OBSERVING THE STRESSED BRAIN

Magnetic Resonance Imaging of the Neural Correlates of Hypothalamic Pituitary Adrenal Axis Function

By Najmeh Khalili-Mahani, M.Eng. M.A.

Department of Neurology and Neurosurgery

McGill University, Montreal, QC, CANADA

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ABSTRACT

The Hypothalamic Pituitary Adrenal (HPA) axis is the coordinator of adaptive responses to physical and psychological stress. The central nervous system plays a key role in modulation of both basal and adaptive HPA axis functions. In fact, since long ago, animal studies have shown that acute and chronic exposure to glucocorticoids (a stress hormone released due to HPA axis activation, cortisol in humans) affects the function and the morphology of brain areas such as the hippocampus and the cingulate cortex. This thesis is based on novel neuroimaging methodologies used to investigate the interactions of psychological stress, cortisol and the brain. It consists of three functional studies and a morphometric one. In the first functional study we show that the hippocampus (where glucocorticoid receptors are most abundant) plays a role in initiation of an HPA axis stress response. In the second study, we provide evidence that besides hippocampus, the neural activity in the so-called "default mode network" (DMN), especially the anterior cingulate cortex (ACC), relates to interindividual variations in HPA axis response to psychological stress. In the third study we have investigated the cortisol-modulation of the DMN. Again, we provide evidence for a role of the ACC and the orbitofrontal cortex in negative feedback inhibition of the HPA axis activity. Finally, we show a morphological link between the ACC and the cortisol response to awakening which is an index of basal HPA axis activity. Overall, our findings confirm the critical role of the ACC and mesolimbic system in HPA axis regulation. These findings also draw attention to the interactions between functional subregions of the medial prefrontal cortex and states of HPA axis function prior to stress onset—suggesting an interplay of the monitoring and the executive planning roles of the

medial prefrontal cortex in behavioral adaptation to stress. Beyond stress research, our findings offer a framework for combining neuroimaging and neuroendocrinology to better understand the interindividual variances in behavior, and perhaps to better identify subgroups at risk of psychological disorders.

RESUMÉ

L'axe Hypothalamo-Hypophyso-Surrénalien (Hypothalamic Pituitary Adrenal ou HPA) coordonne de manière adaptative les réponses de l'organisme au stress physique et psychologique. Son activité de base, comme dans des conditions de stress, est toutefois modulée par le système nerveux central (SNC) qui déploie à son tour sa propre réponse au stress. En effet, les recherches sur des modèles animaux ont depuis longtemps établi que la fonction et la morphologie des aires mésolimbiques comme l'hippocampe et le cortex cingulaire sont affectées par des taux élevés et chroniques de glucocorticoïdes, équivalent du cortisol chez l'humain. Cette thèse se base sur de nouvelles méthodes de neuroimagerie pour examiner les interactions entre l'axe HPA et le SNC dans la réponse au stress chez l'humain. Elle comporte trois études fonctionnelles et une étude morphologique. La première étude fonctionnelle montre que l'hippocampe, la structure où les récepteurs aux glucocorticoïdes abondent le plus, joue un rôle dans l'initiation de la réponse au stress de l'axe HPA. La deuxième étude fonctionnelle montre qu'en plus de l'hippocampe, l'activité neuronale dans le cortex cingulaire antérieur (Anterior Cingulate Cortex ou ACC, une autre structure du SNC), et plus généralement dans le mode par défaut du réseau (Default Mode Network ou DMN), est apparentée à des variations interindividuelles dans les niveaux de cortisol en réponse au stress psychologique. La troisième étude fonctionnelle examine la modulation des niveaux de cortisol liés au stress dans l'axe HPA par le DMN. Elle met en évidence le rôle de l'ACC et du cortex orbito-frontal dans l'inhibition négative en retour de l'activité de l'axe HPA. L'étude morphologique démontre l'existence d'une relation entre l'ACC et le niveau du cortisol à l'éveil qui est un index de l'activité de base de l'axe HPA. L'ensemble confirme le rôle critique

