallo, 2001), and is capable of blunting emotional responses to some stimuli (Reuter, 2002). Previous studies have also shown that

exogenous cortisol can directly induce both depression and mania (Ling et al., 1981). Endogenous cortisol levels have also been shown to correlate with depressed mood (Van Honk et al., 2003). Furthermore, experimental drugs that inhibit the synthesis of cortisol (Wolkowitz et al., 1999) or act as cortisol receptor antagonist (DeBattista et al., 2006) have been shown to have antidepressant effects.

The effects of cortisol are accomplished, on the molecular level, through a variety of genomic and non-genomic mechanisms (Rose, 2000; Karst et al., 2002; Mikics et al., 2005; de Kloet et al., 2008a). Evidence from animal studies suggests that behaviors as complex as risk assessment can be modulated by coricosterone through a non-genomic pathway within minutes (Mikics et al., 2005). Therefore, potential effects of cortisol on emotion could manifest within minutes through a non-genomic mechanism, or over much longer time courses, suggestive of a genomic mediated mechanism.

Despite long-standing evidence that cortisol plays a critical role in emotional disorders like depression, alters emotional behaviors, and affects brain regions involved in emotion, to date, little work has been done to explore what functional effect cortisol has on the activity of emotion related brain regions.

The current double-blind placebo-controlled experiment has been designed to characterize how a single exposure and an extended exposure to elevated cortisol levels may affect basic emotional processes in healthy subjects. We theorized that the high levels of endogenous cortisol observed in depression may partially explain brain activity pattern changes observed in patients with depression, such as hyperactivity in the

subgenual cingulate (Mayberg et al., 1999) and amygdala (Sheline et al., 2001; Drevets et al., 2002). Therefore, we hypothesize that in healthy subjects, exposure to elevated cortisol levels would modulate activity in these regions. Specifically, we hypothesized that while viewing sad stimuli exposure to cortisol would result in increases in the amygdala and subgenual cingulate activity while decreasing medial prefrontal cortex activity in regions traditionally associated with emotion regulation (Ongur and Price, 2000; Taylor and Liberzon, 2007). We also predicted that while viewing happy stimuli salience related brain regions, such as the ventral striatum, would exhibit decreased activation during exposure to elevated cortisol levels.

Methods

Subjects

Sixty healthy (30 male and 30 female), right-handed subjects, ages 18-30 years old, were recruited from the local population and signed a comprehensive written consent form, as approved by the local ethics committee. Exclusion criteria included a history of endocrine disorders, head injury, psychiatric or neurologic disorders, presence of an acute medical condition, medication use, recent major surgeries, a history of traumatic life events, current illicit drug use or dependence, smoking, and current exposure to excessive psychological stress. Subjects meeting MINI criteria for axis-1 psychiatric disorders or scoring more than 7 points on the Beck Depression inventory-II were excluded. All female subjects were free of hormone-based contraceptives and fMRI scanning was scheduled to coincide with the luteal phase of their menstrual cycle.

Subjects were matched by age, sex, weight, and ethnic/racial background. Subjects were randomly assigned to one of three groups (placebo, single dose 100 mg hydrocortisone, extended dose 25 mg/day hydrocortisone over the course of five days), forming a 2x3 (gender X dose) factorial design. Subjects and the experimenters were blind to group assignment until the completion of data collection for that subject.

Cortisol Administration

Cortisol was administered in a double blind fashion, either as a single oral dose of 100 mg hydrocortisone given two hours prior to fMRI scanning or as an extended dose, which was a split dose of 25 mg/day oral hydrocortisone given as a 3:2 dose ratio (15 mg at 8 a.m. and 10 mg at 8 p.m.) over 5 days. A single placebo group acted as an experimental control for both the extended dose group and the single dose group by giving the single dose group and the placebo group placebos at 8 a.m. and 8 p.m. over 5 days in the same fashion as the extended exposure regimen.

fMRI Task

A passive emotion induction task was used to study feelings of happiness, sadness, and neutrality. This design was chosen to observe the effects of cortisol on emotion processes relevant to the persistent sadness and anhedonia common in depression.

Happy, sad, and neutral International Affective Picture System (IAPS) stimuli and images of facial expressions of emotion (Gur et al., 2002) were presented in a variable length block design. Each stimulus was presented for 6 seconds (3 repetition times (TR)). Blocks of like stimuli were presented for 18, 24, or 30 seconds (3, 4, or 5 stimuli/block, respectively). Each block of emotional stimuli was flanked by 12 seconds of presentation

of a black screen with a white fixation cross. These blocks were then assembled into 6 runs, lasting approximately 7 minutes and 12 seconds per run (216 TR). Block order was pseudo-randomized to control for the effects of order of emotions presented, order of stimulus type presented (IAPS or facial expression), order of the gender of facial expressions being presented, and the order of presentation of particular facial identities. Each run contained identical and proportional representation of each emotion condition.

Subjects were instructed to "Look at each picture, and feel whatever you feel". In order to verify that subjects remained on task, subjects were also instructed to make a button press using their right index finger at the onset of each emotional stimuli or fixation cross. This button press was logged for each experimental stimulus in order to verify compliance.

Measurement of Emotional States

Subjective emotional states were monitored over the 5 days using the positive and negative affect schedule (PANAS). The PANAS consists of 60 adjectives that are endorsed to reflect a subject's current subjective emotional experience. PANAS adjectives are rated on a 1 (very slightly) to 5 (extremely) scale to indicate the intensity of the experience. These 60 adjectives are subdivided into 11 subscales. Included subscales are attentiveness, fatigue, fear, guilt, hostility, joviality, sadness, self-assuredness, serenity, shyness, surprise. The PANAS was completed daily at 8 a.m., 12 p.m., 4 p.m. and 8 p.m. for 5 days. Data from the PANAS was analyzed using a multivariate repeated measures ANOVA with *a priori* planned contrasts designated

between each cortisol group and the placebo group for the joviality and sadness subscales.

Measurement of Cortisol

Cortisol was assayed from saliva samples, on the day of scanning, using a salivette system and quantified using standard direct, non-extraction, Coat-A-Count tube, RIA kit from Diagnostic Products Corporation (Los Angeles, CA). Saliva samples were collected from subjects every 20 minutes between 2 p.m. and 4 p.m., at which point subjects underwent fMRI scanning. Additional saliva samples were collected 0 and 20 minutes after fMRI scanning (5:30 p.m. and 5:50 p.m.), for a total of nine saliva samples per subject. Assays of circulating cortisol levels were analyzed using a repeated measures ANOVA model. Planned contrasts were designated *a priori* between each cortisol group and the placebo group.

MRI Image Acquisition

MRI imaging was performed on a 3.0 Tesla GE Signa system using a standard radio frequency coil. A T1 weighted image was acquired for land marketing and positioning of subsequent scans. Whole brain, functional scans were acquired using a T2* -weighted, single shot, reverse-spiral pulse sequence (TR = 2000 ms, TE = 30 ms, flip angle = 90°, FOV = 22 cm, slice thickness = 3 mm, number of the slices = 40) to minimize susceptibility artifact (Yang et al., 2002). Functional scanning was divided into 6 runs. At the beginning of each functional run, 4 functional volumes (8 seconds) were collected and subsequently discarded to allow for T1 equilibrium. High-resolution, T1 weighted, inversion recovery SPGR anatomical images were also collected for each

subject (TR = 10.5 ms, TE= 3.4 ms, flip angle = 25°, FOV = 24 cm, slice thickness = 1.5 mm, number of slices = 106) to allow for normalization of functional volumes to standard anatomical space (MNI)

Ratings of Emotion induced by Stimuli Presented During Scanning

After completion of fMRI scanning, subjects rated each of the 144 stimuli that were presented to them in the magnet on a series of 1-to-9 Likert scales for happiness, sadness, neutrality, valence and arousal. All of these scales, with the exception of valence, were unidirectional, where a rating of 1 indicates a low magnitude of the measured emotional experience and 9 indicates a high magnitude of the emotional experience. For the valence scale, a rating of 1 indicated a negative emotional experience, a rating of 5 indicated a neutral experience and a rating of 9 indicated a positive emotional experience. Both the valence and arousal ratings scales use the self-assessment manikin system (Lang et al., 1999). Planned contrasts were designated *a priori* between each cortisol group and the placebo group comparing ratings of the magnitude of happiness elicited by happy stimuli and ratings of sadness elicited by sad stimuli. Additional planned contrasts compared valence and arousal ratings elicited by happy and sad stimuli separately.

fMRI Analysis

Functional volumes were slice-time corrected, realigned, co-registered within the native anatomy, then normalized to standard anatomical space and smoothed using a 6 mm^3 Gaussian kernel to correct for individual variability within the gyral anatomy. Additional preprocessing and statistical analysis were performed using SPM version 5

(Wellcome Institute of Cognitive Neurology, London) and Matlab (www.mathworks.com Natick, MA.).

Functional imaging data was analyzed using a general linear model approach, with parameters associated with each of the psychological conditions (happy facial expressions, neutral facial expressions, sad facial expressions, happy IAPS pictures, neutral IAPS pictures, and sad IAPS pictures), for each run. Each experimental run was also modeled as a parameter to control for systemic variability between runs. This resulted in a total of 6 (condition) x 6 (runs) + 6 (identity parameters) for a total of 42 parameters. Using the functional scans as input to the model, parameter estimates were generated across the whole brain for each subject, in the form of beta images. Contrast images were then generated for each subject by applying linear contrasts between beta images, or between beta images and the implicit baseline.

Using the standard hierarchical model approach, a second level, random effects, full factorial model was created with subject groups (placebo, single dose 100 mg, extended dose 25 mg/day) and gender (male, female) permutations as parameters to be estimated, and using first level contrast images of psychological conditions, from the first level, as input. This serves to estimate variability between subjects, thus allowing population inference. All reported second level contrasts met a conventional minimum voxel-wise threshold above $p<0.005$ in a cluster threshold minimum of 5 voxels, although activation clusters within our regions of interest were often significant at much lower thresholds.

Regions of interest, hypothesized to be sensitive to the effects of cortisol and involved in

emotion processes, were identified *a priori*. These regions included the amygdala, ventral medial prefrontal cortex, and subgenual cingulate. Regions of interests were defined in standard anatomical space (MNI) using anatomical labels included in the MNI Space Utility (http://www.ihb.spb.ru/~pet_lab/MSU/MSUMain.html) and Anatomical Automatic Labeling software (http://www.cyceron.fr/freeware). Contrast value averages were then extracted using custom Matlab code, based on SPM5 code and the Neurotools extension (Adrian Imfeld, http://www.aimfeld.ch/neurotools/neurotools.html). Contrast value extractions were performed for each region of interests for each subject. These values were subsequently analyzed in a multivariate ANOVA model using SPSS version 15.

Results

Experimental Blinding

In a forced choice situation, 76.2% of subjects taking the single dose of cortisol and 50% of subjects taking the extended dose of cortisol thought that they were taking placebos. This indicates that cortisol did not produce subjective effects that could be easily noticed and thus interfere with experimental blinding. Although experimental blinding does seem to be more effective for a single dose of cortisol than the extended dose of cortisol, few subjects in the extended dose group reported any noticeable effects. (See Table 1)

Table 1 Confusion matrix table indicates that subjects were poor at distinguishing between taking hydrocortisone and taking a placebo. Columns indicate the group assignment. Rows indicate the group the subject thought they were in. Cells indicate the rate of correct identification of group (diagonals) or misidentification of group (off diagonals).

	Placebo	Single Dose	Extended Dose
Placebo	16 (80.0%)	16 (76.2%)	10 (50.0%)
Single Dose	2 (10.0%)	3 (14.3%)	5 (25.0%)
Extended Dose	2 (10.0%)	2 (9.5%)	5 (25.0%)

Circulating Cortisol Levels Elevated by Cortisol Administration

Data from 2 subjects in the placebo group and 1 subject in the extended dose group were corrupted during the analysis of the saliva samples. Overall there was a significant group effect on circulating cortisol levels [$F(2, 53) = 30.623$, $p < 0.001$]. This effect was driven by a robustly significant difference between the placebo and single dose group [$p < 0.001$] and no significant difference in circulating cortisol levels between the placebo and extended dose group [$p = 0 .992$] (See Figure 4). Administration times (8 a.m. and 8 p.m.) for the extended dose group were planned to allow clearance of enough cortisol so that circulating levels had returned to baseline. The data verify that the final exogenous cortisol dose in the extended dose group, administered at 8 a.m. had, indeed, fully metabolized to baseline levels. It is noteworthy that there was a high degree of individual variability in circulating cortisol levels measured over time.

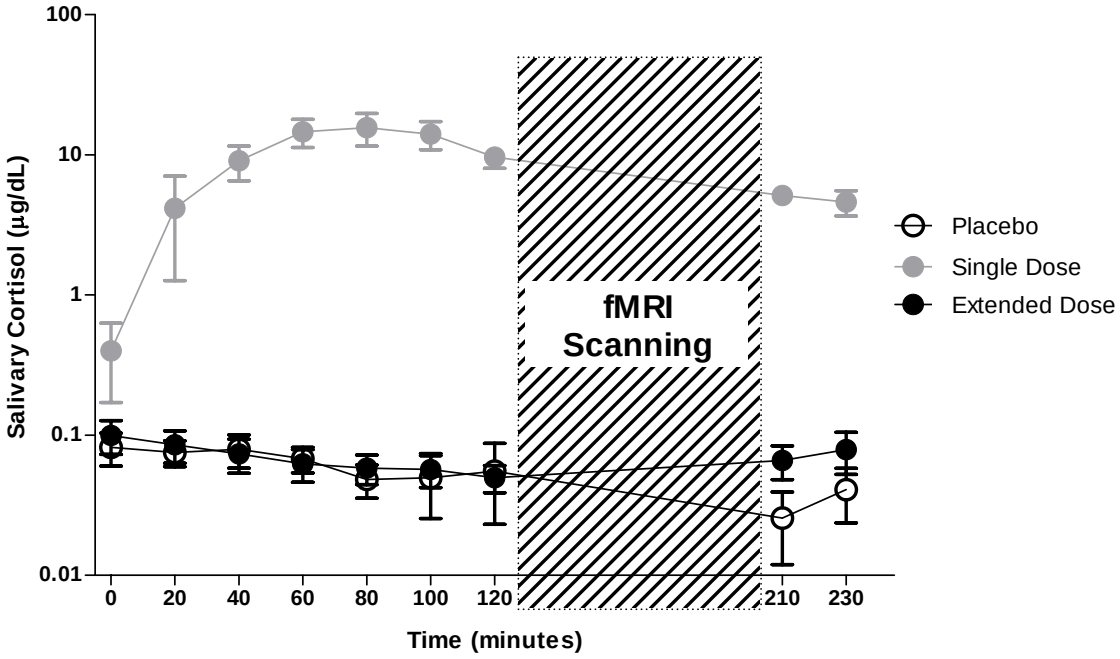

Figure 4 Circulating cortisol concentrations as measured in saliva. The single dose group was administered 100mg oral hydrocortisone at time 0. The extended dose group was administered 25mg hydrocortisone/day over 5 days, ending 6 hours prior to time 0.

The maximum circulating cortisol value was 0.082(± 0.022) µg/dL for the placebo group, 16.22 (± 3.81) µg/dL for the single dose group, and 0.109 (± 0.027) µg/dL for the extended dose group. The fMRI scanning session did not take place until after the peak circulating cortisol levels for the single dose group (male peak=60 min; female peak = 80 min). However, at the time of fMRI scanning session circulating cortisol levels were still robustly increased [T (37) = 7.07, p <0.001] in the single dose group compared to the placebo. Average circulating cortisol levels during the fMRI scanning session were 0.040 (± 0.023) µg/dL for the placebo group, 7.71(± 1.030) µg/dL for the single dose group, and 0.058(± 0.012) µg/dL for the extended dose (See Figure 5).

