Psychological Stress and Vulnerability for Major Depressive Disorder:

Cortisol, brain structure, function, and cognitive processing in young adults

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Table 1: Characteristics of the study population. Continuous variables are displayed as mean values ± SD. Apart from BDI levels, which were obtained at the time of the recruitment, all other psychological assessment were conducted at the time of the MRI scanning. BDI: Beck Depression Inventory; MADRS-S: Montgomery-Asberg Depression Rating Scale Self-Assessment HDI: Hamilton Depression Inventory; STAI-trait: Spielberger Trait Anxiety Inventory; TICS: Trier Inventory for the assessment of Chronic Stress; §= comparison with the control group; #=comparison with the subclinical group; *=p<.05; **= p<.01; ***=p<.001

List of Abbreviations

ACTH	adrenocorticotropic hormone
AG	amygdala
CRH	corticotropin-releasing hormone
fMRI	functional magnetic resonance imaging
GR	glucocorticoid receptors
HC	hippocampus
HPA	hypothalamus–pituitary–adrenal
MIST	Montreal Imaging Stress Task
MR	mineralocorticoid receptors
PET	positron emission tomography
PFC	prefrontal cortex
PVN	paraventricular nucleus
TSST	Trier Social Stress Test

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Contributions of Authors

The following thesis includes four articles (two which are published, one which has been accepted, and one which is in preparation). As the first author in all four, I conducted the literature review, developed study and task designs, recruited subjects, completed endocrine, behavioral and anatomical and functional MRI data analyses, wrote and edited manuscripts (***my contribution to each manuscript is outlined in italics below***). Overall, the contribution of co-authors included consultation on study designs, assistance with tasks development and testing, as well as consultation on the style and content of the manuscripts, and supervision of the projects (for specific contributions for each manuscript, please see bullet points below).

Manuscript 1:

Dedovic K, D'Aguiar C, Pruessner JC (2009) What stress does to your brain: a review of neuroimaging studies. <u>Can J Psychiatry</u>. Jan;54(1):6-15

Literature review, manuscript writing, editing: 80%

Contribution of co-authors:

- Catherine D'Aguiar: initial literature search and proof reading

- Jens Pruessner: supervision and editing

Manuscript 2:

Dedovic K, Rexroth M, Wolff E, Duchesne A, Scherling C, Beaudry T, Lue SD, Lord C, Engert V & Pruessner JC. (2009) Neural correlates of processing stressful information: An event-related fMRI study" <u>Brain Res.</u> 2009 Oct 13;1293:49-60. Epub 2009 Jun 23

Recruitment and testing of subjects, principle investigator for the event-related Montreal Imaging Stress Task (event-MIST), task development, behavioral, endocrine and fMRI data analyses, manuscript writing, editing: 80%

Contribution of co-authors:

- Miriam Rexroth, Elisabeth Wolff: assistance with study preparation, research assistant for the event-MIST, data entry

- Annie Duchesne, Carole Scherling: research assistant for the event-MIST, study design consultant

- Thomas Beaudry: programming of Design Matrix Generator for processing of event-MIST log files

- Sonja Damika Lue: re-analysis of fMRI data for the key contrasts for independent data quality verification

- Catherine Lord, Veronika Engert: research assistant for the event-MIST, study design consultant

- Jens C. Pruessner: project supervision, study design consultant, event-MIST task development, manuscript writing and editing

Manuscript 3:

Katarina Dedovic, Veronika Engert, Annie Duchesne, Sonja Damika Lue, Julie Andrews, Simona Efanov, Thomas Beaudry, Jens C. Pruessner (accepted in Biological Psychiatry) Cortisol awakening response and hippocampal volume: Vulnerability for Major Depressive?

Study design, recruitment and testing of subjects, behavioral, endocrine and structural MRI data analyses, manuscript writing, editing: 90%

For contribution of co-authors please see below.

Manuscript 4:

Katarina Dedovic, Annie Duchesne, Veronika Engert, Sonja Damika Lue, Julie Andrews, Simona Efanov, Thomas Beaudry, Jens C. Pruessner (in preparation) An fMRI study investigating psychological stress processing and vulnerability for depression in healthy young adults.

Study design, modified Montreal Imaging Stress Task (MIST) development, recruitment and testing of subjects, principle investigator for the MIST, behavioral and functional magnetic resonance imaging data analyses, manuscript writing, editing: 90%

Please note that the contributions of co-authors are equivalent in these last two manuscripts and are as follows:

- Veronika Engert, Annie Duchesne: MIST research assistant, consultant on manuscript content and style

- Sonja Damika Lue, Julie Andrews, Simona I. Efanov: MIST research assistant

- Thomas Beaudry: programming MIST task design modifications, programming Design Matrix Generator for processing of attentional bias log files and MIST log files

- Jens C. Pruessner: project supervision, study design consultant, manuscript editing

Abstract

Psychological stress has an important impact on one's physical and mental health. Activation of the hypothalamic-pituitary-adrenal (HPA) stress axis and the subsequent increase in the stress hormone cortisol constitutes the organism's main response to stress. Individual differences in stress response contribute to one's vulnerability and resilience to a host of physical and psychological ills. Understanding the regulatory networks underlying stress processing in both healthy and vulnerable populations is essential. The work presented in this thesis aimed to investigate neural correlates of psychological stress processing and the HPA axis function in samples of healthy individuals as well as those with distinct vulnerability to a stress-related illness, Major Depressive Disorder. Our literature review revealed that only studies using serial subtraction or the Montreal Imaging Stress Task (MIST), a task that combines mental arithmetic and negative social evaluation components, were able to induce a significant cortisol stress response. Deactivation in orbitofrontal regions and the limbic system were most consistently observed in response to psychological stress. Exposing healthy subjects to a new, event-related version of MIST revealed that reduction of brain activity in the limbic system observed previously was specifically associated with the processing of social evaluative threat, a key component of psychological stress. We then examined HPA axis function (both basal and reactive) and the HPA regulatory brain areas for evidence of dysregulation in a sample of healthy young adults who showed varying levels of depressive tendencies, but at subclinical levels. This was the first time that these concepts were assessed in a

subclinically depressed population. The subjects with increased subclinical levels of depression showed impairments in HPA function (in a form of blunted cortisol awakening response and blunted stress response), as well as impairment in certain key regions within the HPA axis regulatory network (for e.g. small hippocampal volumes and dysregulated medial orbitofrontal cortex). I conclude the thesis by proposing a basic model of a neural network underlying stress processing in a healthy population, and also outline nodes at which this network might be affected in subclinically depressed populations. Some research avenues for future studies are also highlighted.

Résumé

L'expérience de stress psychologique peut compromettre la santé mentale et physiologique d'un individu. L'activation de l'axe hypothalamo-surrénalien, caractérisée par la libération subséquente de cortisol, constitue la principale réponse physiologique de stress. La susceptibilité ou la résilience pour un ensemble de maladies d'ordre physiques ou psychologiques est influencée par la variabilité interindividuelle dans la réponse de stress. Il est donc essentiel de comprendre le fonctionnement des systèmes régulateurs de la réponse de stress comparativement chez des sujets sains et vulnérables. Le travail présenté dans cette thèse investigue les processus neuronaux et endocrinologiques du stress psychologique chez des sujets sains exprimant divers degré de susceptibilité à la dépression majeure, une psychopathologie reliée au stress. Notre revue de la littérature suggère que l'exposition à une épreuve de soustraction en série de même que l'exposition au *Montreal Imaging Stress Task* (MIST), une épreuve de calcul mental dans lequel le sujet est évalué négativement, peuvent induire une augmentation significative de cortisol. Au niveau neuronal, la réponse de stress psychologique se manifeste par une réduction de l'activité du cortex orbitofrontal et des régions du système limbique. L'exposition de sujets sains à une nouvelle version du MIST, employant un paradigme événementiel, a démontré que la réduction de l'activité du système limbique était spécifiquement associée aux éléments de menace psychosocial, une composante clé dans l'induction de la réponse de stress. Nous avons ensuite étudié l'activité de l'axe hypothalamo-surrénalien, (basale et réactive) en relation avec les régions cérébrales régulatrices

afin d'observer certaines irrégularités chez de jeunes adultes sains qui présente divers degré de susceptibilité au développement de trouble dépressifs tout en demeurant sous le seuil clinique. Les sujets présentant un profil dépressif sous clinique élevée on démontrer un dysfonctionnement de l'axe hypothalamo-surrénalien, (une suppression des niveaux de cortisol à l'éveil et en réponse de stress) ainsi que l'altération de régions cérébrales régulatrices de la réponse de stress (volume hippocampique réduit, dysfonctionnement de l'activité du cortex orbitofrontale médial). Je conclue cette thèse en proposant un modèle d'interaction cérébrale impliqué dans la réponse de stress chez des sujets sains en soulignant les possibles sites de dysfonctionnement chez les sujets présentant un seuil dépressif sous clinique élevée. Finalement, quelques projets futurs seront présentés.

Chapter 1: INTRODUCTION

Introduction

As everyone could attest, stress is a fact of daily life. Stress has an important impact on an individual's physical and mental health (McEwen 2000; Chrousos 2009). The main stress axis in both animals and humans is the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1) (Brown 2000). In response to perceived threat, an HPA cascade of hormone release is initiated leading to the eventual increased release of glucocorticoids (corticosterone in animals and cortisol in humans), the main stress hormone. With respect to psychological stress, key situational components (such as elements of novelty, unpredictability and/or presence of social evaluative threat) have been shown to lead to a strong increase in cortisol secretion (Mason 1968; Dickerson and Kemeny 2004). Yet, individual differences in the stress response to psychological stress are often observed (for example (Kirschbaum, Prussner et al. 1995; Pruessner, Gaab et al. 1997; Kirschbaum, Kudielka et al. 1999; Kudielka, Hellhammer et al. 2009). The individual's appraisal of the demands of a given situation and his or her resources to cope seem to contribute to these differences (Lazarous 1993).
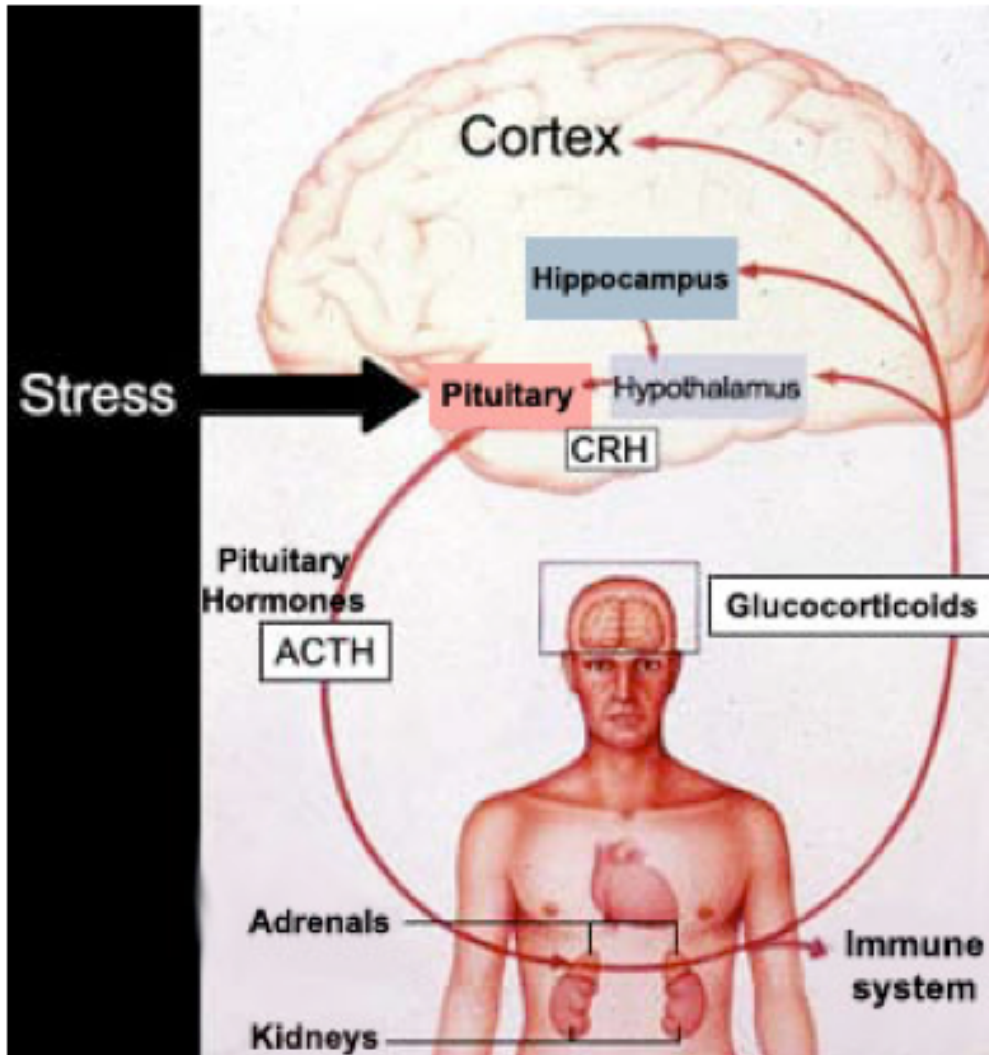
Figure 1: The Hypothalamic Pituitary Adrenal (HPA) axis. In response to perceived threat, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary to release adrenocorticotropin hormone (ACTH), which, in turn, leads to increased release of glucocorticoids (corticosterone in animals and cortisol in humans) from the adrenal glands. Illustration taken from http://www.chronicprostatitis.com/ forum/ viewtopic.php?f=4&t=5529.

It is important to note that the stress response is meant to be an adaptive response of an organism to a threat (McEwen 1998; Lupien, Maheu et al. 2007;

McEwen and Gianaros 2010). At the level of the central nervous system, the stress response facilitates arousal and attention, inhibits vegetative functions, and activates counter-regulatory feedback loops (Chrousos and Gold 1992; Chrousos 2009). At the level of the periphery, energy resources are rerouted to the brain, heart and skeletal muscles, there is an increase in metabolism, cardiovascular tone and immunosuppression (Chrousos and Gold 1992; Chrousos 2009). However, when this response is inappropriate (either inadequate, excessive and/or prolonged), a strain is placed on a range of central and peripheral systems thus increasing the likelihood of development of a host of physical and/or psychological illnesses (McEwen 2000; Chrousos 2009). Factors such as the individual's genetic constitution, early life experiences, present adverse or protective environmental circumstances, represent one's vulnerability or resilience factors and interact with HPA axis functioning, both at rest and in response to stress, to also over time determine the development and course of various pathologies (Chrousos 2009).

Thus, understanding regulatory networks underlying stress processing in both healthy and vulnerable populations is essential.

Animal studies as well as pharmacobehavioral human studies have provided a body of evidence outlining a central framework of HPA axis regulation (Herman, Prewitt et al. 1996; Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005; Kudielka, Hellhammer et al. 2009; Abelson, Khan et al. 2010). However, recent advances in neuroimaging techniques now allow for

noninvasive investigations of changes that are taking place in the central nervous system in response to an acute psychological stressor directly in humans.

Thesis Objectives

The work presented in this thesis investigates neural correlates of HPA axis regulation and psychological stress processing in samples of healthy individuals and those with distinct vulnerability to a stress-related disorder, Major Depressive Disorder, by using Magnetic Resonance Imaging (MRI) techniques.

The key objectives of the research presented within this thesis are the following:

(1) To examine neural networks underlying processing of psychological stress in order to further understand sources of individual differences observed in stress response in normal populations.

(2) To assess HPA axis function and neural regulatory network subserving psychological stress processing in a sample of healthy young adults who show varying levels of depressive tendencies, but at subclinical levels. Here, I specifically aimed to:

  a. Assess whether some of the abnormalities associated with HPA axis function and HPA regulation mechanisms seen in Major Depressive Disorder can already be shown in a vulnerable population prior to depression onset.

b. Evaluate possible differences in neural correlates of psychological stress processing in vulnerable populations in comparison to a control group.

What is Stress?

Until 1936, the term "stress" was employed primarily in engineering circles to mean the forces applied to exert a strain on any given object. In 1936, Dr. Hans Selye introduced "stress" within the realm of medicine to define a non-specific phenomenon representing a collection of symptoms produced by the body in response to various noxious stimuli, or "stressors" (Selye 1998). For Selye, stress was a physiological response to a wide-variety of stimuli.

Since then, other definitions of stress have been proposed (reviewed in Pacak and Palkovits 2001). At present day, stress is most often conceptualized as a threat, real or implied, to homeostasis (McEwen 2000; Lupien, Maheu et al. 2007; McEwen and Gianaros 2010). Homeostasis, a notion first introduced by Walter Cannon (Cannon 1932), represents a complex dynamic equilibrium of an organism both with respect to physical and emotional realms.

Therefore, in keeping with the original sense of the word "stress" and the present-day conceptualization of this phenomenon, in the present work, the words "stress" or "stressor" refer to a situation which poses a real or implied threat to an organism and leads to a set of physiological responses. The "stress response" is defined as the organism's response to a stressor. Specifically, I focus on the

increased release of cortisol as the main (biological) indicator of the stress response.

Importance of Psychological Stress

Selye posited that the determinants of stress response are non-specific (Selye 1998). However, he primarily investigated the effects of physical stressors (such as pain, cold, immobilization) on the HPA axis. Yet, it is psychological and experiential factors that are among the most powerful of stressors and are the most potent activators of the HPA axis (Mason 1968; McEwen 2000; Dickerson and Kemeny 2004). The studies from this field revealed that there are specific characteristics of a given situation that are most likely to elicit a strong HPA axis response.

Specific determinants of one's response to psychological stressors

In 1968, John Mason reviewed the responses of the HPA axis to a set of psychological stressors (for example aircraft flight, final examination, mental calculations) and established that there are key situational components that contribute to a given psychological situation being able to elicit a physiological reaction; these elements were novelty, unpredictability and lack of control over a situation (Mason 1968).

In 2004, a thorough meta-analysis by Dickerson and Kemeny (Dickerson and Kemeny 2004) reviewed 208 laboratory studies of acute psychological

stressors and revealed that tasks that combined a motivated performance task with elements of uncontrollability and especially, social evaluative threat components induced the largest HPA response, most consistently (Dickerson and Kemeny 2004). The elements of social evaluative threat included permanent recording of the performance, presence of evaluative audience during the task (main experimenter and at least one more individual), and presence of negative social comparison (either real or mocked) (Dickerson and Kemeny 2004).

Although there are specific components of a given situation that contribute to the activation of the HPA axis, there are great individual differences with respect to the HPA axis' response to such a threat (for example, Kirschbaum, Prussner et al. 1995; Schommer, Hellhammer et al. 2003; Kudielka, Buske-Kirschbaum et al. 2004; Kudielka, Hellhammer et al. 2009). Factors such as intensity and context of the threat, and presence of vulnerability and protective factors in the individual and social environment can account for some of the differences observed in the magnitude of the response (Dickerson and Kemeny 2004). Another key component is the appraisal of a given situation. A prominent stress theory postulates that when an individual perceives the demands of a particular event to exceed the available resources, the stress response ensues (Lazarous 1993).

Physiological stress response and subjective psychological distress

An underlying assumption with respect to the response to a psychological stressor is that the level of subjective psychological distress that an individual

perceives or feels in a given situation is reflected in the individual's physiological response (HPA axis activation and cortisol release) to that same situation. Overall evidence shows that subjective psychological distress and physiological stress response do reflect the same construct, however, the relationship between these two concepts is not a simple one (Hellhammer, Wust et al. 2009).

Indeed, several studies investigating the association between subjective measures of distress and cortisol levels in response to several challenges have reported inconsistent findings (Al'Absi, Bongard et al. 1997; Buchanan, al'Absi et al. 1999; Oswald, Mathena et al. 2004). For example, a study found that although negative mood increased following a public speaking task and a mental arithmetic task, the change in anxiety, anger and depression was positively correlated only with cortisol response to the public speaking task, but not the mental arithmetic task (Al'Absi, Bongard et al. 1997). In contrast, a study by Buchanan and colleagues did not find any associations between cortisol and negative affect in response to a public speaking task (Buchanan, al'Absi et al. 1999), while Cohen et al (2000) reported negative correlation between anxiety levels and cortisol response to a public speaking task (Cohen, Hamrick et al. 2000).

A recent study by Schlotz and colleagues revealed that there seems to be a time component to the association between subjective measures and cortisol measures that might explain the inconsistency in findings (Schlotz, Kumsta et al. 2008). Using cross-correlational analyses, the authors found that subjective psychological responses preceded same-direction changes in the HPA axis activity with a maximum lag between anxiety levels and cortisol to be around

17.5 minutes in response to a psychological task (Schlotz, Kumsta et al. 2008).

It is important to note that psychological stressors involve higher order brain areas such as the limbic system and the prefrontal cortex (Herman, Ostrander et al. 2005; Kudielka and Kirschbaum 2005). Therefore, another source of inconsistencies may be the intricate connections that exist between limbic system structures and prefrontal brain areas and are involved in monitoring, evaluating and regulating internal and external environmental and situational demands and regulating the HPA axis. These connections may complicate the association between subjective and physiological measures of stress response and could perhaps account for conservative associations between these two variables reported thus far.

In addition, the limited association may also be due to the assessment methods of perceived stress by self-report questionnaires (Hellhammer, Wust et al. 2009). For example, many of these investigations have focused on measures of anxiety and tension as reflective of experience of stress, while recently it has been suggested that the key feeling to stress response to a psychosocial stressor may rather be a feeling of embarrassment and shame in the presence of social evaluative threat (Dickerson and Kemeny 2004).

Therefore, although the association between psychological distress and physiological mediators of stress is a conservative one, these concepts are still thought to represent a similar construct. In the following work, I focus mainly on the physiological measures (i.e. levels of cortisol) as primary indicators of perceived threat and measure of individual's stress response to a psychological

challenge, particularly since it is the physiological response that still represents the key step of connecting experience with vulnerability or resilience to an illness (Lupien, Ouelle-Morin et al. 2006)

Hypothalamic-Pituitary-Adrenal axis: in times of stress and rest

HPA axis in times of stress

In response to a perceived threat, the hypothalamic-pituitary-adrenal (HPA) axis is activated. When the HPA axis is triggered, neurons from the paraventricular nucleus of the hypothalamus secrete corticotropin releasing hormones (CRH) in order to stimulate the pituitary gland (Carrasco and Van de Kar 2003). Specifically, parvicellular neurons of the hypothalamic PVN synthesize CRH and arginine vasopressin. These neurons project to the median eminence of the hypothalamus. From there, CRH enters hypopohyseal portal veins and stimulates corticotroph cells of the anterior pituitary to release the adrenal corticotropic hormone (ACTH) (Brown 2000). Vasopressin for its part stimulates ACTH very weakly although it does potentiate the effect of CRH (van Praag, de Kloet et al. 2004). The released ACTH travels through systemic circulation and binds to the receptors on the adrenal gland. In response to ACTH, the adrenal cortex secretes cortisol. The adrenal medulla, which is surrounded by the adrenal cortex, releases catecholamines (adrenaline and noreadrenaline). The secretion of catecholamines is under the control of the autonomic nervous system (Brown 2000). The secreted hormones then enter the blood circulation. Peak in

cortisol in saliva secretion generally appears between 10-30 minutes after the cessation of a psychological stressor (Foley and Kirschbaum 2010).

The majority of the circulating cortisol in the blood is actually bound to corticosteroid binding globulin (CBG), sex-hormone-binding globulin and albumin (Levine, Zagoory-Sharon et al. 2007). The unbound cortisol constitutes between 5% and 10% of all cortisol and is referred to as "free" cortisol (Westphal 1983). It is assumed that only the free cortisol is biologically active and exerts its effects on the target cells (Westphal 1983). It should be noted however that a recent review cautions against this simplification as it suggests that CBG-bound cortisol may also have an impact on target tissues (Levine, Zagoory-Sharon et al. 2007).

Cortisol levels can be assessed in many ways: through urine, blood or saliva sampling. Unlike other methods, saliva sampling offers stress-free, noninvasive method of assessment, and can be conducted in a multitude of settings. In addition, cortisol measured from saliva represents the free active cortisol portion (although some have reported that about 14% of salivary cortisol is bound (Chu and Ekins 1988; Levine, Zagoory-Sharon et al. 2007). Salivary cortisol levels are also highly correlated with total plasma values and circulating free cortisol, despite the fact that salivary cortisol levels are generally lower compared to plasma due to enzymatic conversion of some of the salivary cortisol into cortisone (Levine, Zagoory-Sharon et al. 2007; Hellhammer, Wust et al. 2009). Due to the complexity of HPA axis function and regulation mechanism, there is a degree of dissociation between salivary cortisol levels and CRH and

ACTH levels, in that a linear relationship between these factors does not necessarily exist (Hellhammer, Wust et al. 2009). Nevertheless, salivary cortisol assessments are widely used in neuropsychoendocrine research, and salivary cortisol is considered an important biomarker of HPA axis function (Hellhammer, Wust et al. 2009). Therefore, in the present work, we used saliva sampling as a method of choice for assessment of cortisol levels.

HPA axis in times of rest

At rest, the HPA axis shows pulsatile rhythmicity and is characterized by infradian and ultradian cycles, with a most distinct circadian rhythm (Weitzman, Fukushima et al. 1971). During basal condition, the CRH is released approximately every 60min, leading the adrenals to produce hourly secretory bursts of cortisol (de Kloet and Sarabdjitsingh 2008). The amplitude of these ultradian peaks varies across the 24 hrs and can distinguish between different secretory episodes that then define the circadian profile of cortisol (Lightman, Wiles et al. 2008). The 24 hour cycle circadian oscillation of basal cortisol is characterized by the highest levels in the morning after awakening, followed by a subsequent decline over the course of the day, and the nadir achieved around midnight (Hellman, Nakada et al. 1970).

Cortisol Awakening Response

A distinct phenomenon above that of the circadian oscillation, the cortisol awakening response (CAR), is a sharp rise in cortisol following awakening and typically peaks at about 30min following the awakening (Pruessner, Wolf et al. 1997; Wilhelm, Born et al. 2007). It has been hypothesized that this additional surge of cortisol following awakening may be due to a reduced pre-awakening adrenal sensitivity to ACTH, followed by an increased post-awakening adrenal sensitivity to ACTH (Clow, Hucklebridge et al. 2009). Interaction between the regulatory mechanisms controlling the HPA axis (for example, input from the hippocampus), as well as extra-pituitary mechanism (e.g., input from the suprachiasmatic nucleus) may underlie this process (Clow, Hucklebridge et al. 2009). The CAR is thought to reflect the sensitivity of the HPA axis to a natural challenge (the awakening) and can be differentially affected by stress and psychopathologies (reviewed in (Chida and Steptoe 2009; Fries, Dettenborn et al. 2009).

Glucocorticoid receptor types

Released cortisol has a widespread impact on several systems of the body such as cardiovascular, metabolic, immune and reproductive systems, as well as cognitive and emotional processes. In addition, cortisol regulates its own secretion through negative feedback at the level of hypothalamus and pituitary, and also at regulatory sites within the central nervous system (CNS), in particular, the hippocampus, amygdala and prefrontal cortex (Herman, Ostrander et al. 2005).

Two types of nuclear receptors make this possible: mineralocorticoid receptors (MR; Type I) and glucocorticoid receptors (GR; Type II) (McEwen, De Kloet et al. 1986; de Kloet, Joels et al. 1991). These receptors are ligand-driven transcription factors that regulate gene transcription (de Kloet, Joels et al. 2005; Joels, Karst et al. 2008).

Role of the genomic MR and GR receptors

The genomic MR controls basal HPA activity, exerts tonic inhibition in times of rest and determine the sensitivity or threshold of the stress system, while genomic GR contributes to adaptation to a stressor, contributes to the negative feedback loop, facilitates recovery from stressor-induced disturbances, as well as exerts negative feedback during the peak circadian activity (van Praag, de Kloet et al. 2004). The ratio of MR and GR occupancy has been suggested to contribute to differences in cognitive function during the day and in times of stress (Lupien, Maheu et al. 2007). The differential involvement of these receptors in the actions of cortisol is due to their differential affinity to cortisol and their distribution in the body and the brain. The MR has very high affinity for glucocorticoids, about 6-10 times higher than that of GR (De Kloet, Vreugdenhil et al. 1998). During low levels of circulating cortisol more than 90% of MR is occupied, but only about 10% of GR. However, at times of increased levels of cortisol (such as the circadian peak or in times of stress), MR is completely saturated, and occupation of GR is at about 67-74% (Lupien, Maheu et al. 2007). The MR is highest in density in the hippocampus, lateral septum, dentate gyrus, brain stem, entorhinal,

insular cortices, amygdala and frontal cortices (Brown 2000; Dalman 2000). However, the MR is absent from the hypothalamus and pituitary gland, which have a high concentration of GR. The GR is also localized in the hippocampus and the prefrontal cortex regions. In addition, GR is distributed in every cell type in the organism (De Kloet, Vreugdenhil et al. 1998; Dalman 2000).

Role of the non-genomic MR and GR-like receptors

There have also been recent reports of membrane MR receptors (Karst, Berger et al. 2005), as well as GR-like membrane receptors (Di, Malcher-Lopes et al. 2003; de Kloet, Fitzsimons et al. 2009), which would allow cortisol to exert fast, non-genomic actions on its target cells. These membrane-embedded receptors seem to be of similar type as the nuclear receptors, except that, in case of membrane MR, they seem to show lower affinity to glucocorticoids (Karst, Berger et al. 2005).

The GR-like receptors in the cell membrane of the hypothalamus seem to exert a similar role as the nuclear GR receptors (i.e. inhibit hypothalamic hormone secretion) (Di, Malcher-Lopes et al. 2003; Tasker, Di et al. 2006). However, membrane MR receptors show both similar and distinct functions from nuclear MR receptors. Firstly, the low affinity of hippocampal membrane MR suggests that, unlike its nuclear counterpart, membrane MR would be activated in times when cortisol levels are increased, namely during stress and at the peak of ultradian pulse (Joels, Karst et al. 2008), or the peak of circadian activity (Atkinson, Wood et al. 2008). In addition, preliminary data also seem to suggest

that hippocampal membrane MR may first amplify enhanced excitability induced by stress hormones and synergize with other mediators in the primary stress reaction, such as CRH, (nor)adrenalin or vasopressin (Kruk, Halasz et al. 2004; Joels, Karst et al. 2008). However, activation of membrane MR was also shown to lead to an enhanced glutamate release in the hippocampus. Given that the hippocampus exerts its inhibitory control of the HPA axis by stimulating the inhibitory connections surrounding the PVN (described in more detail below), it may be possible that hippocampal membrane MR also contributes to inhibitory tone exerted by the hippocampus on the HPA axis (a function that so far was ascribed to nuclear MR) (Joels, Karst et al. 2008). The field is just beginning to understand the functional implications of the non-genomic MR and GR receptors.

Importantly, proper functioning and balance between all of these receptor types are essential for an adaptive and healthy profile of the HPA axis output both at times of rest and of stress (De Kloet, Vreugdenhil et al. 1998; Joels, Karst et al. 2008; de Kloet, Fitzsimons et al. 2009).

Regulatory network of the HPA axis

As previously mentioned, cortisol exerts negative feedback at the level of the pituitary and the hypothalamus of the HPA axis, and it also affects other brain regions that form the regulatory circuit of the HPA axis, most notably hippocampus, amygdala and prefrontal cortices (Brown 2000).

Involvement of these areas in the regulation of the HPA axis seems to be influenced by several factors, one of which is stressor type (reviewed in Dedovic, Duchesne et al. 2009). Findings from animal literature suggest that reactive stressors, those that increase the demand on the system through a real sensory stimulus (pain, bodily injury or an immune challenge) would implicate brainstem, the bed nucleus of stria terminalis and specific hypothalamic nuclei, all which have direct connections to the PVN (Herman, Figueiredo et al. 2003). Anticipatory stressors, those that tap into innate or memory programs (such as social challenges or unfamiliar situations) seem to involve the limbic system areas and monosynaptic connections. The hippocampus and prefrontal regions have primarily inhibitory connections with the PVN of the hypothalamus, although specific components of the prefrontal cortex may play quite different roles in the regulation of the cortisol secretion and these may be stressor specific (Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005). Amygdala for its part seems to promote activation of the HPA axis. While in animals it responds to both physical and psychological stressors, in humans it has been suggested to underlie a response primarily to the physical threat (Pruessner, Dedovic et al. 2008; Dedovic, Duchesne et al. 2009).

Limbic structures do not seem to innervate the hypothalamic PVN directly, but rather influence the HPA axis through neurons located in the peri-PVN area (Herman, Ostrander et al. 2005; Jankord and Herman 2008). These neurons are primarily GABAergic and therefore exhibit an inhibitory influence on the PVN (Cullinan, Ziegler et al. 2008). Excitatory glutamate inputs from

hippocampus and specific prefrontal regions to peri-PVN contribute to the inhibition of the HPA axis, while GABAergic connection from amygdala nuclei to peri-PVN promote HPA activation through disinhibition (Herman, Ostrander et al. 2005).

Modes of cortisol action

All in all, the HPA axis and its regulatory network serve to allow an organism to respond to threat in an adaptive manner. Specific modes of cortisol action (permissive, feedback and preparatory) make this possible (Sapolsky, Romero et al. 2000; van Praag, de Kloet et al. 2004). For example, permissive actions prime the defensive mechanism prior to stress onset, and these effects are seen on cardiovascular, immunological, metabolic and cognitive functions (Sapolsky, Romero et al. 2000). Suppressive or feedback actions of cortisol are observed an hour or more following the stressor and function to restrain stress reaction from becoming damaging to the organism (for example, immune response to infection or neurochemical reaction psychosocial stressor are curtailed) (van Praag, de Kloet et al. 2004). Finally, preparatory action, as the name implies, prepares the organism's response to subsequent stressors (for example acts on disposition of glycogen and facilitates storage of information) (Sapolsky, Romero et al. 2000; van Praag, de Kloet et al. 2004).

However, when the organism is not able to orchestrate and regulate such a response, over time, physical and psychiatric illnesses may precipitate (Engelmann, Landgraf et al. 2004). For example, hypertension, metabolic

syndrome, gastrointestinal symptoms, insomnia, anxiety, cognitive deficits are all common in response to severe or chronic stress (Chrousos 2009). While the link between cortisol dysregulation and physical illnesses is an intuitive one, one may however ask how could the dysregulation of a hormone that seems to have primarily metabolic effects lead to the development of a psychiatric illness as well? As it has been suggested previously, cortisol readily penetrates the brain to contribute to its own regulation and also to impact functions of key brain areas that underlie cognitive and emotional regulation (Herman, Ostrander et al. 2005; Joels, Karst et al. 2008). Along with CRH, cortisol also interacts with several neurotransmitter systems such as the serotonin, dopamine and noradrenalin (Dinan 1994; van Praag, de Kloet et al. 2004), which are all involved in neurobiology of psychiatric illnesses, such as Major Depressive Disorder (MDD) (Belmaker and Agam 2008).

Due to the tight coupling between psychological stress, HPA axis dysfunction and MDD (Mazure 1998; Gold and Chrousos 2002; Hammen 2005; Monroe and Reid 2009), I have chosen to investigate the HPA function and its neural regulatory network in a population with distinct vulnerability to developing depression, the subclinically depressed individuals.

Below I outline some of the findings relating MDD and dysregulation of the HPA axis function and regulatory processes. Further, I elaborate on the choice of investigating a sample of subclinically depressed individuals, as an example of a population vulnerable to develop depression.

Depression and Stress

Major Depressive Disorder (MDD) is a severe and a debilitating illness that is complex in nature. Worldwide, depression has been projected to become the leading cause of burden of disease over the next two decades (World Health Organization 2008). In Canada, 9% of men and 15% of women will meet criteria for depression during their lifetime (Government of Canada 2006).

The onset and the development of MDD is often, though not always (for example, Monroe and Reid 2009), preceded by periods of extreme or chronic stress (e.g. Hammen 2005). For example, a positive association exists between severity and number of stressful life events and probability of depression onset (Kendler, Karkowski et al. 1998). However, not everyone who experiences high stress develops depression. Therefore, it is clear that vulnerability and resilience factors interact with life stress experiences and influence depression onset (Kendler, Kessler et al. 1995; Kendler, Karkowski et al. 1998; Heim and Nemeroff 2001; Caspi, Sugden et al. 2003). Interestingly, however, although severe stress life events often precede the first episode of depression, subsequent recurrences of the illness are less likely to be preceded by a severe stress event (Monroe and Harkness 2005). The first depressive episode therefore, may have "scarring" or sensitizing effects on the brain, that allow for the recurrence of depression without a clear triggering event (Monroe and Reid 2009). Clearly then, it is imperative to understand whether there are difference in stress processing in the population with a distinct vulnerability to develop their first depressive episode.