de l'ACC dans la régulation des réponses de l'axe HPA, tout en spécifiant les rôles des subdivisions fonctionnelles régionales du cortex médial préfrontal dans les états fonctionnels de l'axe HPA présents avant l'initiation expérimentale de la réponse au stress, suggérant un double rôle pour la région du cortex médial préfrontal de supervision de la réponse au stress de l'axe HPA et de planification de la réponse comportementale de l'organisme dans l'adaptation au stress. Au delà de la recherche sur le stress, nos résultats établissent un cadre méthodologique et conceptuel pour combiner la neuroimagerie et la neuroendocrinologie dans le but de mieux comprendre les variations comportementales interindividuelles, et probablement de mieux identifier des sous-groupes à risque dans l'adaptation psychologique a su stress.

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To Reza ...
October 30, 2008

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"Life exists by maintaining a complex dynamic equilibrium, or homeostasis, that is constantly challenged by intrinsic or extrinsic adverse forces or stressors. Stress is, thus, defined as a state of threatened homeostasis, which is reestablished by a complex repertoire of physiologic and behavioral adaptive responses of the organism. The adaptive responses may be inadequate for the reestablishment of homeostasis or excessive and prolonged; in either case a healthy steady state is not attained, and pathology may ensue."

Chrousos, 1997 Hanse Selye Memorial

"To understand the mechanisms of stress gives physicians a new approach to the treatment of illness, but it can also give us a new philosophy to guide our actions in conformity with natural laws."

Selye, The Stress of Life, 1956

CHAPTER 1

INTRODUCTION

Research Context

Over seventy years ago, Hanse Selye introduced the concept of stress as a General Adaptation Syndrome: an adaptive response of the organism to a non specific 'noxious' threat to its homeostasis (Selye, 1936). Selye considered stress as the common denominator of all adaptive reactions in the body manifested in a quantifiable syndrome (Selye, *Stress of Life*, pp 54-56). He pinned the quantity of stress syndromes on the amplitudes of hormones secreted from adrenal cortex, which seemed critical for regulation of homeostasis. Selye posited that balanced secretion of adrenal hormones was the key to the organism's health. Imbued with Selye's initial concept, years of following research confirmed that: a) the Hypothalamus Pituitary Adrenal (HPA) axis was the central coordinator of the "stress response" and b) stress syndromes would manifest not only in the presence of physiological disturbances, but also psychological ones (Selye, 1950). The study of psychophysiology of "stress" was particularly promoted in the aftermath of the World War II and the Vietnam

war when psychiatrists wished to understand and treat the psychosomatic symptoms such as 'combat fatigue' and 'war neurosis' (Jones, 1987). Another incentive for 'stress study' was to identify psychological characteristics of 'good' soldiers who would not be vulnerable to developing such conditions (Brunner, 1991; Holt, 1949). Early observations established that psychological modulation of the HPA axis activation is stimulus specific (Mason, 1968); and very heterogeneous across populations (Folkman et al., 1987; Lazarus, 1974; Opton and Lazarus, 1967). Thus a field of research opened that, to date, has been seeking the correlates of health states in psychoneuroendocrinological substrates. The term "allostatic load"—i.e. the toll of unbalanced and prolonged physiological responses to stress hormones—is now regularly used in relation to aging process, illness and mental health (McEwen, 2007).

Today, modern medicine considers 'stress' as an effector of health and disease—both physical and mental. Increased co morbidity of chronic stress with immune dysfunction (Bauer, 2005; Black, 1994), diabetes (Black, 2006; Rosmond, 2003), obesity (Black, 2006), cardiovascular disease (Bjorntorp, 1997), depression (Brown et al., 1986; Munce et al., 2006), substance abuse (Brady and Sinha, 2005), and dementia in later years of life (Wilson et al., 2007) increases the impetus for stress research. By McEwen's account, in the US, the economic cost of stress surmounts 200 billion dollars per year (McEwen, *The End of Stress as We Know it,* p.3). Calling stress a 21st-century concern, he writes: "[S]tress is not necessarily the same thing as hardship and the stressors are on the rise. We are besieged daily with more information than a staff of 20 can keep up with, threatening our sense of control over our own lives." (p 15) Not only the quotidian hassles of modern life are on the rise, current world events such as America's war in several fronts, global anxiety with climate