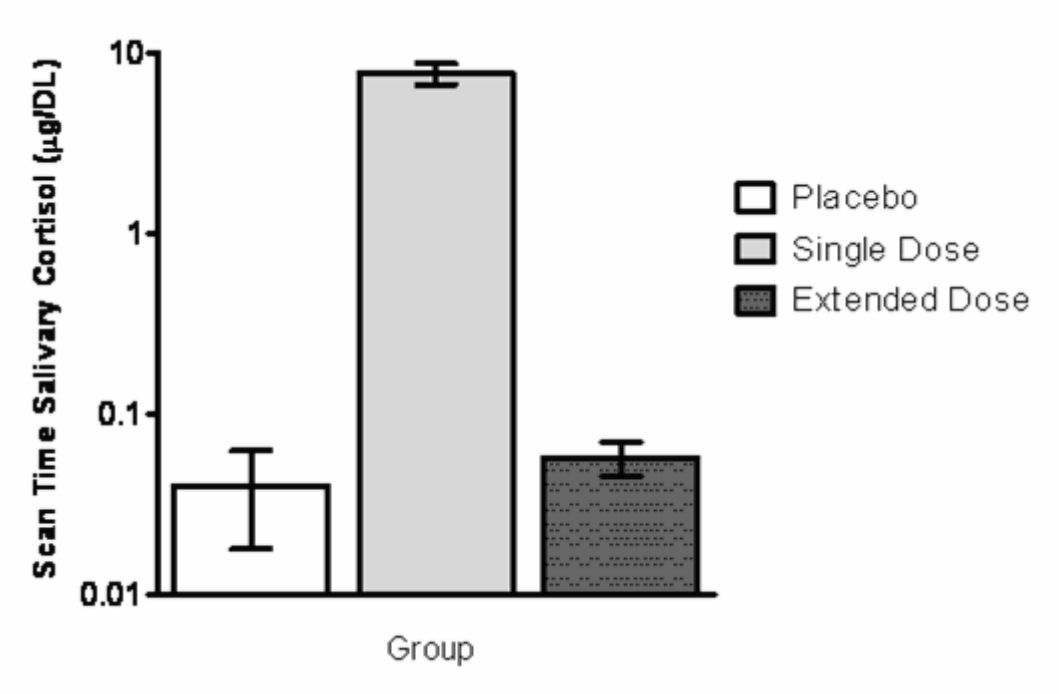

Figure 5 Average circulating cortisol during fMRI scanning was robustly elevated in the single dose group.

Cortisol Produces a Trend Level Increase in Sad Mood

Cortisol did not significantly affect ratings on any of the 11 PANAS subscales, collected over the course of the 5 days. However, there was a trend level increase in the sadness subscale for the extended dose compared to the placebo group [T (38) = 1.81, p = 0.074].

Cortisol Increases Arousal Ratings of Sad Stimuli

The arousal ratings of sad emotional stimuli, collected after the fMRI scanning session, demonstrated an overall group effect [F (2, 58) = 3.313, p = 0 .044] confirming one of our *a priori* hypotheses. Planned contrasts revealed that this effect was driven by a significant difference between the placebo group and the extended dose group [T (38) = 2.56, p = 0.013] and that the difference between the placebo group and the single dose

group was not significant [T (38) = 1.06, p = 0 .293]. No other *a priori* or post-hoc effect of group, gender, or group by gender interaction was significant.

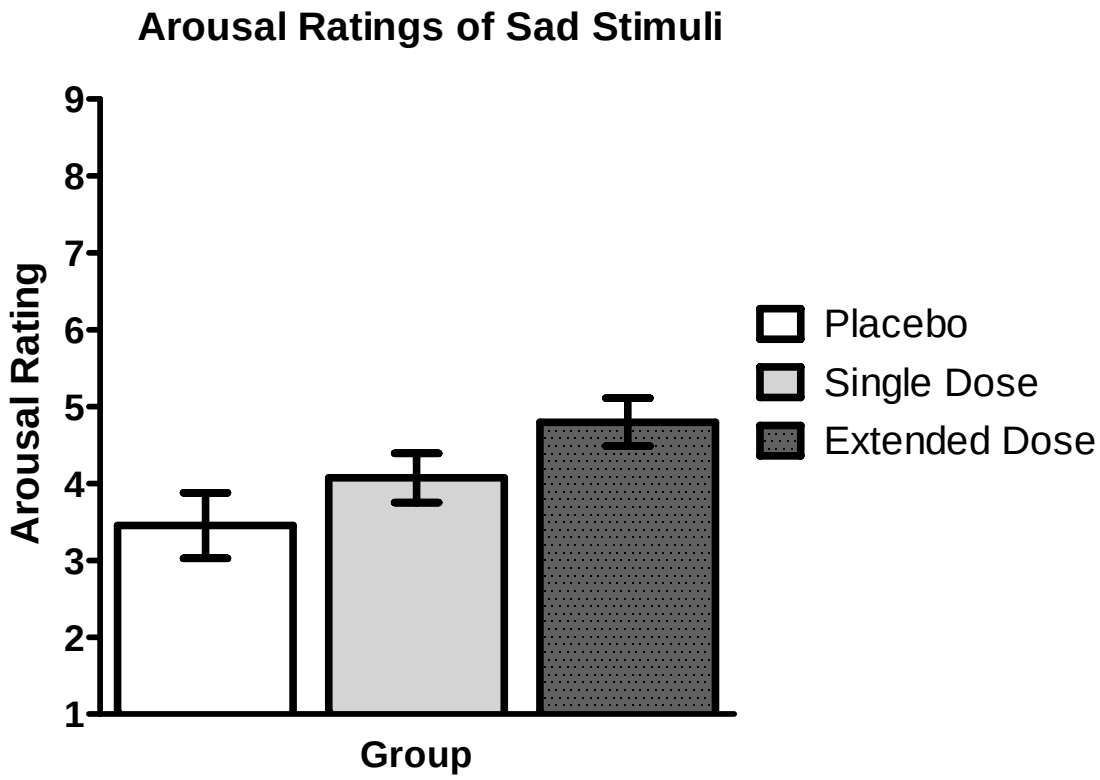

Figure 6 Arousal ratings in reaction to viewing sad stimuli were significantly elevated in the extended dose group but not the single dose group.

Emotional Stimuli Robustly Activate Subcortical and Cortical Brain Regions

When compared to the baseline fixation cross, typical (Phan et al., 2002) and robust emotion related brain activity was induced by the passive viewing of emotional stimuli. Large clusters of elevated activity were observed centered on the visual cortex, thalamus, superior colliculus, amygdala, hippocampus, caudate, putamen, dorsal medial prefrontal

cortex, dorsal lateral prefrontal cortex and ventral medial prefrontal cortex (See Figure 7 red color spectrum). In addition, a few brain regions exhibited decreased activity during the emotion manipulations. These regions include the superior temporal gyrus with a cluster spanning the lateral sulcus, insula, rostral anterior cingulate, dorsal lateral prefrontal cortex, and posterior cingulate/precuneus (See Figure 7 blue color spectrum). Among these regions, the superior temporal gyrus and the posterior cingulate/precuneus are typical deactivations that are observed in many different types of tasks. These regions are components of what is often termed a "default mode" of brain function (Raichle et al., 2001).

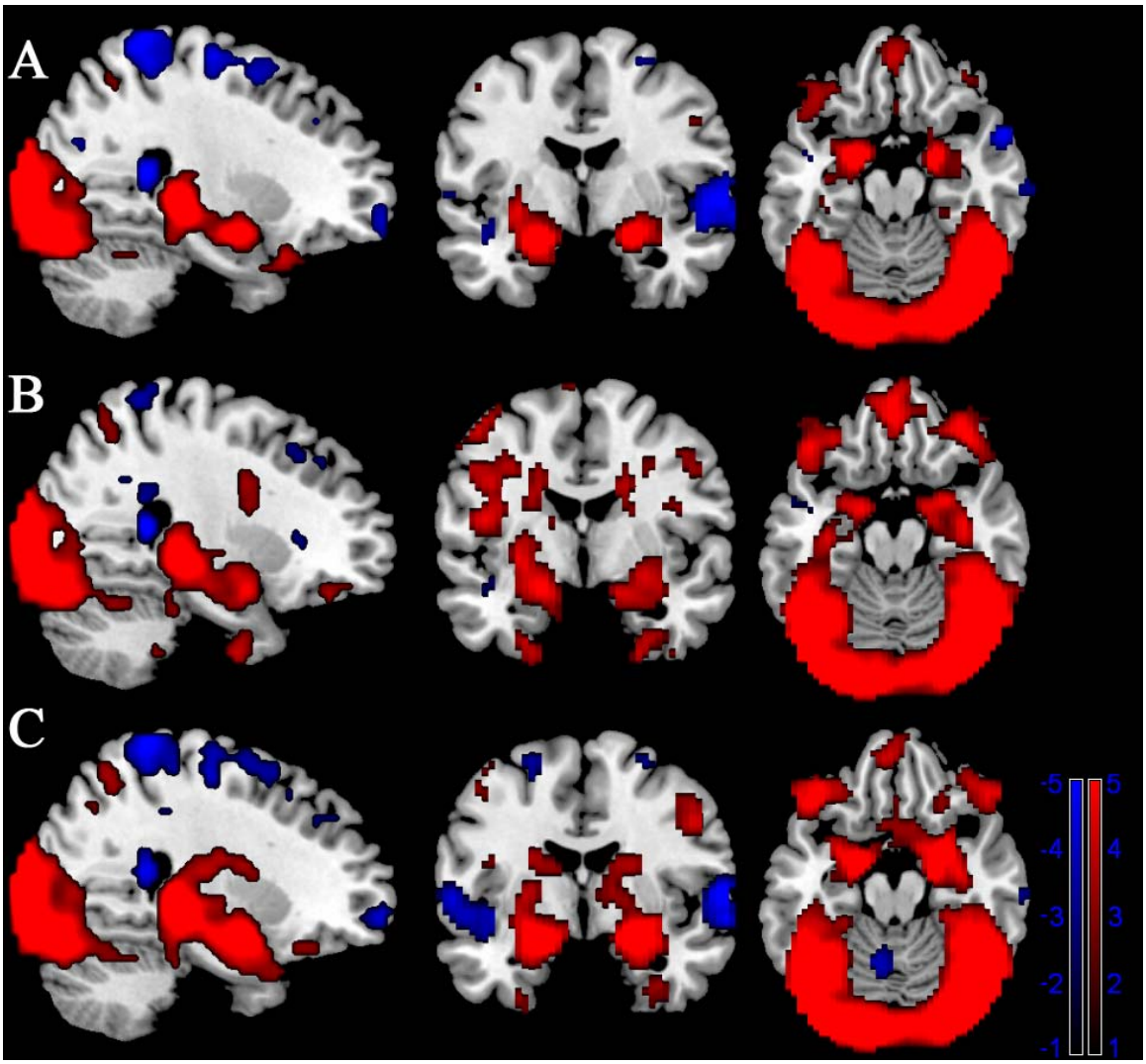

Figure 7 Brain Activity Changes Resulting from the Viewing of Happy (Row A), Neutral (Row B), and Sad (Row C) stimuli. All emotion condition activations/deactivations are relative to fixation cross for the placebo group only.

Cortisol Modulates Brain Activity Elicited by Emotional Stimuli

Within our *a priori* regions of interest, subgenual cingulate activity was inhibited by both the single dose [T (39) = -2.36, p = 0.022] and the extended dose regimen [T (39) = -2.89, p = 0.006] (See Figure 8). This subgenual cingulate inhibition was specific to the

sadness condition (See Figure 9). Circulating salivary cortisol measures within the single dose group were also negatively correlated with subgenual cingulate activity during the sadness condition [r^2= 0.22, p = 0.038] (See Figure 10), confirming our between groups observation that cortisol functions to inhibit activity in subgenual cingulate during sadness. It is also notable that subgenual cingulate activity during sadness is correlated positively with arousal ratings of sad stimuli, but only in the cortisol groups [single dose: r^2= 0.32, p = 0.008; extended dose: r^2= 0.31, p = 0.013], where arousal ratings are elevated and subgenual cingulate activity is suppressed.

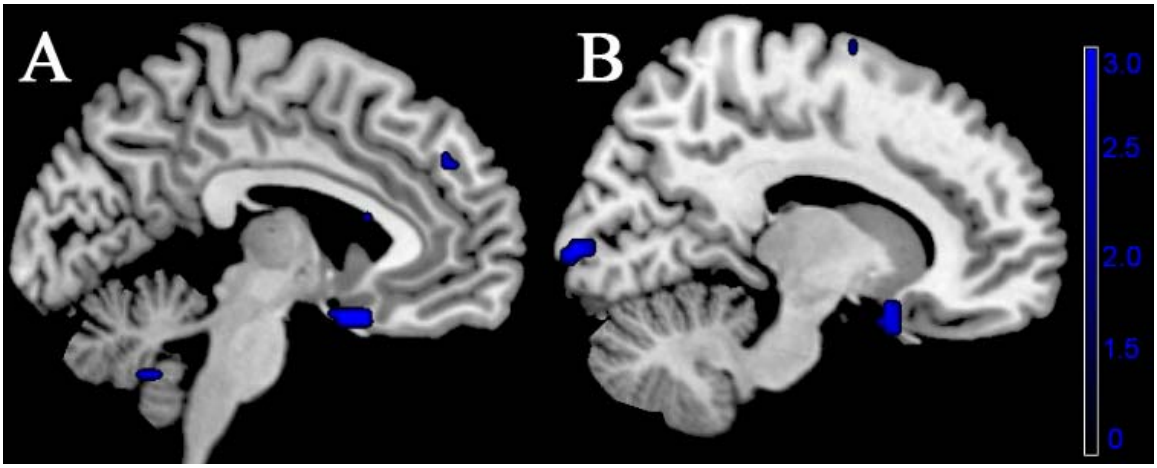

Figure 8 Subgenual cingulate suppression by a single dose (A) and an extended dose (B) of cortisol relative to the placebo group.

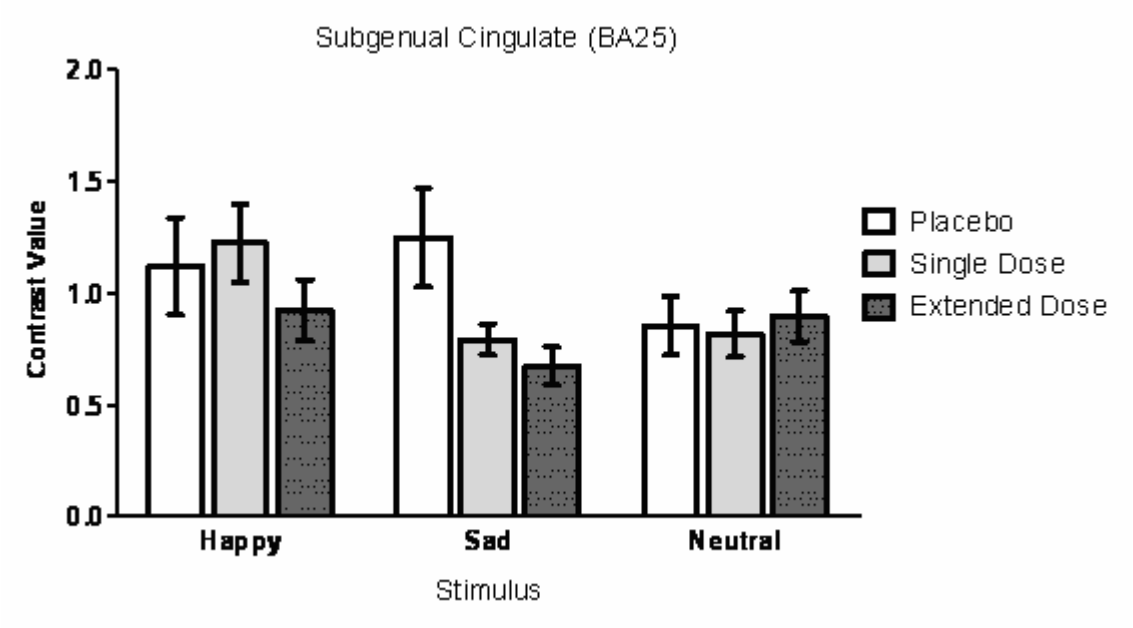

Figure 9 Cortisol induced subgenual cingulate activity suppression is observed only during the sadness condition.