Types of Depression

MDD is a heterogeneous disorder. Although past literature has distinguished between endogenous depression (occurring without presence of a clear triggering event) or exogenous (reactive) depression (representing a response to, for example, a stressful life event), in most recent years, the focus has rather been on distinguishing between melancholic and atypical depression (Hammen 2005). Melancholic depression is associated with a state of hyperarousal characterized by high anxiety levels particularly related to self, feelings of worthlessness, and ruminating over past transgressions and failures, with the depressed mood being the worst in the morning (Lam and Mok 2008). Melancholic depression is physiologically associated with weight loss, insomnia (most often early morning awakening), suppression of growth hormone and reproductive axes (reviewed in Gold and Chrousos 1999; Gold and Chrousos 2002). Atypical depression on the other hand, is characterized by feelings of disconnectedness and withdrawal from the social world. Individuals suffering from atypical depression experience high levels of fatigue, excessive sleepiness, increased appetite and weight, and depressive symptoms which worsen as the day progresses (Gold and Chrousos 1999). Patients with atypical depression are also more likely to be highly sensitive to interpersonal rejection (Lam and Mok 2008). Interestingly, these subtypes seem to show different profile of HPA axis dysregulation: in melancholia, HPA axis seems to be hyperactive, while atypical depressed seem to show hyporesponsive HPA function (reviewed in Gold and Chrousos 2002) (see details below).

Dysregulation of the HPA axis in depression

Major Depressive Disorder (MDD) has been associated with dysregulation of both basal and stress-related regulation of the HPA axis (Burke, Davis et al. 2005; Binder and Nemeroff 2010). It is however important to note that findings are often contradictory due to differences in depression severity and subtype, populations evaluated, and cortisol sampling methodology.

Dysregulation of basal function of the HPA axis in depression: Diurnal cortisol

Several studies have examined plasma cortisol levels over the course of 24 hours in depressed populations (Deuschle, Schweiger et al. 1997; Weber, Lewicka et al. 2000). A study evaluating depressed men reported increased plasma cortisol over the 24 hr period, with increased frequency of cortisol pulses in the evening, and reduced time of quiescence of cortisol release in patients compared to controls (Deuschle, Schweiger et al. 1997). Similarly, more recent studies evaluating the course of the circadian rhythm in both the severely depressed men and women also found increased plasma cortisol secretion over the 24hr cycle (Weber, Lewicka et al. 2000). However, other groups investigating chronic depression or subgroup of atypical depressed patients found either decreased (in chronic patients) or no alterations of plasma cortisol (in atypical subgroup) in the patient groups compared to controls (Watson, Gallagher et al. 2002; Stewart, Quitkin et al. 2005; Veen, van Vliet et al. 2010). It has been suggested that the difference may be due to the distinction between melancholic and atypical

depression. For example, several studies reporting an increase in cortisol during the typical quiescent period of the circadian rhythm found this effect specifically in melancholic depression (Wong, Kling et al. 2000; Gold, Wong et al. 2005; Carroll, Cassidy et al. 2007), while these nocturnal cortisol levels did not change in a sample with atypical depression (Antonijevic 2008).

Studies examining salivary cortisol levels during the day showed that more severe levels of depression were associated with flatter diurnal cortisol patterns (decreased levels following the awakening, and increased levels in the afternoon/evening) (Hsiao, Yang et al. 2009). Veen and colleagues have shown a positive association between severity of anhedonic depression and salivary cortisol secretion in the afternoon/evening (Veen, van Vliet et al. 2010). If depression was evaluated as a categorical variable, depression state according to DSM IV was associated with overall higher cortisol and steeper slope compared to controls (Veen, van Vliet et al. 2010).

Therefore, overall, more severe depression seems to be associated with an increase in cortisol secretion, particularly in the evening. However, atypical subtype of depression is most likely associated with basal cortisol secretion profile which is similar to that of healthy controls.

Dysregulation of basal function of HPA axis in depression: Cortisol

Awakening Response (CAR)

As with diurnal cortisol levels, findings of both increased and blunted cortisol awakening response (CAR) have been reported in depression. A study comparing medication-free recovered depressed patients to a matched healthy control group reported a higher increase in CAR in the patient sample (Bhagwagar, Hafizi et al. 2003). Similarly, a large study investigating current and remitted middle-aged depressed subjects found that, in comparison to control subjects, patients showed a higher CAR (Vreeburg, Hoogendijk et al. 2009). On the contrary, a smaller study examining young adults reported that depressed patients had a blunted CAR (Stetler and Miller 2005). Further, in an outpatient population, more severe levels of depression were more likely associated with flattened diurnal cortisol patterns (Hsiao, Yang et al. 2009), while a lower CAR has been observed in depressed patients compared to patients with other psychiatric diagnoses (Huber, Issa et al. 2006). A recent study investigated association between depression levels, assessed both as a DSM-IV categorical variable and a dimension measure of a mood questionnaire, and CAR, in groups of outpatients (depressive, anxiety, and comorbid depressive and anxiety disorders) and controls (Veen, van Vliet et al. 2010). This approach revealed no group differences with respect to CAR when categorical distinctions between groups were applied. However, when non-linear association was assessed across the whole sample, there was an inverted U shape function explaining the association between anhedonic depression levels and area-under-the-curve (AUC)

of the CAR, while controlling for presence of different outpatient groups. Specifically, individuals with mild anhedonic depression levels showed similar CAR AUC as controls, those with moderate levels showed an increase, while those with severe levels showing a decrease in CAR AUC compared to controls (Veen, van Vliet et al. 2010). However, it is important to note that a recent meta-analysis found a negative association with severity of depression and CAR, specifically with respect to the area-under-the-curve-increase (AUCi) or absolute increase score assessment of the CAR (Chida and Steptoe 2009).

Dysregulation of the HPA axis in depression in response to pharmacological stimulation

Although this thesis does not investigate pharmacological challenges to the HPA axis, it is necessary to discuss this body of literature with respect to depression, given that most of the evidence for the generally-held belief of a hyperactive HPA axis in depression stems from these paradigms. Additional studies evaluating concentration of CRH levels in cerebrospinal fluid, and postmortem studies of CRH mRNA in hypothalamus lend further support (Nemeroff and Evans 1984; Banki, Bissette et al. 1987; Raadsheer, van Heerikhuize et al. 1995).

Three tests have been used to challenge the HPA axis: CRH test, dexamethasone (DST) test and DST/CRH test. All three act at the level of the pituitary (Schommer and Heuser 2007). The DST is a synthetic glucocorticoid with 25 times higher binding affinity to GR receptors than cortisol itself. DST has

difficulty crossing the blood-brain barrier, and so it is used to assess negative feedback sensitivity of the HPA axis at the pituitary level (Schommer and Heuser 2007).

Dexamethasone Test (DST test)

The DST test involves swallowing a small dose of DST (1mg) at 11pm, and assessing the plasma cortisol concentrations at several time points the following day. A normal response to DST involves suppression of the ACTH secretion and subsequent decrease in the synthesis and release of cortisol. Depression has been associated with non-suppression, which is suggestive of impaired feedback regulation and hyperactivity of the HPA axis (Gillespie and Nemeroff 2005). However, DST non-suppression is not seen in all depressed patients. It is more likely for endogenous/melancholic or psychotic depression (occurrence rate 40-55%) than in non-melancholic outpatients with major depression (13-30%) (Arana, Wilens et al. 1985; Nelson and Davis 1997). Furthermore, some have even reported an exaggerated negative feedback response to DST in women with atypical symptoms (Levitan, Vaccarino et al. 2002).

Corticotropin-releasing hormone test (CRH test)

The CRH test consists of the administration of an intravenous dose of 1 μg/kg ovine CRH or 100 μg of human CRH, and the assessment of ACTH and cortisol in 30min intervals in 2-3 hrs periods following the injection. Here,

healthy subjects show increased secretion of ACTH and cortisol, while depressed subjects show blunted ACTH, but normal cortisol (Kathol, Jaeckle et al. 1989; Heim, Newport et al. 2001; Gillespie and Nemeroff 2005). It has been suggested that blunted ACTH in depressed is due to down regulation of the CRH receptors at the level of pituitary (as a response to overproduction of endogenous CRH). The normal cortisol levels in response to this decreased ACTH pulse may be due to hyperactivity of the adrenals, where more cortisol is released per pulse of ACTH (van Praag, de Kloet et al. 2004).

Combined dexamethasone/corticotropin-releasing hormone test

(DST/CRH test)

Finally, the DST/CRH challenge involves pretreatment with DST at 11pm, and giving 100μg infusion of CRH the following day (Holsboer, von Bardeleben et al. 1987). Here, depressed patients tend to show an enhanced ACTH and cortisol response compared to healthy individuals (Ising, Kunzel et al. 2005; Watson, Gallagher et al. 2006). It has been proposed that DST leads to a decrease in ACTH and endogenous cortisol level; this in turn reduces the negative feedback by cortisol on the hypothalamus and therefore stimulates the production of CRH. The additional shot of CRH the following day would then override the down-regulation of CRH receptors and lead to an increased ACTH response and even greater cortisol response in patients compared to controls (van Praag, de Kloet et al. 2004). Some have however suggested that the impairment profile

observed in depression in response to DST/CRH may be secondary to early-life trauma given that a study has shown that an increase in cortisol was found only depressed men with history of early-life trauma, while non-early life trauma depressed subjects showed cortisol profile similar to controls (Heim, Newport et al. 2008).

Although these tests cannot distinguish between subtypes of depression, or from depression and other illnesses (such as panic disorders, schizophrenia, eating disorders, etc) (van Praag, de Kloet et al. 2004; Antonijevic 2008), they provide solid evidence of how the HPA axis is affected in depression. However, these pharmacological challenges can only reveal abnormalities at the levels of the HPA axis specifically (from pituitary onward). They cannot reveal abnormalities at higher regulatory areas such as hippocampus, amygdala or prefrontal cortices. As it was previously mentioned, psychological stressors involve these central nodes, and therefore investigations of the HPA axis response to psychological stressors can provide additional insight into the dysregulation of mechanisms underlying processing of stress in depression.

Dysregulation of the HPA axis in depression in response to psychological stress

Although the link between psychological stress and depression is often discussed, there are actually very few studies that have investigated the stress

response in depressed populations in response to psychological stressors (Breier 1989; Trestman, Coccaro et al. 1991; Croes, Merz et al. 1993; Gotthardt, Schweiger et al. 1995; Ravindran, Griffiths et al. 1996; Heim, Newport et al. 2000; Young, Lopez et al. 2000; Heim, Newport et al. 2002). A recent meta-analysis evaluated studies investigating cortisol response to psychological stress in depression (Burke, Davis et al. 2005). A total of nine studies were included. The meta-analysis revealed that in afternoon studies (eight studies), depressed patients compared to controls had higher cortisol levels at baseline, and during the recovery period following the stressor (Burke, Davis et al. 2005). However, in response to a laboratory psychological stress task specifically, no significant differences in cortisol levels were observed between depressed and controls. In fact, when cortisol stress levels were adjusted for baseline effects, depressed patients showed a relatively blunted cortisol response to stress (Burke, Davis et al. 2005). Another study evaluating cortisol response to daily hassles and negative events found that depressed participants also exhibited a blunted cortisol response (Peeters, Nicholson et al. 2003). Similarly, a recent review concluded that in response to a psychological stress, cortisol release is either similar to those of healthy individuals (in case of plasma cortisol) or somewhat blunted (salivary cortisol) (Handwerger 2009).

These findings are also supported by additional research studies published recently. In middle-aged women remitted from recurrent major depression, psychosocial protocol elicited a blunted response in serum cortisol and ACTH levels compared to controls (Ahrens, Deuschle et al. 2008). Similarly, in a

population study of 725 middle-aged men and women, cortisol reactivity to a collection of psychosocial tasks was found to be lower in those with mild-to-severe depression compared to controls (de Rooij, Schene et al. 2010). However, a recent study investigating sex differences in stress response in a population with chronic depression revealed that while depressed men showed a blunted peak salivary cortisol response to a psychological stressor compared to healthy men, depressed women had an overall higher cortisol secretion in response to the stressor compared to healthy women (Chopra, Ravindran et al. 2009).

It is important to note here that unlike pharmacological challenge studies of depression, studies having exposed depressed populations to a psychological challenge did not assess whether their participants were presenting melancholic versus atypical features of depression. Therefore, it is unclear which subtype is more likely to display this blunted cortisol response to a psychological stressor.

Neural correlates of stress processing in depression

Although there is a paucity of neuroimaging studies investigating specifically functional neural correlates of psychological stress processing in depressed populations or populations with distinct vulnerability for depression, there is a great number of studies that have examined the neural correlates of mood regulation and treatment response. Not surprisingly some of the brain areas previously discussed to play an important role in HPA axis regulation have also been found to contribute to mood regulation.

For example, a major body of work has focused on investigating the role of the hippocampus in depression. Here, findings of smaller hippocampal volumes in the depressed populations compared to control groups have consistently been reported, although there is an ongoing debate with respect to the origin of this abnormality, i.e. is it a cause or a consequence of the illness (for example, Frodl, Meisenzahl et al. 2002; McKinnon, Yucel et al. 2009). In addition, specific regions such as the prefrontal cortex (particularly medial prefrontal cortex), amygdala, and cingulate cortex, also feature prominently in several of the proposed models of neural networks underlying mood dysregulation in depression (for example, Mayberg 2003; Drevets, Price et al. 2008).

The findings in regard to the association between depression and the hippocampus, as well as the proposed models of neural network abnormalities, are outlined below, as these concepts are relevant for the second objective of this thesis. This objective is to assess whether some of the abnormalities associated with the HPA axis function and the HPA regulation network seen in Major Depressive Disorder can already be present in a vulnerable population prior to depression onset. The main assumption is that evidence of abnormalities and dysregulation of this system in the subclinical sample found prior to onset of clinical depression would be suggestive of these impairments reflecting vulnerability factors rather than the consequence of a long battle with clinical illness.

Depression and the Hippocampal volume

The hippocampus has intricate connections to prefrontal and cingulate cortex, amygdala, basal ganglia, anterior thalamic and septal nuclei and of course, the hypothalamus; thus, not only is the hippocampus part of the HPA axis regulatory network (Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005), but it also contributes to the neuroanatomical network of mood regulation (Drevets 2001).

In human studies, hippocampal volume is used as a proxy measure of hippocampal integrity. Namely, it has been suggested that hippocampal volume (HC volume) might reflect differential neuronal and glial packing density, as well as differences in neuronal soma sizes (Stockmeier, Mahajan et al. 2004).

Studies investigating recurrent or treatment resistant depression most often report bilateral HC volume reductions (for example, Sheline, Wang et al. 1996; MacQueen, Campbell et al. 2003; Sheline, Gado et al. 2003; Caetano, Hatch et al. 2004; Hickie, Naismith et al. 2005). In addition, several meta-analyses conducted over the last decade have concluded that unipolar depression has a negative effect on bilateral HC volume (Campbell, Marriott et al. 2004; Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009). This line of evidence suggests that a reduced HC volume is a result of depression and represents a burden of illness. Namely, it has been suggested that reduced HC volume may reflect a neurotoxic effect of increased cortisol levels (hypercortisolemia) observed in some depressed patients, as well as stress-

induced reduction of neurotrophic factors and stress-induced reduction in neurogenesis or glial cell loss (Sheline, Gado et al. 2003).

However, none of the above mentioned studies sampled blood, urine or saliva from their participants in order to verify the presence of hypercortisolemia. In those studies where cortisol measures were available, no relationship was found between cortisol levels and HC volume in the patient population (Vythilingam, Vermetten et al. 2004). In addition, several studies found only unilateral HC atrophy (Bremner, Narayan et al. 2000; Mervaala, Fohr et al. 2000; Steffens, Byrum et al. 2000; O'Brien, Lloyd et al. 2004). However, how a systematic process such as neurotoxicity-induced cell apoptosis could affect the hippocampus differentially in two hemispheres is unclear and remains to be elucidated.

Furthermore, in humans suffering from Cushing's disease, increased levels of cortisol have been shown to exhibit clear neurotoxic effects and are responsible for the reduction in the HC volume (Starkman, Giordani et al. 1999). However, this effect is reversible upon stabilization of cortisol levels (Starkman, Giordani et al. 1999). In depression, smaller-than-normal HC volume persists after remission of the depressive episode and the normalization of cortisol levels (Hoschl and Hajek 2001). It remains to be seen whether changes in cortisol regulation could affect the same structure differently depending on the illness.

To further contribute to the contradictory nature of this field, several studies failed to find any differences in the HC volume between patient and

control groups (Vakili, Pillay et al. 2000; von Gunten, Fox et al. 2000; Rusch, Abercrombie et al. 2001; Posener, Wang et al. 2003; Hastings, Parsey et al. 2004; Vythilingam, Vermetten et al. 2004).

A new line of studies investigating first episode depressives has emerged in recent years and some of these findings stand to challenge the view of smaller HC volumes found in depressed populations representing a consequence of illness burden. Namely, patients diagnosed with a first episode of depression and thus a very short lifetime duration of the illness, already show a reduced HC volume - pointing to smaller HC volume as risk factor for, rather than a consequence of, the illness (Frodl, Meisenzahl et al. 2002). While few studies have found no differences in the HC volume between first episode depressives and controls (MacQueen, Campbell et al. 2003; Milne, MacQueen et al. 2009; van Eijndhoven, van Wingen et al. 2009), others report reduced left HC volume in first episode male patients (Frodl, Meisenzahl et al. 2002; Kronmuller, Schroder et al. 2009), as well as in first-episode female drug naïve subjects (Kaymak, Demir et al. 2009), and a group of drug-naïve men and women (Zou, Deng et al. 2009). In addition, in these latter studies, HC volume did not correlate with illness duration (Kaymak, Demir et al. 2009; Kronmuller, Schroder et al. 2009; Zou, Deng et al. 2009). Furthermore, a study of healthy volunteers has shown that there is a great variability in the HC volume within a healthy population, with percent difference between mean and the lowest quartile amounting to 16% for 25-40 years age range, which is greater than what is observed between clinical and healthy populations (Lupien, Evans et al. 2007).

Taken together, even though the prevailing thought in the field is that depressive episodes contribute to the reduction in HC volume, recent studies provide support to the idea that small HC volume could precede MDD onset. It is thus possible that in such an event, dynamics of crucial systems, such as HC connections with the HPA axis, amygdala and prefrontal cortex, could be negatively affected and could contribute to disturbances in mood and emotional regulation, typical of depression.

Models of mood regulation in depressed populations

Numerous studies have investigated the effect of MDD on broader neural networks (for example, Drevets, Videen et al. 1992; Mayberg, Brannan et al. 2000; Videbech, Ravnkilde et al. 2001; Canli, Sivers et al. 2004). Based on these findings, several models have been proposed to explain the cognitive, emotional, endocrine, and neurochemical dysregulation observed in depressed patients (Drevets, Price et al. 2008; Phillips, Ladouceur et al. 2008; Mayberg 2009). Despite the fact that these studies did not assess stress regulation in depressed populations, all models include brain areas that also subserve HPA axis regulation and function, such as the hypothalamus, the hippocampus and the medial orbitofrontal cortex.

Mayberg's Model

Helen Mayberg proposed a four-compartment model of cortical-limbic dysregulation underlying MDD (Mayberg 1997; Mayberg 2003; Mayberg 2009). The dorsal cortical compartment, which subserves attention, appraisal and execution (all basic exteroceptive cognitive processings), includes prefrontal cortex (BA 46/9), premotor area, parietal cortex (BA 40), dorsal cingulate cortex (BA24) and dorsal posterior hippocampus. The ventral limbic compartment that is involved in introceptive processes (autonomic function, circadian rhythm) contains subcallosal cingulate gyrus (BA 25), anterior insula, hypothalamus, brain stem and ventral-anterior hippocampus. Two other compartments are also presented. The medial frontal cortex (BA 10/9), medial orbital frontal cortex (BA 11) and pregenual ACC (BA 24) form the mood regulation network involved in the cognitive and active control of affective states and underlying self-relevance, prioritization, contingencies and reinforcement. Finally, mood monitoring compartment includes amygdala, ventral striatum-caudate, midbrain-ventral tegmental area and dorsomedial thalamus. All regions within the compartments have strong anatomical connections, and are also connected across the compartments. The functions within these compartments are influenced by changes in mood and various treatments (Mayberg 2009).

Drevets' Model

Drevets and colleagues proposed a model that emphasizes the role of the medial orbitofrontal area and amygdala in explaining various depression symptoms (Drevets, Price et al. 2008). It has been suggested that dysfunction

within the orbital/medial prefrontal cortex leads to the disinhibition of the limbic system transmission mainly through the amygdala and thus contributes to the key symptoms of depression with respect to cognition, emotion, an endocrine function, as well as autonomic and neurochemical features of depression (Drevets, Price et al. 2008). Namely, through its connection to amygdala and other limbic system structures, a dysregulated medial orbitforontal cortex may affect output signals from the central nucleus of amygdala for example; this, in turn, may modulate amygdala connections to the hypothalamic nuclei, locus ceruleus, raphe nuclei, and other important nuclei, together leading to disturbance of neurochemical, neurotranmitter, autonomic and endocrine systems underlying stress and emotion (Drevets, Price et al. 2008).

Phillips' Model

Finally, the model proposed by Phillips et al focuses on automatic and voluntary emotion regulation (Phillips, Ladouceur et al. 2008). Here, dorsolateral prefrontal cortex and ventrolateral prefrontal cortex subserve voluntary emotion regulation and are suggested to operate by feedback mechanisms. On the other hand, dorsal, rostral, and subgenual anterior cingulate, orbitofrontal cortex and hippocampus underlie automatic subprocesses and operate by feedforward mechanisms. Orienting and perception of emotion would be underlined by ventral striatum, thalamus, and amygdala; which in turn may modulate both feedforward and feedback processes (Phillips, Ladouceur et al. 2008).

Brain activity changes observed in depression

With a multitude of studies evaluating resting state, mood induction, and treatment response, utilizing a variety of neuroimaging methods and paradigms, the findings with respect to changes observed in these cortical and subcortical regions have been inconsistent. A recent meta-analysis has attempted to quantitatively synthesize the findings from these studies (Fitzgerald, Laird et al. 2008), and these results found in depressed patients are presented below.

At rest, depressed patients seem to show decreased activity in pregenual and dorsal cingulate cortex, bilateral middle frontal gyrus, insula and superior temporal gyrus. Increased activity was observed in deep brain structures, such as the thalamus and the caudate, and cortical areas such as for example the superior frontal gyrus. In response to treatment, the pregenual and subgenual cingulate as well as the left middle frontal gyrus and superior frontal gyrus, putamen and hippocampus/parahippocampus gyrus decreased in activity. The dorsal and posterior cingulate as well as part of the midbrain and parietal and precentral gyrus also increased.

Positive affect induction was associated with decreased activity in the pregenual and posterior cingulate, the left orbitofrontal, the medial and lateral temporal and the posterior cerebellum. Increases were found in the subgenual and posterior, as well as the lingual and precentral gyrus. Negative affect decreased pregenual, dorsal and posterior cingulate, bilateral middle frontal gyrus, insula and superior temporal gyrus, anterior and posterior cerebellum. It also increased

activity in the posterior cingulate, the right middle frontal, the lateral temporal, parietal, amygdala and the putamen (Fitzgerald, Laird et al. 2008).

Subclinical depression as a choice for vulnerability assessment

Depression is a complex and heterogeneous disorder with several risk factors contributing to its onset and development.

For example, depression is a sexually dimorphic illness affecting almost twice as many women compared to men (Fava and Kendler 2000). In addition, personality traits such as increased neuroticism and depressive coping style, as well as reduced self-esteem and mastery, have all been identified as vulnerability factors for depression (Ormel, Oldehinkel et al. 2004). In a study examining monozygotic twins in women, low optimism and current stressful life events discriminated significantly between affected and non-affected twin pairs (Kendler and Gardner 2001). A prospective study also reported that experiencing an elevated level of stress predicted development of an episode of depression and elevated depression severity scores over time in a sample of men and women (Lewinsohn, Hoberman et al. 1988). Moreover, family history of depression and difficult family dynamic during early life also represent vulnerability factors: children of depressed parents are at an increased risk to develop depression (Sullivan, Neale et al. 2000; Schreier, Hofler et al. 2006), and individuals who have experienced adversity in early life are more likely to develop depression later on in life (Kendler, Kessler et al. 1993; Kendler, Bulik et al. 2000; Nelson, Heath et al. 2002).

Importance of subclinical levels of depression

In the present thesis however I focus on individuals with subclinical levels of depression as an example of a population with distinct increased vulnerability and risk for Major Depressive Disorder. Subclinical or subthreshold depression has been defined in various ways, from scoring above a cut-off point on a self-rating scale, to having a depressed mood with one or more additional symptoms of a mood disorder, or as meeting the criteria for minor depression in DSM-IV (Cuijpers and Smit 2004). In this thesis, subclinical depression was assessed as currently scoring above a cut-off point on a self-rating depression inventory. A recent study revealed that assessment of subthreshold depression either via symptom counting method or assessment of symptom severity was associated with functional impairment in daily life; however, the symptom severity assessment was found to be more suitable to measure clinically relevant subthreshold depression (Karsten, Hartman et al. 2010).

Importantly, despite the heterogeneity in definition of the subclinical population, a consistent pattern has been observed of increased incidence of MDD among subjects with subclinical depression compared to those without it (Cuijpers and Smit 2004). Furthermore, several studies put forth evidence that the subclinical depression might represent a milder condition on the depression continuum (Solomon, Haaga et al. 2001; Lewinsohn, Klein et al. 2003; Rivas-Vazquez, Saffa-Biller et al. 2004). Some have even suggested that subclinical depression may represent the precursor for the full disorder (Shankman, Lewinsohn et al. 2009). Indeed, this 15-year longitudinal study, showed that

subthreshold depression at time 1 in adolescence (defined as an episode of depressed mood or loss of interest or pleasure lasting at least 1 week, plus at least two of the seven associated symptoms), was predictive of developing a full syndrome diagnosis over the course of a 15 year follow up. In addition, subthreshold depression was specific to predicting development of a full syndrome depression disorder even after adjusting for comorbidity (Shankman, Lewinsohn et al. 2009). It is important to note that the risk of developing MDD is larger in current subthreshold depression compared to last year or lifetime (Cuijpers and Smit 2004).

Therefore, a subclinical depression population would allow us a unique opportunity to investigate the HPA axis function and neural regulatory networks in a population that is at a direct risk of developing depression, but who has not as yet succumbed to the full clinical syndrome. This population will allow us to investigate the vulnerability hypothesis and assess whether impairments in the HPA axis regulatory system may already be present prior to onset of clinical depression.

Chapter 2: What stress does to your brain: a review of neuroimaging studies

Katarina Dedovic (BSc), Catherine D'Aguiar (BA), Jens C Pruessner (PhD)

Preface to Chapter 2

The HPA axis' response to psychological stress is processed and regulated by an intricate neural network including key brain areas in the prefrontal cortex and the limbic system (Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005). Although animal studies and human pharmacological and behavioral studies have revealed some of the mechanisms underlying these processes, advances in neuroimaging technology now allow for non-invasive investigations of the changes in brain function taking place during processing of psychological stress directly in human subjects.

However, it is important to note that translation of behavioral psychological stress paradigms into those suitable for the restrictive neuroimaging environment was and still remains a challenge. For example, the Trier Social Stress Test (TSST), a behavioral psychosocial stress task that has been shown to reliably induce a strong stress response in majority of subjects involves delivering a 5-minute job talk followed by performing serial subtraction for 5 minutes out loud, all in front of a panel of judges trained to maintain a neutral face (Kirschbaum, Pirke et al. 1993). Such a design is difficult to implement in a neuroimaging environment given that minimal head movement is an important prerequisite in obtaining high quality functional brain images. In addition, impact of presence of social evaluative threat (i.e. presence of judges) is highly reduced in the neuroimaging environment, in particular in the Magnetic Resonance Imaging (MRI) environment where the subject's whole body is in the bore of the magnet.

Therefore, in the following chapter (Dedovic, D'Aguiar et al. 2009), we take the time to review human neuroimaging studies that had aimed to investigate changes in neural activity in response to an acute psychological stressor with a particular emphasis on the neuroimaging stress task design. As we summarize the key findings from these studies, we also assess the appropriateness of the different stress tasks used while keeping in mind the findings of the psychological stress literature as to what is stressful (Dickerson and Kemeny 2004).

We also discuss the neuroimaging stress task developed in our lab, the Montreal Imaging Stress Task (MIST) (Figure 1) (Dedovic, Renwick et al. 2005). The MIST combined mental arithmetic tasks with components of uncontrollability (induced failure) and presence of social evaluative threat in order to induce a stress response.
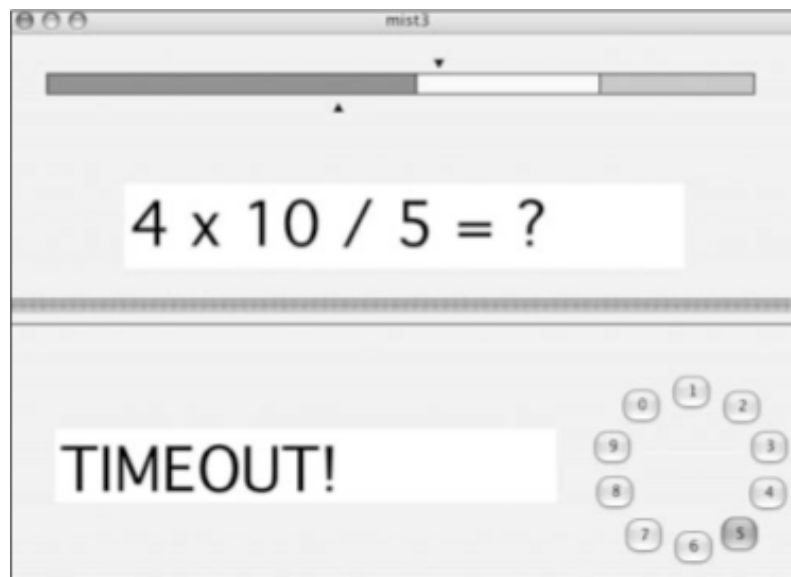
Figure 1: Graphical user interface of the Montreal Imaging Stress Task (MIST). From top to bottom, the figure shows the performance indicators (top arrow = average performance, bottom arrow = individual subject's performance),

the mental arithmetic task, the progress bar reflecting the imposed time limit, the text field for feedback, and the rotary dial for the response submission. (Reproduced with permission from (Dedovic, Renwick et al. 2005).

The MIST was developed as a block design task for usage in Positron Emission Tomography (PET) and fMRI environment. In comparison to the TSST, it is considered a mild stressor. In addition, there is significant heterogeneity in individual cortisol responses (Figure 2), allowing us to investigate brain activity changes in the group of responders, those who show a significant stress response, and non-responders, those who do not (Figure 3) (Pruessner, Dedovic et al. 2008).
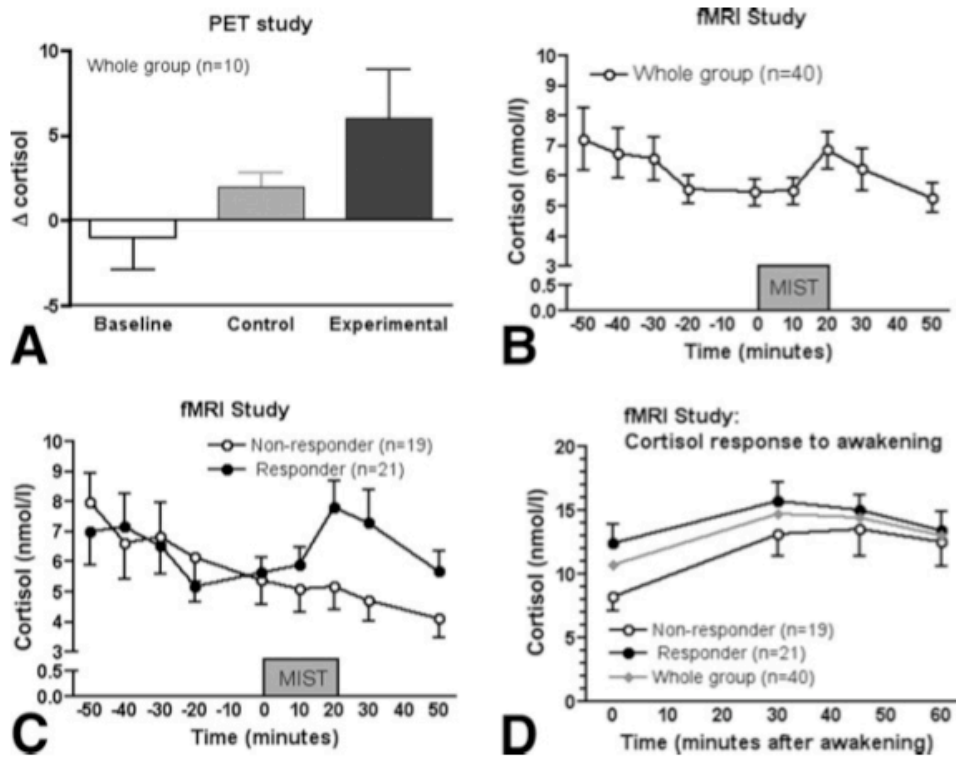
Figure 2: Cortisol levels in the different experiments and subgroups. (A) Post-pre cortisol levels of the three testing conditions rest, control and experimental in the positron emission tomography (PET) study (n = 10). (B) Cortisol levels (whole group n = 40) during the fMRI experiment. (C) Cortisol

levels in the responder (n = 21) and nonresponder (n = 19) subgroups during the fMRI experiment. (D) Cortisol levels during the first hour after awakening on a separate day from the fMRI experiment. Error bars shown are SEM. fMRI, functional magnetic resonance imaging. (Reproduced with permission from (Pruessner, Dedovic et al. 2008).
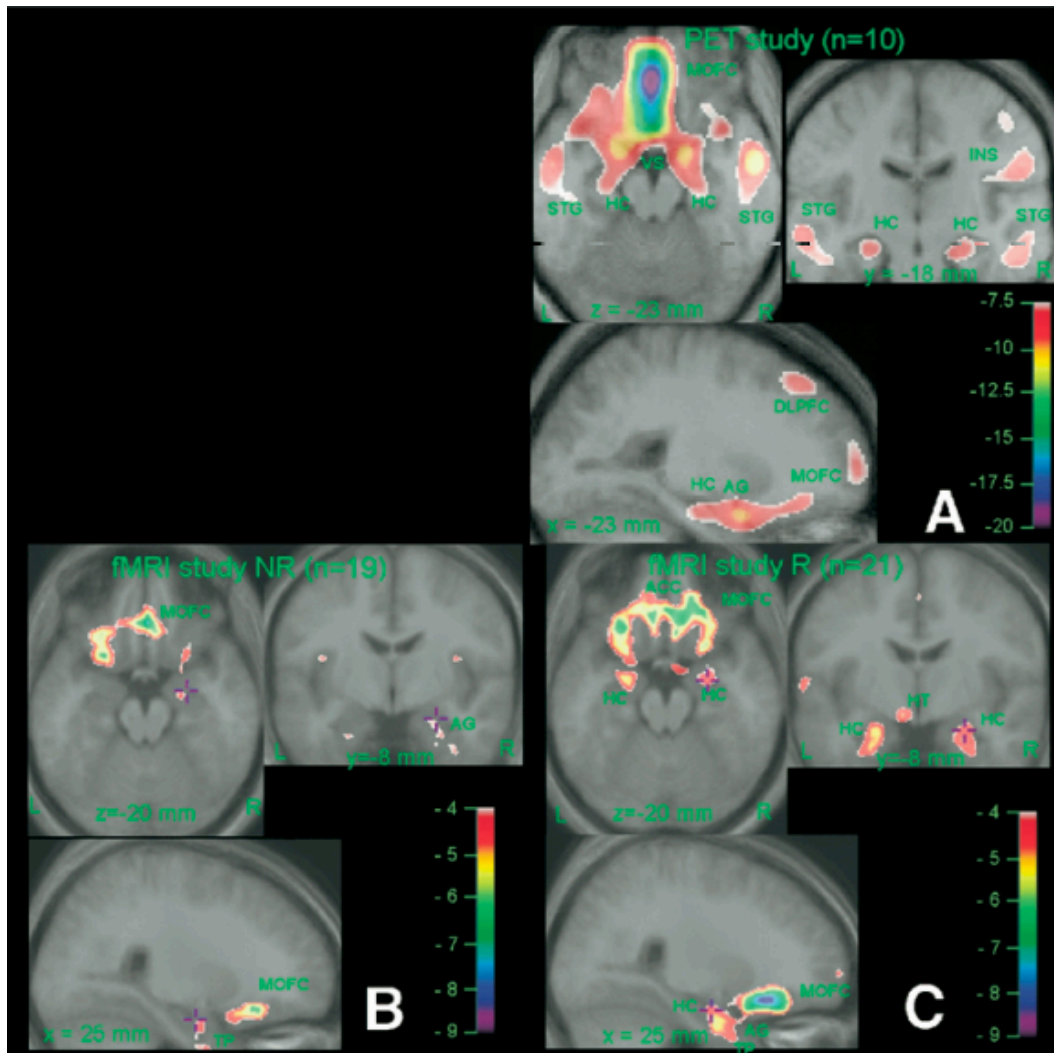
Figure 3: Significant deactivations in the (experimental minus control) contrast in the two neuroimaging experiments. (A) PET deactivations, whole group. (B) fMRI study nonresponder (n = 19). (C) fMRI study responder group (n = 21). x, y, z = sagittal, coronal and horizontal view in world coordinates. L, left; R, right;MOFC, medio-ordbitofrontal cortex;STG, superior temporal gyrus; HC, hippocampus; INS, insula;DLPFC, dorsolateral prefrontal cortex; AG, amygdala;

VS, ventral striatum; HT, hypothalamus; TP, temporal pole; ACC, anterior cingulate cortex; PET, positron emission tomography; fMRI, functional magnetic resonance imaging. (Reproduced with permission from (Pruessner, Dedovic et al. 2008).