change or the high likelihood of an economic depression add to the weight of stress experienced by average North Americans. The popular awareness of the impact of mental states upon physical wellbeing thus provides political incentive[1] for scientific investment in understanding the mechanisms of resilience or vulnerability to stresses of life.

Today's problematic of stress research is not different from those Selye counted half a century ago. "The tweezers of stress have three prongs", he wrote:
"1. The *stressor,* the external agent which started the trouble, for instance by acting directly upon the skin, kidney or the mind.
2. The *defensive measures*, such as the hormones and nervous stimuli which encourage the body to defend itself against the stresses as well as it can, [either by physiological or emotional and psychological barricades].
3. The *mechanisms for surrender,* such as hormonal or nervous stimuli, which encourage the body not to defend itself." (Selye 1956, p261)

Interindividual heterogeneity in stress-sensitivity (e.g. how easily one gets stressed, how easily one recovers, and how susceptible one is to negative health outcomes of stress) poses one of the greatest challenges of stress research. Early on, Selye recognized that even animals differed in baseline "adaptation energy", i.e. the energy consumed during continued adaptive work (Selye 1956, p87-89).

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[1] The World Health Organization considers "work stress" a leading cause of mental and health problem and has dedicated resources to train organizations to treat work related stress as a major health risk factor. See **Protecting Workers' Health Series** No. 3 (*Work organization and stress*, by Stavroula Leka, Pr Amanda Griffiths, Pr Tom Cox, Institute of Work, Health and Organizations, United Kingdom ISBN 92 4 1590475 © 2003 World Health Organization) and No. 6 (*Raising awareness of stress at work in developing countries*; by Irene Houtman and Karin Jettinghoff, TNO Work & Employment, The Netherlands, and Leonor Cedillo, Occupational Health researcher, Mexico © World Health Organization 2007; ISBN 92 4 159165 X).

He hypothesized that interindividual differences in stress resilience were related to innate and perhaps inherited characteristics. Indeed, emerging evidence strongly suggest that genes and environment interact on the development of HPA axis sensitivity to stress (Meaney et al., 2007; Szyf et al., 2007). Given the heterogeneity of the human condition, differences in psychological traits or developmental factors add extra levels of difficulty when studying the causality of relationships between any stressors, the stressed organisms, or the allostatic load. Therefore, the need for developing a unified theoretical framework that lends itself to objective and controlled experimentation becomes paramount.

Stress and the Brain

In 1968, Bruce McEwen discovered that the stress adrenal hormone crosses the blood brain barrier to target corticosteroid receptors of the limbic system (McEwen et al., 1968). Ever since, much attention is given to the role of the CNS and cognitive and emotional variants in dynamics of HPA axis activity. The stress system in humans is complex. The HPA axis integrates autonomic signals from the internal milieu with limbic and paralimbic signals that depend on executive, cognitive and emotional complexities of the external stimuli *vis a vis* the "individual". The adaptive responses of the HPA axis are dependent on myriad physiological and psychological traits particular to an individual (Chrousos, 1998). The end product of the HPA axis activation, glucocorticoid (cortisol in humans) is important for glucose production and also for the breakdown of proteins and lipids that provide metabolites (for baseline homeostatic functions) and also extra energy for countering "stress" or a threat to homeostasis (Brillon et al., 1995). Glucocorticoids cross the blood brain barrier and act on CNS structures such as the hippocampus (McEwen et al., 1968), which is important for alertness (Gray and McNaughton, 2003) and

learning (Squire, 1992; Stern and Hasselmo, 1999) and also for negative feedback inhibition of the HPA axis activity (Jacobson and Sapolsky, 1991; Sapolsky et al., 1984). As the energetic bases of the brain seem to be linked to behavioral factors (Raichle and Gusnard, 2005; Shulman et al., 2004), the awareness in effect of glucocorticoids on brain function grows (Peters et al., 2004).