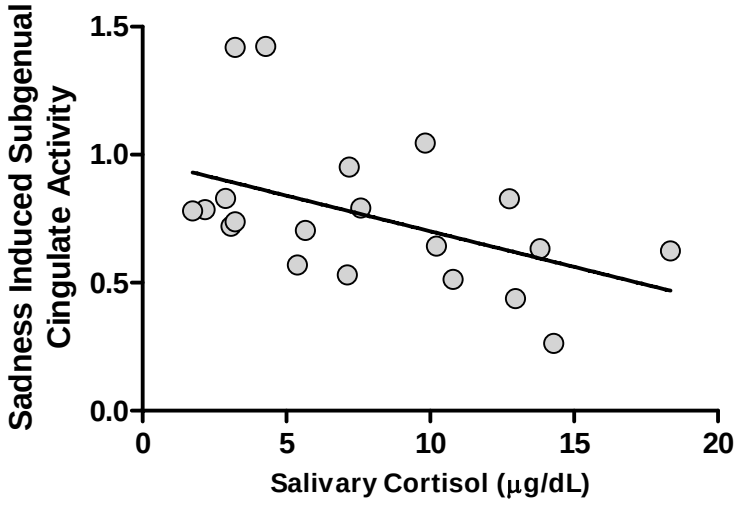

Figure 10 Sadness induced subgenual cingulate activity in the single dose group is negatively correlated with circulating cortisol levels at the time of scanning.

Ventral medial prefrontal cortex activity was also suppressed by the extended dose [T (39) = -2.54, p = 0.014], but single dose suppression was only significant at a trend level [T (39) = -1.70, p = 0.094] (See Figure 11). This ventral medial prefrontal cortex suppression is also specific to the sadness condition.

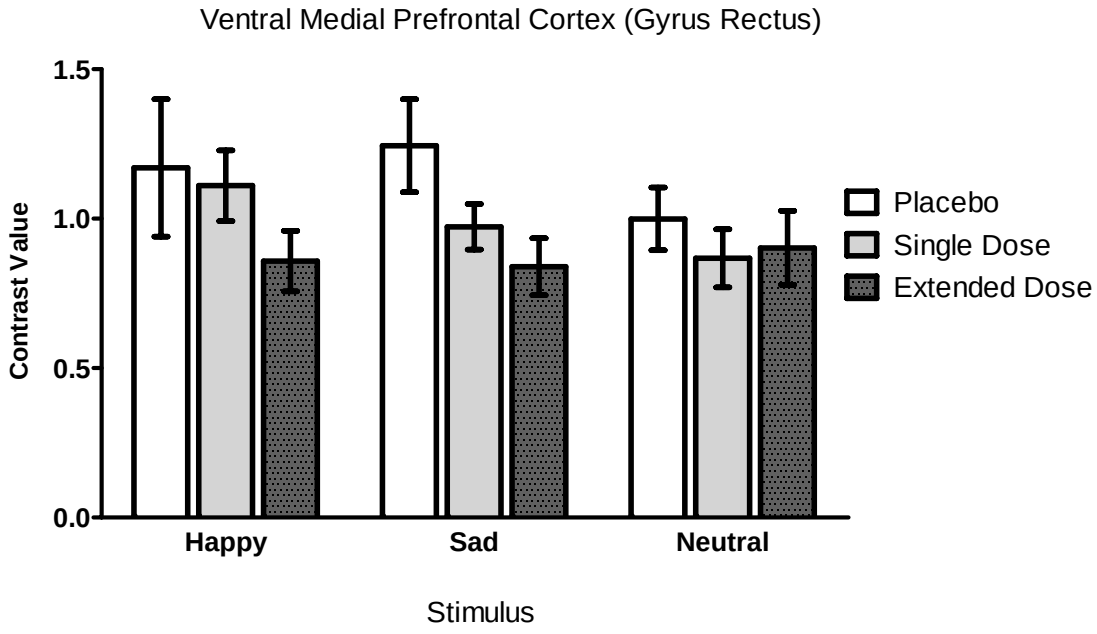

Figure 11 Cortisol induced VMPFC activity suppression is observed only during the sadness condition. The single dose produces only trend level suppression, whereas, the extended dose produces significant suppression.

Amygdala activity was not significantly affected by either the single dose [Left Amygdala T (39) = - 0.93, p = 0.355; Right Amygdala T (39) = -1.52, p = 0.134] or the extended dose regimen [Left Amygdala T (39) = -1.59 , p = 0.117; Right Amygdala T(39) = -1.01 , p = 0.316] during the sad, happy or neutral conditions (See Figure 12).

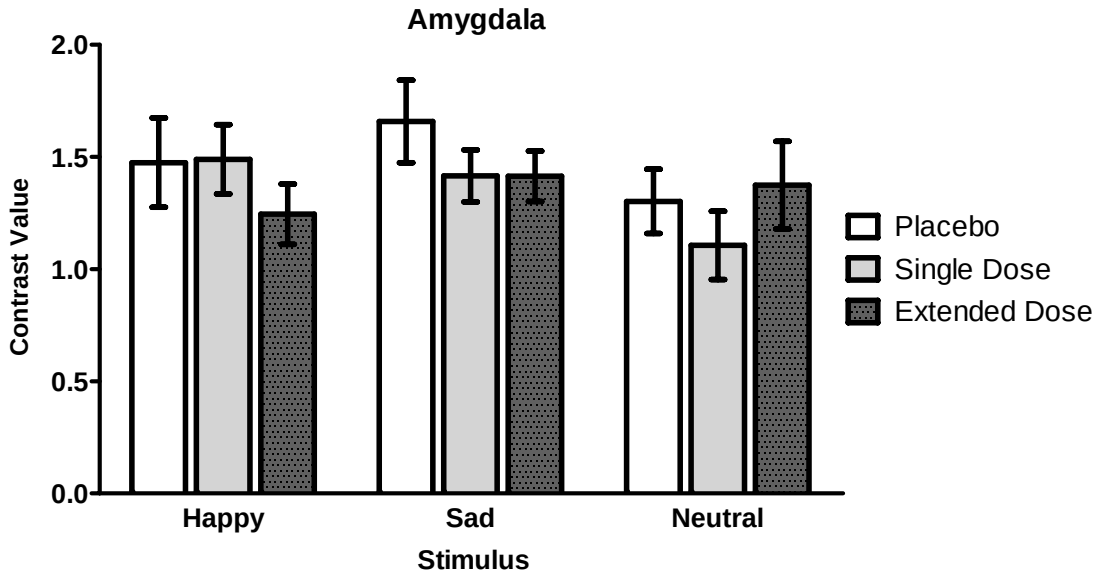

Figure 12 Amygdala activity in response to emotional stimuli is not significantly altered by either the single dose or the extended dose of cortisol.

Interestingly, parahippocampal/periamygdaloid cortex activity was significantly increased by cortisol administration. Clusters of single dose induced increases in activity were seen during the sadness condition [Z= 3.17, p = 0.0008, k = 5, XYZ = -18, -18, -24] (See Figure 13 A). In addition, clusters of extended dose induced increases in activity were seen in the sadness [Z= 3.04, p = 0.001, k = 12, XYZ = -18, -15, -21] (See Figure 13 B), happiness [Z= 3.12, p = 0.0009, k = 13, XYZ = 33, -3, -30] (See Figure 13 D), and the neutral conditions [Z= 3.25, p = 0.0006, k = 28, XYZ = -48, 3, -33] (See Figure 13 C).

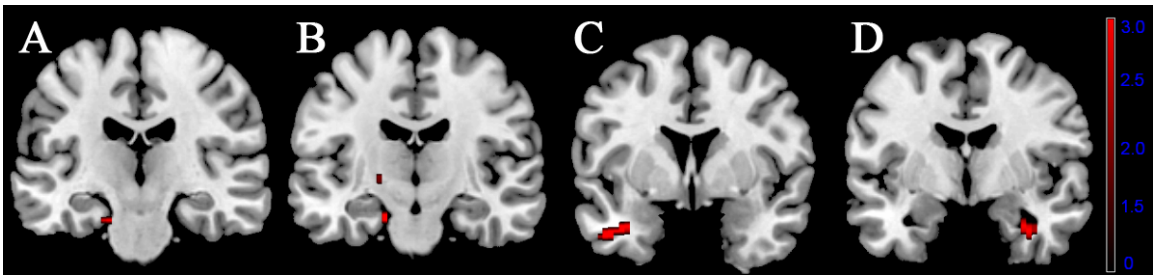

Figure 13 Parahippocampal/perimamygdala activity is increased in the single dose group during sadness (A) and the extended dose group during sadness (B), neutrality (C), and happiness (D).

Sublenticular extended amygdala (SLEA, substantia inominata) activity during sadness was significantly decreased in the extended dose group [Z= 3.25, p = 0.0006, k = 42, XYZ = 15, 12, -21]. This cluster was connected to, but distinct from, the large subgenual cingulate cluster (See Figure 14).

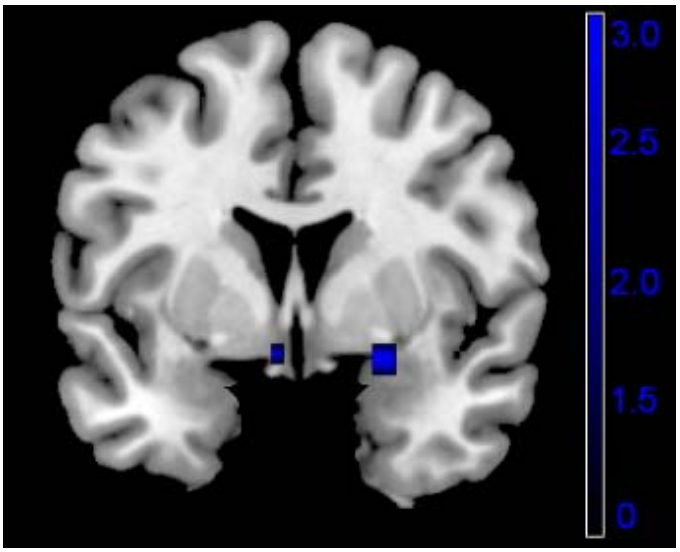

Figure 14 Extended dose induced suppression of SLEA activity during sadness.

Visual cortex was modulated by cortisol administration in ways that varied by subregion, emotion condition and dose. During the sadness condition the single dose regimen resulted in clusters of increased activity in visual cortex Brodmann areas 17 and 19.

However it also produced clusters of reduced activity in Brodmann area 18 and a separate subregion of Brodmann area 19. The extended dose regimen however had no effect on visual cortex during the sadness condition. During the happiness condition the single dose regimen resulted in clusters of increased activity in Brodmann area 19 but also decreased activity in Brodmann area 18. The extended dose produced clusters of decreased visual cortex activity in Brodmann area 17, 18, and 19. During the neutral condition the single dose regimen produced clusters of increased activity in Brodmann area 19, but reduced activity in Brodmann area 18 and in a separate subregion of Brodmann area 19. The extended dose regimen produced clusters of decreased visual cortex activity in Brodmann areas 17, 18, and 19.

Thalamus and superior colliculus activity during sadness was decreased by administration of the single dose of cortisol (See Figure 15). Clusters of decreased activity were also present just below the threshold of significance in the extended dose group.

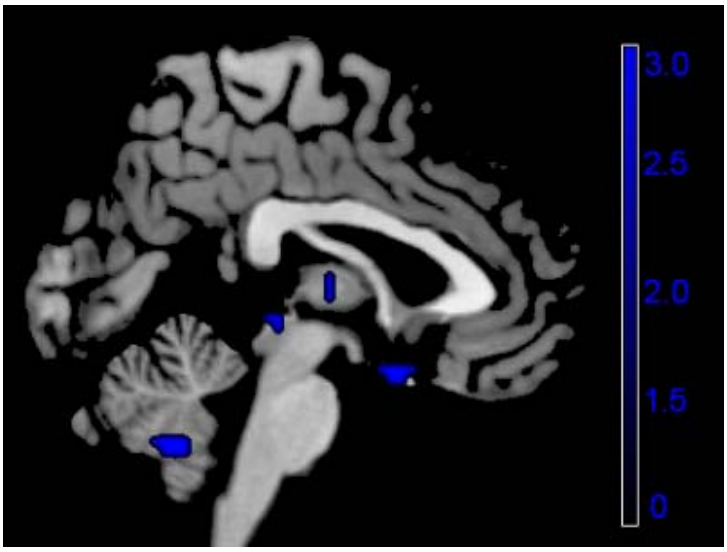

Figure 15 Superior colliculus and thalamus activity is suppressed by a single dose of cortisol.

Table 2 Table of significant group differences in brain activity while viewing sad stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of $p < 0.005$, uncorrected; Extend threshold k=5 voxels

Sad Stimuli	**Region(x,y,z)[a]**	**Cluster[b]**	**Z-Score[c]**
Extended Dose > Placebo			
Cerebellum (R)	30 -33 -30	6	3.15
BA38 (R)	21 0 -45	10	3.07
Parahippocampal Gyrus (L)	-18 -15 -21	12	3.04
Lentiform Nucleus (L)	-21 -15 0	5	2.82
Placebo > Extended Dose			
BA11 (R)	3 39 -30	9	3.40
BA23 (L,R)	-3 -45 21	19	3.26
BA25 (R)	15 12 -21	42	3.25
BA40 (L)	-45 -51 57	36	3.25
BA39 (L)	-48 -51 6	10	3.20
BA17/18(R)	12 -96 3	25	3.12
BA9 (L)	-36 30 39	14	3.08
BA40 (R)	42 -48 57	14	3.03
BA11 (L)	-9 18 -21	9	2.96
BA19 (R)	33 -69 -21	20	2.96
BA45 (L)	-57 30 3	5	2.94
BA9 (R)	36 18 42	16	2.91
BA6 (R)	24 15 63	14	2.88

Superior Frontal Gyrus (R)	15 -3 72	6	2.80
BA11/25 (L)	0 30 -18	6	2.78
Single Dose > Placebo			
BA19 (L)	-12 -90 33	30	3.57
BA 5/7 (R)	9 -48 63	20	3.17
Parahippocampal Gyrus (L)	-18 -18 -24	5	3.17
BA19/7 (R)	24 -87 36	32	3.13
BA 13 (L)	-45 -45 21	15	3.12
Superior Temporal Gyrus (L)			
Inferior Parietal Lobule (L)			
Superior Temporal Gyrus (L)	-21 6 -45	5	3.09
BA 6 (L)	-42 -12 54	5	3.04
BA 37 (L)	-57 -66 3	10	2.97
Cerebellum (L)	-24 -54 -30	6	2.91
BA19 (L)	-27 -78 30	8	2.89
Precuneus(L)			
BA 39 (L)	-39 -63 12	10	2.84
BA 7 (L)	-9 -78 51	7	2.79
Precuneus(L)			
Placebo > Single Dose			
BA 11/25 (L)	-9 15 -21	31	3.36
BA 18/19 (R)	27 -96 9	20	3.35
Cerebellum (R)	15 -54 -42	83	3.29
Midbrain (R)	18 -21 -9	48	3.25

Superior Colliculus (R)			
BA 9 (L)	-9 45 33	6	3.15
BA33/Anterior Cingulate (R)	6 6 27	42	3.14
Caudate (L)	-12 -15 21	22	3.14
Caudate (L)	-9 9 21	14	3.07
BA 9 (R)	33 21 33	8	3.01
Thalamus (L)	-3 -12 3	8	2.98
Caudate (R)	15 -12 21	20	2.92

Table 3 Table of significant group differences in brain activity while viewing happy stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of $p < 0.005$, uncorrected; Extend threshold k=5 voxels

Happy Stimuli	**Region(x,y,z)[a]**	**Cluster[b]**	**Z-Score[c]**
Extended Dose > Placebo			
Middle Occipital Gyrus (L)	-33 -63 6	16	3.28
Parahippocampal Gyrus (R)	33 -3 -30	13	3.12
BA 37 (R)	54 -54 -6	8	2.94
Middle Temporal Gyrus (R)	51 -36 -9	7	2.92
Cerebellum (L)	-24 -51 -36	8	2.70
Placebo > Extended Dose			
BA 17/18 (R)	15 -96 3	48	3.40
Lingual Gyrus (R)			