MANUSCRIPT

Title:

What stress does to your brain: a review of neuroimaging studies

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Abstract

Objective: Recent neuroimaging studies aimed at investigating effects of psychological stress on the neural activity have used a range of experimental paradigms to elicit an acute stress response. The goal of this review is to, first, summarize results from these studies, from a perspective of task design, and, second, assess the appropriateness of the different stress tasks used.

Method: We completed a PubMed search on recent articles that have examined the effects of psychological stress on neural processes in a neuroimaging environment. Selected articles were arranged according to the stress task used into the following categories: script-driven stress stimuli, Stroop Color-Word interference task, speech in front of an audience, serial subtraction, and Montreal Imaging Stress Task (MIST).

Results: Only studies using serial subtraction or the MIST were able to induce a significant cortisol stress response in their participants. Most consistent findings include decreased activity in orbitofrontal regions in response to stress. Additional findings of note are increases in activity in the frontal lobes, particularly the anterior cingulate cortex, as well as deactivation of the limbic system, particularly the hippocampus.

Conclusion: Research to date is beginning to outline the involvement of prefrontal and limbic regions in perception and modulation of psychological stress. However, additional research is needed in designing a neuroimaging stress task that will yield a significant cortisol stress response consistently, across populations and labs.

Clinical Implications

- Individual differences exist regarding stress reactivity.

- Sex differences in neural activity in response to stress may underlie differential vulnerability to psychiatric illnesses between men and women.

- Neuroimaging stress tasks have a potential of identifying people at risk to develop stress-related disorders at both neural and physiological levels.

Limitations:

- To date, only a few neuroimaging stress task designs have been able to reliably elicit a stress response.

- Comparability of results generated via differential neuroimaging methods is limited.

- Only a few studies have compared men and women specifically regarding stress-related changes in neural activity.

Key Words: neuroimaging studies, psychological stress, stress tasks, orbitofrontal, anterior cingulate cortex, hippocampus, fMRI, perfusion fMRI, PET, near-infrared spectroscopy

Abbreviations used in this article

ACC   anterior cingulate

ACTH   adrenocorticotropic hormone

BOLD   blood oxygenation level dependent

AG   amygdala

CAD   coronary artery disease

CBF	cerebral blood flow
CRF	corticotropin-releasing factor
DLPFC	dorsolateral prefrontal cortex
fMRI	functional magnetic resonance imaging
HC	hippocampus
HPA	hypothalamus–pituitary–adrenal
MIST	Montreal Imaging Stress Task
MPFC	medial prefrontal cortex
oxy-HB	oxygenated hemoglobin
PET	positron emission tomography
PFC	prefrontal cortex
PVN	peri-paraventricular nucleus
TSST	Trier Social Stress Test

Introduction

Psychological stress has numerous physiological, metabolic, and behavioural consequences. All of these are triggered when a particular situation is perceived as stressful. A prominent stress theory postulates that this perception is associated with the appraisal of the situation: when the demands of the particular event are perceived to exceed the available resources, the feeling of stress ensues (Lazarus 1993). However, besides the appraisal, there are specific situational circumstances that contribute to stress perception. A thorough meta-analysis of a little more than 200 human studies of psychological stress induction revealed that situational characteristics facilitating the generation of a stress response include an atmosphere of high achievement, social evaluation, and little or no controllability (Dickerson and Kemeny 2004). This finding supports the social self-preservation theory, which posits that humans have a strong need to preserve their social self (one's social values, esteem, and status), and are vigilant to threats that may jeopardize this identity. Interestingly, in neuroimaging studies, the network that has been associated with self-referential thought is similar to the network of structures observed in association with the phenomenon of psychological stress.

Psychological stress is a potent trigger of the most important neuroendocrine stress system in animals and humans, the HPA axis. In response to perceived stress, hypothalamus releases CRF, which induces secretion of ACTH from the pituitary. Circulating ACTH targets the adrenal cortex and induces synthesis and secretion of glucocorticoids (cortisol in humans,

corticosterone in rats) from the adrenal cortex. Released glucocorticoids exert their effects on several target systems throughout the organism, including the central nervous system, metabolic, immune and cardiovascular systems, all with an aim to increase the availability of energy substrates and to allow optimal adaptation to heightened demands from the environment. Moreover, glucocorticoids impact on subsequent HPA axis activation via negative feedback exerted on the axis at the level of pituitary and hypothalamus. An additional regulatory network is formed by structures that are also high in glucocorticoid receptors, namely, HC, PFC, and AG.

The HC exercises a primarily inhibitory input to the HPA axis, through a network of interneurons connecting to the PVN of the hypothalamus (Smith and Vale 2006). In addition, the HC has been implicated in an assessment of stressor intensity (Herman, Dolgas et al. 1998; Figueiredo, Bruestle et al. 2003; Herman, Ostrander et al. 2005). Similar to the HC, the MPFC has been associated with the inhibition of the HPA axis, again through the Peri-PVN. Bilateral lesions of the ACC and prelimbic cortex increase ACTH and glucocorticoid responses to stress (Figueiredo, Bruestle et al. 2003). Thus, both HC and PFC play a role in the glucocorticoid-mediated feedback inhibition of the HPA axis. In contrast, the AG is believed to potentiate HPA axis activity. However, similarly to HC and PFC regions, the influence of the AG on the HPA axis has also been reported to be stressor specific (Fuchs and Flugge 2003; Herman, Ostrander et al. 2005).

Until recently, animal and human pharmacobehavioural studies were the method of choice to increase knowledge about the involvement of particular brain

areas in stress processing (Brody, Preut et al. 2002; Herman, Figueiredo et al. 2003; Soderpalm, Nikolayev et al. 2003; Herman, Ostrander et al. 2005; Fries, Hellhammer et al. 2006; Uhart, Chong et al. 2006). However, with the recent advances in neuroimaging research it has become possible to examine changes that are taking place in the central nervous system in response to an acute psychological stressor, noninvasively, and in real time. A series of studies have been published over the past decade describing the experimental paradigms and their results. However, the results are quite divergent, and an overall interpretation of the effects of stress on neural network activity at this time is rather difficult. Most likely this is due to the significant variability in the experimental paradigms used to elicit an acute stress response. Thus, the goal of this review is 2-fold. First, we want to summarize results from recent neuroimaging studies on the neural activity in response to an acute psychological stressor, from the perspective of task design. Second, we want to assess the appropriateness of the different stress tasks used, keeping in mind the findings of the psychological stress literature as to what is stressful.

Script-Driven Stress Stimuli

Two recent studies have investigated neural circuits that underlie emotional stress processing by using script-driven stress stimuli. Sinha et al (2004) developed 3 personalized stress and 3 neutral imagery scripts on a per subject basis, just prior to a fMRI session. In the scanner, 6 trials were employed: 3 stress and 3 neutral trials, in a randomized fashion. Each trial lasted about 5

minutes. In their sample of 8 adult subjects ($n = 7$ men, $n = 1$ woman) they reported that stress imagery resulted in an increased activation in the right MPFC, and the ventral ACC. In addition, they reported increased activation in specific limbic and midbrain regions: left striatum, thalamus, bilateral caudate and putamen, left hippocampal and parahippocampal regions, and the posterior cingulate. The authors did not sample cortisol throughout the fMRI experiment.

Yang et al (2007) used pictures from the International Affective Picture System to investigate sex differences in the hemodynamic response of the prefrontal area to emotional stress. Thirty volunteers ($n = 11$ men, $n = 19$ women) viewed 2 sets of pictures: an emotionally neutral set of pictures of household objects, and a negative set of pictures of mutilated or bloody bodies and accident situations. Each picture was presented to a volunteer for 5 seconds and each condition contained 20 stimuli. Oxy-HB changes in the prefrontal areas were measured using a 16-channel near-infrared spectroscopy system. As in the previous study, an increase of oxy-HB occurred in the PFC during the stress pictures period, compared with the neutral condition. Further, there was a significant interaction between the task and sex of the participants: oxy-HB increase induced by the emotionally negative pictures was present only in women, but not in men. The study concluded that sex differences observed within prefrontal regions reflect distinct hemodynamic responsiveness in men and women to stress pictures. These authors also did not sample cortisol before, during or after the fMRI experiment.

While the tasks used in these 2 studies are referred to as emotional stress paradigms, they are more likely to activate areas involved in emotional memory

processing (first study) or negative affective processing (first and second study) (Table 1). Moreover, none of these studies measured cortisol release, thus it is unclear whether the paradigms led to a significant activation of the HPA axis. Finally, in the second study, owing to the limitation of the near-infrared spectroscopy, the authors were only able to show changes in prefrontal regions, and thus could not draw any conclusions about possible changes in medial temporal lobe limbic areas like HC and AG.

Stroop Color-Word Interference Task

Studies that investigate blood pressure reactivity and cardiovascular disease often use the Stroop Color-Word interference task. Here, the participants are required to identify the colour of the target word, which is either congruent or incongruent with the colour that the target word names, by selecting 1 of 4 identifier words (which can again be in either congruent or incongruent colour) that name the colour of the target word. Gianaros et al (2005) applied the Stroop Color-Word interference task in an fMRI setting to investigate neural correlates of blood pressure during stress (incongruent words with time restraint were used as a stress condition, and congruent words as a control task). Results from 20 subjects ($n = 9$ men, $n = 11$ women) showed that blood pressure increased from the congruent to the incongruent condition. In addition, the increased mean arterial pressure correlated with activation in the perigenual and mid-anterior cingulate cortex, bilateral anterior and mid-insular cortex, and medial and bilateral PFC. Activation in DLPFC, basal ganglia, thalamus, and cerebellum were also reported.

Interestingly, BOLD activation in perigenual and ACC accounted for moderate percentage of the variance in mean arterial pressure. The authors concluded that their study allowed them to characterize cortical and subcortical brain systems that regulate cardiovascular reactions to behavioural stressors in humans. Again, these authors also did not sample cortisol throughout the fMRI experiment.

A follow-up study with a similar task on menopausal women ($n = 50$), found that incongruent minus congruent condition comparison elicited an increased activity of the DLPFC, ACC, supplementary motor area, parietal cortex, occipital cortex, caudate, and cerebellum (Gianaros, Jennings et al. 2007). Increase in blood pressure from baseline to the incongruent trials was correlating positively with activation in left anterior insula, the posterior cingulate cortex, bilateral lateral PFC, and the left cerebellum (Table 1). The authors concluded that their study demonstrated that an acute psychological stressor can effect neural activation in cingulate, orbital prefrontal, insular, and even cerebellar areas, and that these correlated with concurrent changes in autonomic and cardiovascular reactivity.

While both studies implicated DLPFC and ACC in the psychological stress processing and cardiovascular reactivity, the major limitations of this stress task paradigm are the lack of social evaluative threat component and the lack of cortisol measures. It could be argued that these studies simply observed neural activation changes in response to increased cognitive load, one of the main differences between the congruent and incongruent tasks. Increases in heart rate and blood pressure only point to the involvement of the cardiovascular system,

which is unspecific to stress; thus no firm conclusions can be drawn from these experiments.

Speech in Front of an Audience:

In the behavioural studies of psychosocial stress induction and processing, one of the most established tasks to reliably induce stress and to consistently elicit a significant cortisol response is the TSST (Kirschbaum, Pirke et al. 1993; Kudielka, Buske-Kirschbaum et al. 2004; Kajantie and Phillips 2006). The TSST is traditionally composed of a public speaking component (5 minutes; usually a mock job interview) and mental arithmetic (5 minutes; serial subtractions) in front of an audience.

Tillfors et al (2001) attempted to adapt the public speaking task to a ^{15}O water PET environment to investigate CBF during a stressful task in a group of subjects with social phobia. The subjects were asked to speak on a subject of travel or vacation either alone (control condition) or in the presence of a 6- to 8-member audience (stress condition). When they compared data of 18 social phobia subjects ($n = 10$ men, $n = 8$ women) to 6 healthy controls (3 women), they found CBF increases in the social phobia group in the right AG complex, extending into the HC. These increases were positively correlated with self-reported fear, and were absent in the control group. The CBF decreased in people with social phobias, and increased in control subjects more in the insular cortex and right temporal pole, while CBF increased in controls in perirhinal and retrosplenial cortices. Orbitofrontal cortex tended to decrease more in the social

phobia group than in the control subjects. The authors concluded that the activity in these areas may reflect emotional dysregulation linked with failure to inhibit negative affect.

A subsequent study from the same group investigated CBF associated with anticipation of public speaking in participants with social phobia (Tillfors, Furmark et al. 2002). In an anticipation condition, participants spoke alone prior to speaking in front of an audience, and in a control condition, they spoke alone subsequently to speaking in front of an audience. Increases in state anxiety were found to be associated with the anticipation condition and led to enhanced CBF in the right DLPFC, left inferior temporal cortices, and left AG-HC region. CBF was lowered in the left temporal pole and bilaterally in the cerebellum, in the anticipation group. The authors speculated that perfusion in the right DLPFC reflects affective working memory, and may be critical when a person is anticipating future affective outcomes.

These 2 studies are successful and impressive examples of adapting public speaking like in the TSST to the constraints of a neuroimaging environment. Missing cortisol measures likely reflect a limitation of these studies, because it is again difficult to conclude whether these tasks were indeed stressful, and led to the activation of the HPA. Further, the fact that they focused specifically on a population of social phobia patients makes generalizability of these findings slightly more difficult. A finding of note is the reduced activity in the orbitofrontal region, a finding that is consistently reported with other paradigms as well.

Serial Subtraction

As previously stated, the TSST has 2 main components: public speaking and mental arithmetic consisting of serial subtraction. One line of neuroimaging studies has, thus, attempted to incorporate serial subtraction tasks in the scanner to induce psychological stress.

The first study that used serial subtraction as a stress task aimed to investigate the central nervous system effects of stress in patients with CAD using PET (Soufer, Bremner et al. 1998). In the control task, the subjects counted serially backward from 500, while during the stress condition, subjects needed to perform serial subtraction of 7s from a 4-digit number (if subjects were unable to do this, easier subtraction was provided). As an element of uncontrollability, the patients were prompted for faster performance while the base number from which they were subtracting was changed. Ten CAD males and 6 healthy controls performed the ^{15}O infusion PET scanning consisting of 2 baseline, 2 control, and 2 mental stress scans. In patients, compared with the controls, the mental stress condition resulted in an increased activation in left parietal, left ACC, left fusiform, cerebellum, and right visual association cortex. In the group of controls, however, the only significant activation in that contrast was the left inferior frontal gyrus. Decreases in CBF were found in patients, compared with controls, in the right thalamus, right superior frontal gyrus, and right middle temporal gyrus. Within the patient group, those who suffered stress-induced myocardial ischemia, compared with those who did not, had greater activation in the HC and left parietal cortex, left superior and middle frontal gyrus, and right temporal pole.

As expected, outlined regions have been involved in visual and verbal memory, and are integral to performance of mental arithmetic task. In addition, certain areas have also been specifically implicated in stress and emotion (ACC and visual association cortices). The authors interpreted depression of right hemisphere areas as potentially reflecting the underuse of strategies of the right hemisphere, particularly by CAD patients vulnerable to mental stress-induced myocardial ischemia.

Subsequent study by Ito et al (2003) investigated changes in CBF, as well as myocardial blood flow, during mental stress as measured by the dual $C^{15}O$ and $H_2^{15}O$ PET approach. Ten healthy men were asked to perform serial subtraction of 7s from a 4-digit number as quickly and accurately as possible. Because the subjects were asked to provide verbal responses, a head-fixation system with individual molds were used to minimize head movement. The results showed an increase in adrenaline and noreadrenaline in response to stress. CBF of the cerebellum and putamen significantly increased during mental stress; however, no increase was found in absolute CBF in relation to mental stress in the whole cerebrum. Myocardial blood flow significantly increased during the mental stress activity. Relative hyperfusion during the mental stress (measured by anatomic standardization analysis) was observed in the bilateral cerebellum, bilateral thalamus, right insular cortex, right superior temporal gyrus, bilateral inferior frontal gyrus, bilateral precentral gyrus, bilateral ACC, and left angular gyrus. No significant decreases were observed. The authors concluded that the activated

regions may be associated with linguistic function, attention, and working memory.

While these serial subtraction tasks do have a potential to elicit a significant stress response, the social evaluative component introduced here may not be effective enough. As cortisol was not measured in these 2 studies, we cannot conclude anything definite about the stressfulness of these paradigms.

Given that stress plays an important role in the onset and development of psychiatric illnesses such as depression and schizophrenia, Montgomery et al (2006) aimed to investigate a potential contribution of the dopamine system as a neurochemical mediator of these clinical observations. In addition, they investigated influence of maternal care on these associations, because maternal care has been consistently found to influence both cortisol and dopamine responses, throughout life, in animals (Liu, Diorio et al. 1997; Hall, Wilkinson et al. 1999; Seckl 2004). Further, a study by Pruessner et al (2004) found supporting evidence for these associations in human population as well (Pruessner, Champagne et al. 2004). In a [^{11}C] raclopride PET study, 14 volunteers ($n = 9$ women) completed a serial subtraction task. Of importance, all subjects provided serial cortisol samples. They completed 5 blocks of baseline tasks that consisted of subtracting 1 from a 1000. For the stress task, the subjects counted backwards in 7s for 2 blocks from 1000 and 1001, respectively, and then they counted backwards in 13s for 2 blocks from 2000 and 2001; during the break between the 2 blocks of stress, the subjects were told that they should do better. This task was not stressful, as there was no significant difference in absolute cortisol between

stress and nonstress condition, although following stress there was a trend for an increase. Further, no significant changes were observed in binding potential between stress and nonstress condition in the striatum, or the whole brain. Finally, no relation between maternal care and change in binding potential was detected.

Montgomery et al (2006) were not able to replicate findings from Pruessner et al (Pruessner, Champagne et al. 2004). However, their methods differed with respect to the stress task, [^{11}C] raclopride administration, and potential presence and contribution of head movement to signal noise. Moreover, only a small number of subjects ($n = 3$) reported low maternal care.

In 2005, Wang et al (2005) used perfusion functional MRI to investigate CBF during psychological stress. Importantly, salivary cortisol levels were obtained during scanning. Their psychological stress task consisted of serial subtraction of 13 from a 4-digit number. Twenty-three subjects ($n = 11$ men, $n = 12$ women) were required to give their answers verbally. They were also prompted for faster performance and were required to restart the task if an error occurred. In a low stress condition, the subjects counted aloud backward from 1000. The scan began with a baseline condition, followed by low and high stress conditions, and ended with a second baseline condition. The stress task was effective in inducing a stress response as salivary cortisol increased to reach a peak 10 minutes after the end of the high-stress task. Further, a positive correlation was found between CBF and perceived stress scores in right ventral PFC, as well as left insula–putamen. Lasting effects of psychological stress (baseline 2 minus baseline 1 condition) also correlated with right ventral PFC, as

well as ACC and the right insula–putamen. Moreover, significant correlations between CBF changes during stress and area-under-the-curve measures of cortisol (reflecting cumulative cortisol change) were found in the anteromedial PFC. Random effects model of high, compared with low, stress condition revealed increased CBF in the right insula–putamen, DLPFC–ACC, precuneus–superior parietal gyrus, and left inferior temporal region. Suppressed CBF was observed in the left ventrolateral PFC, and orbitofrontal cortex.

The authors conducted further analyses including perceived anxiety scores from the stress task. A strong correlation between changes in CBF (high–low stress) and subjective anxiety rating during stress were found in left insula–putamen–amygdala, and superior temporal regions. Positive correlations between CBF changes and perceived anxiety level during stress tasks were also evident in right putamen, AG, HC, and right superior temporal regions. The central finding of this study was that right ventral PFC activation is specifically associated with psychological stress, and this activity persists even beyond the stress task period. The authors interpreted the persistence of the right ventral PFC activation, even after completion of stress tasks, as potentially reflecting a prolonged state of heightened vigilance and emotional arousal that is elicited by stressors.

Recently, Wang et al (2007) added to the investigation of neural responses to psychological stress by conducting a study on sex differences. By using the same perfusion fMRI stress task as in their previous publication, Wang et al investigated 32 subjects ($n = 16$ men, $n = 16$ women). Overall, the task was successful in eliciting a cortisol stress response. Regarding perceived stress, men

reported a greater increase in perceived stress, compared with women. In addition, in men only, there was an increase in CBF in the right PFC during the stress condition and afterwards. Further, the authors reported a suppression of the left orbitofrontal–inferior frontal cortex in men, both during the stress task and at baseline 2. In women, the reduction in the activity of this region was only significant during the performance of stress task. Regarding the limbic regions activity during the stress task, men did not exhibit any stress related brain activation. In women, the task was associated with CBF increase in the basal ganglia structure, namely, left insula–putamen, right insula, bilateral ventral striatum, including caudate and globus pallidus. Further, the hippocampal CBF was positively correlated with perceived stress during tasks in the female group, but negatively associated with perceived stress in the male group.

After the stress task, persistent ACC, PCC, and right insula increases were associated with increased stress. In male subjects, baseline CBF increase in the right PFC and CBF reduction in the left orbitofrontal–inferior frontal gyrus were correlated with AUC measures of salivary cortisol. Significant cortisol related CBF increases were observed in the dorsal ACC and left thalamus only in the female but not the male group. Right PFC was proposed as a clear factor that separates the male and female group as a neural correlate of stress. In terms of behavioural measures of anxiety, neither sex nor the interaction of sex and experimental condition showed a significant effect. Regression analysis of CBF data with perceived anxiety revealed primary limbic activation in both sexes. (For the summary of findings, see Table 1.)

These studies have yielded support for the use of serial subtraction as stress task and have added highly interesting findings to the literature regarding the involvement of neural activity in the ACC and orbitofrontal regions during stress. Remaining issues include the use of vocalized subtraction, leading to concerns about head movement and its impact on integrity of the imaging data. Another reservation that remains is the use of the perfusion fMRI technique. It is a relatively new technique that has seen only limited use to date, and it restricts the comparability of results with other fMRI studies. Compared with traditional BOLD, perfusion MRI has improved sensitivity for slow changes in neural activity, reduced intersubject variability, more specific functional localization and generally reduced susceptibility effects (Aguirre, Detre et al. 2002; Detre and Wang 2002; Wang, Short et al. 2003). Conversely, it has reduced magnitude of the signal change for perfusion, comp2ared with BOLD, as well as an inferior image coverage in arterial spin labelling methods, not ideal for whole brain studies (Detre and Alsop 1999; Aguirre, Detre et al. 2002).

The Montreal Imaging Stress Task

The MIST is composed of a series of computerized mental arithmetic tasks with an induced failure algorithm. A social evaluative threat component is built into the program, but is also implemented by the investigator providing negative feedback between scanning sessions (Dedovic, Renwick et al. 2005).

Using the MIST, in 2004, we investigated dopamine release in response to a psychological stress in a [^{11}C] raclopride PET study (Pruessner, Champagne et

al. 2004). During the stress scan, subjects were shown mental arithmetic tasks on a computer screen that they needed to solve within a given amount of time. In addition, the computer screen also displayed information concerning the total number of errors, expected average number of errors, time spent on the current problem, and performance feedback for each problem. Further, the algorithm adapts to individual user performance, producing slightly more difficult equations than what the subject is capable of solving, resulting in a poor performance. The stress session was contrasted to a rest session, where subjects would look at an empty screen. Prior to the task, we had screened 120 subjects for self-reported parental care during the first 16 years of their lives; we invited 5 subjects with high and 5 subjects with low parental care for the scanning sessions. Data analysis from high and low mother care groups revealed a significant release of dopamine in the ventral striatum in response to stress only in the low maternal care subjects. In addition, the magnitude of the salivary cortisol response to stress was significantly associated with the reduction of $[^{11}C]$ raclopride binding in the ventral striatum. The data from this study suggested that psychological stress may be associated with mesolimbic dopamine release in humans with increased stress sensitivity. Further, this study suggested that the MIST may be a suitable method to investigate psychological stress in neuroimaging environments.

Soliman et al (2008) followed up on this study by investigating stress-induced dopamine release in humans at risk of psychosis. Ten normal healthy control subjects and 16 subjects reporting high schizotypical behaviour were subjected to the same procedure as in Pruessner et al (Pruessner, Champagne et al.

2004). Both groups showed significant increases in self-reported stress and cortisol secretion between stress and control conditions. When subjects were divided into control, positive schizotypy, and negative schizotypy groups, significant dopamine release in response to stress was only seen in negative schizotypy group, highest in the ventral striatum, and then in putamen and caudate. Analysis was also conducted with respect to maternal care, and it was established that changes in [^{11}C] raclopride binding potential were significantly related to maternal care scores across all subjects. Further, subjects reporting low maternal care showed the greatest stress-induced dopamine release, directly replicating the previous findings. Further, these findings suggest a heightened sensitivity of the dopamine system in negative symptom schizotypy.

Finally, we investigated brain activation changes associated with perception of and metabolic response to stress in both PET and fMRI environments (Pruessner, Dedovic et al. 2008). Ten male subjects were tested in a ^{15}OH$_2$O PET study, while 40 subjects ($n = 20$ men, $n = 20$ women) performed the MIST during an fMRI scan. The task was modified to now include rest, control, and experimental-stress condition. The task was designed so that in the experimental condition, the difficulty of the equations was manipulated to generate a 45% to 50% performance range. Further, the subjects were exposed to a mock user performance indicator that implies a poor performance of the subject in comparison with the average user. Also, negative feedback regarding the performance was provided both by the program and the investigator after each run. The control condition contained mental arithmetic tasks similar in difficulty,

but without the social evaluative threat and negative feedback components. During the rest condition, only the user interface is displayed without mental arithmetic tasks being shown. This task elicited a significant cortisol stress response in both the PET and fMRI study. However, in the fMRI study, there was a significant heterogeneity in the individual cortisol responses. Subjects were split into those who showed a cortisol increase (responders), and those who did not react with respect to cortisol changes (nonresponders). Analysis of the neuroimaging data revealed that there was a profound deactivation of the limbic system including HC, hypothalamus, medioorbitofrontal cortex, and ACC in subjects who showed a significant stress response (Table 1). Moreover, in the fMRI study, the level of deactivation in the HC correlated with the release of cortisol in response to the stress task. We have proposed that the observed limbic system deactivation during stress may be observed owing to a heightened baseline activation of the HC (default mode network activity). As the HC inhibits the HPA via the Peri-PVN, it is therefore plausible that the reduction in limbic system activity as observed during stress activates the HPA axis, and initiates the stress response.

The advantage of the MIST is that it does not require verbal responses from the subject and therefore reduces the contribution of the head movement to the signal noise. Also, by adapting the task difficulty to individual user performance, differences in aptitude across subjects are being controlled for (or, to refer back to Lazarus, the demands of the situation always exceed the available resources). In addition, it incorporates social–evaluative threat components

through program and investigator feedback. However, despite these components, it seems to be only effective in subgroups of people, for example, subjects with low maternal care, or low self-esteem. Further, it is very much an achievement-oriented task, in that it requires the subjects to perform a task according to certain expectations. However, not all subjects may be equally susceptible to these kinds of stress tasks.

Discussion

Recent neuroimaging studies aimed at investigating effects of psychological stress on the neural activity have applied a range of different experimental paradigms to elicit an acute stress response. However, owing to various methodological limitations, only a few have been successful in eliciting and recording an accompanying hormonal stress response (Pruessner, Champagne et al. 2004; Dedovic, Renwick et al. 2005; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008; Soliman, O'Driscoll et al. 2008). These studies have put forth interesting evidence regarding the involvement of prefrontal and limbic regions in psychological stress processing. The most consistent finding is that of reduced CBF in the orbitofrontal regions in response to stress (Tillfors, Furmark et al. 2001; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008). The orbitofrontal cortex has been involved in gathering and integrating sensory information from the body and from the environment (Gusnard and Raichle 2001), participating in voluntary emotional control (Fredrikson, Wik et al. 1995), as well as representing

and updating the value of possible future outcomes (Amodio and Frith 2006). Therefore, it may have a potential role in the initial stress perception as well as perseverance of the stress response.

A change in CBF in ACC is also consistently reported (Soufer, Bremner et al. 1998; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008). Neural activation changes in the ACC have been implicated in stress, cognitive and emotional regulation, and even ruminative thinking. However, the fact that the direction of change in ACC seems dependent on the specific task leaves its exact role in stress processing still open to interpretation. Finally, evidence has been reported for the role of HC in the stress response (Pruessner, Dedovic et al. 2008). Upon perception of stress, deactivation of the HC may lead to a disinhibition of the HPA axis and initiation of the stress hormone release, a finding in line with animal studies investigating the regulatory role of the HC in HPA axis activity (Herman, Ostrander et al. 2005).

In conclusion, numerous designs have been developed to study neural effects of psychological stress task in a neuroimaging environment. Nevertheless, more research is needed to develop a neuroimaging stress task that reliably induces stress, across populations and across laboratories, without the use of deception. Only when this goal is achieved will we be able to fully understand the neural effects of stress.

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Tables

Task	Increase in Brain Activity	Decrease in Brain Activity
Script-driven stress stimuli	-R medial PFC -Ventral ACC -PCC -Bilateral basal ganglia and L striatum, -Thalamus -L hippocampus and parahippocampal regions	------
Stroop color-word interference task	-Medial and bilateral PFC -DLPFC -Perigenual and mid-ACC -PCC -Bilateral insular cortex -Parietal cortex -Basal ganglia -Thalamus -Cerebellum	------
Speech in front of an audience	-R DLPFC -Amygdala-hippocampus complex -Perirhinal and retrosplenial cortices -Insular cortex -Temporal cortices	-Orbitofrontal cortex -Insular cortex -R temporal pole
Serial subtraction	-R ventral PFC -DLPFC -ACC -PCC -L parietal regions and angular gyrus -Inferior, middle and superior frontal gyri -Bilateral precentral gyrus -R insular cortex -Basal ganglia and ventral striatum -Temporal cortices -Hippocampus -Thalamus -Cerebellum	-R superior frontal gyrus -R middle temporal gyrus -R thalamus -L ventrolateral PFC -Orbitofrontal cortex.
Montreal Imaging Stress Task	-L medial PFC -Cingulum -Occipital cortex -L premotor area -Dopamine release in ventral striatum and basal ganglia	The limbic system including: medio-orbitofrontal cortex -ACC -hippocampus -hypothalamus,

Table 1: Summary of reported changes in the brain activity based on the neuroimaging stress task applied. ----- = no data; L: left, R: right

Chapter 3: Neural correlates of processing stressful information: an event-related fMRI study.

Dedovic K, Rexroth M, Wolff E, Duchesne A, Scherling C, Beaudry T, Lue SD, Lord C, Engert V, Pruessner JC.

Preface to Chapter 3

From the previous chapter we can conclude that while many groups had aimed to investigate neural correlates of psychological stress processing, only a few had implemented the situational components which are key to psychological stress (motivated performance task, uncontrollability, and social evaluative threat) in their task design. Of those who had, a certain overlap of reported brain areas underlying psychological stress processing could be found. Specifically, deactivation of the orbitofrontal cortex was most consistently featured, and there was evidence for involvement of hippocampus and anterior cingulate cortex in processing of stress.

Furthermore, the review revealed that often times in these designs, the stress condition differed from the control condition not only in the presence of a social evaluative threat, but also with respect to the math difficulty that the subjects were required to complete (for example, Dedovic, Renwick et al. 2005; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008). With respect to the MIST specifically, although math tasks were similar between the experimental stress and control condition, there was no time limit set for completion of the math tasks in the control condition, therefore making the task more feasible and not stressful. Of course, all the evaluative components were also taken away in the control condition. Thus, it still remained unclear whether the deactivation observed in the limbic regions in the responders was due

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to possible differences in math processing or due to differences in processing of social evaluative threat components.

In order to address this limitation, we designed an event-related version of the Montreal Imaging Stress Task (eventMIST) (Dedovic, Rexroth et al. 2009). The eventMIST allowed us to dissect the specific components of the MIST and evaluate the processing of mental arithmetic and social evaluative threat components separately.

Unexpectedly, the eventMIST also proved to be a unique stress task, in that it elicited a significant cortisol secretion only in those individuals with high self-concept. This is unlike the block design MIST where responders tended to have lower levels of self-esteem compared to non-responders (Pruessner, Dedovic et al. 2008).

MANUSCRIPT

Title:

Neural correlates of processing stressful information: An event-related fMRI study

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Abstract (max 250 words):

Recent neuroimaging studies investigating neural correlates of psychological stress employ cognitive paradigms that induce a significant hormonal stress response in the scanner. The Montreal Imaging Stress task (MIST) is one such task that combines challenging mental arithmetic with negative social-evaluative feedback. Due to the block-design nature of the MIST, it has not been possible thus far to investigate which brain areas respond specifically to the key components of the MIST (mental arithmetic, failure, negative social evaluation). In the current study, we developed an event-related MIST (eventMIST) in order to investigate which neural activation patterns are associated with performing mental arithmetic versus processing of social evaluative threat. Data was available from twenty healthy university students. The eventMIST induced a significant stress response in a subsample of subjects, called the responders (n=7). Direct comparison between brain activity changes in responders *versus* non-responders, in response to the challenging math, revealed increased activity bilaterally in dorsomedial prefrontal cortex (PFC), left temporal pole, and right dorsolateral PFC. In response to negative social evaluation, responders showed reduction of brain activity in limbic system regions (medial orbitofrontal cortex and hippocampus), which was largely lacking in non-responders. Direct comparison between the groups for this contrast did not reveal any significant difference, probably due to small number of events available. This is the first study to use an event-related paradigm to investigate brain activity patterns in relation to challenging math and social evaluative threat separately.

Introduction:

An individual's response to a psychological stressor is determined by specific situational and personality factors (Dickerson and Kemeny 2004; Pruessner, Baldwin et al. 2005). Over the past four decades, numerous behavioral studies have identified which situational (e.g., uncontrollability of the situation, social-evaluative setting;(Mason 1968) and personality factors (e.g., self-esteem;(Kirschbaum, Klauer et al. 1995) contribute to the stress response in standardized laboratory settings (for reviews, see (Biondi and Picardi 1999; Dickerson and Kemeny 2004). In contrast, neuroimaging studies have only recently begun to investigate the neural correlates of the stress response. While these studies were able to identify the specific brain networks underlying the processing of a stressful situation (Pruessner, Champagne et al. 2004; Dedovic, Renwick et al. 2005; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Kern, Oakes et al. 2008; Pruessner, Dedovic et al. 2008; Dedovic, D'Aguiar et al. 2009), they have not as yet examined which of these specific brain areas underlie the processing of each of the key elements of the stressful situation. Thus, in the present study, we aimed to investigate the neural correlates of the different components of a stressful situation.