Precuneus (L)	-12 -63 39	15	3.07
BA 18/19 (R)	27 -72 -18	34	3.06
Fusiform/Lingual Gyrus			
BA 6/9 (L)	-3 48 36	11	3.05
BA 8/9 (L)	-39 33 42	17	2.85
Middle Frontal Gyrus (L)			
BA 23 (L)	-15 -12 -51	5	2.78
Posterior Cingulate (L)			
BA 40 (R)	54 -42 24	10	2.75
Inferior Parietal Lobule (R)			
Single Dose > Placebo			
BA 21/22/41/42 (R)	57 -18 6	85	4.34
Superior and Transverse Temporal Gyrus (R)			
BA 19/7 (L)	-12 -90 36	101	3.75
Cuneus and Precuneus (L)			
BA 38 (R)	54 15 -21	13	3.60
Superior Temporal Gyrus (R)			
Cerebellum (L,R,C)	3 -45 -12	42	3.47
BA 19/7 (R)	18 -78 30	95	3.35
Cuneus, precuneus,			
Superior Occipital Gyrus (R)			
BA 2/3/4 (L)	-45 -18 33	36	3.32
Precentral gyrus (L)			

Postcentral gyrus (L)			
Cerebellum (L)	-18 -51 -30	14	3.27
BA 4/6 (L)	-42 -9 54	11	3.18
Precentral gyrus (L)			
BA 22 (R)	66 -54 12	9	3.17
Superior Temporal Gyrus (R)			
Temporal Pole (R)	30 21 -42	5	3.17
Inferior Temporal Gyrus (R)	36 -54 -3	12	3.04
BA 44/45 (L)	-54 21 21	19	3.01
Inferior Frontal Gyrus (L)			
Cerebellum (R)	18 -39 -30	6	2.97
Putamen (R)	24 15 -12	6	2.94
Caudate/Globus Pallidus (R)	12 6 -3	7	2.93
Cerebellum (L)	-30 -39 -30	32	2.93
BA 19 (L)	-30 -84 33	8	2.80
Claustrum/Insula (R)	39 -6 6	6	2.79

Placebo > Single Dose

Precuneus (L)	-15 -60 36	13	3.33
BA 6 (L)	-15 21 63	5	3.07
BA 18 (R)	27 -96 9	7	3.01
Middle Occipital Gyrus (R)			
Parietal Cortex (R)	27 -51 24	7	2.97

Table 4 Table of significant group differences in brain activity while viewing neutral stimuli. [a] Stereotactic coordinates from MNI152 reference, left/right, anterior/posterior and superior/inferior, respectively. [b] Cluster size in voxels. [c] All foci meet min threshold of p< 0.005, uncorrected; Extend threshold k=5 voxels.

Neutral Stimuli	**Region(x,y,z)[a]**	**Cluster[b]**	**Z-Score[c]**
Extended Dose > Placebo			
Cerebellum (L)	-21 -48 -27	95	3.46
Anterior Cingulate (R)	12 39 3	17	3.44
BA 19 (L)	-33 -63 9	30	3.42
Middle Temporal Gyrus (L)			
Thalamus (R)	15 -30 18	9	3.39
BA 13 (L)	-42 -45 18	18	3.36
Insula (L)			
Superior Temporal Gyrus (L)			
BA 21 (L)	-48 3 -33	28	3.25
Fusiform Gyrus (L)			
Inferior and Middle Temporal Gyrus (L)			
Caudate (R)	21 24 6	7	3.19
BA 13/29/40 (R)	48 -24 15	12	2.97
Insula (R)			
Cerebellum (R)	18 -39 -30	8	2.70
Placebo > Extended Dose			

Pons (L)	-12 -12 -42	28	3.62
BA 17/18 (R)	15 -93 3	23	3.05
BA 8 (R)	33 24 57	5	3.00
BA 19 (R)	30 -72 -18	16	2.99
Fusiform Gyrus (R)			
Cerebellum (R)			
BA 8 (L, R)	-3 48 48	5	2.84
BA 9 (L)	-48 24 39	5	2.80
BA 13/29/40 (R)	-36 6 66	5	2.76

Single Dose > Placebo

BA 19 (L)	-12 -90 33	27	3.34
Parahippocampal Gyrus (L)	-30 -51 -6	6	2.84
BA 19 (R)	21 -90 27	5	2.70
Cuneus (R)			

Placebo > Single Dose

BA 20/21 (R)	66 -45 -15	27	4.46
Inferior and Middle Temporal Gyrus(R)			
Pons (L)	-15 -15 -33	26	4.13
BA 4/44/6 (L)	-51 0 15	25	3.76
Inferior Frontal and Precentral Gyrus (L)			
Temporal Pole (L)	-27 -15 -45	14	3.57
BA 20 (L)	-48 -27 -24	16	3.46

Inferior Temporal Gyrus(L)			
Fusiform Gyrus(L)			
BA 10 (L)	-3 66 0	8	3.44
Superior and Middle Frontal Gyrus (L)			
BA 6 (L)	-33 -3 36	54	3.39
Precentral Gyrus(L)			
BA 20/36 (R)	54 -24 -27	23	3.29
Fusiform Gyrus(R)			
Inferior Temporal Gyrus (R)			
Middle Frontal Gyrus (L)	-24 27 30	12	3.29
BA 10/11 (R)	27 54 -9	9	3.29
Superior and Middle Frontal Gyrus (R)			
Inferior Parietal Lobule (L)	-39 -30 27	24	3.26
BA 40 (R)	51 -45 57	18	3.23
Inferior Parietal Lobule (R)			
BA 18/19 (R)	27 -96 9	17	3.19
Middle Occipital Gyrus (R)			
BA 10/11 (R)	6 57 -6	13	3.15
Supramarginal Gyrus (R)	51 -54 27	26	3.14
Caudate (R)	21 0 30	16	3.02
BA 37 (R)	60 -60 -15	8	3.02
Inferior Temporal Gyrus(R)			
Anterior Cingulate (L)	-18 36 -3	7	3.02
BA 11 (R)	15 48 -21	6	2.99

Superior and Middle Frontal Gyrus (R)			
BA 8 (L,R)	0 48 48	11	2.96
Middle Frontal Gyrus (R)	30 18 36	10	2.91
BA 10 (R)	36 48 18	8	2.85
Superior and Middle Frontal Gyrus (R)			
BA 11 (R)	33 45 -15	5	2.66
Superior and Middle Frontal Gyrus			

Discussion

The main novel findings from this study are that cortisol administration to healthy subjects increases feelings of arousal elicited by sad stimuli and inhibits subgenual cingulate activity induced by sad stimuli. Cortisol administration also resulted in modulation of activity in additional brain regions associated with emotional processing, and that have abberent activity in depressed patients. These regions have been suggested to be part of a network of brain regions that are affected in depression and form an extended visceromotor network (See (Drevets et al., 2008) for a review). Regions of the extended visceromotor network affected by cortisol include the subgenual cingulate, ventral medial prefrontal cortex, parahippocampal/periamygdaloid cortex, SLEA and thalamus. Cortisol also exerted affects on regions of the brain involved in the processing of visual stimuli, such as the superior colliculus, thalamus and visual cortex. These findings are particularly interesting because of their potential relevance to the pathophysiology of depression. In separate bodies of literature, both the dysregulation of cortisol secretion and alterations in subgenual cingulate functioning have been noted in

depressed patients. This study provides initial concrete evidence that these biological components of the symptomatology of depression may be linked to each other and potentially also linked to psychological perturbations in emotion processes.

Cortisol induced exaggerated arousal in the extended dose group is a particularly notable finding for two reasons. First, since the experimental blinding of subjects in this experiment was successful and few subjects reported any awareness of an altered emotional state, yet ratings of arousal feeling evoked by viewing sad emotional stimuli were significantly increased. This seems to indicate that the emotional change may be subtle or outside of the realm of subjective awareness. Secondly, circulating cortisol levels for the extended dose group had returned to baseline at the time of the ratings, whereas for the single dose group, there was a robust elevation in circulating cortisol at the time of the ratings. However, only the extended dose group, and not the single dose group, had elevated arousal ratings of the sad stimuli. This may indicate that cortisol effects on arousal may be partially mediated through mechanisms that require longer time courses.

Inhibition of the subgenual cingulate by exogenous cortisol in this study is a robust finding and is replicated both between groups (See Figure 9) and, in a parametric fashion, within the single dose group (See Figure 10). However, cortisol induced inhibition of the subgenual cingulate may seem counterintuitive, given that elevated cortisol and elevated subgenual cingulate activity have both been reported in depression (Carroll et al., 1976; Carroll, 1980; Mayberg et al., 1999). One possible reconciliation of these observations is that subgenual cingulate activity may adapt over the course of long term

exposures to endogenous hypercortisolemia. In the current study, the longest exposure to exogenous cortisol levels was over five days. However, exposure to elevated cortisol levels from endogenous hypercortisolemia may occur over weeks, months or even years. Another possible interpretation is that decreased sensitivity to cortisol in depression could be underlying increases in both cortisol and sadness. Decreased sensitivity could be preventing the normal operation of cortisol inhibition of subgenual cingulate, observed here, resulting in high levels of activity. Future studies may consider longer exposures to exogenous cortisol, in order to elucidate possible subgenual cingulate adaptations to elevated cortisol levels over time and studies directly involving depressed patients.

Increased activity in the parahippocampal/periamygdaloid cortex suggests that cortisol activity may be influencing cortical processing of amygdala or hippocampus afferents and efferents. Decreased activity in the VMPFC and SLEA suggest that cortisol may not be influencing, in a straightforward manner, the projection pathways from medial frontal cortex to the amygdala thought to code emotion regulation signals (Ongur and Price, 2000; Taylor and Liberzon, 2007).

Inhibition of activity in the thalamus and superior colliculus during emotion processing is particularly notable because these brain regions are key nodes on a sensory processing pathway thought to transmit emotional information to the amygdala in a coarse but expeditious manner. This raises an intriguing question as to if cortisol may be affecting aspects of the sensory experience, leading to increases in the perceptibility of certain kinds of emotional stimuli (See Chapter 4).

Since the discovery of abnormal regulation of cortisol release in depression in the 1970's (Carroll et al., 1976) and the mass production of efficient and convenient ways to measure cortisol in the blood and saliva, a great deal of research has been done characterizing associations between cortisol levels and a host of personality, cognitive, behavioral and emotional measures. However, despite evidence that hypercortisolemia can alter emotional states and evidence that cortisol receptors are located in brain regions known to process emotional material, surprisingly little work has been done to attempt to establish the directionality of association between cortisol and measures relevant to depression. Is hypercortisolemia simply a biomarker of psychological and physiological processes at work in depression? Is cortisol actively contributing to the maintenance or etiology of these processes? Here we provide direct evidence that cortisol may be driving subjective emotional changes similar to those observed in depression and that activity in brain regions implicated in depression are effected by cortisol. We also provide initial evidence, from the extended dose group, suggesting these effects persist even when circulating cortisol levels have returned to baseline.

Limitations:

Several limitations need be considered when interpreting these results. In any study incorporating cortisol administration one can not ignore its numerous and wide ranging effects. Among these is the inhibition of corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). CRH, like cortisol, has several receptors with wide distributions in the brain. Without the ability to experimentally maintain consistent levels of CRH in the brain, any effects ascribed here to exogenous cortisol may also be due to changes in central CRH. Nevertheless, endogenous cortisol also

decreases CRH. Therefore, cortisol released in endogenous hypercortisolemia would also typically serve as an inhibitory signal to CRH. However, endogenous hypercortisolemia can arise from reduced sensitivity to cortisol at sites of inhibitory feedback, leading to the uncoupling of the cortisol inhibition of CRH. Thus, two limitations must be acknowledged. First, the normal physiological effects of cortisol described here could be secondary effects arising from cortisol effects on CRH. Second, extrapolations of this data to endogenous hypercortisolemic states could be complicated by potential abnormalities in the cortisol-CRH dynamic.

An additional limitation is that cortisol receptors do not exclusively bind cortisol. The human glucocorticoid receptor binds similar shaped hormones such as progesterone, aldosterone, testosterone and estradiol, although with much weaker (all <22%) affinity than for cortisol itself (von Langen et al., 2005). These alternative ligands rarely produce all the appropriate contacts with the amino acids of the protein that are required for a transcriptionally active receptor conformation (von Langen et al., 2005). Thus endogenous levels of these hormones could be functioning as competitive agonists reducing the magnitude of the cortisol signal. However, with doses in this study producing high physiological and supraphysiological concentrations of circulating cortisol, the effect of competitive agonists is likely to be minimal.

Another issue that should be considered is that the extended dose exposure to cortisol in this study was only for 5 days and was conducted by repeated bolus. This may or may not adequately approximate the effects of endogenous hypercortisolemia. Endogenous hypercortisolemia likely occurs over weeks, months or even years and is secreted through

pulsatile release (Windle et al., 1998; Young et al., 2004), rather than a series of regularly timed boluses.

Future studies may consider longer exposures to elevated cortisol to attempt to reconcile the cortisol induced inhibition of subgenual cingulate, observed here, with the increased activity observed in the subgenual cingulate of depressed subjects. However, investigators should proceed with caution to avoid overtly inducing depression or mania, as well as many other possible side effects of longer term administration of cortisol.

In conclusion, we present concrete evidence suggesting that cortisol exerts influence over activity in the subgenual cingulate specifically during sadness and that it affects the subjective emotional experience of sadness. These findings have significant implications for the study of the pathophysiology of depression. We demonstrate for the first time that elevated levels of cortisol can cause both changes in the subjective emotional experience of sadness and changes in activity in the subgenual cingulated during sadness. This suggests a possible directionality of emotional and neurophysiological changes occurring in depression. Endogenous hypercortisolemia could be responsible for changes in the subjective experience of emotion and changes in subgenual cingulate brain activity patterns observed in depressed patients.

Chapter 4: The Effect of Cortisol Administration on the Time Course of Early Detection and Identification of Emotional Facial Expressions

Introduction

There is evidence to suggest that cortisol may be an important factor in pathological alterations in emotional processes. Excessive secretion of cortisol, poor regulation of cortisol, and increased adrenal gland volume are common phenomena observed in depression (Carroll et al., 1976; Carroll, 1980; Rubin et al., 1995) . In post-mortem brains of depressed subjects there is decreased GR mRNA expression in several cortical and subcortical structures (Webster et al., 2002) likely due to repeated activation or hyperactivation of the HPA axis and/or breakdown of regulatory mechanisms. The successful amelioration of the hypercortisolemic condition in depressed subjects is associated with recovery from depression and resistance to relapse (Greden et al., 1980; Holsboer et al., 1982; Zobel et al., 1999; Zobel et al., 2001). Animal studies have demonstrated that corticosterone,increases depression-like behaviors (Kalynchuk et al., 2004) and anxiety (Mitra and Sapolsky, 2008) . However, it is still unknown if hypercortisolemia is a consequence of depression or a contributing factor to depression.

There is also evidence to suggest that cortisol may play an important role in non-pathological emotion modulation. Animal studies demonstrate that corticosterone

has direct modulatory effects on the physiological responsiveness of neurons in neural structures associated with emotion, such as the hippocampus (De Kloet et al., 1998), amygdala (Karst et al., 2002), and ventral tegmental area (Cho and Little, 1999). High levels of corticosterone in animals have also been shown to cause dendritic reorganization in emotion related brain regions such as the hippocampus (Woolley et al., 1990) and prefrontal cortex (Wellman, 2001). In humans cortisol administration has been shown to enhance memory for selective types of emotional material (Buchanan and Lovallo, 2001) and have effects on the subjective experience of emotion (See Chapter 3). Cortisol levels have also been shown to correlate with negative mood (Van Honk et al., 2003). It has also been shown that cortisol is capable of blunting emotional responses to some stimuli (Reuter, 2002). Cortisol receptors are also expressed in high levels in brain regions associated with emotional processing such as the hippocampus (Watzka et al., 2000a), amygdala (Sarrieau et al., 1986), frontal and temporal lobes (Watzka et al., 2000b).

In Chapter 3, we demonstrate that cortisol administration is capable of altering subjective reactions to sad stimuli. We also demonstrate that it can modulate brain activity elicited by emotional stimuli in the superior colliculus, thalamus, periamygdaloid cortex, and the visual cortex. Each of these brain regions is also thought to be involved in early perceptual processing of emotional stimuli (LeDoux, 1994). Brain lesions in patients resulting in “blindsight” suggest that the early perception of emotion may follow a colliculus-thalmo-amygdala route (Morris et al., 2001). Rapid and masked presentations of emotional stimuli during human neuroimaging studies also suggest that early perceptions of emotion are routed through the amygdala (Morris et al., 1998;

Whalen et al., 1998; Monk et al., 2008). It has been hypothesized that this colliculus-thalmo-amygdala represents a fast yet coarse resolution signaling of the emotional stimuli (LeDoux, 1994). We theorized that the effect of cortisol on the colliculi, thalamus, periamygdaloid cortex, and the visual cortex may be biasing early perceptual processing of emotional stimuli. This effect might then result in manifestation of well documented mood congruent emotion processing biases observed in depression (Bradley et al., 1995; Murphy et al., 1999; Murray et al., 1999; Elliott et al., 2000; Erickson et al., 2005). It could also alter the subjective experience of emotion to produce and/or reinforce depression symptoms such as persistent sadness and anhedonia.

In order to test this theory we used rapidly displayed, backwards masked, images of human facial expressions to test for emotional perception biases in the early visual processing stream induced by cortisol administration. We reasoned that performance biases in perceptual processing of particular emotions could be observed by testing the accuracy and sensitivity of the detection and identification of rapidly displayed emotional faces. We hypothesized that cortisol administration would lead to greater accuracy and sensitivity to sad facial expressions and lower accuracy and sensitivity to happy facial expression under conditions that limit the subjective awareness of the faces.

Methods

Subjects

Thirty-six healthy, right-handed male subjects, ages 18-30 years old, were recruited from the local population and signed a comprehensive written consent form, as approved by the local ethics committee. Potential subjects were excluded from the study based on a

history of endocrine disorders, head injury, psychiatric or neurologic disorders, presence of an acute medical condition, medication use, recent major surgeries, a history of traumatic life events, current illicit drug use, depression, smoking, and current exposure to excessive psychological stress. Subjects were screened for Axis-1 psychiatric disorders using the MINI. Subjects were screened for drug use using urine drug test kits (Jant Pharmaca Encino, California). Subjects were also screened for acute medical conditions using a general health questionnaire, screened for Depression symptoms using the Beck Depression inventory-II. Subjects scoring more than 7 points on the Beck were excluded from the study. Subjects were matched by age, weight, and ethnic/racial background and randomly assigned to either the placebo or extended dose 25 mg/day hydrocortisone group.

Cortisol administration and Measurement:

Cortisol was administered in a double-blind fashion as a split oral dose of 25 mg/day given as a 3:2 dose ratio (15 mg at 8 a.m. and 10 mg and 8 p.m.) over the course of 5 days. A placebo control group was administered placebos at 8 a.m. and 8 p.m. to match cortisol group. Cortisol was assayed from saliva samples using the salivette system. Saliva samples were collected from subjects on the 5th day of the study at 8 a.m., 10 a.m., 12 p.m., 4 p.m., and 5 p.m., in order to estimate the effect of exogenous cortisol on circulating cortisol levels.

Measurement of Emotional States

Subjective emotional states were monitored over the 5 days using the PANAS. The

PANAS consists of 60 adjectives that are endorsed to reflect a subject's current subjective emotional experience. PANAS adjectives are rated on a 1 (very slightly) to 5 (extremely) scale to indicate the intensity of the experience. These 60 adjectives are subdivided into 11 subscales. Included subscales are attentiveness, fatigue, fear, guilt, hostility, joviality, sadness, self-assuredness, serenity, shyness, surprise. The PANAS was completed daily at 8 a.m., 12 p.m., 4 p.m. and 8 p.m. for 5 days. Data from the PANAS were analyzed using a multivariate repeated measures ANOVA with *a priori* planned contrasts designated between the cortisol group and the placebo group for the joviality and sadness subscales. The remaining subscales of the PANAS data and measures of the ratings of emotional stimuli were subject to post-hoc analysis with Bonferroni correction for multiple comparisons.

Equipment

Experimental stimuli were presented using Eprime version 1.1 (Psychology Software Tools, Pittsburgh, PA) on a ViewSonic Professional Series p225f CRT monitor connected to a Dell Pentium 4, 3.6 GHz Optiplex GX620, with 2GB of RAM and a custom installed ATI Radeon X800 PCI-express graphics card. Refresh rate of 166 Hz (6ms frame rate) was achieved using PowerStrip version 5.1 (EnTech Taiwan http://www.entechtaiwan.com) software, and was validated using an oscilloscope and attached photo-detector.

Stimuli

Images of evoked facial emotions were used as stimuli (Gur et al., 2002). Images of

faces expressing happiness, sadness and no emotion (neutral) were identified for 48 individual actors within this stimulus set. The faces were then extracted from the background, rotated as needed so that the left and right eyes are on the same horizontal plane, and equalized for luminance across expressions using Photoshop CS2.

Design

Two separate tasks, a face detection task and a facial expression identification task were completed by each subject. Each of these tasks incorporated backwards-masking of emotional faces to limit subjective awareness of the stimuli. Masks consisted of a scrambled emotional face with identical pixel value distributions and spatial limits of the emotional faces. A mask was presented for 100ms immediately following each of the emotional faces. At the beginning of each trial a blank screen was presented for a random duration of 300-500ms to reduce the predictability of onset for each trial. Feedback on overall experimental performance, in the form of a percentage correct and percentage incorrect, was given to subjects every 30 trials and a 2-minute compulsory break was given every 210 trials.

The face detection task consisted of 480 trials (2 emotions x 6 time points x 20 signal trials + 20 noise trials) of a standard yes-no signal detection task, using happy and sad facial expressions. An emotional face, which served as signal, or a clockwise swirled image, which served as noise, was presented for 6, 12, 18, 24, 30, or 36 ms. This was followed immediately by the mask (See Figure 16). After the presentation of each masked face, subjects were instructed to indicate "yes" if they saw a face or "no" if they did not see a face.

The emotion identification task consisted of 420 trials (3 emotions x 7 time points x 20 trials) in a three alternative forced choice task (3AFC) with displays of happy, sad, or neutral emotional expressions. An emotional expression was presented for 12, 18, 24, 36, 54, 72, or 90 ms, followed immediately by the mask (See Figure 17). The order of presentation of types of emotional expression and the duration of the stimuli were randomized. After the presentation of each masked expression, subjects were instructed to indicate if the facial expression was happy, neutral, or sad. Subjects were instructed that if they had the experience of not seeing a face at all, they were to "use their intuition to attempt to guess the correct answer, and not simply guess the same way each time". These instructions served to limit the natural bias toward answering neutral under uncertain conditions.

After the completion of these tasks, subjects classified each of the 144 face stimuli as happy, sad, or neutral, without viewing time restrictions. These data were used to eliminate from the analysis, on a subject-by-subject basis, any face stimuli that did not effectively communicate the targeted emotion. Three subjects classified large numbers of stimuli as something other than the targeted emotion, suggesting noncompliance or extremely poor emotion recognition ability. These subjects were excluded from the analysis.

Subjects completed both tasks twice, once before the start of the placebo or cortisol administration, and once on the 5th day of drug administration. Both experimental sessions were conducted at 4 p.m., near the circadian nadir of endogenous cortisol

production.

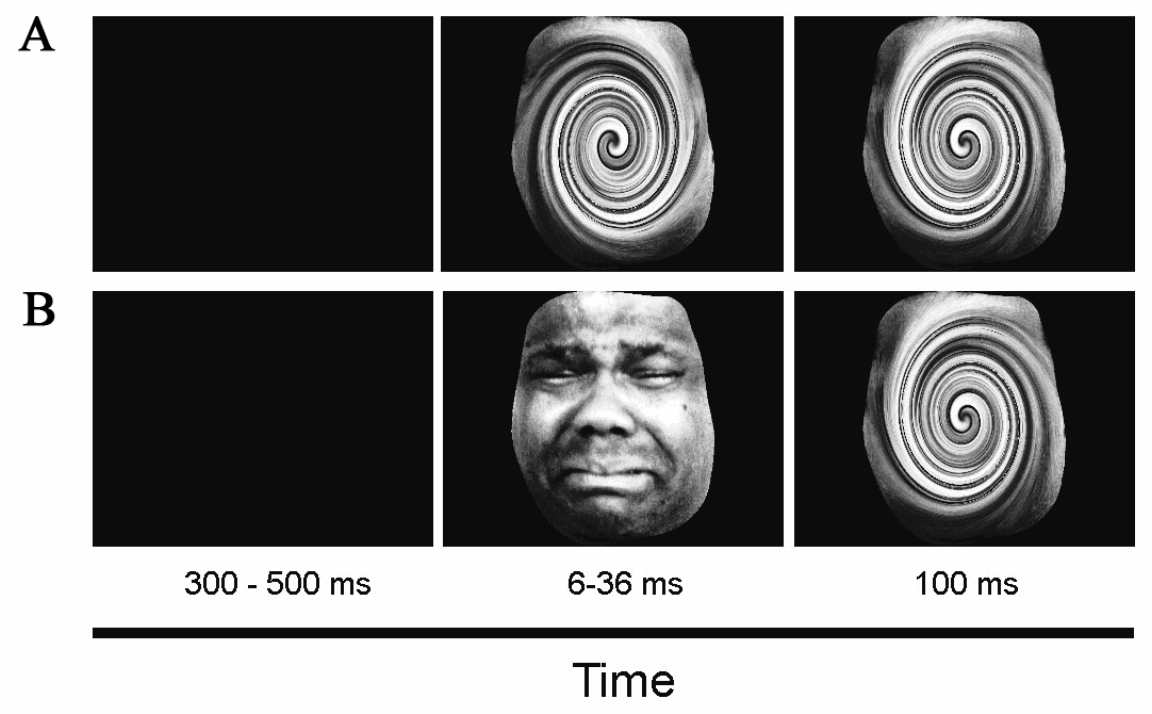

Figure 16 Presentation time course of noise (Row A) and signal (Row B) trials in the face detection task. Each target face was preceded by a blank screen with a 300-500ms duration and then followed by a 100ms perceptual mask.

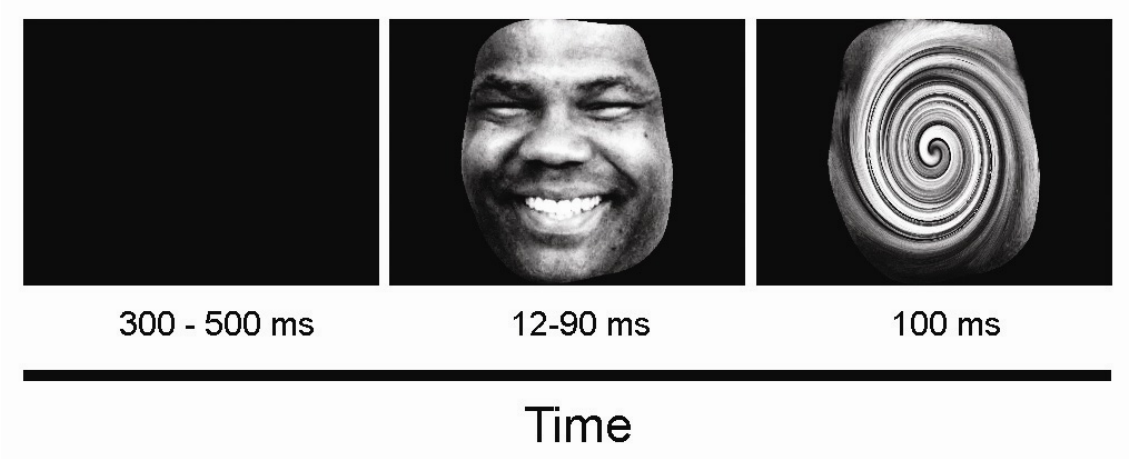

Figure 17 Presentation time course of identification trials. Each target face was preceded by a blank screen with a 300-500ms duration and then followed by a 100ms perceptual mask.

Analysis

Standard accuracy-based analyses and signal detection theory based data analyses were used for both the detection task and the identification task. The signal detection theory based analyses allowed for the calculation of the sensitivity (d'), by considering proportions of both hits and correct rejections. For the detection task accuracy and d' values were calculated from subjects' response proportions. In the identification task the values for d' were calculated from available statistical tables for 3AFC designs. For both the detection and identification tasks, a standard correction equal to half a trial was applied to the d' calculation in order to correct for contingencies that included 0% or 100% hits or false alarms rates. Accuracy and d' values were subsequently entered into repeated measures ANOVA models and analyzed with SPSS.

Results

Experimental Blinding

When given a forced choice question regarding their group assignment 65% of the subjects in placebo group though they were in the placebo group and 75% of subjects in the cortisol group thought they were in the placebo group. This indicates that cortisol administration did not produce detectable subjective effects that affected experimental blinding.

Cortisol Administration does not affect Overall Emotional State

Cortisol administration over 5 days did not significantly affect sadness, joviality, or any other PANAS mood subscale, indicating that cortisol does not significantly alter subjective mood ratings.

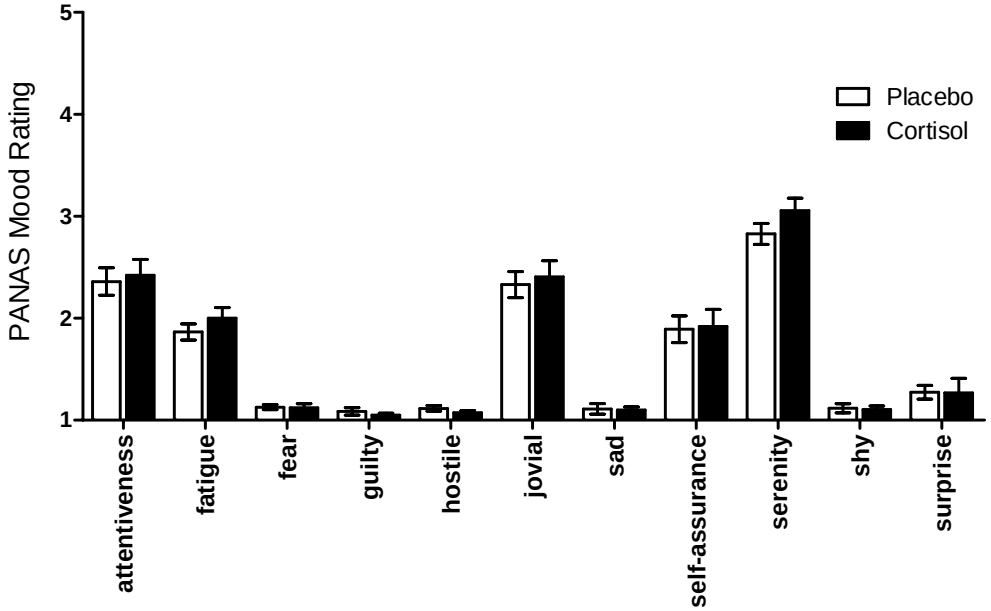

Figure 18 Cortisol administration does not significantly alter any of the 11 PANAS mood subscales.

Circulating Cortisol Levels Elevated by Cortisol Administration

Twenty five subjects (14 placebo, 11 cortisol) collected samples at each of the appropriate time points. As in Chapter 3, dose timing was planned to allow clearance of enough cortisol so that circulating levels would return to baseline by the time the experimental testing session was conducted. Circulating cortisol levels were significantly elevated at 10 a.m. 2 hours after the final dose of cortisol [T(24)=3.746, p=0.001]. No significant group differences were present at any other time point, indicating that cortisol levels had returned to baseline within 4 hours of the final dose.