The increased secretion of the hormone cortisol in response to stress is a consequence of the activation of the hypothalamic-pituitary-adrenal axis (HPA), the major stress axis in humans. Upon perception of a stressful stimulus, corticotropin-releasing hormone (CRH) is secreted from the hypothalamus and travels to the anterior pituitary (Brown 2000). At this level, it induces the release

of adrenocorticotropic hormone (ACTH) into the bloodstream; ACTH eventually reaches the adrenal cortex, where it initiates the synthesis and secretion of glucocorticoids (cortisol in humans, corticosterone in rats;(Brown 2000). The released cortisol, in turn, targets multiple sites related to metabolic, immune, cardiovascular and central nervous system (CNS) functions, which can mostly be summarized as serving to increase energy availability. Cortisol further contributes to its own regulation (McEwen 1998; Buckingham 2006; Lupien, Maheu et al. 2007), by binding to key feedback sites in the CNS: at the level of the pituitary and hypothalamus, as well as hippocampus, amygdala and prefrontal cortex (PFC; (Feldman and Weidenfeld 1995; Herman and Cullinan 1997; Herman, Ostrander et al. 2005).

A recent meta-analysis of over 200 behavioral studies suggests that completing an uncontrollable motivated performance task while being socially evaluated will reliably elicit a hormonal stress response (Dickerson and Kemeny 2004). The presence of social evaluation (considered social-evaluative threat by the authors), in particular, seems to be a key ingredient for a strong, significant activation of the HPA axis (Dickerson and Kemeny 2004).

Neuroimaging studies aiming to investigate the neural correlates of stress have faced a major challenge: needing to employ paradigms that integrate the key elements of psychological stress paradigms within the constraints of neuroimaging environment and, thus, be able to reliably induce a hormonal stress response (for a review, see (Dedovic, D'Aguiar et al. 2009)). The problem here is that some of the elements of stressful situations (e.g., social evaluation) are

difficult to implement when the subject is submerged in the Magnetic Resonance Imaging scanner, and isolated in the scanner room. Successful neuroimaging stress tasks have thus employed serial subtraction with verbal feedback similar to that used in the Trier Social Stress Test (Kirschbaum, Strasburger et al. 1993; Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007), and computerized mental arithmetic with built-in social evaluation (Dedovic, Renwick et al. 2005).

Studies employing serial subtraction and social evaluation reveal an increased cerebral blood flow (CBF) in dorsolateral PFC / anterior cingulate cortex (ACC) region, along with increases in precuneus-superior parietal gyrus, insula/putamen, and inferior temporal region (Wang, Rao et al. 2005), when contrasting low from high stress conditions. Suppressed CBF was found in left ventrolateral PFC and orbitofrontal cortex (Orb; (Wang, Rao et al. 2005).

Our own development, the Montreal Imaging Stress Task (MIST; (Dedovic, Renwick et al. 2005), allows investigation of interindividual differences in stress responsivity by distinguishing between responders and non-responders (usually 50% of the sample shows a significant stress response; (Dedovic, Renwick et al. 2005; Pruessner, Dedovic et al. 2008). Using the MIST, in line with findings from other serial subtraction tasks, we could show that psychological stress is associated with reduced activity in the medial orbitofrontal cortex (mOrb) and the ACC, reduced activity in dorsolateral PFC, and a distinct deactivation of a cluster of limbic system structures, including hippocampus, hypothalamus and amygdala (Pruessner, Champagne et al. 2004; Wang, Rao et al. 2005; Soliman, O'Driscoll et al. 2007; Wang, Korczykowski et al. 2007;

Pruessner, Dedovic et al. 2008).

While the previous neuroimaging stress studies revealed which brain areas are involved in stress processing in general, the employed paradigms were not suitable to identify specific neural correlates of each of the situational components of the stress tasks. For example, in the MIST, the stress condition is a combination of an increased cognitive demand, a social evaluative threat, and the processing of failure in the presence of a success expectation (Dedovic, Renwick et al. 2005). Due to the limitations of the block design, it has not been possible to differentiate specific situational characteristics from each other with regard to the resulting neural activation correlates.

To overcome this limitation, we recently developed an event-related version of the Montreal Imaging Stress Task: the eventMIST. The eventMIST was created as a rapid onset event related functional Magnetic Resonance Imaging design (Burock, Buckner et al. 1998). Within such a design, rest, control, and experimental task components, together with their respective feedbacks are presented in a randomized order. Consequently, the investigation of brain activity patterns associated with the different task components (difficult math vs. control math as variations of cognitive load) and the different social evaluative components (negative feedback vs. positive feedback) became possible. Based on our previous findings and what is known about the functional correlates of the involved structures, we hypothesized that the previously observed deactivation of limbic system structures, particularly the mOrb regions and the hippocampal area, is linked to the negative social evaluation, while the activity in the ACC and

lateral PFC areas may be linked to the cognitive task itself. In addition, we aimed at replicating earlier results of interindividual differences in stress responsivity by detecting groups of responders and nonresponders. Thus, we also planned to examine differences in neural activity patterns and personality traits between these two groups of subjects.

For this study, we exposed 28 subjects to two runs of the eventMIST. Throughout the procedure, we sampled their saliva for subsequent cortisol analysis, starting at 40 minutes before to 60 minutes after the onset of the stressor, in ten to twenty-five minute intervals. During the eventMIST, we recorded brain activation changes associated with experimental and control math, and positive and negative feedback, which subsequently defined specific event types. In addition, we obtained personality measures to be able to covary endocrine response types with specific personality traits.

Results

Behavioral:

Cortisol Stress Responses:

Upon inspecting descriptive statistics of the cortisol data, an outlier (+ 3 SD from the mean) was identified and excluded from all subsequent analyses. Cortisol values for the whole group were not normally distributed, thus we used Friedman's ANOVA for these analyses. Results revealed that there was no significant effect of time on the cortisol levels for the whole group (F_r=7.863, p>0.05), indicating that subjects overall did not show an increase in cortisol

levels. Based on the results of previous studies employing the MIST, we expected the presence of responders and nonresponders within the sample. In order to establish a meaningful separation of the total group, we employed a k-means cluster analysis using the cortisol samples just prior and during the MIST and up to 30 minutes after (Wishart 1998). This two-group cluster solution resulted in 10 responders and 17 nonresponders. However, due to further data loss during transfer (three subjects), excessive head movement (two subjects), lack of events (one subject) and an error during stimulus presentation (one subject), the final analyses could only be performed on 7 responders and 13 non-responders.

As the cortisol values for the remaining subjects were normally distributed, we performed a two factor mixed design (group x time) ANOVA. The analysis revealed a significant group x time interaction (F=2.607, p=0.048, Greenhouse-Geisser corrected; Figure 1). Simple main effects showed that responders and non-responders differed on each time point (all F≥5.89, all p≤0.024) except for time point one (F=2.49, p=0.130), and that there was an effect of time in the responder group only (F=3.90, p=0.001). Within the responder group, we then further investigated whether there was a difference between the eventMIST baseline (sample taken just prior to eventMIST), and the peak of cortisol secretion (sample taken 15 min following the completion of eventMIST). A paired t-test confirmed a significant difference (t=-3.87, p=0.006).

Personality parameters

Responders and nonresponders did not differ on measures of depression, parental bonding, chronic stress levels or coping styles. With respect to self-

esteem, no differences were found using the Rosenberg scale (Rosenberg 1965). However, for the locus of control measure, the mixed design ANOVA revealed a significant group x Questionnaire of Competence and Control (QCC) scores interaction (F=4.848, p=0.012; Greenhouse-Geisser corrected). Subsequent simple main effects analysis showed that the groups differed in QCC self-concept scores, with responders showing higher scores compared to nonresponders (F=18.32, p<0.001). In addition, there was a significant difference between responders and nonresponders with respect to QCC others control subscale. Here, responders showed lower scores than nonresponders (F=4.44, p=0.047).

We additionally tested subjects' state and trait anxiety levels before and after the eventMIST. For this analysis, we performed a three-factor mixed design ANOVA (group x anxiety x time) and found a significant time x anxiety interaction (F=6.116, p=0.023), as well as a significant group x anxiety interaction (F=7.369, p=0.014). Simple main effects analysis of the former interaction revealed that there was a difference in trait anxiety scores across time, with pre-eventMIST trait anxiety scores being higher compared to post-eventMIST trait anxiety scores (F=5.69, p=0.028). Furthermore, after the eventMIST runs, all subjects scored higher on the state anxiety compared to the trait anxiety (F=15.39, p=0.001). Simple main effects analysis of the latter interaction (group x anxiety) revealed that the responders had overall higher state anxiety scores compared to trait anxiety scores (F=14.24, p=0.001). Moreover, there was a trend for nonresponders to score higher than responders on trait anxiety (F=3.44, p=0.079). Finally, we investigated whether there was any effect of eventMIST on anger and

depression/dejection facets of POMS by applying a three factor mixed design ANOVA (group x mood x time). A trend could be detected for time x mood interaction (F=3.944, p=0.062).

Neural Correlates of stress

Our imaging analysis concentrated on three aspects: first, replicating the block design results by combining all math and feedback events for the whole group; second, investigating the neural correlates of performing difficult math by contrasting the difficult math from the control math; lastly, analyzing the neural correlates of perceiving and processing social evaluative threat by contrasting the negative evaluation events from the positive ones (for the full list of event types see Table 1).

First, in order to compare the eventMIST to the previously reported block design analyses (Pruessner et al., 2008), we combined all experimental math and feedback event types and contrasted control math task and control feedback from these. As shown previously, for the whole group in the experimental minus control condition, we found increased activity in the left ventrolateral prefrontal cortex, occipital lobe, cerebellum and cingulum (FDR corrected p<0.01), while significant deactivations were observed bilaterally in frontal poles, left ventral medial and lateral orbitofrontal cortex, as well as temporal poles and posterior insula and right hippocampus (for detailed list see Table 2). We additionally observed bilateral activation in the anterior insula and right hippocampal tail, as well as deactivation in left dorsolateral prefrontal cortex, left medial ventral

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prefrontal cortex, and bilaterally in putamen, posterior cingulate cortex and precuneus (Table 2, Figure 2).

Second, to investigate specifically the neural correlates of processing complex math problems, we contrasted performing control math task from performing experimental math, irrespective of the subsequent performance. In this contrast, responders showed primarily increased activity in brain areas associated with both cognitive and emotional processing. These areas included increased bilateral activity in the dorsomedial PFC, the anterior insula, and the ventral anterior cingulate cortex, as well as the right dorsal anterior cingulate cortex. Further, the right subiculum also showed increased activity (for detailed list see Table 3). Deactivations were limited to the right ventral precuneus, left dorsolateral PFC and right temporal pole (all FDR corrected $p<0.01$) (Figure 3A).

In contrast, the nonresponders t-map was characterized by both activation and deactivation patterns. The non-responders showed strong activations in the dorsal anterior cingulate and ventrolateral PFC, as well as thalamus and hippocampal tail end. Furthermore, increased activity could be observed in left superior parietal lobule, superior colliculi and supramarginal area. Deactivations were found in the area of the prefrontal cortex: specifically, the frontal poles, as well as the dorsal and ventral medial PFC. Finally, the left temporal poles, the left middle temporal gyrus, and the bilateral posterior cingulate cortex and right ventral precuneus also showed decreased activity (all FDR corrected $p<0.01$) (Figure 3B).

A between-group contrast of non-responders vs. responders revealed bilateral activation in dorsomedial PFC, reflecting the opposing recruitment of this area by responders (activated) and non-responders (deactivated) during difficult math. Furthermore, we observed activation in the left temporal pole reflecting greater deactivation of this area in non-responders compared to responders. Similarly, activation in right dorsolateral PFC was observed, reflecting a significant deactivation in this area in non-responders, which was absent in responders (all FDR corrected $p<0.01$) (Figure 3C). No significant deactivations were observed for this contrast.

Finally, we wanted to examine neural correlates of negative feedback and evaluative threat. To accomplish this, we contrasted correct feedback within the stress condition from timeout feedback also within the stress condition (incorrect feedback events could not be used since there were too few events across subjects). Interestingly, in this contrast, in responders, we found primarily decreases in brain activity, unlike to what had been observed during math processing. Indeed, in responders, increased activity could only be found in the left supramarginal gyrus. Decreased activity, on the other hand, was extensive, encompassing the left ventral and dorsal medial PFC and dorsolateral PFC, as well as left medial ventral and lateral orbitofrontal cortex, and the left ventral anterior cingulate cortex (Table 4). Bilateral basal ganglia, anterior middle temporal gyrus, left fusiform gyrus and right hippocampal body were also deactivated (all FDR corrected $p<0.01$) (Figure 4A).

In comparison, in nonresponders, both increased and decreased activity was observed. Activations were found in left dorsolateral PFC, and right ventrolateral PFC, as well in left lateral orbitofrontal cortex. Additionally, bilateral activations were observed in temporal poles and piriform gyrus, as well as posterior medial superior frontal gyrus. The deactivation pattern in nonresponders included the left frontal pole, bilateral basal ganglia and lateral orbitofrontal cortex. In addition, the right insula and right middle cingulate gyrus, as well as the left subiculum showed decreased activity (all FDR corrected $p<0.01$; Figure 4B).

Despite these differential patterns of brain activity between responders and nonresponders for processing of negative feedback, the direct statistical comparison of responders vs. nonresponders did not reveal any significant differences, probably due to the lack of power (Table 4). Conducting an additional analysis of the contrast between the two feedback types while also modeling for the presence of math tasks prior to the feedback yielded more constricted but similar pattern of results as outlined above, suggesting that the spill over from the math task was minimized by task design and analysis procedure.

Discussion

The present study employed a novel event-related neuroimaging stress paradigm, called eventMIST, in order to distinguish between brain activity

patterns associated with motivated task performance and those underlying the processing of social evaluative threat, in a group of young healthy subjects.

The eventMIST paradigm employed here proved to be a milder stressor as compared to the block-design version: it was able to elicit a significant stress response in only 35% of the sample (dubbed 'responders'), as compared to on average 50% that the original version routinely achieves. With respect to neuroimaging results, the analysis revealed a complex set of findings. In general, and as expected, performing a difficult motivated performance task implicated ventral and dorsal ACC, lateral and medial PFC areas, as well as posterior brain regions. Importantly, direct comparison between the groups (responders>non-responders) for the motivated performance task contrast revealed an increased activity in bilateral dorsomedial PFC, left temporal pole and dorsolateral PFC. In response to negative feedback, in addition to the involvement of medial and lateral PFC areas, we observed changes in activity in medial and lateral orbitofrontal areas, as well as basal ganglia, and right hippocampus. It is worth noting that, only in responders, decreased activity in medial orbitofrontal areas and right hippocampus was observed in response to social evaluative threat components. These two areas have been consistently found in stress processing in our previous studies (Dedovic, D'Aguiar et al. 2009). Direct comparison between responders and non-responders for the social evaluative feedback contrast did not reveal significant differences, perhaps due to a gradient of neural activation in response to stress, as discussed previously (Pruessner et al., 2008).

Interestingly, the responders had a personality profile that differed from what was expected from the literature, and our own previous findings. The responders scored higher on measures of self-concept and lower on external locus of control when compared to the non-responders. Perhaps the eventMIST with its constant randomization and rapid cycling of stress, control and rest conditions, appeals more to individuals who have high cognitive appraisals of their abilities and of their control over outcomes. It may very well be that only these individuals would expect from themselves to do well on this complex task and, when this goal remained unattainable, became stressed. This could then also explain why the responders' higher scores on state anxiety compared to trait anxiety for the duration of the experiment might reflect a state of vigilance, or threat, as the subjects may already be anticipating that they will need to be performing well in the mental arithmetic task. However, given that we did not assess subjective reports of participants' expectations and impressions of the task, these propositions remain hypotheses to be tested in future studies.

Despite the fact that the eventMIST is a different stressor compared to the block design MIST, we were able to replicate some of the previous findings of changes in the BOLD signal; we observed increased activity in the left ventrolateral prefrontal cortex, occipital cortex, cerebellum and cingulum, and decreased activity in frontal poles, orbitofrontal cortex, temporal poles and insula,

for the whole group. In addition, we observed a number of areas both activating and deactivating, which were not previously seen with the block design.

Furthermore, our specific interest was to compare responders and non-responders in their brain activity patterns with respect to processing mental arithmetic versus processing negative feedback. For the motivated task performance contrast, comparison between the groups (responders>non-responders) revealed increased activity in bilateral dorsomedial PFC (reflecting the fact that this region was activated in the responders and deactivated in the non-responders), activation of the temporal pole (reflecting greater deactivation in the non-responders compared to the responders), and activation in dorsolateral PFC (due to the fact that the non-responders showed deactivation in this region, which was absent in the responders).

Previous studies have reported involvement of dorsomedial PFC during both task-related and self-focused attention (Castelli, Happe et al. 2000; Gusnard and Raichle 2001; Paulesu, Sambugaro et al. 2009), where increases from baseline were usually associated with self-focused attention, while decreases from baseline were usually observed in association with externally focused attention (Gusnard and Raichle 2001). Increased activity in this area in responders and decreased activity in non-responders might thus reflect differential involvement of the self-focused attention during task processing. Responders might have had self-

relevant cognitions in the face of a difficult task where they expected to do better, while non-responders did not engage in self-relevant thought during the task. Alternatively, studies have also associated increased activity in dorsomedial PFC with increased levels of worry, both in normal controls and in general anxiety disorder patients (Paulesu, Sambugaro et al. 2009). However, the interpretation could be quite similar in that only the responders worry about their poor performance. The findings of the present study may thus reflect differential appraisal associated with completing a more difficult math task compared to a control task.

Increased activity in temporal poles has been observed when subjects are engaged in mental state attributions (Frith and Frith 2003), memory retrieval (particularly autobiographical memory), recognition of familiar objects, as well as in generating, on the basis of past experience, a wider context for the material currently being processed (Steinbeis and Koelsch 2009). Responders deactivated this region in response to difficult math less so than the non-responders. Differential recruitment of this area relative to the control math task in the two groups may thus reflect differing levels of familiarity with the more difficult math tasks between the two groups, or a stronger effort to find matching past experiences in the group of responders.

Similarly, deactivation of the dorsolateral PFC area in the non-responders when performing difficult math task, may represent a failure to recruit dorsolateral PFC for the effortful manipulation of the more complex information

in this subgroup (Crone, Wendelken et al. 2006). Lateral PFC regions, in general, are involved in working memory including maintenance and manipulation of the information. Specifically, while ventrolateral PFC has been associated with online maintenance of information, dorsolateral PFC engages when additional manipulation of the information is needed (Owen, Evans et al. 1996; D'Esposito, Postle et al. 1999; Smith and Jonides 1999; Wagner, Maril et al. 2001).

Thus, taken together, by using the eventMIST paradigm, we were able to identify specific brain areas that are underlying the completion of difficult math, i.e. a motivated task performance. In addition, we found some indication that processing negative feedback (receiving negative social evaluation) may be associated with deactivation in the medial orbitofrontal region and the hippocampus, among other areas.

However, despite the information gains achieved with this eventMIST, there are a number of drawbacks associated with this paradigm, and the current study: First, as previously mentioned, the number of available events for each particular condition is rather small, especially for the social evaluative components contrast, limiting the available statistical power. Specific to this study, the overall number of subjects was also rather small. Although we had targeted a group of thirty subjects, we were able to only scan 28 due to subject attrition. The study then further suffered from an unusual large amount of data

exclusion due to outliers or data processing problems, resulting in quality data from only twenty subjects.

Second, even thought the eventMIST is designed to identify neural activity patterns associated with the differential components of the task, there is a possibility that neural activity involved in processing the math task may influence the neural activity that we subsequently model for the negative feedback. However, when modeling for the presence of math task prior to the feedback, and then within that set-up examining the contrast related to the feedback, we observed more constricted but similar results as compared to simply modeling for the presence of feedback only. Thus, even though we cannot fully exclude that possibility, our results seem to suggest that the spillover is rather limited.

Third, the constant change of event type, from control to experimental task, and from correct to incorrect or timeout answer type, while a necessity for this type of design, might not be optimal for the induction of stress in majority of the population. Here, the block design might simply be a better stressor, with its typical two-minute sessions of experimental task type during which subjects experience constant threat.

Nevertheless, this first event-related neuroimaging stress task enabled the investigation of the neural correlates of performing difficult mental arithmetic, as well as processing social evaluative threat. The eventMIST further created a situation where subjects with high self-concept and low external locus of control would show the stronger stress responses, an important addition to the literature. Thus, future studies might be able to use this task to gain a better understanding of

personality characteristics that may contribute to stress-related illnesses in association with the specific task type.

Experimental Procedure:

Subjects:

We recruited 28 male university students to participate in this study (age range: 23 ± 4.48 years) by posting online classified ads on McGill University website. Subjects were required to complete screening questionnaires via e-mail prior to being admitted into the study. Subjects were excluded if they had a history of neurological or psychiatric illness or were presently suffering from a psychiatric illness. Participants were non-smokers and did not use recreational drugs. In addition, subjects who were using medication that could influence cortisol secretion were excluded. All subjects were right-handed and had no metal fragments, pacemaker, heart/vascular clip, aneurysm, or prosthetic valve. Further, they had no current diagnosis or history of claustrophobia or Axis I disorders. The Institutional Review Board (IRB) of McGill University had approved the study, and informed consent was obtained prior to participation in accordance with the requirements of the McGill IRB.

Psychological Assessment:

Subjects completed several psychological questionnaires to assess their personality profiles. These questionnaires included the Beck Depression Inventory (BDI) (Beck, Brown et al. 1987), the Diagnostic Inventory of

Depression (DID) (Zimmerman, Sheeran et al. 2004), the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger 1983), Profile of Mood States (POMS; (McNair, Lorr et al. 1992), the Parental Bonding Inventory (PBI; (Parker, Tupling et al. 1979), the Rosenberg Self-esteem questionnaire (Rosenberg 1965), and the Questionnaire of Competence and Control (QCC; (Krampen 1991), which provides a measure of locus of control. It should be noted that STAI and POMS were administered twice, before and after the scanning session. Furthermore, we administered the Trier Inventory of Chronic Stress (TICS; (Schulz and Schlotz 1999) and the Ways of Coping questionnaire (WAYS; (Vitaliano, Russo et al. 1985) to assess subjects' stress levels in variety of specific situations and their coping styles.

Behavioral neuroimaging task:

The eventMIST task was built to reflect a rapid onset event related fMRI design (Burock, Buckner et al. 1998) allowing for an investigation of blood-oxygen-level-dependent (BOLD) signal changes during task performance and processing of social evaluative components. Therefore, although the eventMIST has retained many of the user interface features of the block design MIST (Dedovic, Renwick et al. 2005), the presentation of the rest, control, and experimental tasks and respective feedbacks was randomized and specific jitters were included in order to attempt to distinguish between task and feedback, and reduce an overlap distortion between events. The order of task presentations, subject's responses, and scanner signal onsets were recorded in a log file to be

used for subsequent data analysis. The program was developed using the SuperCard application for MAC OS X (Solutions Etcetera, Pollock Pines, California).

During the rest condition, the user interface was displayed including a color performance bar on the top of the screen, empty task and feedback fields, as well as a rotary dial that was to be used in the control and experimental tasks for response submission. The rest card remained on the screen for 5000 msec, and was followed by a cross card, which jittered in duration between 1000 to 3000 msec.

During the control condition, the math task was displayed until the subject submitted the response or 10000 msec had passed. Following response submission or time-out, the user interface remained on display for a period between 100 and 300msec (randomized), allowing for event separation between task and feedback. The subjects were then exposed to feedback for their performance on that particular task for a period of 1000ms. Importantly, during the control condition, the evaluation of the task performance ("correct", "incorrect", timeout") was accompanied by a "Not Recorded" statement, thus presenting a safety signal. This addition was designed to reduce or eliminate perceived social evaluative threat during this condition. The feedback was followed by a cross card which jittered in duration between 1000-3000 msec.

During the experimental condition, the subjects were exposed to difficult math task that they were required to complete within a given time limit, as indicated by a time advance bar on the screen. Following response submission or

a time-out ($\leq$ 5000 ms), the user interface remained on display for a period ranging between 100 and 300msec (again randomized). The subjects then received feedback on their performance to that particular task ("correct", "incorrect", timeout"), along with a "Recorded" statement, indicating that their performance was being evaluated and recorded. In addition, they were able to see how they compared to an "average" user by examining the color bar that showed two arrows (top arrow representing performance of an "average" user, and bottom arrow that of the subject). Importantly, during experimental condition tasks, the program dynamically adjusted to subject's performance in order to induce a 45-55% correct response rate, by either increasing task difficulty or decreasing time available for task completion. Therefore, the subject's performance arrow was eventually entering the red zone on the performance bar, while the simulated average user performance was oscillating within the green zone. Similarly to the control condition, at the end of the immediate feedback, the cross card was shown for a variable time period.

Finally, additional negative feedback was provided by the investigator between the scanning runs: the subject was reminded of the fact that he was being evaluated, that he was showing a poor performance, and that his performance should have been at the level of the average user if the data were to be used in the study.

Endocrine Measurement and Analysis:

Saliva samples were taken via salivettes (Quebec City, Quebec, Canada) throughout the experiment in order to assess levels of cortisol. A baseline saliva

sample was acquired 40 min prior to the first eventMIST run. The following sample was taken just prior to the structural scan, which preceded the first eventMIST run by 25 min. Additional three samples were collected while the subject was in the scanner, just before the start of the first eventMIST run, between first and second eventMIST run, and right after the second eventMIST run, respectively. This was achieved by moving the scanner bench outside the cylindrical tube to the point that the investigator could reach the subject's head. After the sampling, the subject was returned to the original position in the cylindrical tube since the exact coordinates were stored in the scanner's memory. Subjects completed the final three samples outside of the scanner in 15 min intervals, for a total of eight saliva samples. Cortisol measures were established by using a time-resolved fluorescence immunoassay. Intra- and inter- assay variability were less than 10% and 12%, respectively (Dressendorfer, Kirschbaum et al. 1992).

<u>Functional Imaging Data Acquisition and Analysis:</u>

fMRI acquisition

All subjects were scanned in a 3T Siemens Magnetom (Erlangen, Germany) MRI scanner at the Montreal Neurological Institute. For each subject, a T1-weighted 3D gradient-echo high-resolution anatomical scan was acquired (slice thickness, 1mm; 160 sagittal slices; repetition time (TR), 23ms; echo time (TE), 7.4ms; flip angle, 30°; field of view, 256mm). During each functional run,

300 whole-brain BOLD Mosaic 64 T2*-weighted echoplanar images (EPI) were acquired transversely along the direction of anterior commissure to posterior commissure line minus 30° (slice thickness, 4mm; 32 slices; TR, 2s; TE, 30ms; flip angle, 90°; matrix, 64 x 64; FOV, 256mm).

Data analysis

Log files generated during the eventMIST runs were analyzed and each TR was matched with a corresponding event type (Table 1). An event type had to be dominantly represented within a given TR in order to be assigned to that TR. For the match to occur, an event type needed to fulfill two conditions: 1) the dominant event type had to account for at least 50% of the duration of the TR, and 2) the dominant event type duration had to represent a majority of the TR duration. Subsequently, design matrices were designed depending on the desired contrasts (see Tables 1-3).

Functional data were preprocessed and analyzed using Neurolens (Hoge 2006). Preprocessing included motion correcting the raw data to the third frame in each run (Cox and Jesmanowicz 1999), as well as spatially smoothing the data with a 6mm full-width-half-maximum Gaussian kernel to reduce noise. The first level statistical modeling was executed for each run, for each subject separately. The first three frames of each run were excluded since they might not represent steady-state images. The stimulus design matrix for each run and each contrast was convolved with the default hemodynamic response function (Glover 1999), and slice-timing correction was applied.

The resulting effect files and standard error for effect files were then transformed from the native space into the standard Montreal Neurological Institute (MNI) space. Namely, the preprocessed file (which had been motion corrected and smoothed) for a given run was linearly aligned to the standard target (40-subject average 3T EPI target file in MNI space). The obtained transformation parameters were used to resample effect files and standard error for effect files for that respective run, for a given subject.

During the second-level analysis we combined the two runs for each participant by considering only fixed effects (Worsley, Liao et al. 2002). Resulting effect and standard error for effect files from this analysis were then grouped across subjects by using mixed effects analysis to generate the group t map file. This approach is based on smoothing the ratio of random effects variance divided by the fixed effects variance, to achieve 100 degrees of freedom (Worsley 2005). Similarly, the comparisons between responder and nonresponder group for each specific contrast were done at this level as well.

The threshold for the final group t map image was calculated according to the False Discovery Rate because of the low number of subjects and events, and the resulting limitations in statistical power (Genovese, Lazar et al. 2002). We chose a rate of 0.01 as the expected portion of false positives among the voxels above the calculated threshold. Anatomical regions were identified by manual inspection of the brain atlas (Mai, Voss et al. 2008).

Acknowledgements:

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Figures and Figure Legends:
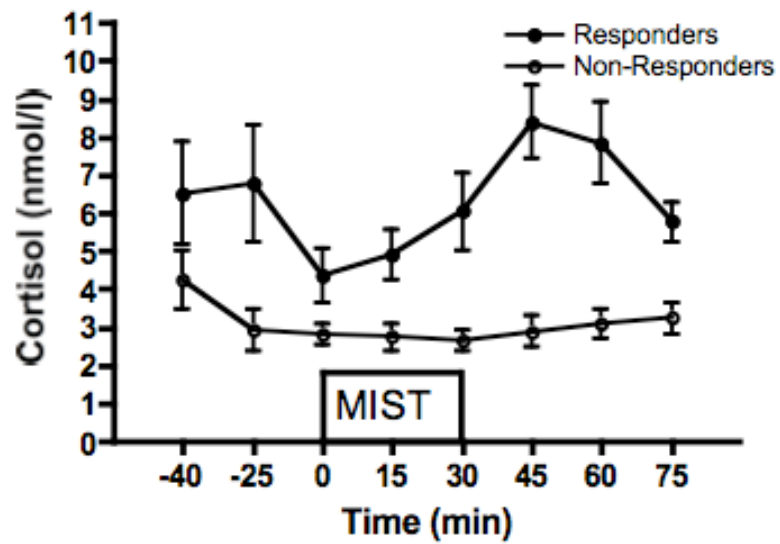
Figure 1

Figure 1: Cortisol levels in response to eventMIST in the responders and the non-responders. Error bars show SEM.
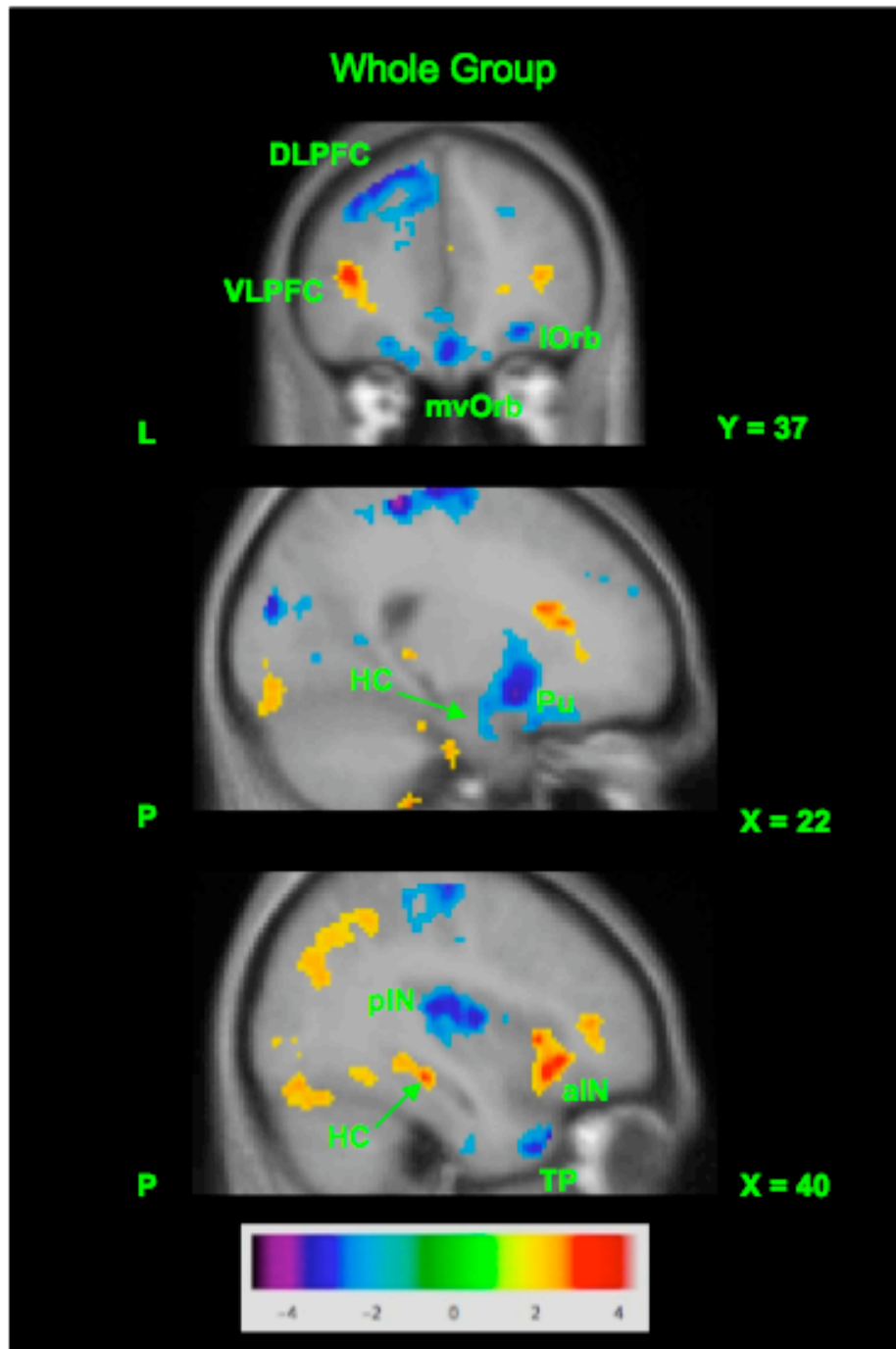
Figure 2.

Figure 2: Brain activity pattern in the whole group (n=20) in response to stress

using the block replication contrast ((all experimental task math and feedback)–(control task math and feedback)). All activity shown is significant after controlling for the False Discovery Rate of p=0.01. x, y, z = saggital, coronal, and horizontal view in world coordinates. Activations are represented in red and deactivations in blue. The underlying anatomical image is an average of the subjects' anatomical files in MNI space. PFC: prefrontal cortex ;VLPFC: ventral lateral PFC; DLPFC: dorsal lateral PFC; mvOrb: medial ventral orbitofrontal cortex; lOrb: lateral orbitofrontal cortex; HC: hippocampus, TP: temporal lobe; pIN: posterior insula; aIN: anterior insula; Pu: putamen; L: left; P: posterior.

Figure 3.

Figure 3: Brain activity pattern in (A) the responders (n=7), (B) the non-responders (13), (C) the comparison between the two groups (responders > nonresponders), in response to difficult mental arithmetic (all experimental task math − control task math) contrast. All activity shown is significant after controlling for the False Discovery Rate of p=0.01. x, y, z = saggital, coronal, and horizontal view in world coordinates. Activations are represented in red and deactivations in blue. The underlying anatomical image is an average of the subjects' anatomical files in MNI space. PFC: prefrontal cortex; VLPFC: ventral lateral PFC; DLPFC: dorsal lateral PFC; DMPFC, dorsal medial PFC; HC: hippocampus, TP: temporal pole; aIN: anterior insula; FP: frontal pole; PrC: precuneus; vACC: ventral anterior cingulate cortex; dACC: dorsal anterior cingulate cortex; PCC: posterior cingulate cortex, BA: Brodmann's area; L: left; P: posterior.

Figure 4.

Figure 4: Brain activity pattern in (A) the responders (n=7), (B) the non-responders (13), in response to negative feedback (experimental timeout negative feedback – experimental correct feedback) contrast. All activity shown is significant after controlling for the False Discovery Rate of p=0.01. x, y, z = saggital, coronal, and horizontal view in world coordinates. No significant activation and deactivation were found in the responders > nonresponders for this contrast (not shown). Activations are represented in red and deactivations in blue. The underlying anatomical image is an average of the subjects' anatomical files in MNI space. PFC: prefrontal cortex; DLPFC: dorsal lateral PFC; HC: hippocampus; vACC: ventral anterior cingulate cortex; MTG: middle temporal gyrus; SM: supramarginal gyrus; mvOrb: medial ventral orbitofrontal cortex; preCent: precentral gyrus; lOrb: lateral orbitofrontal cortex; SubC: subiculum BA: Brodmann's area; L: left; P: posterior.

EVENT TYPE	AVERAGE number of events per run
Experimental task – correct (expC)	26
Experimental task – incorrect (expIC)	11
Experimental task - time out (expTO)	23
Experimental task - not recorded (expNR)	33
Experimental feedback – correct (expCF)	11
Experimental feedback – incorrect (expICF)	4
Experimental feedback - time out (expTOF)	10
Control task – correct (ctrlC)	22
Control task – incorrect (ctrlIC)	5
Control feedback – correct (ctrlCF)	9
Control feedback – incorrect (ctrlICF)	1
Rest	28
Cross Card	86

Table 1: Event types and the average number of events per run

List of event types and their respective occurrences (expressed as average number of events per run) during the eventMIST.

Contrast	Whole group	
	Activations	Deactivations
Block replication expC + expIC + expTO + expCF + expTOF - [ctrlC+ctrlCF]	-VLPFC L (-40, 37, 13)	-Frontal pole L & R (9, 65, -12)
	-Anterior Insula L & R	-DLPFC L (-11, 57, 39)
	(-39, 22, -9)	-MVPFC L (-10, 51, -1)
	-Cingulum L& R (14, 27, 24)	-vmOrb L & R (2, 41, -18)
	-Inf. Front. G. premotor (57, 3, 31)	-LOrb L& R specs (-25, 39, -16)
	-HC tail R (41, -29, -10)	-Sup. Front. G. L (-16, 33, 55)
	-HC tail fimbria L (-29, -39, 5)	-HC head R (20, -6, -20)
	-Supramarginal/Angular G. R (54, -45, 52)	-Putamen L & R (-22, 11, -3)
	-Occipital Lobe/Cerebellum (-40, 77, -18)	-Temporal pole L & R (-53, 7, -28)
		-Posterior Insula R & L (40, -8.5, 15)
		-PCC L & R (8, -15, 51)
		-Precuneus L & R (-5, -69, 33)

Table 2: Localization of activations and deactivations for all participants in response to stress using the block replication contrast.

Anatomical locations and coordinates (x, y, z, World coordinates) of brain activations and deactivations in the whole group of participants (n=20) in response to block design replication contrast (contrasting control math and feedback from experimental stress math and negative feedback). All activity is significant after controlling for False Discovery Rate of $p<0.01$. PFC: prefrontal cortex; VLPFC: ventral lateral PFC; Inf. Front. G: inferior frontal gyrus; DLPFC: dorsal lateral PFC; MVPFC: medial ventral PFC; vmOrb: ventral medial orbitofrontal cortex; lOrb: lateral orbitofrontal cortex; Sup. Front. G: superior frontal gyrus; PCC: posterior cingulate cortex; HC: hippocampus; L: left; R: right

Contrast		Responders	Nonresponders	Resp>Nresp
All experimental task math – control task math (expC + expIC + expTO) - ctrlC	**A C T I V A T I O N S**	-DMPFC L&R (BA10) (7,57,23) -vACC L & R (8, 41, 15) -dACC R (7, 23, 37) -DLPFC R (BA 46) (52, 32, 15) -VLPFC R (BA 47/12) (48, 32, -4) -anterior insula R & L (37, 23, -4) -Internal capsule (11, 5, 2) - Mid. Front. G. precentrally L (-35,-2.5,50) -Thalamus L & R (2, -17,19) -Brainstem colliculi (10, -29,-11) -Supramarginal G. L (-40, -45,48) -HC Subiculum R (22, -27, -10) -Cerebellum L&R (27, -41, 39)	-dACC L & R (11, 23, 41) -VLPFC L & R /(BA 45A) (-45, 47, 6) -anterior insula L & R (-35, 23, 3) -Internal capsule L & R (13, -3, 11) -Thalamus L&R (9, -13, 10) -Sup. Front. G. precentrally L&R (27, -7, 54) -HC tail end L&R (40, -22,-10) -Brainstem colliculi (8, -31, -5) -Supramarginal G. L&R (-51, -33, 45) -dPrC L (-12, -67, 58) -Sup. Parietal lobule L (-29, -65, 52) -Occipital Lobe L & R (-29, -97, -12)	-DMPFC L & R (3, 53, 24) -DLPFC R (BA 9) (15, 49, 45) -Temporal pole L (-36, 19, -29)
	D E A C T I V A T I O N S	-DLPFC L (-38, 29, 43) -Temporal Pole R (33, 9, -35) -vPrC R (8, -70, 43)	-Frontal pole (-5, 67, -7) -DMPFC L (BA 9) (-4, 55, 35) -DLPFC L & R (BA 9) (13, 51, 44) -VMPFC L (BA 11) (-4, 53, -6) -posterior insula (-41, -22, 21) -Temporal pole L (-38, 21, -31) -PCC L & R (-2, -49, 30) -vPrc R (3, -59, 28) -Angular G. L & R (-55, -62, 31) -Sup. Front. G. posterior L (-12, 33, 57) -Mid. Temp. G L (-63, -11, -20)	**NS**

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Table 3: Localization of activations and deactivations for the responders and the nonresponders and the difference between these two groups, in response to performing complex mental arithmetic task.

Anatomical locations and coordinates (x, y, z, World coordinates) of brain activations and deactivations in the responders (n=7), non-responders (13) and comparison between the two groups (responders > nonresponders) in response to all experimental task math – control task math contrast. All activity is significant after controlling for False Discovery Rate of $p<0.01$. PFC: prefrontal cortex; VLPFC: ventral lateral PFC; Inf. Front. G: inferior frontal gyrus; DLPFC: dorsal lateral PFC; DMPFC: dorsal medial PFC; MVPFC: medial ventral PFC; vmOrb: ventral medial orbitofrontal cortex; lOrb: lateral orbitofrontal cortex; Sup. Front. G: superior frontal gyrus; Mid. Front. G.: middle frontal gyrus; Sup. Temp. G.: superior temporal gyrus; PCC: posterior cingulate cortex; vACC: ventral anterior cingulate cortex; dACC: dorsal anterior cingulate cortex; vPrC: ventral precuneus; dPrc: dorsal precuneus; HC: hippocampus;BA: Brodmann's area; NS: no significant activity; L: left; R: right

Contrast		Responders	Nonresponders	Resp>Nresp
Negative feedback within stress expTOF-expCF	A C T I V A T I O N S	-Supramarginal G. L (-62, -41, 38)	-DLPFC L (BA46) (-35, 59, 23) -lOrb L (-27, 31, -18) -Sup. Front. G L & R (-1, 19, 57) -VLPFC R (BA 45A) (61, 19, 12) -Temporal pole L & R (-36, 13, -36) -Piriform L & R (34, 17, -21)	**NS**
	D E A C T I V A T I O N S	-DMPFC L (BA10) (-11, 61, 28) -VMPFC L (-3,49, -13) -lOrb L (-24, 43, -13) -DLPFC L (BA9) (-15, 37, 53) -mvOrb L&R (-.3, 43, -20) -HC R body (31, -27, -7) -vACC L (-4, 41, -2) -Caudate L & R (-16, 17, 7) -Anterior Insula L (-34, 13, 15) -Putamen L & R (22, 13, 0) -Insula (44, -5, 5) -Mid. Temp. G. anterior L & R (-60, -9, -21) -PCC L (-0.1, -43, 39) -Precentral G. L& R (-45, -19, 59) -Fusiform G. L (-33, -29, -25) -vPrC L&R (3, -62, 48) -Angular G. L & R (-53, -71, 14) -Occipital Lobe R (37, -85, -9)	-Frontal pole L (12, 70, -4) -DLPFC L (BA9) (-14, 41, 53) -lOrb L & R (-38, 45, -13) -Caudate L & R (-11, 19, 7) -Putamen L & R (26, 9, -3) -Insula R (44, -5, 14) -MCC R (2, -3, 45) -Cingulum L (-20, -17, 40) -Subiculum HC, L (-19, -29, -12) -Postcentral G. R (51, -29, 59) -Angular G. R (13, -66, 59) -Occipital Lobe R (49, -81, 4)	**NS**

Table 4: Localization of activations and deactivations for the responders, nonresponders and the difference between the two groups, in response to negative

feedback.

Anatomical locations and coordinates (x, y, z, World coordinates) brain activations and deactivations in the responders (n=7), non-responders (13) and comparison between the two groups (responders > nonresponders) in response to experimental negative timeout feedback – experimental positive correct feedback. All activity is significant after controlling for False Discovery Rate of p<0.01. PFC: prefrontal cortex; VLPFC: ventral lateral PFC; Inf. Front. G: inferior frontal gyrus; DLPFC: dorsal lateral PFC; DMPFC: dorsal medial PFC; MVPFC: medial ventral PFC; vmOrb: ventral medial orbitofrontal cortex; lOrb: lateral orbitofrontal cortex; Sup. Front. G: superior frontal gyrus; Mid. Front. G.: middle frontal gyrus; Sup. Temp. G.: superior temporal gyrus; PCC: posterior cingulate cortex; MCC: middle cingulate cortex; vACC: ventral anterior cingulate cortex; dACC: dorsal anterior cingulate cortex; vPrC: ventral precuneus; dPrc: dorsal precuneus; HC: hippocampus; BA: Brodmann's area; NS: no significant activity; L: left; R: right

Chapter 4: Cortisol awakening response and hippocampal volume: Vulnerability for Major Depressive Disorder?

Katarina Dedovic, Veronika Engert, Annie Duchesne, Sonja Damika Lue, Julie Andrews, Simona I. Efanov, Thomas Beaudry & Jens C. Pruessner

Preface to Chapter 4

In the last two chapters we firstly reviewed the neuroimaging studies investigating neural correlates of stress processing in healthy populations and then we examined which brain areas are involved in processing mental arithmetic and which in processing social evaluative threat components of a psychological stress task, in a group of healthy young men. The overall goal was to understand the neural regulatory network that may underlie individual differences in stress response in healthy populations.

Previous studies have shown that maladaptive responses to psychological stress may contribute to various psychopathologies (e.g. Chrousos 2009). Therefore, while the investigations of normal population provide important evidence for the regulatory networks involved in psychological stress and HPA axis function, they are limited in examining how dysregulation of some of these areas may contribute to development of specific psychopathology.

Given that onset and development of Major Depressive Disorder (MDD) in particular is closely associated with psychological stress (e.g. Hammen 2005), we have chosen to investigate HPA axis function and neural regulatory network subserving psychological stress processing in a sample of healthy young adults who show varying levels of depressive tendencies, but at subclinical levels. This population was chosen as it had been shown that those with current levels of subclinical depression are at a higher risk to develop MDD (Cuijpers and Smit 2004).

Therefore, this second portion of the thesis investigates HPA axis function both basal and reactive, as well as structural and functional integrity of key brain areas that are part of the regulatory network of the HPA axis.

For this part we recruited 64 (33 controls (17 women; 16 men) and 31 subclinicals (17 women; 14 men) right-handed, healthy university students (mean age 21.9±2.5 years). The subjects were recruited based on the Beck Depression Inventory (Beck, Brown et al. 1987), and visited the testing facilities at two occasions. During the first meeting, the subjects were given saliva-sampling kits for the assessment of basal cortisol levels at home. When the home samplings were completed and the scanner was available, the subjects came for the second visit. During the second visit, the participants completed a set of psychological questionnaires, and were subjected to an attentional bias task, structural scan, and the MIST. Saliva samples were taken throughout.

In chapter 4, we specifically assess whether abnormalities in basal HPA axis function and hippocampal volume observed in Major Depressive Disorder patients in previous studies can already be present in this sample of healthy young adults who show varying levels of depressive tendencies at subclinical levels.

MANUSCRIPT

Title:

Cortisol awakening response and hippocampal volume:

Vulnerability for Major Depressive Disorder?

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Introduction

The hypothalamic pituitary adrenal (HPA) axis, the major endocrine stress axis in humans, is dysregulated in Major Depressive Disorder (MDD) both basally and in response to a challenge (Gold and Chrousos 2002; Burke, Davis et al. 2005). Structural abnormalities of specific brain regions associated with the regulation of the HPA axis, namely the amygdala, the prefrontal cortex, and the hippocampus in particular, have also been reported in subjects afflicted with depression (reviewed in Campbell and MacQueen 2006; Koolschijn, van Haren et al. 2009)). However, it remains unclear whether some of these impairments are a characteristic of the illness state, or whether they may represent a vulnerability marker existing prior to the illness onset. Thus, in the present study, we investigated whether there is evidence of basal HPA axis dysregulation and of abnormalities of the hippocampal (HC) volume in a sample of healthy young adults showing varying levels of depressive tendencies, but at a subclinical level.

Basal cortisol levels show a 24hr circadian oscillation (Hellman, Nakada et al. 1970). A distinct phenomenon above that of the circadian oscillation, the cortisol awakening response (CAR), is a sharp rise in cortisol following awakening which typically peaks at about 30min following awakening (Pruessner, Wolf et al. 1997; Wilhelm, Born et al. 2007). The CAR is thought to reflect the sensitivity of the HPA axis to a natural challenge such as awakening and can be differentially affected by stress and psychopathologies (reviewed in Chida and Steptoe 2009; Fries, Dettenborn et al. 2009). In depression, findings of both increased and blunted CAR have been reported. A recent study found a higher

increase in CAR in medication-free recovered depressed patients when compared to a matched healthy control group (Bhagwagar, Hafizi et al. 2003). Similarly, a large study investigating current and remitted middle-aged depressed subjects found that, in comparison to control subjects, patients showed a higher CAR (Vreeburg, Hoogendijk et al. 2009). In contrast, a smaller study on young adults reported that depressed patients had a blunted CAR (Stetler and Miller 2005). Further, in an outpatient population, more severe levels of depression were more likely associated with flattened diurnal cortisol patterns (Hsiao, Yang et al. 2009), while a lower CAR has been observed in depressed patients compared to those with other psychiatric diagnoses (Huber, Issa et al. 2006). The inconsistent findings may be due to differences in illness stages assessed, as well as methodological differences in cortisol sampling across the studies (Chida and Steptoe 2009). It is noteworthy, however, that when specifically looking at the area-under-the-curve-increase (AUCi) or absolute increase score assessment of the CAR, a recent meta-analysis found a negative association with severity of depression (Chida and Steptoe 2009).

Structural abnormalities of the hippocampus have also been extensively investigated with respect to MDD, with bilateral HC volume reductions found most often in studies investigating recurrent or treatment resistant depression (for example, see Sheline, Wang et al. 1996; MacQueen, Campbell et al. 2003; Sheline, Gado et al. 2003; Caetano, Hatch et al. 2004; Hickie, Naismith et al. 2005). Several meta-analyses over the last decade have concluded that unipolar

depression leads to a reduction of bilateral HC volume (Campbell and Macqueen 2004; Videbech and Ravnkilde 2004; McKinnon, Yucel et al. 2009). This line of evidence suggests that reduced HC volume is a result of depression, and represents a burden of illness.

However, a new line of studies investigating HC volume integrity in first episode depressives reported findings that contrast this view. Indeed, it was shown that patients diagnosed with a first episode of depression and thus a very short lifetime duration of the illness, already present with reduced HC volume - pointing to smaller HC volume as a risk factor for, rather than a consequence of, the illness (Frodl, Meisenzahl et al. 2002). While few studies reported no differences in HC volume between first episode depressives and controls (MacQueen, Campbell et al. 2003; Milne, MacQueen et al. 2009; van Eijndhoven, van Wingen et al. 2009), others reported reduced left HC volume in first episode male patients (Frodl, Meisenzahl et al. 2002; Kronmuller, Schroder et al. 2009), as well as in first-episode female drug-naïve subjects (Kaymak, Demir et al. 2009), and a group of drug-naïve men and women (Zou, Deng et al. 2009). Furthermore, a study of healthy volunteers has shown that there is a great variability in the HC volume within a healthy population (Lupien, Evans et al. 2007). Together, these findings suggest that smaller HC volume as it is found in depressed subjects may already be present at the onset of the illness and may actually represent a vulnerability for developing this illness.

However, to date, the majority of studies on this topic have been conducted with inpatient clinical populations. Others have also investigated

populations that are believed to be at higher risk for developing the illness but who do not as yet present any of the symptoms (for example, healthy individuals with family history of MDD). With respect to the vulnerability question, both approaches are therefore limited by their temporal proximity and distance, respectively, from the illness proper.

Therefore, for the present study, we focused on a subclinical population, defined here as currently scoring above the cut-off point on a self-rating depression inventory. We chose the subclinical population since it has been suggested that subclinical depression might represent a milder condition on the depression continuum (Solomon, Haaga et al. 2001; Lewinsohn, Klein et al. 2003; Rivas-Vazquez, Saffa-Biller et al. 2004). A consistent pattern has been observed of increased incidence of MDD among subjects with subclinical depression compared to those without it, despite the heterogeneity in definition of the subclinical population (Cuijpers and Smit 2004). Some have even suggested that subclinical depression may represent the precursor for the full disorder (Shankman, Lewinsohn et al. 2009). Therefore, a subclinical depression population provides a unique opportunity to investigate the vulnerability hypothesis in a population that is at more direct risk of developing depression, but who has not as yet succumbed to the full clinical syndrome.

Given the findings from clinical populations, we hypothesized that dysregulation of the HPA axis and HC volume abnormalities would represent vulnerability factors for depression. Specifically, we hypothesized that the CAR would be lower in the subclinical group compared to control subjects, and that the

subclinical participants would also have smaller HC volumes compared to controls.

Methods and Materials

We recruited sixty four (30 men, 34 women) right-handed, healthy university students (mean age 21.9±2.5 years). Subjects were recruited via online classified ads on the McGill University website. Subjects completed several screening questionnaires prior to the inclusion in the study. Exclusion criteria included prior and/or present neurological or psychiatric illness, cigarette smoking and use of recreational drugs on a regular basis. Subjects were also excluded if they were taking any medication that could influence cortisol secretion. All subjects met the standard safety requirements for participation in a functional Magnetic Resonance Imaging (fMRI) study. Further, they had no current diagnosis or history of claustrophobia or Axis I disorders. Their family history of psychiatric illness was also assessed. Finally, the subjects were included in the study based on their scores of the Beck Depression Inventory (BDI; (Beck, Brown et al. 1987). Based on the published BDI cut-off scores (Beck, Brown et al. 1987), subjects were assigned to either a control group (BDI ≤ 9; n=33) or a subclinically depressed group (10 ≤ BDI ≤ 18; n=31; Table 1).

Subjects came to the Montreal Neurological Institute at two occasions. During their first visit, they were given a saliva sampling kit for home use, to collect diurnal cortisol (at the time of awakening, +30 min, +60 min, at 4pm, and at 9pm) over a span of three non-consecutive workdays. Saliva was collected

using salivettes (Sarstedt Inc, Quebec City, Quebec, Canada). The participants were provided with detailed instructions on proper sampling and storing of saliva samples. Samples were analyzed via a time-resolved fluorescence immunoassay. Intra- and inter-assay variability was less than 10% and 12%, respectively (Dressendorfer, Kirschbaum et al. 1992).

During their second visit, the participants completed several questionnaires to evaluate their psychological profile, and underwent functional and structural MRI scans. The results related to the functional data will be described elsewhere (Dedovic et al., in preparation). A crosscheck for BDI depression levels obtained at the time of recruitment was established by having subjects complete the Hamilton Depression Inventory (Reynolds 1995), and the Montgomery-Asberg Depression Rating Scale Self-Assessment (MADRS-S) (Svanborg and Asberg 1994) at the time of the second visit. Subjects also completed the Spielberger Trait Anxiety Inventory (STAI) (Spielberger 1983), the Trier Inventory for the assessment of Chronic Stress (TICS); (Schlotz, Schulz et al. 2004), the Parental Bonding Instrument (PBI); (Parker, Tupling et al. 1979), and the Childhood Trauma Questionnaire (CTQ); (Bernstein and Fink 2003), two retrospective measures of levels of parental care and levels of childhood maltreatment.

The Institutional Review Board of McGill University approved the study, and informed consent was obtained prior to participation in accordance with the requirements of the McGill IRB.

<u>Image acquisition and processing</u>

Subjects were scanned in a 1.5T Siemens Magnetom SonataVision scanner at the Montreal Neurological Institute (MNI). A standard 3D gradient-echo pulse sequence was used, with a field of view of 256mm, isotropic voxel size of 1mm, TR=22ms, TE=9.2ms and flip angle=30°.

The native anatomical images were processed prior to manual volume segmentation in order to first correct for intensity non-uniformity (Sled, Zijdenbos et al. 1998). Then, all images were registered into the MNI normalized brain template using the ICBM 152 model brain (Evans, Collins et al. 1994). This procedure corrects for differences in head size (Collins, Neelin et al. 1994).

The HC volumes were assessed using a manual volume segmentation protocol via the interactive software DISPLAY that allows for simultaneous viewing of the structure in all three orientations (Pruessner, Li et al. 2000). The manual segmentation protocol and the anatomical boundaries have been described in detail elsewhere (Pruessner, Li et al. 2000). An experienced rater assessed all HC volumes while being blind to subjects' characteristics.

<u>Statistical Analysis</u>

Due to presence of depression at clinical levels in some of the subjects, three experimental groups were formed (see the Results section for details). Therefore, group differences in psychological variables were assessed using univariate ANOVA with study group and sex as between factors.

The change in diurnal cortisol was captured by averaging the values for

each time point across the three days. These values were entered in a two-way mixed-design ANOVA, with study group and sex of the subjects as between factors. The change in the CAR was captured in several ways. Similarly to diurnal variation in cortisol, for an assessment of cortisol change across time, values for each morning time point were averaged across the three days, and then used in the mixed design ANOVA. If the subjects did not adhere to the sampling time schedule for some of the days, these values were excluded. For the calculation of the CAR AUCi, a measure that captures the dynamic fluctuation of the system from the awakening baseline (Pruessner, Kirschbaum et al. 2003), we first calculated CAR AUCi for each day, and then averaged across days for each subject. Similarly, the CAR area-under-the-curve-ground (AUCg), a measure of overall cortisol release with respect to zero level, was also assessed.

Measures of right and left HC volumes were entered into a mixed design ANOVA, with laterality as a within, and group and sex as between factors. If appropriate, in some analyses, total HC volume measure was used.

All the ANOVAs, if significant, were followed by Games-Howell post-hoc tests, since this procedure does not assume that population variances or sample sizes are equal (Field 2005).

Results

Study population

Upon inspection of the behavioral, functional and endocrine data, five subjects were excluded due to missing functional data, abnormal cortisol profiles

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or inadequate performance for the functional tasks in the scanner. The final subject number was 59 (29 controls, and 30 subjects with subclinical levels of depression). Women (N=31) in the sample were diverse with respect to their menstrual cycle.

At the time of the second visit, nine subjects scored at clinical levels on either the HDI or MADRS-S (Table 1). Given the short amount of time that passed from the time they were admitted to the study and the second testing session (27.6 days +/- 11.4), we did not exclude these subjects from the study, but rather included them as a separate group representing a group of high-risk subclinical subjects. These subjects were advised to seek professional counsel and were given a referral letter. The final group numbers were 27 controls, 23 subclinicals, and 9 high-risk subclinicals.

As would be expected, univariate ANOVAs revealed that the experimental groups differed on levels of depression, stress and anxiety (Table 1). The subclinical group had higher levels of subclinical depression compared to the control group as assessed by the BDI ($F_{(2,58)}$=82.5, $p<.001$). Furthermore, high-risk subclinical group had higher scores compared to subclinical group who, in turn, had higher HDI ($F_{(2,57)}$=91.4, $p<.001$) and MADRS-S ($F_{(2,58)}$=43.9, $p<.001$) scores compared to controls. There was a main effect of the group ($F_{(2,58)}$=15.9, $p<.001$) on chronic stress levels, with a significant incremental increase in stress levels across the study groups ($p<.02$). Finally, when investigating anxiety levels, we observed a significant main effect of group ($F_{(2,58)}$=21.2, $p<.001$), and a group x sex interaction ($F_{(2,58)}$=3.2, $p=.049$).

Simple main effects revealed that women in the subclinical group had higher anxiety levels compared to women in the control group (t=-2.9, p<.01), while men in the high-risk subclinical group had higher anxiety levels compared to men in the subclinical group (t=-4.5, p<.001). No significant group or interaction effects were found for mother care levels or CTQ total score (all p >0.2).

Reduced cortisol awakening response among the subclinical groups

A mixed-design ANOVA investigating diurnal cortisol variation did not reveal any significant interaction or main effects (all p>.2).

A mixed-design CAR x group x sex ANOVA revealed a significant main effect of CAR (F(2,52)=16.20, p<.001 Sphericity assumed), as well as a significant CAR x group interaction, F(4,52)=3.34, p=.013 (Figure 1A). Further simple main effects analysis of the interaction showed a significant effect of time within each group: controls, F(2,110)=23.29, p<.001; subclinicals F(2,110)=4.11, p=.019; high-risk subclinicals F(2,110)=3.46, p=.04). Within each group, we performed paired samples t-tests to compare the second sample to the awakening sample (the peak following awakening), and the third to the second sample (the return from the peak). For the control group, there was a significant increase from awakening to +30min (t(25)=-5.30, p<.001) and the subsequent return (t(25)=3.78, p=.001). For the subclinical sample, a statistically significant increase was observed, although it did not survive the correction for multiple comparisons (t(22)=-2.76, p=.01). However, the return from the peak was significant (t(22)=4.06, p<.001). With respect to the high-risk subclinical group,

there was no significant increase following awakening (t(8)=-.60, p=.6). Although there was a significant decrease from the peak to the +60 min sample, this difference did not survive the multiple comparison correction (t(8)=3.28, p=.011). There were no differences between the groups within each time point of the CAR (all F<1.66, all p>0.20). There was also a trend for the main effect of gender (F(1,52)=3.2, p=.08), with higher levels in women.

We also assessed differences between the groups with respect to CAR AUCi using univariate two-factor (group x sex) ANOVA. Here, a significant group effect was found (F(2,57)=4.9, p=.01). The Games-Howell post-hoc test revealed that the CAR AUCi was significantly lower in the high-risk subclinical compared to control subjects (p=.02), while there was a trend for the subclinical group to also show lower CAR AUCi compared to controls (p=.08; Figure 1B). Similar analysis investigating differences in CAR AUCg, showed no differences between the groups, although there was a trend for an effect of gender (F(1,57)=2.5, p=.11), with a tendency for women to show greater AUCg.

Reduced total HC volume in the high-risk subclinical group

Mixed-effects (laterality x group x sex) ANOVA, revealed no main effect of laterality, nor an interaction between group and laterality, and sex and laterality (all Fs<1.49, all ps>.23). However, there was a marginally significant main group effect, F(2,53)=2.91, p=.06. Although simple contrasts revealed a smaller total HC volume in subclinical compared to control subjects (p=.05) and a trend for a smaller total HC volume in high subclinical compared to control subjects (p=.06),

running the Games-Howell post-hoc test revealed a significant difference only between the high-risk subclinical group and the control group (p<.05), while the difference between the subclinical group and the control group was only a trend (p=.14).

Assessment of the association between the CAR AUCi and HC volume revealed a trend for positive association in men only (Spearman rho=.32, p=.10; for details see Supplementary Material).

Discussion

By focusing on a healthy population with varying degrees of subclinical levels of depression we aimed to investigate whether there is evidence of dysregulation of the basal cortisol secretion (CAR and diurnal secretion) and changes in HC volume even prior to the onset and progression of MDD. We observed a blunted CAR in the subclinical groups, and a gradual change across groups with respect to the CAR AUCi and the total HC volume. This is the first time, to our knowledge, that these associations have been observed in a subclinically depressed population.

The findings of reduced CAR within the subclinical groups and reduced CAR AUCi in the high-risk subclinical group replicate some of the previous studies examining clinically depressed individuals (Stetler and Miller 2005; Huber, Issa et al. 2006), as well as those studies that have investigated healthy subjects who varied on personality variables associated with vulnerability for

depression (such as self-focused rumination and loneliness) (Kuehner, Holzhauer et al. 2007; Doane and Adam 2010).

Given the heterogeneity of findings in the literature, our results are also contrary to other recent studies (Bhagwagar, Hafizi et al. 2003; Mannie, Harmer et al. 2007; Vreeburg, Hoogendijk et al. 2009; Adam, Doane et al. 2010). With respect to patient populations, Bhagwagar et al. reported greater increase in CAR in remitted depression patients compared to controls (Bhagwagar, Hafizi et al. 2003). Similarly, a large study on both currently depressed and remitted patients also found an increased CAR in the patient samples (Vreeburg, Hoogendijk et al. 2009). In these two studies, saliva samplings were performed only on one day. It has been suggested that CAR measurements on a single day are more influenced by situational factors (Hellhammer, Fries et al. 2007). It could be speculated that the depressed patients are more concerned about the subsequent saliva sampling, which may contribute to a greater CAR at the first and only day of assessment. To that effect, one study has found higher CAR levels in anticipation of exams in a subgroup of undergraduates who may be predisposed to experience higher levels of anxiety (Hewig, Schlotz et al. 2008).

With respect to at-risk but healthy populations, another study investigating young people (19.1 yrs $\pm$ 0.9) found that subjects who had not been depressed themselves but who had a parent with a history of major depression had greater CAR than comparison subjects (Mannie, Harmer et al. 2007). Furthermore, Adams et al. assessed 230 adolescents (17.04 yrs $\pm$ 0.36) and reported that higher CAR at baseline was predictive of having an episode of depression at the follow-

up period a year later (Adam, Doane et al. 2010). These findings, in concert with our own results, raise important questions that will require further investigation.

First, both of these studies have dealt with an at-risk population that is transitioning from late adolescence to young adulthood. Perhaps there is something unique to this transition phase that, when coupled with other depression vulnerability factors, such as high levels of neuroticism or family history of depression, is associated with increased CAR. Upon passing of this transition period, we may observe blunting of CAR in these same at-risk individuals, perhaps representative of exhaustion of regulatory mechanisms. Unfortunately, there is a lack of longitudinal studies not only within the vulnerable populations, but also among the healthy, thus our understanding of potential changes in CAR within the same individual over time is grossly limited.

Second, this collection of results may also highlight the heterogeneous nature of depression. For example, the at-risk sample in the Adam et al. study was over-sampled based on neuroticism (Adam, Doane et al. 2010). Neuroticism, while being a known prospective risk factor for depression (Kendler, Kuhn et al. 2004; Adam, Doane et al. 2010), is also on its own associated with an increased CAR (Portella, Harmer et al. 2005). Therefore, it may be possible that different pathways to depression onset (such as experience of early life adversity, neurotic personality or increased levels of subclinical depression) may actually be associated with different CAR profiles. This will need to be considered by future studies investigating these associations.

The second main finding of this study was reduced total HC volume in the high-risk subclinical group compared to the controls. The total HC volume mean for the subclinical group was in between that of the control and the high-risk group; however, this difference did not reach significance. These findings are in line with previous studies showing reduced HC volume in subjects with a first episode of depression (Frodl, Meisenzahl et al. 2002; Kronmuller, Schroder et al. 2009; Zou, Deng et al. 2009), and suggest that reduced HC volume may be a vulnerability trait. A recent study investigating HC volume size in non-depressed adolescent daughters of depressed moms also corroborates this line of evidence, with preliminary results showing a reduced left HC volume in this population compared to non-depressed adolescent daughters of control moms (Chen, Hamilton et al. 2009). The reduced HC volume as a trait may then either be genetically determined, or may be a result of postnatal adversity, or, most likely, both. Indeed, human twin studies suggest a moderate effect of heritability on HC volume (40-69%) (Peper, Brouwer et al. 2007), while studies examining contribution of early life abuse and maltreatment also find significant impact on HC volume in depressed subjects (Vythilingam, Heim et al. 2002).

The exact role of blunted CAR and smaller HC volume in the etiology of depression is still unclear. Hippocampus is part of a mood regulatory network (Mayberg 2009), and is an important component of a neural circuitry that coordinates behavior with neuroendocrine, immune and autonomic functions for adaptive coping with environmental and psychosocial challenges (McEwen and

Gianaros 2010). A smaller hippocampus may be less efficient in meeting these regulatory demands and may strain the networks over time. At times of stress, the strained regulatory networks may precipitate the onset of depression. Similarly, the blunted CAR may also be an indication of exhausted regulatory mechanisms underlying the CAR (Chida and Steptoe 2009). We have found limited evidence of a positive association between HC volume and CAR, but other factors not considered here may mediate or mask this relationship in the present study, such as genetic polymorphisms (Wust, Kumsta et al. 2009), or heightened sensitivity to social negative feedback (Tops, Riese et al. 2008). Others have suggested that blunted CAR may be an adaptive response prompting an individual to remove one self from the source of stress and promoting social withdrawal rather than engagement (Doane and Adam 2010).

There are several limitations in this study. Firstly, the study is limited by unequal and small group sizes. Also, we did not assess the genetic makeup in our sample, nor did we assess other measures of HPA axis dysregulation. With respect to the CAR, we did not assess variables related to sleep duration and quality for the nights prior to samplings. Although a recent review has suggested that sleep duration or awakenings during the night seem to be unrelated to the CAR (Fries, Dettenborn et al. 2009), this information could have been informative in further understanding the impairment and contribution of sleep-related factors to the CAR in this population. Furthermore, our sample of women was quite diverse with respect to their menstrual cycle phase during the testing day.

However, a recent review has concluded that contribution of gonadal steroids to the CAR is negligible (Fries, Dettenborn et al. 2009). Further, we did not use electronic monitoring device to verify subject compliance with the sampling schedule, which may have affected the results (Kudielka, Broderick et al. 2003). Therefore, although the saliva sampling procedure was explained in detail to the subjects, our control and depression groups might have systematically differed on sampling compliance. However, a study that investigated compliance of outpatient depressed subjects with respect to testing procedure related to the dexamethasone suppression test, found that when people understand the instructions, noncompliance in outpatients is minimal and limited to elderly patients (Remillard, O'Reilly et al. 1993). Considering that our subclinical samples are composed of highly functional, university-educated individuals, it is likely that they would be similar to our controls with respect to compliance. Finally, we specifically focused on the HC volume, which was assessed via manual segmentation protocol based on the 1.5T scanner brain images. Therefore, the study does not evaluate presence or contribution of variability in other brain structures.

In summary, the findings from this subclinical sample suggest that dysregulated CAR and small HC volume may constitute vulnerability factors for MDD. The present literature on this topic would benefit from longitudinal studies applying a prospective design where subjects at risk for depression are followed up over time. In addition, longitudinal studies are also needed within healthy

populations, as we are still lacking normative data for a "normal" CAR. Furthermore, future studies should also consider evaluating flexibility of CAR (for e.g. weekday *versus* week-end samplings) rather than just CAR magnitude, given that a recent study has shown that a more flexible CAR (increased cortisol levels on weekdays and decreased levels during the week-end) was more characteristic of happier, less stressed and less neurotic patients (Mikolajczak, Quoidbach et al. 2010). Finally, there is a clear need for better understanding of the regulatory networks that underlie the CAR. Further multidisciplinary investigations are needed to discern the mechanisms that may underlie these phenomena.