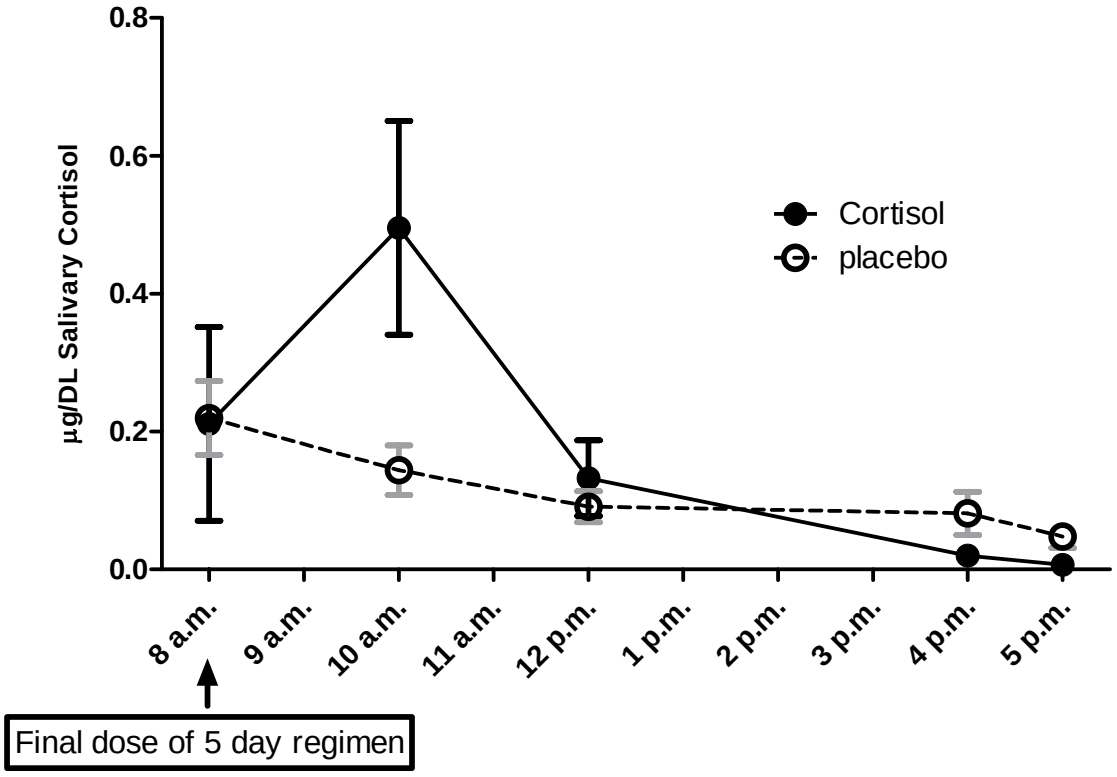

Figure 19 Circulating cortisol levels are significantly elevated 2 hours after cortisol administration.

Type of Emotional Facial Expression Affects Detection Accuracy and Sensitivity But

Cortisol Administration Does Not.

Stimulus durations from 6-36ms were adequate to span the bulk of the range from chance level accuracy and null sensitivity to >90% accuracy and a high degree of sensitivity.

Face detection accuracy and sensitivity response curves were significantly better for happy faces than sad faces [Accuracy $F(5, 155) = 14.42$, $p<0.001$, Sensitivity $F(5, 155) = 8.086$, $p<0.001$] (See Figure 20)

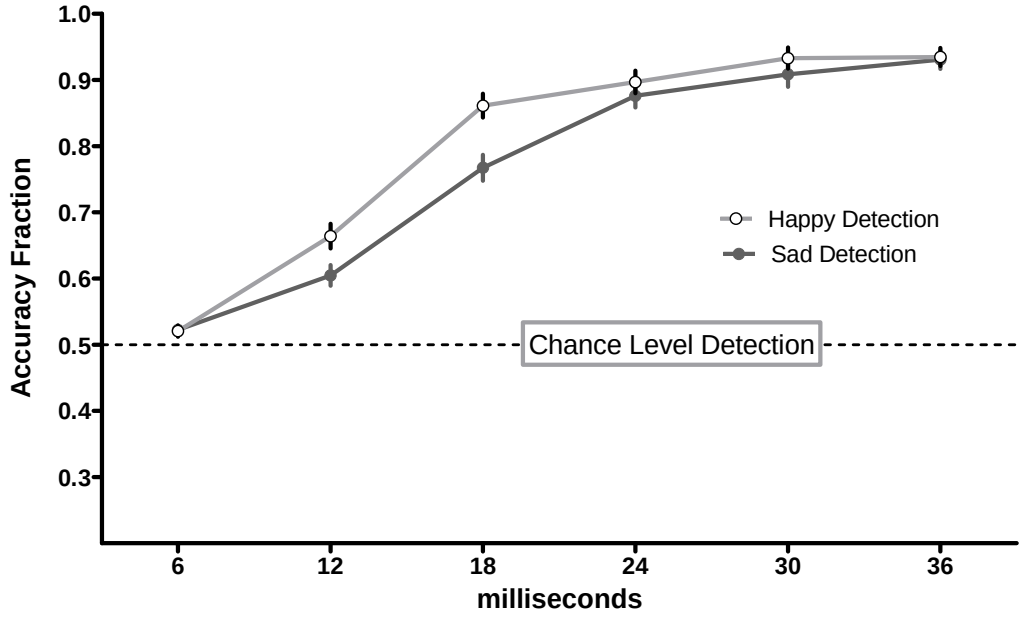

Figure 20 Detection accuracy curve for happy and sad facial expressions.

Accuracy and sensitivity did not significantly improve from session 1 to session 2 for the detection of sad faces or happy faces. This indicates that learning to detect happy or sad facial expressions did not occur (See Figure 26), and suggests that these results may be reflecting the limitations of early perceptual processing.

Cortisol administration did not affect the accuracy or sensitivity with which subjects could detect a happy [accuracy: $F(1,31) = 1.693$, $p=0.203$; d': $F(1,31) = 0.279$, $p=0.601$] (See Figure 21 and Figure 22) or sad faces [accuracy: $F(1,31) = 1.615$, $p=0.213$; d': $F(1,31) = 0.383$, $p=0.541$] (See Figure 23 and Figure 24).

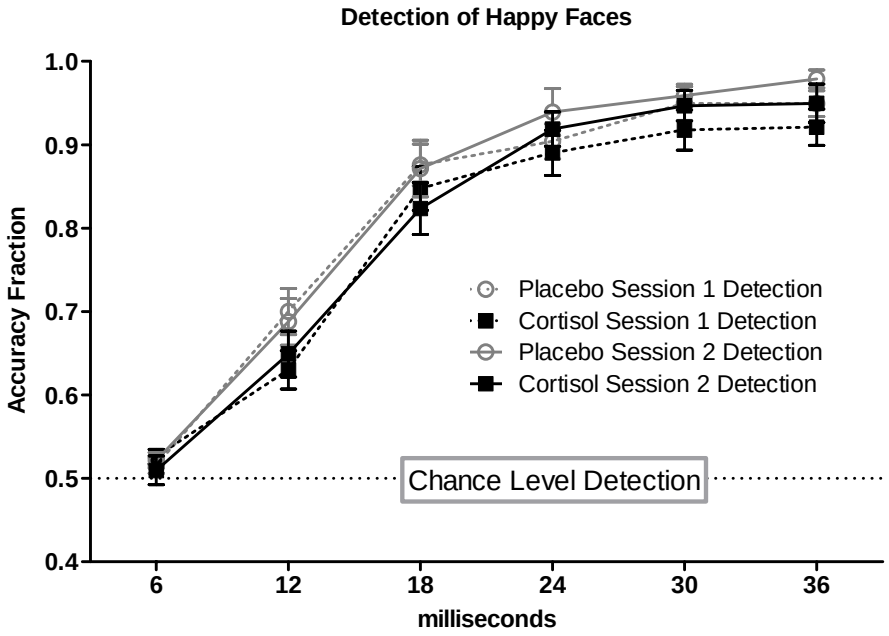

Figure 21 Accuracy of detecting happy facial expression across sessions for the placebo and cortisol groups.

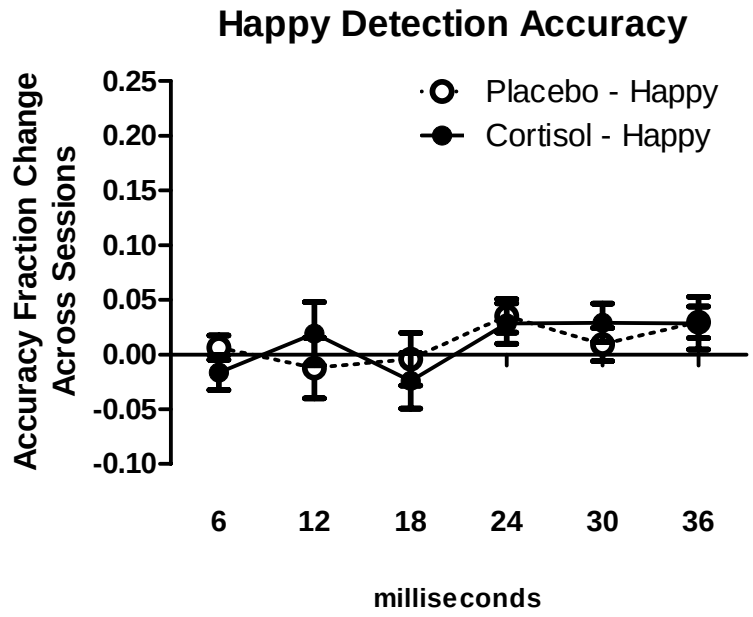

Figure 22 Change in accuracy of detecting happy facial expression across sessions for the placebo and cortisol groups.

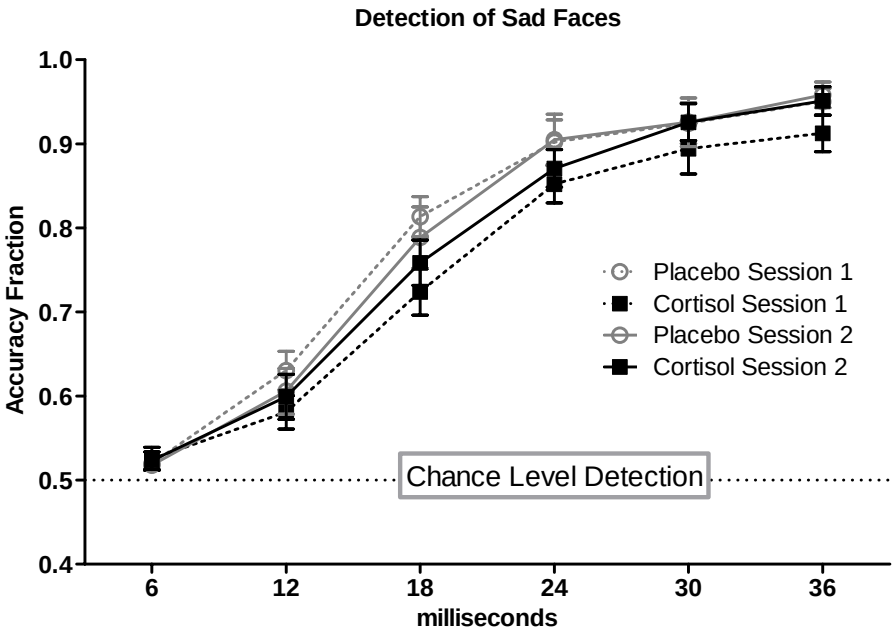

Figure 23 Accuracy of detecting sad facial expression across sessions for the placebo and cortisol groups.

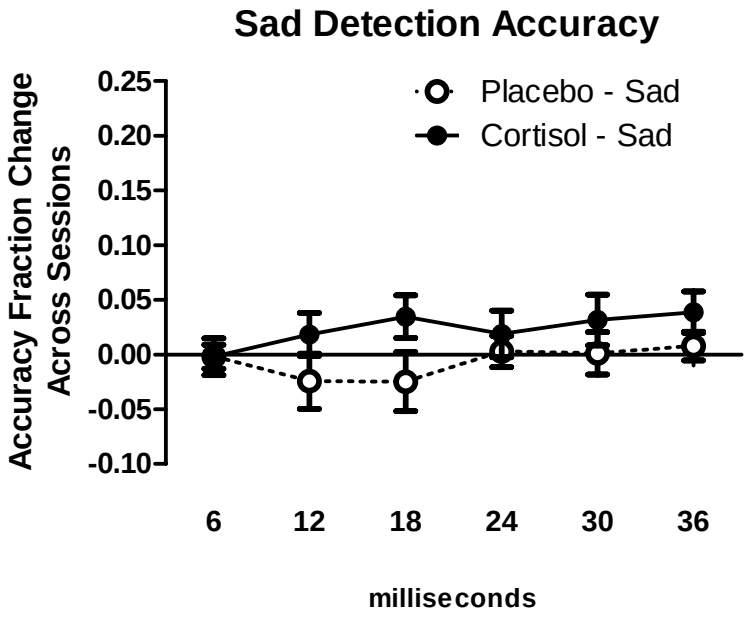

Figure 24 Change in accuracy of detecting sad facial expression across sessions for the placebo and cortisol groups.

Type of Emotional Facial Expression Affects Identification Accuracy and Sensitivity But Cortisol Administration Does Not.

Stimulus durations from 12-90 ms spanned the great majority of the range of response accuracy (See Figure 25) from chance level performance to greater than 80% accuracy. However, happy faces were identified at greater than chance levels at the shortest stimulus duration of 12 ms, indicating that faster presentation times are needed to capture the entire range of responses for happy faces (See Figure 25).

As with the detection task, emotion type affected identification accuracy and sensitivity. Happy faces were identified with greater accuracy and sensitivity than sad [accuracy:

F(1,31) =127.575, p<0.001 ; d': F(1,31) = 141.271, p<0.001] or neutral faces [accuracy: F(1,31) = 113.148, p<0.001 ; d': F(1,31) = 161.295, p<0.001]. Sad faces were identified with similar accuracy and sensitivity as neutral faces [accuracy: F(1,31) = .102, p = 0.751; d': F(1,31) = 0.215, p= 0.646] (See Figure 25)

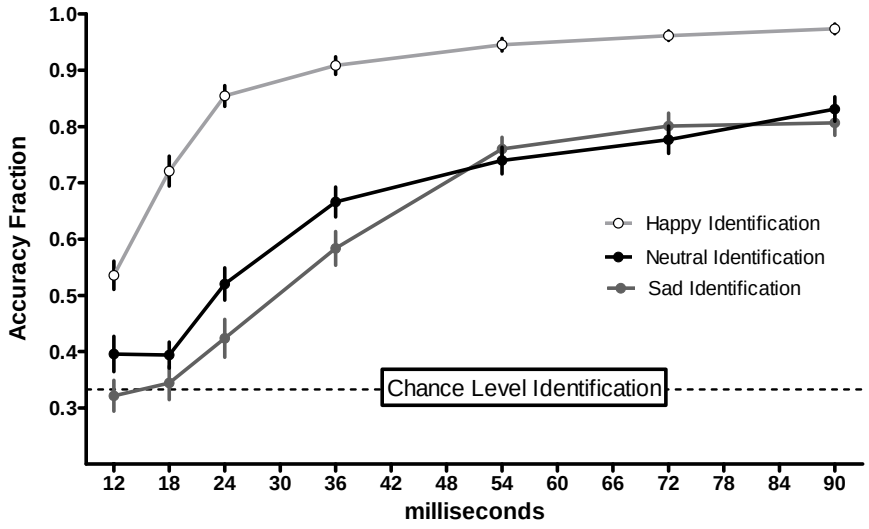

Figure 25 Identification accuracy curve for happy, neutral and sad facial expressions.