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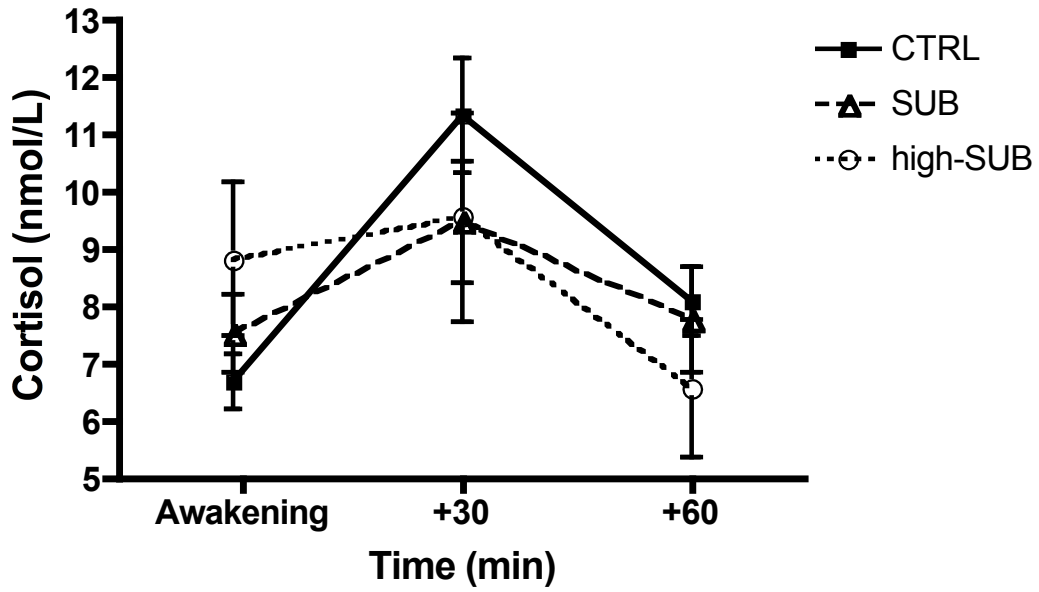
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Figures and Figure captions:

Figure 1

(A)

(B)

Figure 1: Change in Cortisol Awakening Response (CAR) across experimental groups. (A) Significant increase from awakening to +30min (t(25)=-5.30, p<.001) and the subsequent return (t(25)=3.78, p=.001) was observed in the control group. The subclinical group showed a significant decrease from the +30 min peak (t(22)=4.06, p<.001). There was no significant increase following the awakening in the high-risk subclinical group(t(8)=-.60, p=.6). (B) The CAR area-under-the-curve increase (CAR AUCi) was significantly lower in the high-risk subclinical compared to control subjects (p=.04). CTRL: control group; SUB: subclinical group; high_SUB: high-risk subclinical group; Graphs represent mean values ± SEM.

Figure 2
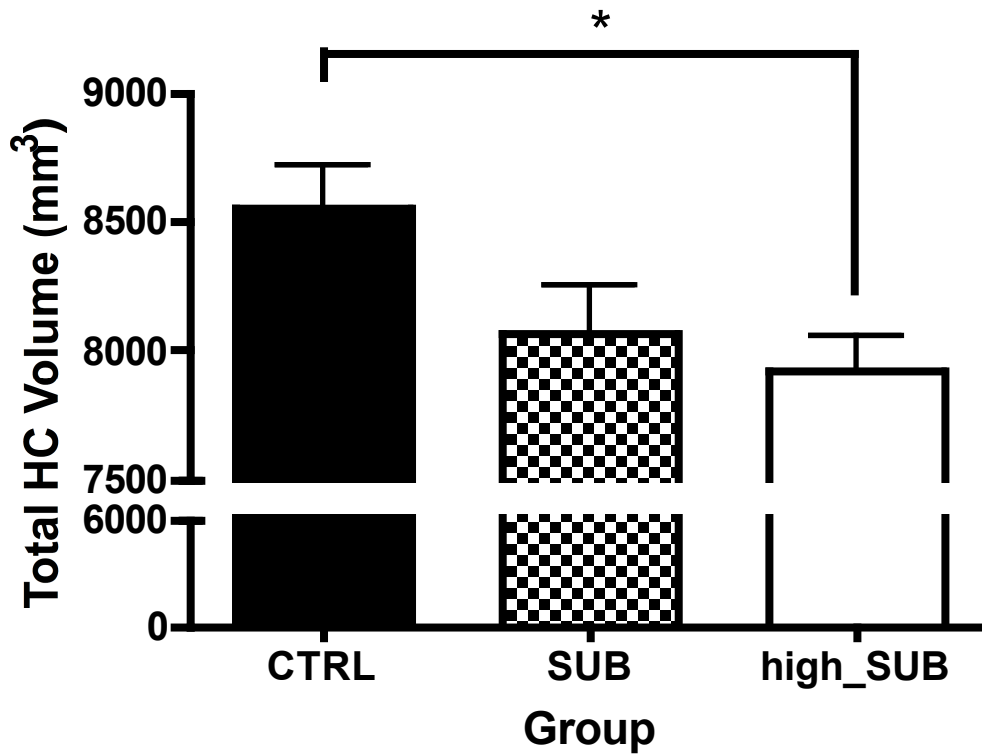
Figure 2: Differences in Total Hippocampal Volume across experimental groups. The high-risk subclinical group had smaller total hippocampal volume compared to the control group (p<.05), while the difference between the subclinical group and the control group was only a tendency (p=.14). HC: hippocampus; CTRL: control group; SUB: subclinical group; high_SUB: high-risk subclinical group. The graph represents mean values ± SEM.

Tables

Group	Control	Subclinical	High-risk Subclinical	Total
Sex Ratio (Male/Female)	12/15	12/11	4/5	28/31
Age (years)	22.6 ± 3.0	21.6 ± 2.1	20.8 ± 1.3	21.9 ± 2.5
BDI (at recruitment)	3.4 ± 2.8	12.1 ± 2.0 §***	12.4 ± 3.3	8.2 ± 5.1
HDI	5.5 ± 3.9	11.4 ± 3.7 §***	26.9 ± 5.9 #***	11.1 ± 8.5
MADRS-S	2.6 ± 2.1	4.6 ± 1.9 §***	10.3 ± 2.9 #***	4.6 ± 3.4
STAI-trait	32.2 ±7.9	39.7 ± 6.9 W§**	49.3 ± 7.7 M#***	37.8 ± 9.5
TICS total	38.1 ± 11.7	51.3 ± 14.8 §*	65.0 ± 9.9 #*	47.3 ± 15.9
Mother Care	27.2 ± 8.5	29.5 ± 5.9	24.2 ± 6.5	27.7 ± 7.4
CTQ total	34.7 ± 10.9	34.5 ± 9.5	41.0 ± 12.0	35.6 ± 10.7
CAR AUCi (nmol/L)	5.4 ±5.4	2.1 ± 4.95	-0.4 ± 4.8 §*	3.2 ± 5.5
HC Left (mm^3)	4316.5 ± 450.9	4041.3 ± 547.9	3983.2 ± 192.5	4158.4 ± 481.7
HC Right (mm^3)	4236.9 ± 431.1	4019.0 ± 430.9	3942.6 ± 287.4	4107.1 ± 424.6
HC total (mm^3)	8553.4± 856.5	8060.4 ± 935.9	7925.8 ± 376.7 §*	8265.5± 868.3

Table 1: Characteristics of the study population. Continuous variables are displayed as mean values ± SD. Apart from BDI levels, which were obtained at the time of the recruitment, all other psychological assessment were conducted at the time of the MRI scanning. BDI: Beck Depression Inventory; MADRS-S: Montgomery-Asberg Depression Rating Scale Self-Assessment HDI: Hamilton Depression Inventory; STAI-trait: Spielberger Trait Anxiety Inventory; TICS: Trier Inventory for the assessment of Chronic Stress; CTQ: Childhood Trauma

Questionnaire; CAR AUCi=Cortisol Awakening Response, area-under-the-curve increase; HC: hippocampus; §= comparison with the control group; #=comparison with the subclinical group; *=p<.05; **= p<.01; ***=p<.001

Supplementary Introduction:

While the exact mechanisms involved in the neural regulation of the cortisol awakening response (CAR) continue to be investigated, past research suggests that the hippocampus plays a major regulatory role. For example, a positive relationship between HC volume and the CAR has been observed in healthy young men (Pruessner, Pruessner et al. 2007). In addition, in patients with unilateral or bilateral damage to the hippocampus (either due to a primary insult or secondary to type 2 diabetes mellitus), a blunted or even absent CAR was found (Buchanan, Kern et al. 2004; Wolf, Fujiwara et al. 2005; Bruehl, Wolf et al. 2009).

Supplementary Results:

Association between cortisol awakening response and the HC volume

We investigated whether the cortisol awakening response was also associated with HC volume in the whole sample. Partial correlations between total HC volume and CAR AUCi (controlling for sex and depression severity) did not reveal a significant association (r=.06, p=.66). (all r<.189, all p>.2). However, given that the previous study that had investigated this association in normal population evaluated only men, we ran an exploratory analysis assessing the correlation, within each study group separately, and for men and women separately.

There was no significant association between total HC volume and CAR AUCi within each study group separately (all r<.00, all p>.66).

Although we found a significant association between total HC volume and CAR AUCi in men (Spearman rho = .39, p=.04), which was not significant in women (r=-.085, p=.66), this association became only a tendency once a suspected outlier was excluded from the analysis (Spearman rho = .32, p=.10).

Supplementary Discussion:

The regulatory mechanisms surrounding the HPA axis activity in general have been extensively investigated with the contributions of the amygdala, the prefrontal cortex and the hippocampus being at the center of these investigations (Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005; Dedovic, Duchesne et al. 2009). However, the exact mechanisms regulating the CAR are still relatively unknown. Given its link to the circadian rhythm, some have suggested that the suprachiasmatic nucleus may be involved (Hucklebridge, Hussain et al. 2005). Others have postulated that it may be an interplay between neocortical networks and the brain stem arousing systems (Chida and Steptoe 2009). Finally, a few studies have investigated the role of the hippocampus and found that this structure may play an important role in the regulation of the CAR, given that the loss of hippocampus abolishes the CAR (Buchanan, Kern et al. 2004), while in healthy young men, a positive association between HC volume and the CAR was observed (Pruessner, Pruessner et al. 2007).

This line of studies suggest that the role of the hippocampus in the contextualization of self-related information in time and space may underlie the CAR: at the time of the awakening self-related information is processed, integrated and the output is the resulting increase of the CAR that prepares one for

the day to come (Fries, Dettenborn et al. 2009). Therefore, the blunted CAR may then result from inefficient information integration particularly at the level of the hippocampus.

In the present study, we observed only a tendency for a positive association between these two variables in men only, and therefore these findings need to be interpreted with caution.

Supplementary References

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Figure 1

Men

(A)
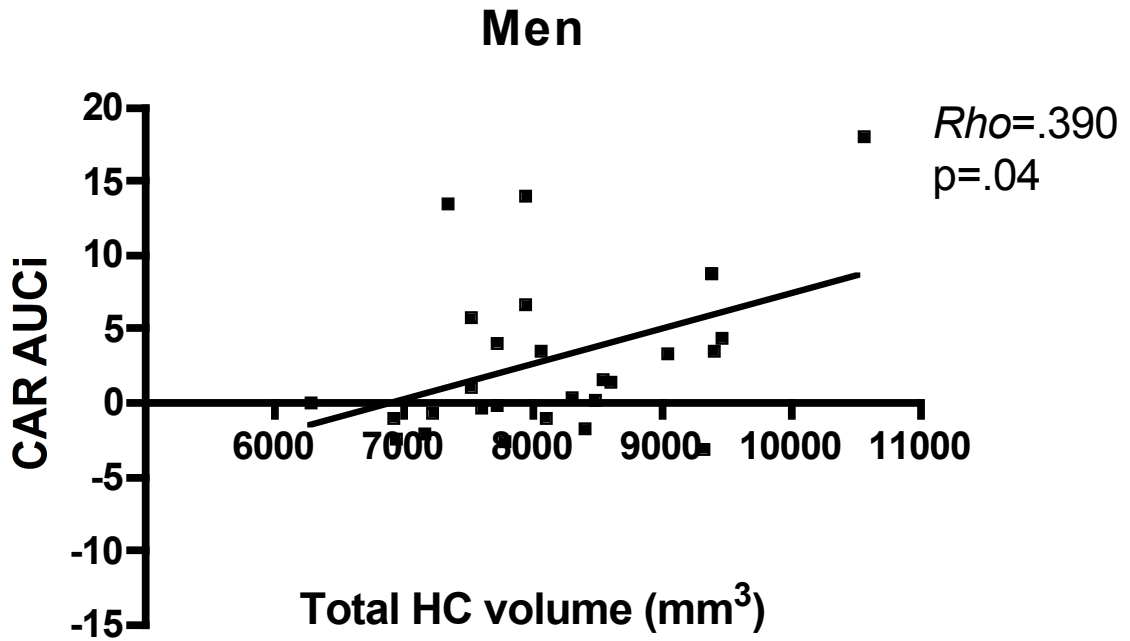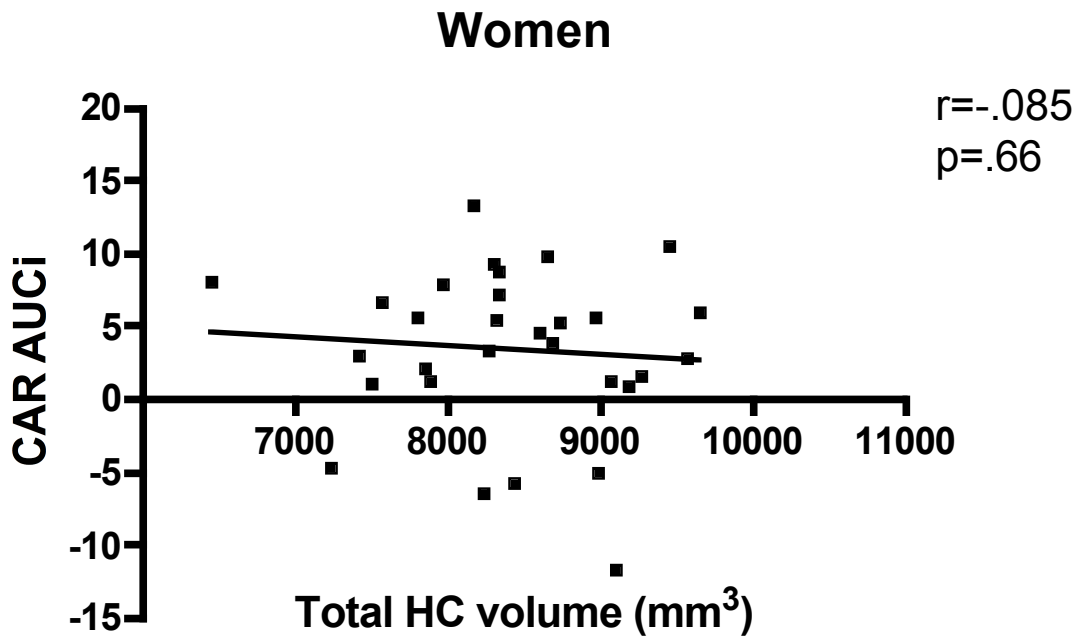
Women

(B)

Supplementary Figure 1: Correlation between the total hippocampal volume and the area-under-the-curve-increase Cortisol Awakening Response (CAR AUCi) in men and women. (A) Significant positive association was observed between the total HC volume and the CAR AUCi in men only (Spearman rho = .390, p=.04). However, once a suspected outlier was excluded from the analysis this correlation was no longer significant (Spearman rho = .32, p=.10) (B) The association was not significant for women (r=-.085, p=.66).

Chapter 5: Endocrine and neural correlates of social evaluative threat processing in healthy young adults with varying levels of depressive tendencies

Katarina Dedovic, Veronika Engert, Annie Duchesne, Sonja Damika Lue, Julie Andrews, Simona I. Efanov, Thomas Beaudry & Jens C. Pruessner

Preface to Chapter 5

The last chapter raised several important points that will also impact the chapter to follow. Firstly, at the time of the second visit, nine subjects scored at clinical levels on the depression questionnaires used to crosscheck group formation based on the Beck Depression Inventory (Beck, Brown et al. 1987). Given the short amount of time that passed from the time the subjects were admitted to the study and the second testing session (27.6 days +/- 11.4), we included these subjects as a separate group representing a group of high-risk subclinical subjects. Although not anticipated, this phenomenon is in keeping with the idea that subclinical depression is on a continuum with MDD (Solomon, Haaga et al. 2001; Lewinsohn, Klein et al. 2003), and that those who show current higher levels of depression at subclinical levels are at heightened risk for developing MDD (Cuijpers and Smit 2004).

Secondly, a gradual decline across groups was observed with respect to the measure of CAR and the total HC volume already in this subclinical sample, suggesting that these abnormalities may represent vulnerability traits for development of depression. Although the CAR is generally assessed with the basal cortisol profile, it is also representative of how the HPA axis can respond to a mild natural challenge, such as an awakening. This is important to keep in mind, as we now investigate, in the same sample of subjects, possible dysregulation of the HPA axis function and its brain regulatory networks in response to a mild psychological task, the modified MIST.

For this portion of the study, we return to the block design MIST. However, we had incorporated a new condition that now allowed us to investigate specifically the neural correlates of social evaluative threat within the block design.

MANUSCRIPT

Title:

Endocrine and neural correlates of social evaluative threat processing in healthy young adults with varying levels of depressive tendencies

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Abstract

Objective: Maladaptive endocrine responses to psychological stress and impaired mechanisms of stress regulation may play an important role in the vulnerability to Major Depression. In the present study, we aimed to investigate whether healthy young adults with varying levels of depressive tendencies at a subclinical level, may already show abnormalities in the endocrine stress response and the corresponding regulatory neural network when exposed to a mild psychosocial challenge.

Methods: We recruited healthy young men and women from a local university. Based on depression scores derived from standard questionnaires, three groups were formed: a control group (N=27), a subclinical group (N=23), and a high-risk subclinical group (N=9). The subjects completed an attentional bias dot-probe task, followed by a structural scan, and two runs of the modified Montreal Imaging Stress Task (MIST), a task that combines mental arithmetic with social evaluative threat components.

Results: The subclinical group showed a blunted cortisol response compared to the control group at specific time point during the modified MIST. Compared to the control group, the subclinical group also showed a greater change in signal intensity in the right occipital lobe in response to social evaluative threat. The change in activity within this region was positively correlated with sad attentional bias in the subclinical group, but with happy attentional bias in the control group. In addition, in the control group only, increase in signal intensity in the right occipital lobe was associated with decreased state depression scores following the

MIST compared to pre-scan measures. Furthermore, the control and the subclinical group both showed significant deactivation in the medial orbitofrontal (mORB) region in response to social evaluative threat processing, which was absent in the high-risk subclinical group.

Conclusions: The present findings suggest presence of dysregulation of both the endocrine profile and the neural network subserving processing of social evaluative threat even prior to onset of clinical levels of depression. Investigating the neural correlates of psychosocial stress in a subclinical population is essential for better understanding the ways in which dysregulation of specific processes may represent a vulnerability in the illness proper.

Introduction

Psychological stress has an important impact on central nervous system regulation, and it has been identified as an important culprit in several physical and psychological illnesses (Chrousos 2009). In particular, there is an intricate relationship between psychological stress and Major Depressive Disorder (MDD), with the onset and the development of MDD being often, although not always (e.g. Monroe and Reid 2009), preceded by periods of extreme, prolonged or chronic stress (e.g. Hammen 2005). Furthermore, dysregulation of the main stress axis, the Hypothalamic-Pituitary-Adrenal (HPA) axis, is common in depression (Gillespie and Nemeroff 2005; Pariante and Lightman 2008). Therefore, investigating individual differences in mechanisms underlying the processing of psychological stress, particularly in vulnerable populations, would be an important step in furthering our understanding of the contribution of stress to the etiology of depression.

However, while several studies have investigated neural networks associated with the psychological stress processing in normal populations (for example, Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008), to our knowledge, no study has directly investigated this in a population with an explicit vulnerability to depression, i.e. subclinically depressed individuals. Therefore, in the present study, we investigated a population of healthy young adults who showed varying levels of depressive tendencies, at a subclinical level. The goal of the study was two-fold: (1) to investigate whether the HPA response to a mild psychosocial challenge differs between healthy

controls and individuals with depressive tendencies at subclinical levels and (2) whether there are differences between these groups with respect to neural correlates of psychosocial stress processing.

We focused on individuals with subclinical depression (defined here as scoring above a certain cut-off on a self-rating depression inventory), since it has been suggested that the subclinical depression may represent a milder condition on the depression severity continuum (Solomon, Haaga et al. 2001; Lewinsohn, Klein et al. 2003; Rivas-Vazquez, Saffa-Biller et al. 2004). Despite some heterogeneity in definition of subclinical depression, studies investigating the incidence of major depression within subclinical populations consistently find increased incidence of MDD among these subjects in comparison to healthy controls (Cuijpers, Smit et al. 2005). Therefore, this subclinical sample represents a unique opportunity to examine possible changes within the stress processing system prior to the onset of the full-blown disorder.

The HPA axis is the key stress axis and is activated in response to a perceived stressful stimulus or situation. Psychological stress tasks that combine a motivated performance task, presence of uncontrollability, and social evaluative threat components are strong triggers of a stress response (Dickerson and Kemeny 2004). Despite this, individual differences in stress response to a psychological stressor have been observed (Kirschbaum, Klauer et al. 1995; Kirschbaum, Prussner et al. 1995; Pruessner, Gaab et al. 1997; Kirschbaum, Kudielka et al. 1999; Kudielka, Buske-Kirschbaum et al. 2004). The severity of the stressor, its context, individual's biological and environmental vulnerability and resilience

factors, as well as individual's appraisal of whether the demands of the situation exceed one's resources underlie these differences. With respect to depression specifically, cognitive factors such as dysfunctional attentional bias or tendency to ruminate over negative events, interact with stressful events to not only contribute to onset of a depressive episode, but also make recurrent bouts of depression more likely (De Raedt and Koster 2010).

The stress response involves the triggering of the HPA cascade and a sequential release of corticotropin-releasing hormone (CRH) from the hypothalamus, adrenocorticotropic hormone (ACTH) form the anterior pituitary, and finally, cortisol from the adrenals (Brown 2000). The released cortisol binds to its receptors, which are located on various sites in the periphery and in the central nervous system. Cortisol can thus regulate its own secretion through negative feedback at each level of the HPA axis, as well as by binding to its regulatory sites located throughout the limbic system, including hippocampus, amygdala and prefrontal cortex (PFC) (Herman and Cullinan 1997; Herman, Figueiredo et al. 2003; Dedovic, Duchesne et al. 2009).

The dysregulation of the HPA axis is common in MDD. Although a depressive state has been associated with a hyperactive HPA axis (Gillespie and Nemeroff 2005), studies using laboratory psychological stressors have reported inconsistent findings (Chopra, Ravindran et al. 2009; Handwerger 2009). A recent review concluded that the cortisol stress response in depressed is either similar to those in control groups (if examining total plasma cortisol levels) or somewhat blunted (when levels of free cortisol are assessed in saliva) in response to a

psychosocial stressor (reviewed in Burke, Davis et al. 2005; Handwerger 2009). Furthermore, a recent study investigating sex differences in the cortisol stress response in chronically depressed patients revealed that while depressed compared to healthy men showed a blunted peak salivary cortisol response to a psychological stressor, depressed compared to healthy women had an overall higher cortisol secretion in response to the stressor (Chopra, Ravindran et al. 2009). Yet, another study evaluating women remitted from recurrent major depression, found blunted serum cortisol levels in the patient compared to the healthy control group (Ahrens, Deuschle et al. 2008). Discrepancies in findings may be accounted for by differences in illness stage and cortisol sampling methods, and potentially by differences in the affected regulatory brain areas. Indeed, processing and regulation of the psychological stress response does not only involve the HPA axis, but also higher-order regulatory brain areas (Herman, Figueiredo et al. 2003; Herman, Ostrander et al. 2005; Pruessner, Dedovic et al. 2008; Dedovic, Duchesne et al. 2009).

Findings from neuroimaging studies on the neural correlates of psychosocial stress processing and regulation in healthy populations suggest that psychological stress processing is associated with deactivation in the orbitofrontal cortex, medial PFC, and the hippocampus, and activation in the ventrolateral PFC (reviewed in Dedovic, Duchesne et al. 2009). The anterior cingulate cortex (ACC) has also been implicated in this process, with both increases and decreases in activity observed in this region in response to stress (Dedovic, D'Aguiar et al. 2009).

Interestingly, some of these areas are also involved in mood regulation and have been implicated in depression etiology (Drevets, Price et al. 2008; Mayberg 2009; Price and Drevets 2010). For example, the orbital prefrontal network has been suggested to play a role in the integration of sensory information as well as coding for affective characteristics of stimuli (Drevets, Price et al. 2008). The medial prefrontal network has strong connections to limbic structures and also controls visceral functions (Ongur and Price 2000). The subgenual ACC seems to underlie autonomic and circadian components of mood regulation (Drevets, Price et al. 2008; Mayberg 2009), while supragenual ACC is related to self-referential processing (Northoff 2007), as well as monitoring of own emotional state and thinking about social attributes of the stimuli (Amodio and Frith 2006). It should be noted that both hypoactivity and hyperactivity in these areas have been reported with respect to depression (Drevets, Price et al. 2008; Mayberg 2009; Price and Drevets 2010).

Based on the previous literature, we hypothesized that in comparison to the control group, the subclinical group would show a different cortisol response to the psychosocial stress task. In addition, we expected to find differences between the groups in brain activity changes in those brain areas that have been previously observed in stress and mood regulation, i.e. hippocampus, orbitofrontal and medial prefrontal areas. Furthermore, given that our previous research has indicated that there are individual differences in stress responding (Pruessner, Dedovic et al. 2008; Dedovic, Rexroth et al. 2009), we expected to find subgroups

of responders, participants showing an increase in cortisol in response to stress, and non-responders, within each of our study groups.

Methods

Subjects

Sixty-four (30 men : 34 women) right-handed, healthy college students (mean age=21.9 ± 2.5) were recruited for this study via online classified ads. Subjects completed screening questionnaires to establish their eligibility for the study. Subjects were excluded if they had prior and/or present neurological or psychiatric illness, if they were regular smokers, used recreational drugs on a regular basis, and if they were taking any medication that could influence cortisol secretion. All subjects included met the safety requirements for participation in a functional Magnetic Resonance Imaging (fMRI) study. Further, they had no current diagnosis or history of claustrophobia or Axis I disorders. The final selection was based on their score on the Beck Depression Inventory (BDI) (Beck, Brown et al. 1987). Following the published BDI cut-off scores (Beck, Brown et al. 1987), the subjects were assigned to either a control group (BDI ≤ 9) (N=33) or a subclinically depressed group ($10 \leq BDI \leq 18$) (N=31) at the time of recruitment.

The Institutional Review Board (IRB) of McGill University approved the study, and informed consent was obtained prior to participation in accordance with the requirements of the McGill IRB.

Procedure

On the testing day, participants arrived at the Montreal Neurological Institute in the afternoon, one hour prior to when the scanning was scheduled. The subjects were given several psychological questionnaires to complete during this resting period. Fifteen minutes prior to entering the scanning room, a research assistant explained the procedure and tasks that would be performed in the functional Magnetic Resonance Imaging (fMRI) scanner. The tasks included an attentional bias task and a challenging mental arithmetic task, a modified version of the Montreal Imaging Stress Task (MIST) (Dedovic, Renwick et al. 2005). Subjects were then introduced to the study investigator and exposed to three functional runs of the attentional bias dot-probe task, followed by a structural scan, and finally two runs of the modified MIST. Although behavioral and fMRI results relating to the attentional bias task will be reported elsewhere (Dedovic et al. *in preparation*), a brief description of the task is provided below as we also investigated whether there was an association between the subjects' attentional bias scores and variables associated with stress processing.

Attentional Bias Dot-Probe task

Attentional bias scores were derived from a classic dot-probe task (Bradley, Mogg et al. 1998) adapted for the neuroimaging environment. The scores are based on the subjects' performance on cue+target trials specifically. During these trials, the subjects were exposed to pairs of faces (neutral and sad; neutral and happy) for 1000ms; once the face pair disappeared, a dot probe (either two dots arranged vertically or horizontally) appeared either on the side where the

emotional face was on (congruent) or on the neutral side (incongruent). The subjects were required to indicate, as quickly and as accurately as possible, by a button press, what type of dot probe they saw on the screen. The attention bias score for each emotion was derived by subtracting the mean reaction time for the congruent trials from the mean reaction time for the incongruent trials. A positive score indicated that a subject had a tendency to attend to a given emotional face, while a negative score indicated that one had a tendency to avoid it. The trials were balanced for male and female faces, position of the emotional face, and dot probe appearing congruent or incongruent to the emotional face.

Modified Montreal Imaging Stress Task

The Montreal Imaging Stress Task is a psychosocial stress task that uses mental arithmetic to combine the key situational components shown to facilitate mounting of a stress response: (1) presence of social evaluative threat (recording of subjects' responses, presence of "average" user responses for comparison, immediate negative feedback, and negative feedback by principle investigator) in (2) atmosphere of high achievement (or challenge) (mental arithmetic tasks) and (3) little or no controllability (math difficulty automatically adjusts to individual performance and induces failure) (Dickerson and Kemeny 2004). The MIST has been described in details elsewhere (Dedovic, Renwick et al. 2005). However, in the current study, the following changes were introduced to this task:

In the modified MIST, subjects were exposed to four conditions: rest (12 acquisitions), control (20 acquisitions), experimental/stress (30 acquisitions), and a new experimental/non-stress condition (30 acquisitions). These conditions were

presented in a block design, and repeated three times, in a pseudo-randomized fashion, over the course of the run.

During the experimental/stress (exp_S) condition, the task window was outlined by a red frame (Figure 1A). The subjects were explained that this was the condition of most importance for the task and it was emphasized that during this time their performance was being evaluated. During this condition, the user interface included: (1) a performance color bar on the top of the screen indicating the subject's performance in comparison to a mock "average" user, (2) the math task that needed to be solved in the middle of the screen, (3) a time advance bar just below the math task indicating the amount of time the subjects had to complete the task, (4) a rotary dial on the bottom right-hand corner where the subject could submit the response and (5) a performance feedback window, where upon the submission of response or timeout, the subject's performance on that task was printed out (Figure 1A).

The modified MIST was programmed to automatically adjust to the subject's level of performance. The program selected math tasks from specified categories ranging in difficulty levels (from simple operations with two or three one-digit integers to those involving several fractions). The answer of a given math expression was always between 0-9. The program used probabilities to determine whether the next question should be more difficult, easy or same as the previous, and whether to reduce or increase the time allowed for answering the question (based on the subject's previous performance and task difficulty). Although the subjects were told that they should be able to perform at about 85%

correct level, the task was designed to induce a performance level of about 50% during this exp_S condition.

The performance bar that ranges in color from red to orange to green, during exp_S condition, also included two arrows, with the top arrow indicating performance of a mock "average" user on this task, while the bottom bar reflected the performance of the subject up until that point. The subject was told that they were expected to perform within the green zone, as well as to be at the level of the average user or slightly above. Inevitably, over the course of the scan, their performance would be below that of an average user, and their performance arrow would regress into and stay within the red zone. Furthermore, the subjects were also provided with an immediate feedback on their performance, with either CORRECT, INCORRECT, TIMEOUT, now printed out along side RECORDED, in order to emphasize that their performance is being continually recorded and evaluated.

The experimental/non-stress (exp_NS) condition was governed by the same programming rules as the exp_S condition with respect to the difficulty of the math task presented and the time restrictions imposed; however, all of the evaluative threat components were removed (Figure 1B). Therefore, the outline of the window was green, the performance bar did not have any performance arrows displayed, the time advance bar was not shown, and the immediate performance was associated with NOT RECORDED, indicating to the subjects that at that time their performance was not recorded or evaluated.

In the modified MIST, the control condition now included math tasks with

lower difficulty compared to the experimental conditions and the subjects were given enough time to complete the given tasks. Like the exp_NS condition, the task window was outlined by green, and no evaluative threat components were shown. In fact, to the subjects, the modified MIST was introduced as having only three conditions: rest, control and experimental. The subjects were not told about differences in math task difficulty; rather it was emphasized that during the "experimental red condition" they would be evaluated and recorded, while during the "control green condition" their performance would not be recorded.

Finally, during the rest condition, the subjects were simply exposed to the user interface including the color bar on the top of the screen and a rotary dial, but no task was presented. They were told that they are not required to do anything during this time.

The key contrast of interest was exp_S > exp_NS, which is thought to capture the processing of social evaluative threat components, a key component in psychological stress processing (Dickerson and Kemeny 2004).

As with the original MIST version, in between each MIST run, the subjects were exposed to negative feedback given directly by the study investigator. During this time, the investigator informed the subject of how they had been performing up until that point in comparison to the average user. The investigator also reminded the subject of the fact that there was a required minimum performance for the task. Subjects were also reminded of the correct use of the response button box. Following the last MIST run, subjects were thanked for their participation and escorted by the research assistant to the

behavioral testing room in order to complete additional questionnaires and saliva samples. The full debriefing was given only after all the saliva samples were collected and all the questionnaires completed.

Saliva sampling

Participants completed eight saliva samples in total in order to assess levels of cortisol. Saliva was collected using the salivette sampling device (Sarstedt Inc, Quebec City, Quebec, Canada). The first saliva sample was taken before the scan, when subjects were seated at the scanning bench. The second saliva sample was taken after the second attentional bias task run, approximately 30min following the first sample. In order for the investigator to be able to reach the subject's head and collect saliva samples while the subject was in the scanner, the scan bench was partially taken out of the scanner bore. In such a way, the investigator was able to reach the subject and gently insert the salivette into the subject's mouth. When the subject was finished with the salivette, he/she pushed out the salivette to the tip of the mouth, so that the investigator could retrieve it. The third saliva sample was measured following the structural scan (about 60min following the first sample), and right before the first MIST run. The forth and the fifth saliva samples were collected after each of the MIST runs, and the final three were sampled outside of the scanner, in 15 min intervals, while the subject was completing additional questionnaires and resting.

Once all the saliva samples were collected and questionnaires completed, the subject was debriefed about the testing procedure. The saliva samples were stored in the laboratory freezer until analysis. Samples were analyzed via a time-

resolved fluorescence immunoassay, of which intra- and inter-assay variability was shown to be less than 10% and 12%, respectively (Dressendorfer, Kirschbaum et al. 1992).

Psychological assessment

Subjects completed the Hamilton Depression Inventory (Reynolds 1995), as well as the Montgomery-Asberg Depression Rating Scale Self-Assessment (MADRS-S) (Svanborg and Asberg 1994) as a crosscheck for BDI depression levels obtained at the time of recruitment. Co-morbid trait anxiety levels were assessed using the Spielberger Trait Anxiety Inventory (STAI) (Spielberger 1983). In addition, we assessed levels of psychological stress within the previous month by administering the Trier Inventory for the assessment of Chronic Stress (TICS) (Schlotz, Schulz et al. 2004). We also assessed state changes in current mood by using the Profile of Mood States (McNair, Lorr et al. 1992). Finally, we investigated the impact of the MIST procedure on the subjects' state levels of performance and social self-esteem via the Current Thoughts Scale (Heatherton and Polivy 1991).

Behavioral Statistical Analysis

Given that several subjects scored at clinical levels of depression on scan day, three experimental groups were formed (see the Results section for details). Therefore, group differences on psychological variables were assessed using univariate ANOVA with study group and sex as between factors. Differences with respect to change in cortisol were assessed in several ways. First, a mixed-design ANOVA was conducted with cortisol levels across the scanning time as repeated

measures, and group and sex as between factors. Since the cortisol values were not normally distributed, we log transformed the data for the statistical analyses. The figures however reflect the non-transformed values for easier interpretation of the data.

We also assessed cortisol output during the MIST in terms of area-under-the-curve (AUC) measures. Here, an AUC increase (AUCi) measure reflects the response of the system compared to the baseline (saliva sample #3 right before the first MIST run; Time=0); while an AUC ground (AUCg) measure represents an overall cortisol output with respect to the level of zero (Pruessner, Kirschbaum et al. 2003).

For the assessment of change in mood and self-esteem across time, we applied mixed-design ANOVA, with levels of mood or self-esteem and time as repeated measures, and group and sex as between factors.

If the sphericity assumption was violated, we applied Greenhouse-Geisser (GG) correction. If main effect ANOVA was significant for the study group factor, this analysis was followed by Games-Howell post-hoc test, a test that does not assume that population variances or sample sizes are equal (Field 2005). In case of significant interactions, ANOVA analysis was followed up by the simple main effects tests, and, if needed, by t-tests, which were Bonferonni-corrected for multiple comparisons.

Functional imaging data acquisition and processing

The subjects were scanned in a 1.5 T Siemens Magnetom SonataVision scanner For the structural images, standard 3D gradient-echo pulse sequence was

used, with the field of view of 256 mm, the voxel size of 1 x 1 x 1mm, TR of 22ms, TE of 9.2ms and a flip angle of 30°. Among other structural preprocessing steps including non-uniformity correction and signal normalization, the structural images were registered into the MNI brain space using the 152 ICBM model brain (Evans, Collins et al. 1994) with non-linear transformation algorithms. The resulting transformation file of each subject was used to then transform each of the subject's functional files into the standard MNI space.