Identification accuracy and sensitivity improved across sessions for neutral [accuracy: F(1,31) =10.174, p = 0.003; d': F(1,31) = 16.526, p< 0.001], and sad faces [accuracy: F(1,31) =22.972, p < 0.001; d': F(1,31) = 24.078, p< 0.001], but not for happy faces [accuracy: F(1,31) =1.856, p < 0.183; d': F(1,31) = 1.887, p< 0.179]. (See Figure 26)

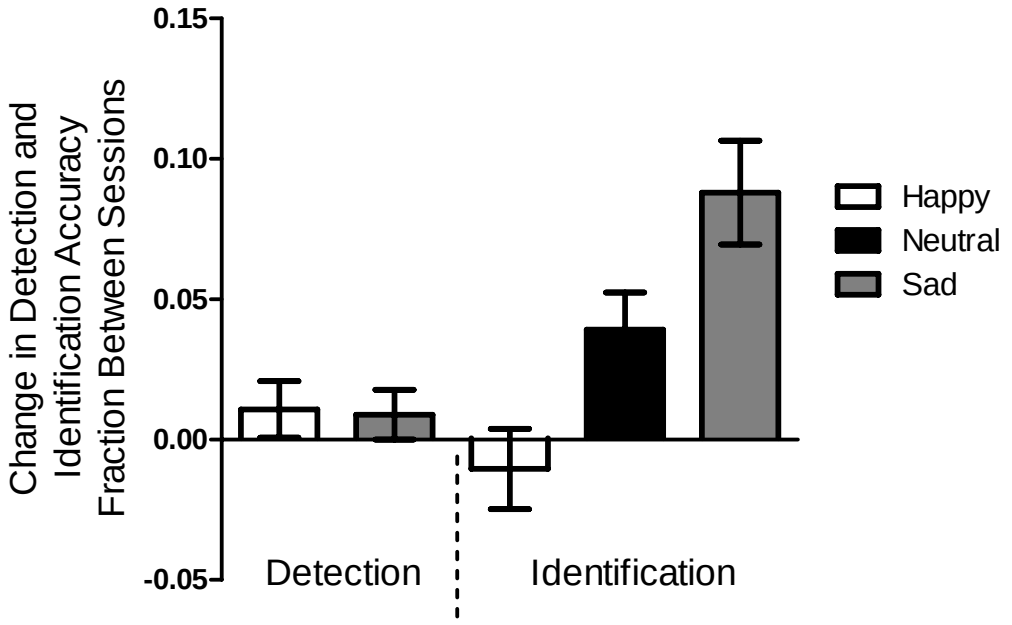

Figure 26 Change in detection and identification accuracy across sessions for each type of emotional facial expression.

Cortisol administration did not change the accuracy or the sensitivity with which subjects could identify happy [accuracy: $F(1,31) = 0.003$, $p = 0.960$; d': $F(1,31) = 0.020$, $p = 0.889$] (See Figure 28, and Figure 27), neutral[accuracy: $F(1,31) = 0.741$, $p = 0.396$; d': $F(1,31) = 1.216$, $p = 0.279$] (See Figure 30 and Figure 29), or sad [accuracy: $F(1,31) = 0.259$, $p = 0.615$; d': $F(1,31) = 0.222$, $p = 0.641$] (See Figure 32 and Figure 31) emotions expressed by a human face. Cortisol administration also did not appreciably affect the rates at which subjects misidentified happy, neutral or sad faces (See Table 5).

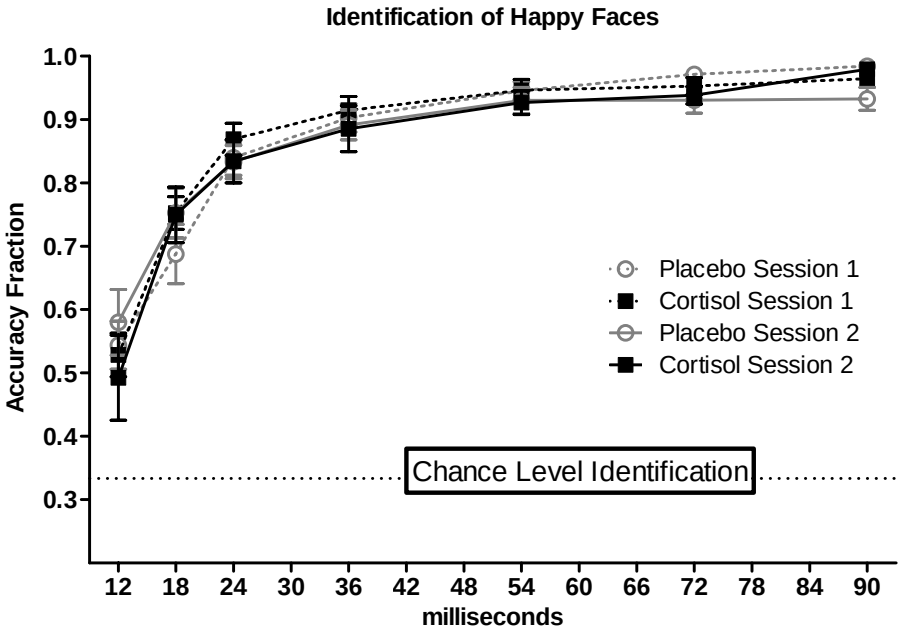

Figure 27 Accuracy of identifying happy facial expressions across sessions for the placebo and cortisol groups.

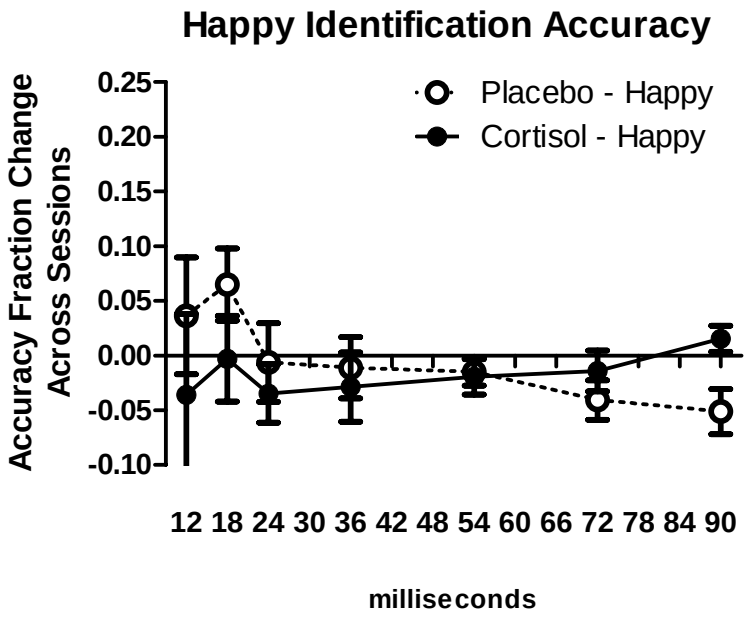

Figure 28 Change in accuracy of identifying happy facial expressions across sessions for the placebo and cortisol groups.

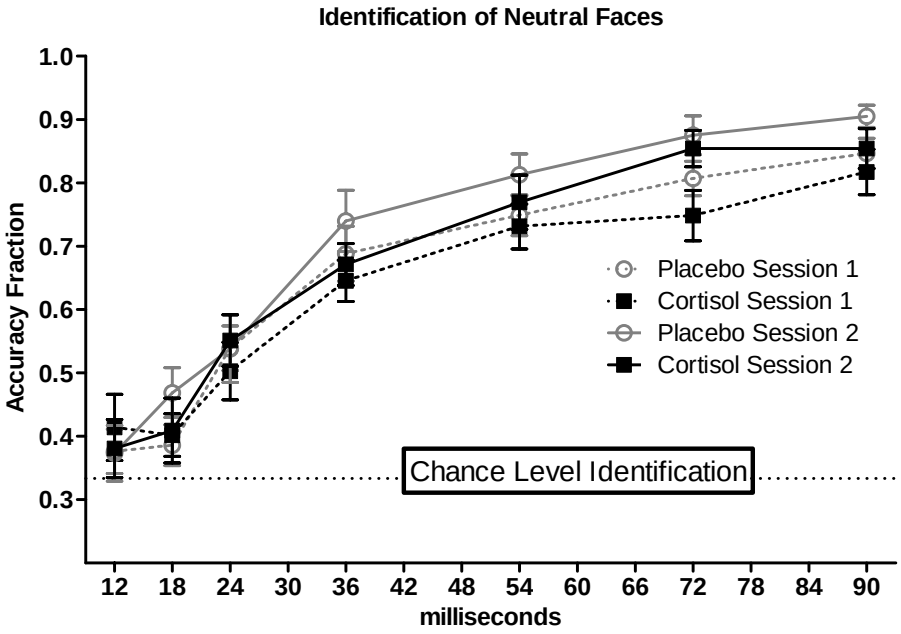

Figure 29 Accuracy of identifying neutral facial expressions across sessions for the placebo and cortisol groups.

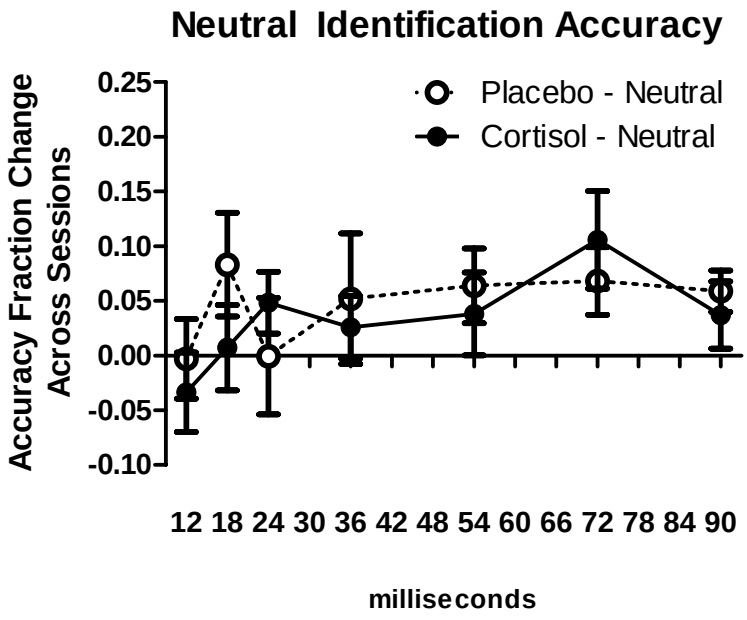

Figure 30 Change in accuracy of identifying neutral facial expressions across sessions for the placebo and cortisol groups.

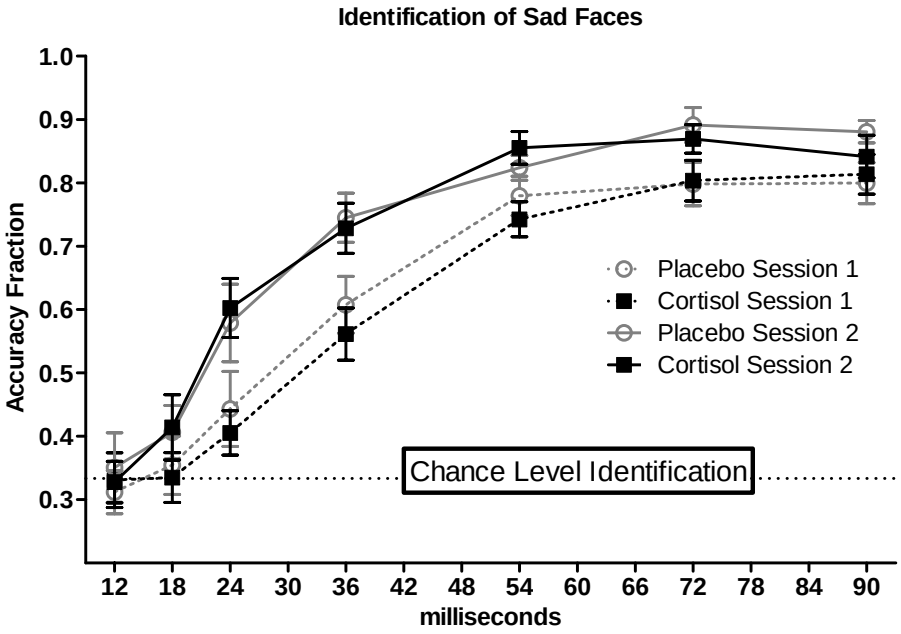

Figure 31 Accuracy of identifying sad facial expressions across sessions for the placebo and cortisol groups.

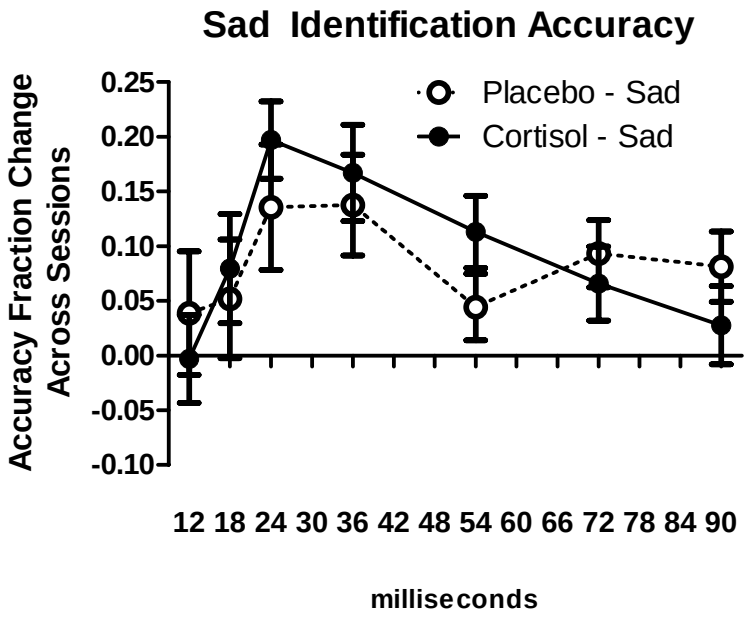

Figure 32 Change in accuracy of identifying sad facial expressions across sessions for the placebo and cortisol groups.

Table 5 The confusion matrix of emotional facial expression. Columns indicate the emotion presented. Rows indicate the response. Cells indicate the rate of correct identification (diagonals) or misidentification (off diagonals).

Placebo Session 1	Happy	Neutral	Sad	**Placebo Session 2**	Happy	Neutral	Sad
Happy	83.50%	12.30%	19.92%	Happy	83.57%	12.04%	16.52%
Neutral	8.08%	62.76%	21.18%	Neutral	5.48%	67.31%	16.78%
Sad	8.44%	24.94%	58.90%	Sad	10.97%	20.68%	66.80%
Cortisol Session 1	Happy	Neutral	Sad	**Cortisol Session 2**	Happy	Neutral	Sad
Happy	84.66%	12.47%	20.14%	Happy	82.93%	11.39%	15.94%
Neutral	7.80%	60.95%	22.94%	Neutral	7.70%	64.40%	17.61%
Sad	7.52%	26.56%	57.00%	Sad	9.39%	24.23%	66.43%

Discussion

The main findings of this experiment is that cortisol does not robustly affect the early detection or identification of emotional facial expressions even though evidence from Chapter 3 indicates that cortisol modulates activity in brain regions thought to compose a fast and coarse representation of emotional stimuli. Despite these negative results, we do demonstrate an interesting effect of emotion type on how facial expressions are detected and identified. Faces expressing happy emotions seem to be easier to detect and identify. We speculate that the display of teeth in the happy faces serve as both as a distinct detection cue and aides in discriminating happy faces from sad and neutral faces. These results indicate that subtle perceptual properties of the emotional facial expressions can affect how easily a face is detected or identified, whereas the cortisol administration regimen used here had little or no effect on the processes of detection or identification.

Effects of Cortisol on Detection and Identification

Evidence from Chapter 3 suggests that cortisol affects arousal on reactive measures of

emotion, such as ratings of feelings elicited by emotional stimuli. Chapter 3 also demonstrates that emotion related and visual perception related brain regions are both modulated by cortisol. This raises the intriguing possibility that cortisol may affect visual perception in such a way that it biases the salience of emotional stimuli to produce an altered subjective experience. However, in the current study we found no evidence of cortisol modulation of the detection or identification of facial expressions. Therefore, it remains unclear what effect, if any, cortisol modulation of the superior colliculus and thalamus has on visual perception of emotional stimuli.