Subjects were exposed to two functional MIST runs. During each functional run, 276 whole-brain BOLD Mosaic 64 T2*-weighted echo-planar images were acquired transversely, along the direction of the anterior commissure to the posterior commissure line minus 30° (voxel size= 4 x 4 x 5 mm; slice number = 28; order of slice acquisition = interleaved; TR = 2370 ms; TE = 50 ms; flip angle = 90°, matrix = 64 x 64, field of view = 256 mm).

Prior to data analysis, functional raw data were motion corrected by alignment to the third frame in each run (Cox and Jesmanowicz 1999), and a 6 mm full-width-half-maximum Gaussian kernel was applied to spatially smooth the data and reduce noise.

The full data analysis was conducted using the *fmristat* program developed at the MNI (Worsley, Liao et al. 2002). The design matrix of block onsets and durations was determined from the log files collected during the MIST runs, and was convolved with the default hemodynamic response function. The first level statistical modeling was executed for each run, for each subject separately. The first three frames in each run were excluded, as they may not represent steady-

state images. In addition, we applied a 3D Gaussian kernel to smooth the autocorrelation of residuals, with a target degrees of freedom of 100. Furthermore, data were converted to percent of whole volume and spatially and temporally detrended. The main contrast of interest was exp_S > exp_NS. We also conducted main effects analyses for each of the conditions (exp_S and exp_NS).

During the second-level analysis, the two runs for each subject were combined by considering only fixed effects (Worsley, Liao et al. 2002). Finally, for the third-level analysis, where we combined data across all subjects, we first resampled effect files and standard-error-for-effect files obtained from the first level analysis into the standard MNI space. The resampled files were then combined across subjects by using a mixed-effects analysis to generate specific group t map files. This approach aims to achieve 100 dfs by smoothing the ratio of random effects variance divided by the fixed variance (Worsley 2005). The direct comparisons between the study groups for exp_S > exp_NS contrast were performed at this level as well.

The threshold of the t-map was calculated using the *stat_threshold* commandof the MNI toolbox, which returns the threshold for local maxima and the cluster size for t-maps generated by the *fmristat* program. Significant clusters were determined by using the methods described by Cao and colleagues (Cao 1999). This procedure established that any cluster greater than 637 mm^3, containing voxels at t-value greater than 3.17, was deemed significant at p=.05 corrected. Automatic determination and localization of the significant peaks and clusters while taking into account varying degrees of freedom for each

comparison was established using the *stat_summary* command. Therefore, unless otherwise specified, the clusters reported are significant at t > 3.17 for activations, and t < -3.17 for deactivations, at p=.05 corrected. We used Neurolens 1.7.3 for visualization of brain activity patterns (Hoge 2006). All t-map files were superimposed over an average structural file of all the subjects from the study.

We created a functional mask for each significant cluster that was detected. The masks were then applied onto the effect files for the exp_S > exp_NS contrast, as well as for the main effects analyses (exp_S and exp_NS), for each subject. The effect size (% signal change) values for voxels within the cluster were then averaged to obtain a mean value of % signal change for each cluster for each subject. These values were then entered into a statistical program and graphed. This was done in order to be able to further characterize the differences in signal intensity changes detected during the fMRI statistical analyses. It should be noted that separate ANOVAs were not conducted on these values. The mean % signal change values for the contrast exp_S > exp_NS were used in the follow-up exploratory correlational analyses.

Results

Study population

Initial inspection of the data revealed that five subjects had to be excluded due to missing functional data, abnormal cortisol profile, or an inadequate performance in the computer tasks, leaving the final subject number of 59 (29 controls, and 30 subclinicals).

On the scan day, several subjects had scored at clinical depression levels either on the HDI or MADRS-S. We did not exclude these subjects since only a short amount of time had passed from when these subjects were admitted to the study (27.6 days +/- 11.4). Instead, they formed a third group representing high-risk subclinical subjects. These subjects were advised to seek professional counsel and were given a referral letter. The final group numbers were 27 controls (12 men; 15 women), 23 subclinicals (12 men; 11 women), and 9 high-risk subclinicals (4 men; 5 women).

Women (N=31) in the full sample varied with respect to their menstrual cycle and contraceptive usage. However, across the study groups, the samples appeared to be well balanced. In the control group, 5 women were in the follicular phase, 2 in luteal, and 7 were on contraceptives. In the subclinical group, 3 were in the follicular phase, 0 in luteal and 8 were on contraceptives. Finally, within the high-risk group, 2 were in follicular, 1 in luteal and 1 on contraceptives. Two women did not provide information on their menstrual cycle (1 from the control group, and 1 from the high-risk subclinical group). Due to small numbers for each menstrual phase within each study group, chi-square analysis could not be conducted.

Behavioral data

The experimental groups differed on levels of depression, stress and anxiety (Table 1). The subclinical group had higher levels of subclinical depression compared to the control group as assessed by the BDI (F(2,58)=82.5,

p<.001). Furthermore, the high-risk subclinical group had higher scores compared to the subclinical group who, in turn, had higher HDI (F(2,57)=91.4, p<.001) and MADRS-S (F(2,58)=43.9, p<.001) scores compared to controls. There was a main effect of the group (F(2,58)=15.9, p<.001) on chronic stress levels, with a significant increase in stress levels across the groups (p<.02). With respect to trait anxiety levels, we observed a significant main effect of the group (F(2,58)=21.2, p<.001), and a group by sex interaction (F(2,58)=3.2, p=.049). Women in the subclinical group had higher anxiety levels compared to women in the control group (t=-2.9, p<.01), while men in the high-risk subclinical group had higher anxiety levels compared to men in the subclinical group (t=-4.5, p<.001).

Overall differences in stress response: the subclinical group shows blunted cortisol levels during the modified MIST

For each time point, we assessed whether there were any outliers with respect to saliva samples. We found an outlier value per time point stemming from 4 subjects (3 controls, 1 subclinical). Rather than discarding these subjects, we applied a correction to bring these values within a 3.29 standard deviation from the mean (Field 2005).

A mixed design ANOVA to examine change in cortisol levels over the whole scan time (with study group and sex as between variables) revealed a main effect of time (F(3.03, 160.39)=11.4, p<.001, GG corrected), a main effect of group (F (2, 53)=3.17, p=.05), as well as a group by time interaction (F(6.05, 160.39)=2.8, p=.013, GG corrected).

The simple main effects revealed that there was a significant difference between the groups after the first MIST run ($F_{(2,56)}=4.92$, p=0.01) and 15min following the second MIST run ($F_{(2,56)}=3.70$, p=.03). A trend for difference between the groups was observed directly after ($F_{(2,56)}=2.77$, p=.07), and at 30min after the second MIST run ($F_{(2,56)}=3.06$, p=.06). All other values were found to be non-significant (all F<2.45, all p>.095). We conducted independent samples t-tests in order to verify whether the controls differed from the subclinical group at the time point after the first MIST run, and also to examine whether the control group differed from subclinical and high-risk subclinical groups at 15 minutes after the completion of the MIST. The control group showed higher cortisol levels compared to the subclinical group after the first MIST run ($t_{(48)}=2.58$, p=.013), which remained significant after correcting for multiple comparisons (Figure 2A). Differences between the controls and the subclinical group for the time point at 15 min following the end of the MIST showed a trend only ($t_{(48)}=1.57$, p=.12); the comparison with the high-risk subclinical group was not significant ($t_{(34)}=1.41$, p=.17).

The simple main effects analysis of the effect of time within each study group revealed a significant effect of time within the subclinical ($F_{(7, 392)}=4.3$, p<.001) and high-risk subclinical ($F_{(7, 392)}=7.6$, p<.001) groups, but not within the control group ($F_{(7, 392)}=1.4$, p=.187). Within the subclinical groups, we were particularly interested in comparing the time point after the first MIST run (Time=+15) to the baseline (Time=0), as well as examining the time point at 15 min following the end of the MIST scan (Time=+45) in comparison to the

baseline measure. In the subclinical group, there was a decrease in cortisol at both time points in comparison to the baseline (Time=+15, t(22)=2.1, p=.047; Time=+45, t(22)=2.6, p=.018). In the high-risk subclinical group there was a significant decrease in cortisol at Time=+45, (t(8)=2.4, p=.046). However, after correction for multiple comparisons, only the time point at +45 min following the baseline, within the subclinical group, remained a trend while all others were non-significant.

We also conducted two univariate ANOVAs (group and sex as between factors) assessing differences with respect to AUCi and AUCg measures related to MIST. There was a tendency for the group effect on AUCgMIST (F(2,53)=2.3, p=.106); however, there was no difference with respect to the AUCiMIST measure (F(2, 53)=1.1, p=.33) (Figure 2B).

Overall, despite the fact that the control group did not show an increase in cortisol over time, there is evidence of lower cortisol levels in the subclinical group compared to the controls at specific time points during the MIST procedure (after the first MIST run and a trend for lower cortisol levels 15 minutes following the MIST).

Stress response subgroups: only responders within the control group show cortisol increase over time in response to the modified MIST

Although there was no overall time effect on cortisol levels within the control group, and only a trend for a decline over time within the subclinical group, from our previous research, we were expecting to find groups of

responders and non-responders (Pruessner, Dedovic et al. 2008; Dedovic, Rexroth et al. 2009). Therefore, we conducted a k-means clustering procedure entering raw data starting at baseline (Time=0), until the end of the testing, requesting a solution of two or three clusters within each group separately in order to be able to capture distinct patterns of stress response. The three-group solution yielded the expected subgroups of responders and non-responders, but allowed us to also capture extreme responders within the study groups. The following subgroups were found: in the control group there were 20 non-responders, 6 responders, 1 high-responder (Figure 2C); in the subclinical group, there was a subgroup of subjects who declined over time (non-responders, N=14), a group of subjects that showed a "flat response" (flat-responders, N=8), and 1 high-responder (Figure 2D). Finally, in the high-subclinical group, there were 5 non-responders, 3 flat-responders, and 1 subject that showed a very uncharacteristic cortisol response that was quadratic in shape (Figure 2E). A flat response was characterized by absence of a trend in the data for a negative slope from the baseline measure. While this is clearly not the expected stress response profile, a recent article has suggested that even the absence of the expected circadian decline in cortisol levels over the course of the experiment may reflect an important disturbance of the normal HPA function (Lovallo, Farag et al. 2010).

Within each study group separately, we conducted a mixed-design ANOVA with time as within factor and subgroups as a between factor (responders and non-responders only, since high-responders could not be included in the analyses). In the control group there was a main effect of subgroup

(F(1,24)=15.86, p<.001, GG corrected), a trend for main effect of time (F(2.89, 69.54)=2.35, p=.082, GG corrected), and a significant time by group interaction (F(2.89, 69.54)=6.95, p<.001, GG corrected). The simple main effects revealed that there was an effect of time within each group, with responders showing an increase between the time point at 15 minutes following the end of MIST and baseline (t(5)=3.61, p=.015), while non-responders showed a significant decline for the same comparison (t(19)=3.26, p=.004) (Figure 2C). In the subclinical group, there was a main effect of time (F(3.47, 69.32)=4.92, p=.002, GG corrected) and group (F(1,20)=17.89, p<.001, GG corrected), with the subgroup of flat responders having overall greater levels of cortisol compared to non-responders (Figure 2D). A trend for a time by group interaction (F(3.47, 69.32)=2.17, p=.09, GG corrected) was also observed. Similarly, in the high-subclinical group, we found a main effect of time (F(2.19, 13.14)=7.20, p=.007, GG corrected), and group (F(1,6)=22.77, p=.003, GG corrected), with the flat responders showing overall higher levels of cortisol compared to non-responders (Figure 2E); however, time by group interaction was not significant (F(2.19, 13.14)=1.53, p=.253, GG corrected).

fMRI results

Examining differences between control, subclinical, and high-subclinical groups in neural correlates of processing social evaluative threat (exp_S > exp_NS)

Direct comparison between control > subclinical group for the exp_S > exp_NS contrast revealed a greater signal change in the subclinical group within a large cluster located in the right occipital lobe (BA 19, highest peak at x, y, z = 45, -84, 11, t=-5.43) (Figure 3A, B). The bar graphs depicting the effect size show differential recruitment of this area in the subclinical group in exp_NS and exp_S conditions (Figure 3C).

Contrasting control group > the high-risk subclinical group for exp_S > exp_NS condition, we observed decreased signal intensities in the control group in the gyrus rectus of the medial orbitofrontal region (BA11, highest peak at x, y, z = 2, 28, -21, t=-5.09) (Figure 4A, C). Similarly, the direct comparison between subclinical group > high-risk subclinical group revealed decreased signal intensities in the subclinical group in this same area (highest peak at x, y, z = 1, 28, -21, t=-5.41) (Figure 3B, C). The bar graphs show that processing of social evaluative threat components is associated with a decrease in signal intensities in both control and subclinical groups (Figure 3D). The high-risk subclinical group showed a distinct pattern of decreased signal intensities for both exp_NS and exp_S conditions (Figure 4D).

Exploratory correlational analyses: associations with attentional bias scores as well as state depression scores following the modified MIST

In order to assess whether differences in signal intensities in the right occipital lobe and the medial orbitofrontal cortex may relate to mood scores following the MIST, in particular state levels of anxiety and depression, we

conducted exploratory analyses with contrast estimates of exp_S > exp_NS for these clusters and the change measure of state anxiety and depression scores (pre-scan measure subtracted from post-MIST measure).

Furthermore, given the role of the occipital lobe in processing of visual information, we ran an exploratory analyses evaluating association between contrast estimates of exp_S > exp_NS within this region and a measure of attention processing, attentional bias scores.

In the control and subclinical groups together, there was a positive association between contrast estimates within the right occipital lobe cluster and both mean happy bias (Spearman Rho=.37, p=.01, N=47) (Figure 5A), and mean sad bias (Spearman Rho=.31, p=.03, N=47) scores (Figure 5B). There were no significant associations with changes in depression and anxiety scores pre-scan and post-MIST (all Spearman Rho < -.27, all p>.07).

We then investigated the correlations within each group (control and subclinical) separately. In the control group, there was a positive association between contrast estimates for the occipital lobe and mean *happy* bias (Spearman Rho=.66, p<.001, N=25) (Figure 5C). In addition, there was a significant negative association between contrast estimates and change in depression scores, i.e. the greater the change in response to social evaluative threat, the lower the depression state score at post-MIST compared to pre-scan (r=-.64, p=.001, N=24) (Figure 5D). In the subclinical group, we found a positive association between contrast estimates for the occipital lobe and mean *sad* bias (Spearman Rho=.69, p<.001, N=22) (Figure 5E).

No associations were found between contrast estimates for exp_S > exp_NS within the medial orbitofrontal cluster and changes in state anxiety and depression measures in the whole group or within each group separately (control, subclinical and high-risk subclinical).

Changes in psychological state measures: current anxiety and fatigue increase, while vigor and performance self-esteem decrease following the modified MIST

We conducted a mixed design ANOVA with current mood (depression, anxiety, anger, fatigue, vigor and confusion) and time (pre-scan and post-MIST) as repeated measures, and group and gender as between factors. The mixed design ANOVA revealed a significant main effect of mood (F(1.96, 88.47)=28.9, p<.001, GG corrected), a time by mood interaction, (F(2.35, 105.66)=7.9, p<.001, GG corrected), and a mood by group interaction (F(3.93, 88.47)=4.3, p=.003, GG corrected). There was a trend for a three-way interaction for mood, group and gender (F(3.93, 88.47)=2.3, p=.064, GG corrected).

The simple main effects revealed that levels of current anxiety and fatigue were higher post-MIST compared to pre-scan (anxiety: F(1,45)=4.08, p<.049; fatigue F(1,45)=4.05, p=.05). Levels of vigor decreased over time (F(1,45)=12.78, p<.001). There was a trend for time difference for levels of anger (F(1,45)=3.31, p=.075). No time difference was observed for depression and confusion scores (all Fs(1.45)<.32, all ps>.575) (Table 2).

Decomposing the current mood x group interaction revealed a significant effect of group on current anxiety levels (F(2,48)=5.60, p=.007), depression

(F(2,48)=5.34, p=.008), anger (F(2,48)=5.18, p=.009) and fatigue (F(2,48)=7.67, p=.001) levels. There was a trend for group effect on levels of confusion (F(2,48)=3.00, p=.059), but there were no significant differences with respect to vigor (F(2,48)=1.94, p=.15). We conducted the simple t-tests as a follow-up and applied the multiple comparisons correction. This procedure revealed that, compared to the control group, the subclinical and high-risk subclinical groups had higher current levels of fatigue (subclinical: t(36.2)=3.3, p=.002, equal variances not assumed; high-risk subclinical (t(34)=5.2, p<.001). In addition, the high-risk subclinical group had higher levels of anxiety compared to the control group (t(32)=4.2, p<.001). Differences between subclinical and high-risk subclinical group did not survive multiple comparisons correction.

A mixed design ANOVA, with self-esteem type (performance, social) and time as repeated measures, and group and gender as between measures, revealed a main effect of time (F(1, 53)=5.9, p=.019, GG corrected), a time x self-esteem interaction (F(1, 53)=23.8, p<.001), and a self-esteem x gender interaction (F(1,53)=7.5, p=.008).

There was also a main effect of group (F(2, 53)=11.3, p<.001). Post-hoc Games-Howell procedure on this main effect revealed that the control group had higher overall self-esteem levels compared to the subclinical group (p=.051), who, in turn, had higher self-esteem levels compared to the high-risk subclinical (p=.05).

The simple main effects of time x self-esteem interaction revealed that state levels of performance self-esteem decreased following the MIST (F(1,

53)=15.2, p<.001) (Table 2). There was no effect of time on state levels of social self-esteem (F(1, 53)=.32 p=.57) (Table 2).

Decomposing self-esteem x gender interaction revealed that only women showed overall higher state levels of social self-esteem compared to performance self-esteem (F(1.57)=7.8, p=.007).

Discussion

In the present study, we focused on a sample of healthy individuals with varying levels of subclinical depression in order to first investigate whether there are abnormalities in the cortisol response to a mild psychosocial challenge prior to clinical stages of depression; and second, to examine whether there are group differences with respect to neural correlates of processing social evaluative threat. This is the first study, to our knowledge, to investigate both the endocrine and neural correlates of social evaluative threat in the sample showing subclinical levels of depression.

Subclinical depression subjects already show a hypoactive HPA axis

Despite the absence of a significant cortisol stress response in the control group, we found evidence of lower cortisol levels in the subclinical group compared to the control group following the first MIST run. In this same sample of subjects, we have previously reported a blunted cortisol response to the natural challenge of awakening in both the subclinical and high-risk subclinical groups compared to the control group (Dedovic et al, in press). In conjunction with those

results, the present endocrine findings are suggestive of a hypoactive HPA axis in the subclinical group in response to a mild psychological stressor.

This result extends the findings from the studies of clinically depressed populations that also showed a blunted cortisol response to laboratory psychological stressors as well as daily life stressors in the depressed patient populations compared to controls (reviewed in Burke, Davis et al. 2005; Handwerger 2009). Therefore, a blunted cortisol response to a psychological stressor seems to be present prior to onset of clinical depression and may be a risk factor. This interpretation is further supported by findings from a recent study in healthy students which showed that greater trait depressive rumination was associated with a more blunted cortisol response in the condition with social evaluation present (Zoccola, Dickerson et al. 2008).

The blunted nature of cortisol response in the subclinical group is further evident in the cortisol profiles of the subgroups of responders and non-responders. Specifically, although the responders and non-responders were found within each study group, only the responders within the control group showed a significant increase in cortisol in response to modified MIST over time. Within the subclinical and high-risk subclinical groups, the responders were characterized by a rather flat cortisol profile, but overall greater cortisol levels compared to the non-responders. Nevertheless, this flat response (or blunted response in the overall subclinical groups) should not be interpreted to mean a non-existent response. More specifically, a recent study comparing stress-induced cortisol levels not only to a baseline measure obtained on the day of the stress task

administration, but also in comparison to a resting control day, suggested that use of only stress-day measures may underestimate stress reactivity, particularly in cases of what may be considered the flat responders (Lovallo, Farag et al. 2010). In that study, the resting control day followed the stress procedure day and involved the subjects simply reading magazines or watching nature programs at the same time of the day and for the same duration as during the stress task procedure, the day earlier. Examining sex differences in the stress response using only the stress day data, the authors found a significant cortisol increase in men in response to public speaking tasks, but a flat cortisol response in women. However, when they compared women's flat cortisol response of the stress day to their resting control day levels, it became apparent that the "flat" cortisol levels are actually much higher compared to what these women's normal circadian decline would have dictated (Lovallo, Farag et al. 2010). To what extent does this type of perturbation of the diurnal cycle impact individual's physical and mental health as well as their vulnerability or resilience to various illnesses requires further investigation.

Therefore, in the present study, the apparent lack of cortisol stress response may still represent a response, although to a much lower degree that what was expected. As we did not have a resting control day, we could not assess whether this was indeed the case.

Presence of a third cluster containing an extreme responder within each of the study groups is representative of individual differences present in both normal and at-risk populations, and shows that within each of these study groups, some

individuals may have an extreme cortisol response even to a mild stressor. Unfortunately, the cluster size which was restricted to N=1 within each study group, precluded inclusion of these subjects in responder/nonresponder related analyses.

But, what may account for the blunted cortisol response observed in the subclinical group? Given that the subclinical group reported greater levels of chronic stress compared to the controls, the blunted response may reflect exhaustion of the regulatory mechanisms of the HPA axis over time (Hellhammer and Wade 1993; Fries, Hesse et al. 2005). For example, it has been suggested that blunted HPA axis activity may occur following an extensive period of hyperactivity, as in situations of chronic stress (Heim, Ehlert et al. 2000). After such a period, the system will then either become non-responsive or may over-adjust (for example, reducing the receptor number at the pituitary in response to a CRH overdrive) (Fries, Hesse et al. 2005). Yet, this explanation does not account for the similarity in cortisol response profile between the controls and the high-risk subclinical group (who had the highest levels of chronic stress). Therefore, the differences between the groups may also reflect additional influence of different genetic make-up on the HPA axis regulation, perhaps at the level of glucocorticoid receptor polymorphisms known to play a role in Major Depression (Spijker and van Rossum 2009). It should however be noted that some have suggested that the hypoactive HPA profile may also represent an adaptive response of the organism to reduce the negative effects of repeated stress-induced cortisol response in these individuals (Fries, Hesse et al. 2005). The hypoactive

HPA profile may also underlie the sickness behavior, which has been proposed to promote recuperation of the organism, particularly in atypical depression (Van Hoof, Cluydts et al. 2003). Therefore, a blunted cortisol response in the subclinical group may also be adaptive.

Alternatively, it may be possible that exposure to the scanner may have triggered the HPA axis during the first part of the scanning period, which could have affected the HPA axis response during the MIST. However, we found no significant associations between the change in cortisol over the first two cortisol measures and the cortisol output during the MIST in each group (data not shown). Finally, it must be kept in mind that the overall absence of cortisol stress response in the control group is suggestive of the modified MIST being an extremely mild stressor and imposes limits on an extensive interpretation of the meaning of group differences in cortisol levels found in the present study.

Although a very mild stressor, the modified MIST did have an effect on the subjects' affective state, with an overall increase in anxiety, as well as overall increase of fatigue and decrease in vigor following the procedure observed in the full sample. In addition, the subjects' state performance self-esteem levels decreased suggesting some internalization of the negative feedback that had been presented to them.

Occipital lobe and attentional bias: a link between cognitive vulnerability to depression and processing social evaluative threat?

The sole region that differentiated between the control and the subclinical group in processing social evaluative components was the right occipital lobe, BA19, with the subclinical group showing a greater increase in signal intensity in response to social evaluative threat. Occipital lobe underlies the processing and integration of visual information. Increased activity in BA 19 specifically, has been reported in processing of both negative and positive adjectives in comparison to neutral ones (Demirakca, Herbert et al. 2009), in third-person perspective taking (Jeannerod and Anquetil 2008), and has also been associated with manipulation of spatial relationships between objects (Haxby, Grady et al. 1991). The components of the exp_S condition may load on some of these functions. The decreased signal intensity in the subclinical group observed during exp_NS condition is suggestive of lack of engagement of this area toward external stimuli during the condition when evaluation is not emphasized.

Importantly, the change in signal intensity in BA19 in response to social evaluative threat components was associated with increased bias for sad information in the subclinical group only. These findings are in line with cognitive vulnerability theory of depression (Clark, Beck et al. 1999), where negative bias in attention, memory and information processing may contribute to the onset of Major Depressive Disorder (reviewed in Mathews and MacLeod 2005), particularly during times of stress. For example, a study of healthy university students found that in individuals experiencing high level of life stress,

greater attentional bias for negative information at baseline was associated with higher levels of dysphoria at follow up 7 weeks later (Beevers and Carver 2003). In the control group in the present study, the change in signal intensity in BA19 was associated with increased *happy* bias, suggestive of a possible protective mechanism at play within this group. Indeed, the change in signal intensity in BA 19 was associated with lower depression state scores following the MIST compared to pre-scan scores.

The information processing within the human visual cortex can be influenced by both bottom-up processes, such as saliency of the visual stimulus, as well as top-down attentional processes generally subserved by frontal-parietal network (Kastner and Ungerleider 2000). Such biasing signals may, for example, contribute to the enhancement of neural responses in visual cortex to attended stimuli (Kastner and Ungerleider 2000). Therefore, the difference in BA19 found here may reflect differences within these higher order brain areas, which may be too subtle to detect at these subclinical levels of depression. These findings are suggestive of BA19 as an important node within the information-processing network that may signal brooding impairments developing in this network even prior to clinical levels of the illness.

Medial orbitofrontal cortex as a key differentiator between the high-risk subclinical group compared to both the control and subclinical groups in response to social evaluative threat

The key region that differentiated the control and subclinical group from the high-risk subclinical group was the gyrus rectus (BA11) of the medial orbitofrontal cortex. The deactivation observed in the controls and the subclinicals in response to social evaluative threat replicates previous findings from our laboratory (Pruessner, Dedovic et al. 2008; Dedovic, Rexroth et al. 2009) and from other groups (Tillfors, Furmark et al. 2001; Wang, Rao et al. 2005). We had previously proposed that the medial orbitofrontal cortex might play a role in initial stress perception and preservation of the stress response (Dedovic, Rexroth et al. 2009). Deactivation observed here without the presence of a strong cortisol stress response could suggest that perhaps the change in signal may need to pass a certain threshold in order to trigger the regulatory cascade that would allow for the significant increase in cortisol levels.

The completely opposite pattern of responses in the high-risk subclinical group within the medial orbitofrontal cortex is suggestive of an abnormal functioning of this area both in the evaluative and non-evaluative conditions. Medial orbitofrontal cortex has been implicated in mood regulation (Phillips, Ladouceur et al. 2008) and is an important player in the limbic-frontal circuitry models of depression (Mayberg 2003). Its connections to subcortical limbic and paralimbic regions (Ongur and Price 2000), as well as to cortical areas implicated in higher-order cognitive and executive processing (Phillips, Ladouceur et al.

2008; Price and Drevets 2010), allow this region to contribute to integration of sensory information from the body and environment (Gusnard and Raichle 2001), participate in automatic and voluntary emotional regulation (Phillips, Ladouceur et al. 2008), and process and update the value of possible future outcomes in order to help select goal-directed responses (Phillips, Ladouceur et al. 2008). The abnormal functioning of this area in both the evaluative and non-evaluative condition may thus reflect impairment in assessment of one's surroundings or represent a disconnection between environmental and internal demands and the regulatory mechanisms. As such, changes in activity pattern in medial orbitofrontal cortex may be an important moderator, at the neural level, of the connection between psychological stress and depression onset.

Untreated depressed state seems to be associated with decreased function in the orbital frontal cortices (BA 10 & BA 11) (Mayberg 2003; Mayberg 2009), which is consistent with the findings in the present study. However, some studies have also shown increased metabolic function in this area (reviewed in Drevets, Price et al. 2008). In addition, it has been suggested that reduced metabolic activity in areas such as subgenual anterior cingulate cortex may be an artifact of volumetric deficits in this area observed in depression. When correcting for partial volume averaging effect some have observed decreases in metabolic activity to revert to increases (reviewed in Price and Drevets 2010). As we did not assess volumetric differences in medial orbitofrontal cortex between our groups, we cannot dismiss this possibility. Nevertheless, even if the direction of change would be reversed, it would have an impact on both experimental conditions;

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therefore, the overall conclusion of dysregulated medial orbitofrontal cortex in high-risk subclinical group compared to the subclinical participants and controls still remains valid.

Differential response between the groups in the medial orbitofrontal cortex might indicate how the mood regulation network may be impacted even in response to a very mild stressor. The maladaptive response may reflect a condition that is pre-existent or a trait characteristic, but it may also represent a change in brain function resulting from repeated insults.

Limitations

The present study suffers from several limitations. Firstly, the fact that the modified MIST failed to induce a clear stress response in the control group limits the interpretation of group differences with respect to cortisol output. The apparent lack of cortisol stress response may be due to the changes we brought into the task. We have modified the original MIST in order to introduce an experimental/non-stress condition that would be exactly the same with respect to mental arithmetic and time-limit imposition as the experimental/stress condition, except for the presence of evaluative components. This, in addition to the control condition, which now contained easy math and ample time to respond, had amounted to a much longer period of time (total 5.93min/run) during which the subjects were exposed to what they knew as the "safe condition" compared to the evaluative condition (total 2.37min/run). While the limited exposure to the evaluative condition was enough to induce mood changes and have an impact of

state levels of performance self-esteem, it was not enough to trigger the expected HPA stress response. Secondly, women in the sample were quite diverse with respect to menstrual cycle phase. Previous studies have shown that menstrual cycle and oral contraceptive usage can influence cortisol response to stress (Kudielka and Kirschbaum 2005). However, the study groups were relatively evenly matched with respect to number of women using oral contraceptives and those in each menstrual cycle phase. Therefore, it is unlikely that the group differences observed were influenced by the diversity of menstrual cycle phase in women within this sample. Thirdly, we did not assess the participants' subjective measures of stress, nor did we assess their subjective perception of being under evaluation. Although we obtained measures of changes in mood and state self-esteem in response to the MIST, these additional subjective measures would have been a valuable asset in further understanding the psychological impact of the MIST manipulation.

Conclusion

This is the first study to characterize impairments, both with respect to endocrine and neural changes, in response to a mild psychosocial mental arithmetic challenge task in a sample of healthy individuals showing varying levels of subclinical depression. Investigating the neural correlates of psychosocial stress in a subclinical depression population is essential for better understanding the ways in which dysregulation of specific stress-related processes may represent a vulnerability for Major Depression.

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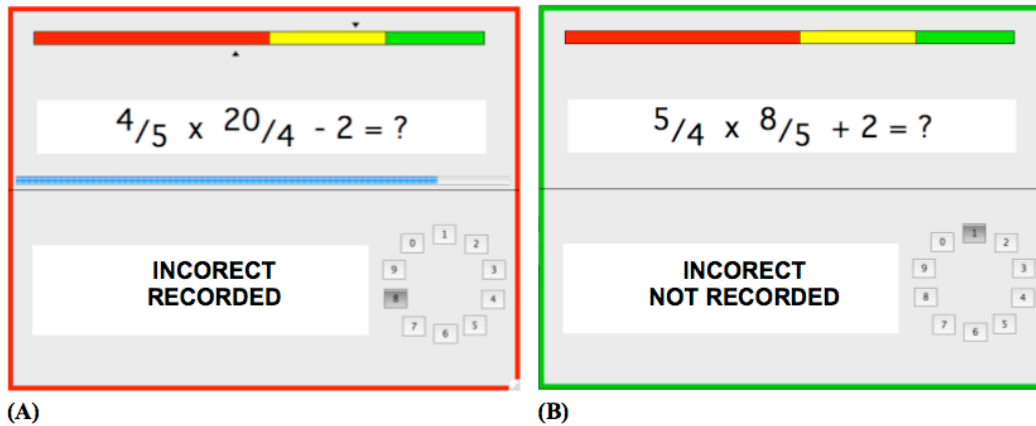
Figures and Figure Legends

(A)　　　　　　　　　　　　　　　　(B)

Figure 1.

Figure 1: The modified MIST user interface. (A) The experimental/stress (exp_S) condition includes performing challenging mental arithmetic in social evaluative setting: a performance color bar, on the top of the screen, indicating the subject's performance (bottom arrow) in comparison to a mock "average" user (top arrow), a time advance bar indicating the amount of time the subjects had to complete the task, and a performance feedback window, emphasizing that the subject's poor performance was recorded. **(B)** The experimental/nonstress (exp_NS) condition contains mental arithmetic task of same difficulty, and same time limit, but social evaluative components are removed.

(A)

(B)

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(D)

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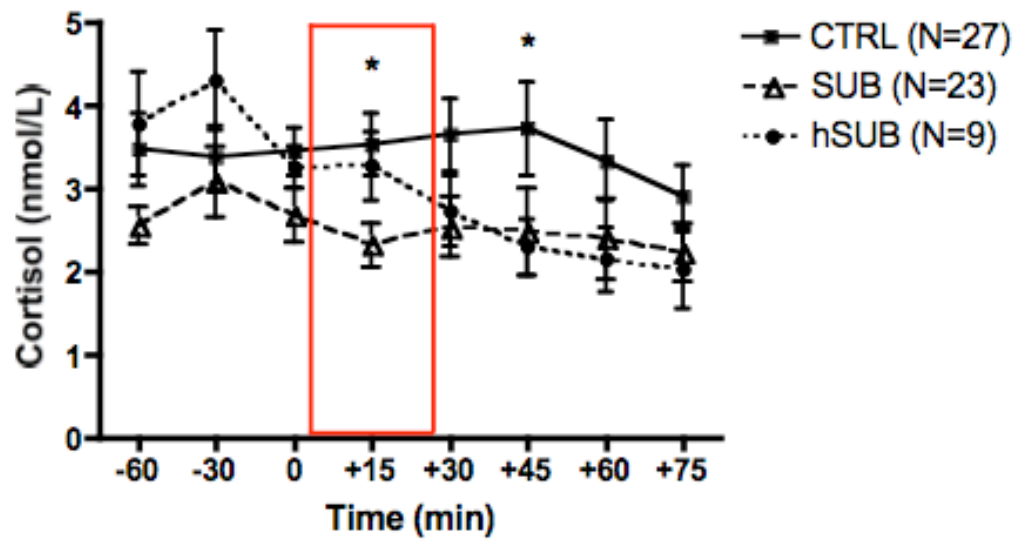
(E)

Figure 2.

Figure 2: Salivary cortisol levels during the scanning session. (A) Despite the lack of cortisol response in the control group, the subclinical group shows decreased cortisol levels at two time points, Time=+15min and Time=+45min, $p<.05$. The rectangle depicts duration of the stress task. Error bars show SEM. **(B)** Group differences with respect to area-under-the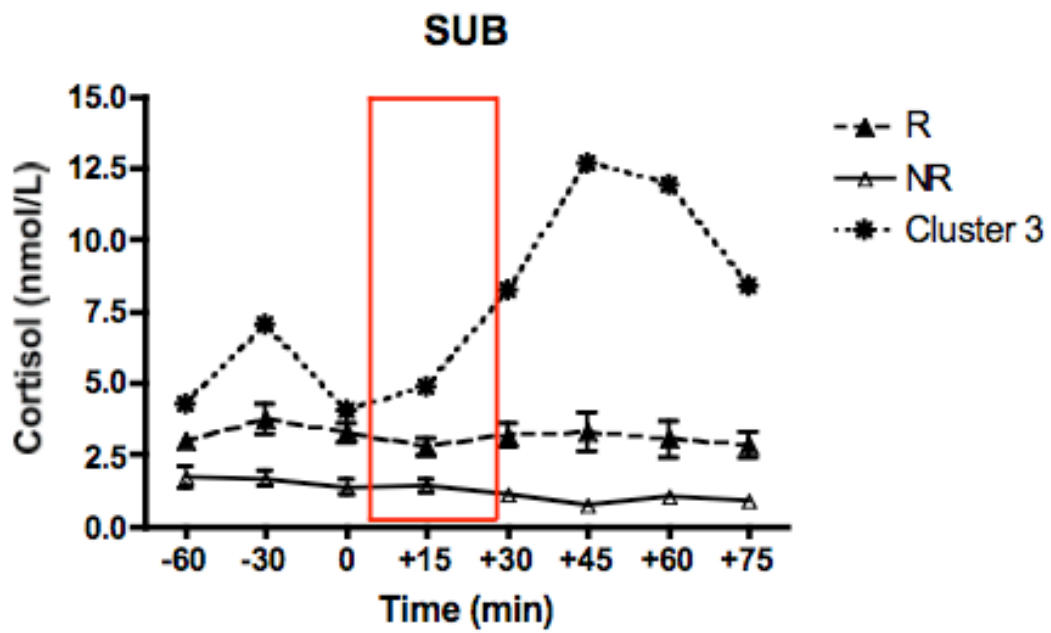-curve increase measure of cortisol output during and following the modified Montreal Imaging Stress Task (AUCiMIST), were not significant despite a pattern of increased blunting in the subclinical groups. **(C)** Cortisol profile in subgroups of responders and non-responders in the group of control subjects. Also shown a high-responder (Cluster 3). There was a significant increase in cortisol at time=+45min compared to the baseline (Time=0) in responders ($p=.015$), and a significant decrease for the same comparison in the non-responders. ($p=.004$) **(D)** Cortisol profile in subgroups of responders and non-responders in the group of subclinical subjects. Also shown a high-responder (Cluster 3). Responders had overall greater cortisol levels compared to non-responders ($p<.001$). **(E)** Cortisol profile in subgroups of responders and non-responders in the group of high-risk subclinical subjects. Also

shown an extreme responder (Cluster 3). Responders had overall greater cortisol levels compared to non-responders (p=.003). For A, C-E, the rectangle depicts duration of the stress task. Error bars show SEM. CTRL: control group; SUB: subclinical group; hSUB: high-risk subclinical group; R: responders; NR: non-responders

Figure 3. Differences between control and subclinical groups in brain activity changes for experimental/stress>experimental/nonstress (exp_S > exp_NS) comparison. (A) Direct comparison between the control and the subclinical group (CTRL > SUB) revealed significant deactivation cluster in the right occipital lobe (Brodmann Area 19). Cluster threshold was set at t<-3.17, p<.05 corrected. x, y, z = sagittal, coronal, and horizontal view in World coordinates. The underlying anatomical image is an average of the subjects' anatomical files in MNI space. **(B)** Bar diagrams show percent signal changes (Effect size) during processing of social evaluative threat components (exp_S >exp_NS contrast) for the control and subclinical group. **(C)** Bar diagrams show percent signal changes (Effect size) for the main effect of exp_NS and exp_S condition separately for the control and subclinical group. CTRL: control group; SUB: subclinical group; L: left; BA: Brodmann Area

Figure 4. Differences between the control and subclinical groups and high-risk subclinical group in brain activity changes for experimental/stress>experimental/nonstress (exp_S > exp_NS) comparison. **(A)** Direct comparison between the control and the high-risk subclinical group (CTRL > hSUB) revealed significant deactivation cluster in the gyrus rectus of the medial orbitofrontal cortex (Brodmann Area 11). Cluster threshold was set at t<-3.17, p<.05 corrected. x, y, z = sagittal, coronal, and horizontal view in World coordinates. The underlying anatomical image is an average of the subjects' anatomical files in MNI space. **(B)** Direct comparison between the subclinical and the high-risk subclinical group (SUB > hSUB) also revealed a significant deactivation cluster in the gyrus rectus of the medial orbitofrontal cortex (Brodmann Area 11). Cluster threshold was set at t<-3.17, p<.05 corrected. **(C)** Bar diagrams show percent signal changes (Effect size) during processing of social evaluative threat components (exp_S >exp_NS contrast) for the control, subclinical and high-risk subclinical group. **(C)** Bar diagrams show percent signal changes (Effect size) for the main effect of exp_NS and exp_S condition separately for the control, subclinical and high-risk subclinical groups. CTRL: control group; SUB: subclinical group; hSUB: high-risk subclinical group; L: left; BA: Brodmann Area

(A)

CTRL+SUB

Spearman *Rho*=.37
p=.01

Happy Bias

Effect size BA 19 (exp_S>exp_NS)

(B)

CTRL+SUB

Spearman *Rho*=.31
p=.03

Sad Bias

Effect size BA 19 (exp_S>exp_NS)

(C)

CTRL

Spearman Rho=.66
p<.001

Happy Bias

Effect size BA 19 (exp_S>exp_NS)

(D)

CTRL

r=-.635
p=.001
N=24

Change in state depression

Effect size BA 19 (exp_S>exp_NS)

(E)

SUB

Spearman Rho=.69
p<.001

Sad Bias

75
50
25
-0.1   0.1   0.2   0.3   0.4   0.5
-25
-50
-75

Effect size BA 19 (exp_S>exp_NS)

Figure 5.

Figure 5. Correlations between the changes in signal intensity in exp_S > exp_NS contrast and psychological parameters. (A) Positive correlation between percent signal change during exp_S > exp_NS contrast in the right occipital lobe (Brodmann area (BA) 19) and happy bias in the control and subclinical groups combined. **(B)** Positive correlation between percent signal change during exp_S > exp_NS contrast in the right occipital lobe (BA 19) and sad bias in the control and subclinical groups combined. Separating the control and the subclinical group revealed: **(C)** positive correlation between percent signal change in response to social evaluative components in the right occipital lobe and *happy* bias in the control; **(D)** also, only in the control group, we observed a negative correlation between percent signal change in response to social evaluative components in the right occipital lobe and change in state depression score from pre-scan to post-MIST; while **(E)** in the subclinical group, we found a positive correlation between percent signal change in the right occipital lobe and *sad* bias. CTRL: control group; SUB: subclinical group; BA: Brodmann Area

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Tables

Group	Control	Subclinical	High-risk Subclinical	Total
Sex Ratio (Male/Female)	12/15	12/11	4/5	28/31
Age (years)	22.6 ± 3.0	21.6 ± 2.1	20.8 ± 1.3	21.9 ± 2.5
BDI (at recruitment)	3.4 ± 2.8	12.1 ± 2.0 §***	12.4 ± 3.3	8.2 ± 5.1
HDI	5.5 ± 3.9	11.4 ± 3.7 §***	26.9 ± 5.9 #***	11.1 ± 8.5
MADRS-S	2.6 ± 2.1	4.6 ± 1.9 §***	10.3 ± 2.9 #***	4.6 ± 3.4
STAI-trait	32.2 ±7.9	39.7 ± 6.9 W§**	49.3 ± 7.7 M#***	37.8 ± 9.5
TICS total	38.1 ± 11.7	51.3 ± 14.8 §*	65.0 ± 9.9 #*	47.3 ± 15.9

Table 1: Characteristics of the study population. Continuous variables are displayed as mean values ± SD. Apart from BDI levels, which were obtained at the time of the recruitment, all other psychological assessment were conducted at the time of the MRI scanning. BDI: Beck Depression Inventory; MADRS-S: Montgomery-Asberg Depression Rating Scale Self-Assessment HDI: Hamilton Depression Inventory; STAI-trait: Spielberger Trait Anxiety Inventory; TICS: Trier Inventory for the assessment of Chronic Stress; §= comparison with the control group; #=comparison with the subclinical group; *=p<.05; **= p<.01; ***=p<.001

Chapter 6: CONCLUSION

Conclusion

Thesis Summary

The overall aim of the work presented in this thesis was to investigate neural correlates of individual differences observed in the hypothalamic-pituitary-adrenal (HPA) stress axis response and subsequent cortisol release to a psychological stressor in healthy normal populations as well as in those showing a distinct vulnerability to Major Depressive Disorder (MDD). This present work stems from two previous key findings: 1) the development of a psychosocial stress task suitable for neuroimaging environment, the Montreal Imaging Stress Task (MIST) (Dedovic, Renwick et al. 2005), and 2) the finding that deactivation in the limbic system seems to underlie the processing of psychological stress (Pruessner, Dedovic et al. 2008).

The first article included in this thesis reviewed the neuroimaging studies to date that had aimed to investigate the neural correlates of psychological stress processing in humans. This article emphasized that a reliable neuroimaging psychological stress task, just like a behavioral psychosocial stress task, needs to include a motivated performance task, with elements of uncontrollability and particularly, social evaluative threat. Indeed, only studies using serial subtraction or the Montreal Imaging Stress Task (MIST), a task that combines mental arithmetic and negative social evaluation components, were able to induce a significant cortisol stress response. Most consistent findings from such neuroimaging studies included deactivation of the orbitofrontal cortex,

involvement of the anterior cingulate cortex and deactivation of the limbic system (particularly hippocampus) in processing of psychological stress.

The second study aimed to investigate whether the deactivation observed in the limbic system areas in response to psychological stress processing is due to completing mental calculations or processing the social evaluative threat components. To this end, we developed an event-related MIST design. With this approach, we were able to observe that in response to mental arithmetic, the responders, those who showed an increase in cortisol, differed in changes in brain activity in dorsomedial prefrontal cortex, left temporal pole, and right dorsolateral prefrontal cortex compared to non-responders. In response to negative social evaluation, the responders showed reductions in brain activity in limbic system regions, which were largely lacking in non-responders. The findings suggested that the social evaluative threat components specifically, were associated with the deactivation of the limbic system areas. An unexpected result was that the event-related MIST protocol yielded responders, who had higher levels of self-concept compared to non-responders. Therefore, the event-related MIST protocol not only allowed for investigation of key components of the stress processing, but also turned out to be a task that may specifically target high self-concept individuals, opening another avenue in stress research.

Given an important contribution of psychological stress to onset and development of Major Depressive Disorder, we evaluated neural regulatory networks of psychological stress processing in a sample of healthy young adults who showed varying levels of depressive tendencies, but at subclinical levels.

Many studies investigating depression vulnerability factors and traits focus either on first episode depressives or populations that are believed to be at higher risk for developing the illness but who do not as yet present any of the symptoms (for example, healthy individuals with a specific genetic profile or those with family history of MDD). With respect to the vulnerability question, both approaches are therefore limited by their temporal proximity and distance, respectively, from the illness proper. Therefore, the subclinical sample offers a unique opportunity to investigate the vulnerability hypothesis in a population at a more direct risk to develop depression, but who has not yet succumbed to the full clinical syndrome.

In this second portion of the thesis I was specifically interested in assessing whether some of the abnormalities associated with the HPA axis function and the brain areas involved in the HPA regulation, such as the hippocampus, seen in MDD can already be present in this vulnerable population prior to depression onset. In addition, we aimed to evaluate possible differences in neural correlates of psychological stress processing in the subclinical population in comparison to a control group.

We found evidence of a blunted cortisol awakening response (CAR) and smaller hippocampal (HC) volume in the high-risk subclinical group compared to the controls. The subclinical group showed levels that were in-between these two groups, but the difference did not reach the level of significance. That was the first time that blunted CAR and smaller HC volume have been observed in subclinically depressed individuals, with the results suggesting that these characteristics might be vulnerability factors for depression.

The final article investigated the neural correlates of psychological stress processing in these same groups of subjects. In this study, we applied the block version of the MIST; however, we have introduced an additional condition that would allow us to isolate social evaluative threat processing in a block MIST as well. In response to this modified MIST, although the control group did not show a significant cortisol increase, we nevertheless observed a blunted cortisol response in subclinical group compared to the control group. In addition, the subclinical and the control groups differed in neural correlates of stress processing in the visual association cortex in the occipital lobe. Interestingly, increases in signal in the occipital lobe in response to social evaluation were associated with increased happy attentional bias in the controls, but increased sad attentional bias in the subclinicals. Furthermore, the high-risk subclinical group differed from the subclinical and control groups specifically with respect to brain activity changes in the medial orbitofrontal cortex. The medial orbitofrontal cortex may therefore be an important moderator of the intricate but strong link between psychological stress (in particular social evaluation) and depression onset. To our knowledge, this is the first study to investigate these concepts in a subclinical depressed population.

Together, these studies provide an important insight into the role of key brain areas in the processing of psychological stress in a healthy population. In addition, the findings reveal in what way some of these areas may be dysregulated and affect the HPA axis profile in those with a distinct vulnerability for depression. The overall puzzle is far from being complete, but certain pieces are

starting to give shape to the overall picture. Below we outline the big picture of the findings presented in this thesis.

Conclusion of the findings in healthy population: a basic framework of brain areas involved in processing psychological stress

The work featured in this thesis (Dedovic, D'Aguiar et al. 2009; Dedovic, Rexroth et al. 2009) as well as the previous work from our lab (Dedovic, Renwick et al. 2005; Pruessner, Dedovic et al. 2008) and other groups (Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Kern, Oakes et al. 2008; Taylor, Burklund et al. 2008) has put forward important evidence that has allowed us to recently propose a basic framework of brain areas involved in processing psychological as well as physical stressors (Dedovic, Duchesne et al. 2009). This model is outlined below and schematically presented in Figure 1a.

(A)

(B)

Figure 1: (A) Basic framework of brain areas involved in processing physical and psychological stressors. The model summarizes data from functional studies in human populations. It is based on a hierarchical integration of physical versus psychological stress processing in central nervous system (Herman, Figueiredo et al. 2003). Animal studies indicate that physical or reactive stressors tend to implicate brainstem, while psychological or anticipatory stressors tend to engage limbic system regions. Given that amygdala has direct connection to key brainstem nuclei, it might play a more crucial role in processing of physical stressors. The influence of the PFC regions on the downstream regulators varies with region and nature of the stimulus. BS: brainstem; HY: hypothalamus; HC: hippocampus; AG: amygdala; PFC: prefrontal cortex; oPFC: orbital PFC; mPFC: medial PFC; vlPFC: ventrolateral PFC, light blue indicates that this regions is found on the lateral surface of the brain; ACC: anterior cingulate cortex; Reproduced with permission from (Dedovic, Duchesne et al. 2009). (B) Key nodes in the basic network that seem to be affected in the subclinical and high-risk subclinical depressed groups. Structures affected in the subclinical group are outlined in pink. Those affected in high-risk subclinical group are outlined in red.

Although the hippocampal volume was smaller both in the subclinical group and in the high-risk subclinical group compared to controls, it is outlined in red given that only the comparison between the high-risk subclinical group and the control group was statistically significant. Although the function of the hypothalamus and CRH levels were not specifically assessed in our studies, the regulatory impairment at this level may account for some of our findings. In addition, others have put forth evidence for the implication of the CRH system (hypothalamic and extra-hypothalamic) in the development of depression (Binder and Nemeroff 2010). Therefore, this area is also highlighted (bright blue) in our model, as an additional site of impairment. Components of physical stressor were removed to improve the legibility of the labels. CRH: corticotrophin releasing hormone; BS: brainstem; HY: hypothalamus; HC: hippocampus; AG: amygdala; PFC: prefrontal cortex; oPFC: orbital PFC; mPFC: medial PFC; vlPFC: ventrolateral PFC, light blue indicates that this regions is found on the lateral surface of the brain; ACC: anterior cingulate cortex; OCC: occipital lobe, visual association area, light blue indicates that this regions is found on the lateral surface of the brain; dashed arrows indicate that functions of these areas are probably affected by the dysregulation of the nodes outlined in the mode, however the exact nature of these impairments is at present unclear.

The most consistent finding in neuroimaging studies of psychological stress processing is the decreased activity in orbitofrontal cortex (Brodmann Area (BA) 11) being associated with increased cortisol secretion in response to a psychological stress task in healthy populations (Wang, Rao et al. 2005; Pruessner, Dedovic et al. 2008). Similarly, increased activity in medial prefrontal (PFC) (BA 9 and BA 10) regions correlate with decreased cortisol secretion (Kern, Oakes et al. 2008). These areas play a role in gathering and integrating

sensory information from the body and the surrounding environment (orbitofrontal; (Gusnard and Raichle 2001), participate in monitoring and control of one's emotional state (medial PFC and orbitofrontal cortex respectively; (Fredrikson, Wik et al. 1995; Amodio and Frith 2006), monitor the perception and judgments of other people (medial PFC; (Amodio and Frith 2006), and therefore these regions may represent candidates for the processing of the stress response, by integrating perception, passive coping and possibly perseverance. Importantly, these proposed functions for orbitofrontal PFC and ventromedial PFC (BA 10) are also supported by their intricate and far reaching projections to the limbic system including the hippocampus (Carmichael and Price 1995), amygdala, hypothalamus, periaqueductal grey region and the brainstem nuclei (Gusnard and Raichle 2001).

When a stressful psychological stimulus is perceived, an increase in cortisol response is observed. One way to achieve this is by curtailing the indirect tonic inhibition of periventricular nucleus of hypothalamus by hippocampus (HC), through HC deactivation (Pruessner, Dedovic et al. 2008). This process, from stress perception to stress response, could be modulated by activity in areas such as the ventrolateral PFC (BA 47), and the anterior cingulate cortex (ACC) (BA32). For example, the ventrolateral PFC is involved in first-order executive processes such as active selection, comparison and judgment of stimuli, as well as processing information under conscious effort (Petrides 2005). Findings of inverse associations between activity in this area and cortisol release (Wang, Rao et al. 2005; Taylor, Burklund et al. 2008), may suggest a role for the ventrolateral

PFC in active control of the cortisol release. Interestingly, while the ventrolateral PFC has scarce projections to the HC (Mohedano-Moriano, Pro-Sistiaga et al. 2007), it has extensive positive connections to the ventromedial PFC (Marsh, Blair et al. 2009). This may be a mechanism that could allow ventrolateral PFC to counteract the decrease in activity in the orbital and medial PFC areas related to stress processing. Here, the inadequate level of control may be associated with prolonged increased cortisol secretion. This would be supported by findings of increased ventrolateral PFC activity linked to lasting effect of stress and with increased cortisol secretion (Wang, Rao et al. 2005; Kern, Oakes et al. 2008).

With respect to the ACC, its pattern of activity varies considerably across studies. Since the ACC plays a role in error monitoring and regulating adaptive behaviors in response to environmental cues (Bush, Luu et al. 2000; Luu and Posner 2003), the variability in the findings might reflect differential error processing for different types of tasks.

Following an overview of animal and human studies (described in detail in (Dedovic, Duchesne et al. 2009), we proposed that changes in the brainstem and amygdala may play crucial roles in processing physical stressors that load more on fear processing rather than social evaluation (Dedovic, Duchesne et al. 2009).

It should be noted that data from animals and humans suggest a hierarchical integration of stress, where the influence of the prefrontal regions on the downstream regulators varies with region and nature of the stimulus (Herman, Figueiredo et al. 2003), and possibly, nature of the regulatory and coping

approach of an individual. The current model outlined above reflects but one possibility of this dynamic integration.

Contribution to the basic framework of psychological stress processing from the study of the subclinical depression population

<u>*HPA axis regulatory network*</u>

Assessment of the HPA regulatory network within the sample of healthy subjects with various degrees of depressive tendencies revealed several regions within this network that showed structural and functional abnormalities in this population. Namely, we observed structural abnormalities of the hippocampus as well as differential changes in brain activity across the study groups in the medial orbitofrontal cortex and the visual association areas in response to social evaluative threat processing (Figure 1b). Below, I present some hypotheses of what may be the impact of these findings on the network proposed.

A small hippocampal volume observed in the high-risk group may represent a vulnerability trait and is most likely the result of a gene by environment interaction. Hippocampal volume is thought to represent the packing density of neurons and glial cells, as well as neuronal soma sizes (Stockmeier, Mahajan et al. 2004). Furthermore, given that the largest compartment that is contributing to hippocampal volume is neuropil (consisting primarily of dendrites and axons, and to a small degree of glial processes) (Tata and Anderson 2010), smaller volume may mean that information processing (input and output) may be impaired and thus affect other regions sharing connections with the hippocampus. Of course, one would suspect that the rest of the network linked to the

hippocampus could adapt to an inefficient hippocampus. However, it may be possible that, in time of stress, such impairment might compromise hippocampal regulation of the HPA axis as well as place overbearing strain on the HPA regulatory network and mood regulation networks, yielding depressive symptomatology. It is important to note here that a direct connection between abnormalities in structure to impairment in function depicted here is an assumption at best, as this structure-function link still remains unclear and is most likely to be more complex than what has been assumed. Clearly, additional studies that examine this question specifically are needed.

Another important finding is that the area that seems to distinguish the control and the subclinical groups from the high-risk subclinical group in processing of social evaluative threat is the medial orbitofrontal cortex. The medial orbitofrontal cortex features prominently in both stress studies of normal populations (Wang, Rao et al. 2005; Wang, Korczykowski et al. 2007; Pruessner, Dedovic et al. 2008; Dedovic, Rexroth et al. 2009), as well as in the models of mood regulation (Drevets, Price et al. 2008; Phillips, Ladouceur et al. 2008; Mayberg 2009), therefore this may be an important node that, at the level of changes in brain activity, underlies the link between psychological stress processing and development of depression.

Interestingly, the orbitofrontal cortex and the hippocampus have bidirectional connections through entorhinal and perirhinal cortices, which are the primary pathways of cortical input to the hippocampus (Rempel-Clower 2007). In addition, it has been proposed that the entorhinal and the perirhinal cortices act as

gates by selectively allowing only relevant information to reach the hippocampus; the input from the orbitofrontal cortex may be the key to the gates, facilitating the encoding of emotionally relevant stimuli (de Curtis and Pare 2004). A small hippocampus, in tandem with an impaired orbitofrontal function (i.e. lack of differential encoding of evaluative and non-evaluative situations), may contribute to a maladaptive contextualization of life events, disconnection from environment, as well as impaired responsivity and regulation of the HPA axis. Importantly, chronic glucocorticoid administration has been shown to induce dramatic reorganization of dendritic arborization in medial prefrontal cortex in rats, with possible functional implications for stress-induced changes in cognition (Wellman 2001). Therefore, the connection between the medial orbitofrontal cortex and the hippocampus may reflect an important pathway in stress processing that may be operating at suboptimal level in the subclinical individuals; in times of chronic stress, this pathway may create a vicious loop that may lead to impairment in information processing and emotional regulation, eventually contributing to the onset of depression.

Finally, we may also add the right visual association area to the neural network model of processing of social evaluative threat as a region that may differentiate the control subjects from the subclinical depressed individuals. Its positive association with happy and sad bias in the control and subclinical group, respectively, is suggestive of a potentially important role in threat perception as well as a potential site for therapeutic intervention. For example, we have previously suggested that the deactivation of the orbitofronal cortex may

contribute to the very initial components of stress processing such as stress perception. But, one's detection of threat would probably be influenced by what one is paying attention to or focusing on in the first place. Given that we have observed differences in the visual association areas between the controls and subclinicals, interventions aiming at modulating these attentional processes may contribute to the prevention of onset of clinical depression. A study has shown that subjects who were exposed to a therapeutic computer game training them to shift their attentional bias on accepting information (searching for a smiling face within a 4 x 4 matrix of frowning faces), compared to those subjects who were simply trained to look for a specific object (five-petal flower in a matrix of six-petal flowers) subsequently showed lower levels of self-reported stress, increased self-esteem levels, as well as lower levels of cortisol release to real-life stressors such as academic exam or holding a telemarketing job (Dandeneau, Baldwin et al. 2007). Therefore, it would be interesting to assess whether this type of training may also have an effect on the cortisol release in response to a neuroimaging stress task and whether it may modify brain activity patterns in visual association cortex and other regions, in response to a stress task. For this, development of a neuroimaging stress task that could be presented multiple times to the subject is the key.

It should be noted that although function of the HPA axis with respect to function of the hypothalamus and CRH secretion was not specifically assessed in the present thesis, the regulatory impairment at this level may account for some of our findings. Indeed, extensive literature has put forth evidence for the

implication of CRH system (hypothalamic and extra-hypothalamic) in development of depression (reviewed in (Binder and Nemeroff 2010). Therefore, this area is also highlighted in our model, as an additional possible site of impairment (Figure 1b).

HPA axis output

Investigation of both basal and reactive HPA axis function in the subclinically depressed groups revealed a dysregulated, hyporeactive HPA axis in these individuals compared to the controls. A blunted HPA output has been observed previously in several disorders such as atypical subtype of depression (at least with respect to basal cortisol or pharmacological challenge), posttraumatic stress disorder (PTSD), chronic fatigue or pain (reviewed in Heim, Ehlert et al. 2000). As it has been suggested by Fries and colleagues (Fries, Hesse et al. 2005), several mechanisms may account for this phenomenon: (1) reduced synthesis or release of key hormones at each level of the HPA axis, (2) hypersecretion of one of the secretagogue in addition to down-regulation on the respective receptors, (3) enhanced sensitivity of cortisol negative feedback, (4) lower levels of free cortisol and/or (5) cortisol resistance of target tissue types (Fries, Hesse et al. 2005).

Studies examining atypical depression suggest the hypothalamic CRH deficiency to be an important player in the down-regulated HPA axis (Gold and Chrousos 2002). In regard to PTSD, hypocortisolemia may be associated with an increased sensitivity of the HPA axis to negative feedback inhibition (e.g. (Yehuda, Giller et al. 1991) but a potentially hyperactive central CRH system (Bremner, Licinio et al. 1997; Yehuda 1997). Chronic stress on the other hand is

characterized firstly by an increase in the HPA output, then by a subsequent decrease of hormone secretion, and lower reactivity of the HPA axis (Heim, Ehlert et al. 2000) suggesting exhaustion of the regulatory mechanisms (Fries, Hesse et al. 2005). Therefore, the blunted HPA axis output may be associated with different mechanisms depending on the illness or even subtype of an illness.

One possible mechanism that may account for the group differences with respect to the cortisol awakening response (CAR) may be potential impairment in adrenal sensitivity to ACTH, extra-pituitary suprachiasmatic nucleus (SCN) pathways, or glucocorticoid receptors (particularly MR) function on the hippocampus. Namely, a recent review suggested that there may be two distinct influences on the magnitude of CAR, one during the pre-awakening and the other during the post-awakening period, both influenced mainly by pathways from SCN, as well as the hippocampus (Clow, Hucklebridge et al. 2009). Prior to awakening there is a steady increase in ACTH secretion and cortisol secretion. However, there is a dissociation between these two measures in that the pre-awakening rise in ACTH is steeper than the rise observed in cortisol (reported in (Hellhammer, Wust et al. 2009)). It has been suggested that, under the influence of SCN extra-pituitary pathways, sensitivity of the adrenal gland to ACTH may be reduced during the pre-awakening. This effect would however be reversed to an increased sensitivity during the post-awakening period in order to be able to produce the marked increase in cortisol usually observed during the CAR (Clow, Hucklebridge et al. 2009). It could be speculated that in the subclinical groups this

process of alternating adrenal sensitivity may be compromised leading to the blunted pattern of CAR observed in our study.

Another way to keep the pre-awakening levels of cortisol in check would be an adequate steady feedback regulation of the pulsatile release from the HPA axis. The genomic MR localized in the hippocampus subserves this function. Interestingly, it has been suggested that, during the pre-awakening period, the hippocampus is active (Balkin, Braun et al. 2002), assumingly contributing to the inhibitory influence on the cortisol secretion. Awakening is associated with the switching off of hippocampal activation (Balkin, Braun et al. 2002), assumingly leading to the release of the break imposed on the HPA axis. Inadequate inhibition at pre-awakening could perhaps impair the magnitude of cortisol response during post-awakening period.

Impairments in the mechanisms at the level of the HPA axis function which may account for the group differences observed in response to stress are more difficult to speculate on, particularly due to the lack of a clear increase in cortisol in the control group.

As this is the first study to investigate HPA axis function in subclinical depressed population, more studies will be needed in order to illuminate some of the potential mechanisms that may be at play here. For example, a potential study could include in-lab 24 hr (or overnight and morning) assessment of ACTH and cortisol secretion in both healthy and the subclinical depression populations, at two instances, comparing CAR response when subjects receive pretreatment with spironolactone, the precursor of the MR antagonist canreonate that is formed in

the body following spirololactone administration (Pariante and Lightman 2008), compared to CAR profile when they receive placebo. In order to verify the blunted HPA output in response to psychological stress, the subclinical subjects could also be exposed to the Trier Social Stress Task (TSST) (Kirschbaum, Pirke et al. 1993), a behavioral stress task that has been shown to reliably elicit stress response in healthy populations.

Limitations

While the work presented here contributed interesting new findings to the present literature, its several limitations need to be acknowledged. Firstly, although the review article concisely summarized some of the key findings in the recent literature on the neural correlates of psychological stress processing, this summary was qualitative in nature. Namely, no formal meta-analysis was conducted as it was deemed to be beyond the scope of the review. Secondly, the eventMIST study results were hindered by the small study sample and in particular, the small number of events of interest. Similarly, the last two articles were also limited by uneven and, in the case of high-risk subclinical group, small, group samples. Thirdly, although we have included measures of mood changes in response to the several versions of the Montreal Imaging Stress Task, lack of assessment of subjective experience of psychological stress was a drawback in these studies. Furthermore, we did not formally assess whether the subclinical and high-risk subclinical subjects presented more atypical or more melancholic features of depression. This could have provided some insight with respect to the

possible mechanisms underlining the differences observed across the groups in the cortisol profiles. Moreover, in the last article, the lack of a strong cortisol response in the healthy controls in response to the modified MIST limited extensive interpretation of the group differences observed. At this point it is also important to note that we did not assess other measures of the HPA axis dysregulation, such as the levels of adrenocorticotropic hormone. In addition, we did not assess the genetic makeup of our subjects.

Future directions

An important aspect of the work presented in this thesis is the ongoing effort for development and improvement of a reliable psychological stress task suitable for the neuroimaging environment. The Montreal Imaging Stress Task (MIST) versions to date have yielded a stress task that is relatively mild compared to the behavioral stress task, such as the TSST. One reason for this difference is that the neuroimaging environment, particularly fMRI environment, significantly reduces the effect of social evaluative threat. However, an advantage of a mild neuroimaging stressor is that it might be more likely to approximate mild stressful experiences encountered in real-life, and therefore reveal networks that are impacted the most on an everyday basis. However, the real-life validity of these tasks still remains to be investigated.

Another important drawback of the neuroimaging stress tasks used to date is the strong element of deceit and negative feedback. With these elements present in the task, debriefing of the subject following the testing session is a must. This unfortunately eliminates a possibility for subjects to undergo multiple exposures

of this procedure. From behavioral TSST studies, we know that there are those who show habituation to the stress protocol, as well as those who show sensitization, and those who do not respond at all (Kudielka and Wust 2010). It would be reasonable to assume that the HPA regulatory network might show different patterns of changes in brain activity depending on the different adaptation profile of the stress response. Importantly, the ways in which the HPA regulatory network might differ among the various adaptation profiles may be more telling of individual differences in vulnerability and resilience for stress-related disorders than differences in regulatory network observed following a one-time only challenge.

In addition, when considering subclinical or clinical populations, particularly depressed populations, it would be important to assess the subjects' HPA responses to a stressor or situations that may be more relevant for this population. For example, exposing these subjects to paradigms of social rejection. Today's social rejection tasks such as Cyberball (a computerized ball tossing game between three participants, where one of the participants (the subject) gets excluded from ball tossing) (Williams, Cheung et al. 2000; Eisenberger, Lieberman et al. 2003) have been shown to not elicit a cortisol response in a healthy population (Zoller, Maroof et al. 2010). However, it would be interesting to investigate whether a subclinically depressed population may show physiological and neural dysregulation in response to such a task given that, in this population, social interactions and social rejection may be an important contributor to the onset of clinical syndrome.

Overall, a push towards the development of a new neuroimaging stress task, both achievement-based and social rejection based, is essential.

Now that we are starting to understand the key players in the neural regulation of stress response in human populations, it would also be important to attempt to explore potential mechanisms that may underlie these associations. Several behavioral studies have already started to look at contribution of specific genetic profiles such as serotonin transporter gene polymorphisms (e.g. Gotlib, Joormann et al. 2008), brain derived neurotrophic factor gene polymorphisms (e.g. Alexander, Osinsky et al. 2010), or glucocorticoid receptors gene variants (e.g. Kumsta, Entringer et al. 2007; van Leeuwen, Kumsta et al. 2010) on basal and reactive HPA axis profiles. It would be important to introduce these variables in the neuroimaging studies of the HPA axis regulation as well. In addition, we know from animal and human studies that neurotransmitters such as glutamate and γ-Aminobutyric acid (GABA) as well as their respective receptors are important agents allowing for connections within and between neural networks (Herman, Mueller et al. 2004; Cullinan, Ziegler et al. 2008; Hashimoto 2009). Therefore, positron emission tomography studies evaluating for example occupation of glutamate and GABA receptors at rest and in times of challenge, in healthy and in vulnerable populations, would be important to investigate.

Furthermore, additional investigation of dysregulation of the HPA axis function and impairments of processing of psychological stress in the subclinical individuals is absolutely necessary. A longitudinal design would be ideal in order

to be able to monitor if and how the subclinical levels of depression fluctuate, and whether this profile of change across time is more related to or is more telling of the impairments in the HPA axis output or differences in brain activity changes in response to stress compared to one-time assessments. In addition, the assessment of other subclinical populations, such as anxiety, would also be of importance. Depression and anxiety are often comorbid and it would be interesting to investigate whether at subclinical levels these illnesses may carry different neural signature in response to psychological stress.

Final remarks

The work presented in this thesis has evaluated the HPA axis function and neural correlates of psychological stress processing in population of healthy and subclinically depressed young adults. The findings from the present studies allowed us to construct a basic model of neural network underlying stress processing in a healthy population. In addition we were able to show that individuals with subclinical levels of depression already show impairments in HPA function, as well as impairments in certain key regions within the HPA axis regulatory network. Importantly, results from this work have generated new interesting questions and hypotheses to be evaluated and answered in future studies.

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Appendix 1: Reprints of published papers included in the thesis

What stress does to your brain: a review of neuroimaging studies

Pg3

Pg4

Pg5

Pg6

Pg7

Pg8

Pg9

Pg10

Pg11

Neural correlates of processing stressful information: an event-related fMRI study.

Pg1

Pg2

Pg3

Pg4

Pg5

Pg6

Pg7

Pg8

Pg9

Pg10

Pg11

Pg12

Appendix 2: Reprints of additional first authored papers not included in the thesis

Contribution of Authors

Below I have included reprints of my other first-authored papers not included in the thesis. For these articles, I conducted the literature review, contributed to the task design, recruited subjects, and completed endocrine, behavioral and anatomical and functional MRI data analyses, wrote and edited manuscripts (*my contribution to each manuscript is outlined in italics below*). Overall, the contribution of co-authors included consultation on study designs, assistance with tasks development and testing, as well as consultation on the style and content of the manuscripts, and supervision of the projects (for specific contributions for each manuscript, please see bullet points below).

Manuscript 1

Dedovic K, Duchesne A, Andrews J, Engert V, & Pruessner JC. (2009) "The brain and the stress axis: the neural correlates of cortisol regulation" Neuroimage. 2009 Sep;47(3):864-71. Epub 2009 Jun 13. Review.

Literature review, writing (introduction, sections on the hippocampus and the prefrontal cortex, the synthesis of stress processing framework, conclusion), overall editing: 65 %

Contribution of co-authors:

- Annie Duchesne: literature review and writing for the section on amygdala, consultant on manuscript style

- Julie Andrews: literature review and writing for the section on physical/physiological stressors, consultant on manuscript style

- Veronika Engert: literature review and writing for the section on development and other neurotransmitter systems, consultant on manuscript style

- Jens C. Pruessner: consultant on manuscript content and style, overall editing

Manuscript 2

Dedovic K, Wadiwalla M, Engert V, Pruessner JC (2009) The role of sex and gender socialization in stress reactivity. <u>Developmental Psychology</u>, Jan;45(1):45-55.

Literature review, writing, editing: 75%

Contribution of co-authors:

- Mehereen Wadiwalla: assistance with literature review, consultant on manuscript content and style

- Veronika Engert: assistance with literature review, consultant on manuscript content and style

- Jens C. Pruessner: consultant on manuscript content and style, assistance with writing

Manuscript 3

Dedovic K, Renwick R, Mahani NK, Engert V, Lupien SJ, Pruessner JC. (2005)

The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. <u>J Psychiatry Neurosci.</u> **30**(5):319-25.

Recruitment and testing of subjects for the fMRI study, fMRI analysis, writing, editing: 65%

Contribution of co-authors:

- Robert Renwick, Jens C. Pruessner: assistance with data acquisition
- Robert Renwick, Najmeh Khalili Mahani, Jens C. Pruessner: assistance with behavioral, endocrine, fMRI analysis
- Veronika Engert, Jens C. Pruessner: assistance with article writing
- Sonja J. Lupien, Jens C. Pruessner: study design

Reprints