Happy Faces Detected and Identified Better Than Sad or Neutral Faces.

There was a robust difference in the time course of detection and identification of different emotions. Happy facial expressions were detected and identified with greater accuracy and sensitivity than other emotional expressions even when displayed for very brief durations.

Other research investigators using the "visual search task", which requires subjects to detect an emotional face amongst distracter faces, have noted that conspicuous perceptual properties and components of facial expressions that convey emotion can confer a performance advantage (for review see (Frischen et al., 2008)). Several previous studies provide evidence that regions around the eyes are important in the detection and identification of facial expression and that this information may be transmitted as part of a coarse representation of a face through the colliculus-thalmo-amygdala route (Whalen et al., 2004). Similarly, we speculate that a large block of white from teeth could

provide an early cue as to the presence of a face and even transmit information regarding the type of emotional expression. The display of teeth, which occurred almost exclusively in happy facial expression in the current experiment, may be driving the relatively high rate of early detection of happy faces even at the 6 and 12 ms time points. Considering the potential early evolutionary advantage of fast detection of predators and threatening conspecifics with forward facing eyes and tooth displays, it is possible that such an adapted threat detection system may have aided in the early detection of the happy facial expressions with prominent tooth displays.

Learning across sessions

The detection of happy and sad faces did not improve across sessions, indicating that previous experience with the task did not improve performance on face detection (See Figure 26). This suggests that the process of detecting facial expression, as tested in this experiment, is not subject to learning. However, the identification of sad and neutral, but not happy, faces did show improvement across sessions. One possible explanation for these results lies in the differences in the amount of information that must be processed in order to accomplish detection and identification.

The detection of a facial expression requires only that a minimum of one face-like cue be perceived, whereas for identification requires considerably more information or higher quality information. In the identification task the perception of a single cue that discriminates one emotion from the rest of the options is required, or alternatively, perception of multiple face-like cues could provide complementary information to permit

discrimination.

Facial expression detection may not have improved across sessions because the time that it takes to recognize a single face-like cue may be subject to the physical limitations of the visual system. However, face identification may have improved as subjects learned to adopt strategies that focus attention on aspects of facial expressions that are more likely provide discriminatory cues. Anecdotally, several subjects explicitly indicated that they adopted strategies focusing on the eyes and mouth.

It is notable that the identification of happy facial expression may not have improved across sessions due to a ceiling effect arising from the ease with which these facial expressions are identified, relative to other emotions. Figure 25 and Figure 28, respectively, demonstrate that approximately 90% of happy faces are identified accurately within 36ms and that little or no improvements across sessions are observed across sessions while performance is at such a high level.

It is also notable that for happy facial expressions, the identification curve lags only slightly behind the detection curve. This suggests that cues that allow the detection of happy faces may be also serving to distinguish between happy expressions and other emotional expression. In contrast, cues that are used to detect sad and neutral faces may not contain adequate information to allow subjects to distinguish which emotion is displayed.

Limitations

These finding suggest that cortisol administration does not produce robust changes in our measures of basic perceptual processing of emotional stimuli. Thus, the principle effects of cortisol on emotion may be occurring during higher order processes subsequent to an unadulterated perceptual process. However, several issues may complicate the straightforward adoption of this conclusion, and will require additional research to clarify.

We demonstrate that the detection and identification of emotional facial expressions is not influenced by cortisol. However, other perceptual processes that were not addressed in this series of experiments could be affected. Studies of the influence of cortisol in gaze dwell times, attention biases, and additional measures of perceptual salience may help to clarify these issues. In addition, only one dose concentration/regimen was used in this study. Cortisol may be exerting effects on perceptual processes that require circulating cortisol levels to be elevated at the time of perception. While the 5 day exposure to elevated cortisol employed in this study would likely activate gene transcription mediated effects, effects of cortisol mediated by membrane bound mechanisms may not have been observable. Considering that the single dose group in Chapter 3 produced the most robust effects on the thalamus superior colliculus, exploration membrane mediated effects of cortisol would be particularly compelling

These experiments also characterize psychophysical curves for the detection and identification tasks and differences amongst these curves between emotion types. However, two critical issues must be considered when interpreting these results. First,

backwards masking procedures are likely sensitive to several experimental parameters, including size, position, and orientation of the stimuli, stimuli duration, stimuli luminance, mask type, mask duration, time between the stimuli and the mask. The psychophysical curves presented here are thus reliable only when using the presentation parameters employed in this study. However, the curves demonstrated provide a useful reference point for future studies and allowed a valid comparison between cortisol and placebo groups in the current study. Second, increased speed of detection and identification of happy facial expressions compared to sad and neutral expressions could indicate that the visual system is sensitive to areas of stimuli containing high contrast, sensitive to indicators of threatening components of stimuli (teeth, and forward facing eyes), or sensitive to certain types of emotional content (positive emotion bias). However, the presence of teeth exclusively in happy facial expressions in these studies confounds our ability to determine the underlying origin of this effect. Previous studies attempting to disentangle similar confounds arising from perceptual properties of emotional faces have employed the use of line drawings to remove such cues or used inverted facial expressions in order to change the way in which stimuli are visually scanned. These experiments have thus far achieved mixed results, suggesting that both emotion type and perceptual properties contribute to the process of detecting emotional faces (See (Frischen et al., 2008)).

In conclusion, these results provide initial evidence that cortisol might not robustly affect the perceptual processes of detecting or identifying happy sad and neutral facial expressions. They also demonstrate that the time course of detection and identification might be influenced by the perceptual properties and/or the emotional content of the

stimuli. However, several unanswered questions remain about the role of cortisol in the perception. What effects, if any, does the cortisol induced changes in superior colliculus and thalamus observed in Chapter 3 have on the perception of emotional stimuli? Would a study sensitive to other aspects of perception besides detection and identification of emotional stimuli reveal the effects of cortisol? Would the use of different dose regimens or concentrations reveal these effects? Additional research studies targeting these questions are required if the behavioral consequences of cortisol inhibition of the superior colliculus and thalamus are to be elucidated.

Chapter 5: Conclusion

In this dissertation we present a series of experiments designed to begin elucidating the potential effects of cortisol on emotional processes relevant to depression. The first set of experiments, outlined in Chapter 2, were developed to verify that cortisol administration does not affect the shape or magnitude of brain hemodynamic responses, thus providing a methodological basis for the use of exogenous hydrocortisone administration in functional MRI experiments, our key experimental approach in Chapter 3. These findings permit valid inferences to be made about how cortisol administration influences emotion related brain activity in Chapter 3. In addition, it allows these brain activity findings to be paired, in meaningful ways, with additional findings from Chapter 3 concerning the effect of cortisol on subjective emotional states and subjective reactions to emotional stimuli. Surprisingly, the findings outlined in Chapter 3 suggested that some brain regions typically associated with visual perception may be susceptible to the modulatory influences of cortisol. Chapter 4 then explicitly investigates the effects of cortisol administration on early perceptions of emotional stimuli in an attempt to discover the functional impact of cortisol on these brain regions. Taken together, findings from Chapters 3 and 4 suggest that cortisol administration affects some specific aspects of the subjective experience of emotion while not affecting the visual perception of an emotional stimulus. These findings also reveal an effect of cortisol that may potentially explain how, in depressed patients, endogenous hypercortisolemia and aberrant subgenual

cingulate activity interact with each other and with the subjective experience of emotion.

In Chapter 3 we demonstrate that cortisol is capable of affecting the subjective emotional reactions that are experienced in response to viewing emotional pictures. Importantly, this effect was specific to arousal ratings of sad stimuli. The effects of cortisol on increasing circulating glucose and altering glucose uptake into some tissues could explain a general increase in feelings of arousal, but the arousal effects detected here were emotion specific. This demonstrates that the effect of cortisol on arousal is not a general effect but a more nuanced effect, increasing arousal to sad stimuli more than other stimuli.

Chapter 3 also provides evidence for direct links between brain activity pattern changes that have been shown to occur in depression and the effects of cortisol administration. We demonstrate that cortisol administration effects subgenual cingulate activity (See Figure 8, Figure 9, and Figure 10). The effect of cortisol on subgenual cingulate brain activity occurs only during sadness. This is a critical finding in that it demonstrates that two of the major physiological markers of depression may be causally related. We suggest that endogenous cortisol in depressed patients with hypercortisolemia may be responsible for the changes in subgenual cingulate activity observed in depression.

In addition to the effects of cortisol on emotion related brain regions, outlined in Chapter 3, several regions associated with visual perception were also noted to have been modulated by cortisol administration. These regions include the thalamus, superior colliculus and visual cortex, each of which is a node in the sensory processing of visual

stimuli. These findings raise the possibility that, in addition to cortisol's effect on the experience of emotion, it may also be exerting influence over how emotional stimuli are perceived on the most rudimentary level..

In order to further investigate the findings from Chapter 3, which indicate that cortisol affects brain activity in visual processing brain regions, Chapter 4 aimed to elucidate how cortisol might be affecting behavioral measures of the perception of emotional stimuli. Classical studies of "blind sight" have implicated the visual cortex in the subjective awareness of visual stimuli. Since we demonstrated cortisol's ability to influence activity in regions earlier in the sequence of visual processing, we reasoned that the behavioral effect could occur prior to explicit awareness. However, no evidence of cortisol induced changes in the early detection or identification (pre or post explicit awareness) of emotional stimuli were found in Chapter 4. This seems to indicate that cortisol does not impact the measures of how emotional stimuli are visually perceived used in our experiments. Several possibilities could explain these results. First, cortisol may be selectively impacting how emotions are experienced but not how they are perceived. It is possible that cortisol effects on superior colliculus and thalamus are impacting neural signals involved in the experience of emotion, and that the neural signals relayed through these regions that are coding for perceptual information is unmodified by cortisol. Another possibility is that cortisol is actually affecting aspects of the perception of emotional stimuli, and we were just unable to observe it because our experiments did not measure the correct aspects of perception.

Limitations:

Several experimental limitations need to be considered when evaluating the results and inferences from individual chapters and how these chapters integrate with each other.

One example of this is that the amount of cortisol that is administered may affect the strength and stability of the findings in each of the chapters. In Chapter 2 and Chapter 3 two dose regimens were used. These doses were a single dose of 100mg and extended dose of 25mg/day over 5 days. Experiments in Chapter 4, however, only incorporated the extended dose regimen. These doses may be sufficient to observe some of the effects of exogenous cortisol but not others. A single dose regimen could potentially reveal perceptual effects of cortisol. Additional research studies will be needed to determine how the effects of cortisol outlined in this body of work react to variations in dose and length of exposure.

A related issue involves the method of cortisol administration. In each of the experiments outlined in chapters 2-4 cortisol is administered in single or multiple oral doses. This method of administration results in a rapid peak in circulating cortisol levels (See Figure 4). Endogenous cortisol, however, is released in a more pulsatile fashion. Differences in the time course of exposure to elevated cortisol could potentially influence the results we outline chapters 2-4.

Another issue is that cortisol also has a wide range of physiological effects that may account for some of the findings discussed in chapters 2-4. One example of this is cortisol induced inhibition of CRH and ACTH. CRH, like cortisol, has several receptors with wide distributions in the brain. Without the ability to experimentally maintain

consistent levels of CRH in the brain, any effects ascribed here to exogenous cortisol might also be due to changes in central CRH. Nevertheless, endogenous cortisol also decreases CRH. Therefore, cortisol released in endogenous hypercortisolemia would also typically serve as an inhibitory signal to CRH. However, endogenous hypercortisolemia can arise from reduced sensitivity to cortisol at sites of inhibitory feedback, leading to the uncoupling of the cortisol inhibition of CRH. Thus, two limitations must be acknowledged. First, the normal physiological effects of cortisol described here could be secondary effects arising from cortisol effects on CRH. Second, extrapolations of this data to endogenous hypercortisolemic states could be complicated by potential abnormalities in the cortisol-CRH dynamic.

Competitive agonist effects from other endogenous hormones, such as progesterone, aldosterone, testosterone and estradiol may also affect the results of each of these studies. The human glucocorticoid receptor binds these hormones, although with weaker affinity and in a manner that is not likely to result in a transcriptionally active signal. Thus, endogenous levels of these hormones could be functioning as competitive agonists reducing the magnitude of the cortisol signal. However, with exogenous cortisol doses producing high physiological and supraphysiological concentrations of circulating cortisol, the effects of competitive agonist are unlikely to be a major influence on these experiments.

Additional and experiment specific limitations should also be considered when interpretations are made regarding the results of Chapter 2 and Chapter 4. In Chapter 2 we present results indicating that cortisol does not alter the HRF in the visual cortex.

However, HRF patterns show some variability across brain regions and it is therefore possible that cortisol has significant effects on the shape of the HRF of brain regions outside the visual cortex. In Chapter 4 no evidence was found of cortisol having a behavioral effect on how emotional stimuli are detected or identified. However, cortisol may still be influencing other components of visual processing of emotion that we were unable to observe by studying only early detection and identification processes.

Significance & Future Directions

The most notable findings from this series of studies occurs in Chapter 3, where we demonstrate that that cortisol administration affects the subjective experience of sadness, and also that cortisol inhibits activity in the subgenual cingulate cortex during sadness. This is the first demonstration that cortisol, a hormone hypersecreted in many depressed patients, can affect activity in the subgenual cingulate, the brain region most strongly linked to sadness and depression. Subgenual cingulate activity in depression and during sadness is typically increased. Indeed, we observe sadness induce increases in subgenual cingulate in the placebo group (See Figure 7 Row C and Figure 9). Curiously, cortisol administration was shown to inhibit this sadness induced increase in activity. In the discussion section of Chapter 3 we present a possible solution to this seemingly counterintuitive finding involving adaptive disinhibition.

In addition to the clinical implications of these findings they also provide new insights into the physiological effects of cortisol on emotion processes that may be occurring during periods of high stress. As a component of the stress response the short term

physiological effects of cortisol can be seen as largely adaptive because they inhibit processes that require ongoing energy investments to yield long term benefit, such as neurogenesis, reproduction, energy storage, and reproductive functions. However, over longer term exposure to cortisol these effects may be more damaging than beneficial (Sapolsky, 1998).

Similarly, the physiological effects of cortisol on emotion processes may provide a useful short term adaptive advantage by increasing arousal associated with negative emotional stimuli and impacting brain activity that produces these subjective effects. However, over the long term these same processes could lead to increased anxiety and depression. Within certain environments some of the behavioral components of depression may be adaptive. In a highly dangerous area for example hypervigilance, emotional biases toward negative emotional stimuli, an easily evoked fear response, decreased movement, decreased consumptive motivation, and decreased interest in social interaction may confer a selective advantage. However, in modern societies humans rarely face the kinds of extreme environmental and predator pressures that may have shaped the physiological systems that produce these effects (with the exception of warfare). The net result of which is vulnerability to decreases in emotional and physical well being, without an obvious advantage of increased survivability.

The ability of cortisol to modulate brain regions implicated in depression underscores its potential as a contributor to depressive symptoms and suggests further investigation within healthy populations and in depressed patients is needed. Future studies may consider parametrically varying the dose and/or the length of time subjects are exposed to

cortisol administration. These studies could uncover a temporal process of the development of depression induced by hypercortisolemia. Furthermore, these studies could bring to light neural mechanisms of the production of symptoms as depression progresses. Along similar lines, studies of neural activity changes brought on by exogenous cortisol in depressed patients at various stages of the development and recovery from depression could prove useful in uncovering how cortisol's neural effects may contribute to the development of depression. Additional studies may also prove useful in addressing the limitations of the studies presented here. Studies incorporating the use of pharmacological agents that robustly activate or inhibit central CRH secretion could be important if used with cortisol administration or cortisol receptor antagonism. By pharmacologically separating cortisol and CRH secretion we could begin to determine if the effects of cortisol on the brain emotion processes are mediated through CRH inhibition or if cortisol has direct effects on neural tissues.

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