Effects of cortisol on the CNS are mediated via two types of corticosteroid receptors in the limbic system: mineralocorticoid (MR, or type I) and glucocorticoid (GR or type II) receptors. The MRs and GRs are co-localized in the limbic system (De Kloet et al., 1998), especially in the hippocampus where the ratio of MR/GR binding capacity is greatest (Chao et al., 1989; Revsin et al., 2005). Acting in concert, MRs—with ten times higher affinity for glucocorticoids (Reul and de Kloet, 1985)—are essential for maintaining basal HPA axis sensitivity and triggering initial HPA axis responses. The GRs on the other hand are important for balancing the initial stress responses (De Kloet et al., 1998). A dominant theory proposed by De Kloet (De Kloet et al., 1998) suggests that the health of an organism depends on efficiency of MRs in appraising situations that necessitate triggering of the HPA axis activation, and the efficiency of GRs in counter balancing the initial stress responses. Another important theory emphasizes that the time course of glucocorticoid actions with respect to the phase of adaptation determines whether glucocorticoids function in a permissive or a suppressive way (Sapolsky et al., 2000). This theory is based on propositions made independently by Tausk[2] and Munck (Munck and Naray-Fejes-Toth, 1992), suggesting that glucocorticoids protect not against the source

[2] Tausk M 1951 Hat die Nebenniere tatsächlich eine Verteidigungsfunktion? Das Hormon (Organon, Holland) 3:1–24

of stress itself, but against the overshooting of the defense reactions that are activated by stress (Sapolsky et al., 2000). Research on cost-benefit effects of suppressive or permissive glucocorticoid actions is ongoing. It seems that effects of glucocorticoids on neurons (function and morphology) follow and inverted u-shape, were two little or too much of them threat the health states (De Kloet et al., 1998; Lupien and Wan, 2004).

Besides glucocorticoids, the neuronal pathways that process and integrate the stress information from external stimuli and internal milieu play a prominent role in HPA axis regulation. A comprehensive review of lesion and electrophysiological animal studies by Herman and colleagues (Herman et al., 2003) lists several neuroendocrine and limbic signaling pathways that interact with the visceral, somatosensory, emotional and cognitive modulation of the HPA axis activity. For instance, the HPA axis receives catecholaminergic (norepinephrine (NE) and epinephrine (E)) signals from the Nucleus of the Solitary Tract (NTS), serotonergic signals from Raphe nuclei (RN), and dopaminergic inputs from the thalamus, which carry excitatory signals to the hypothalamus. Projections between the NTS and the hypothalamic nuclei seem to be important for integration of reactive HPA responses to visceral illness, cytokine/inflammatory challenge, hypoxia and hypotension and pain. However precise links between these signaling pathways and HPA axis regulation is still under investigation. In addition, cerebral forebrain inputs from the subicular hippocampus and the medial prefrontal area arrive at the parvocellular PVN of the hypothalamus via the GABAergic interneurons of the bed nucleus of the stria terminalis (BST). By contrast the basolateral and central nucleus of amygdala innervate the PVN via the NTS (hence excitatory). The lateral septal neurons however innervate PVN-projecting regions that contain both

GABAergic and glutamatergic neurons. The limbic inputs to the PVN are also filtered through structures such as thalamus, which itself receives extensive input from regions like ventral subiculum, infralimbic/prelimbic cortex, BST, and NTS, thus forming complex feedback and feedforwad pathways that are yet ill understood. Therefore the limbic and prefrontal areas play a complex role in inhibition or excitation of the PVN and HPA axis modulation. So far, it seems that the hippocampus and the medial prefrontal cortex play a specific role in regulation of the HPA axis to anticipatory stress—but not physiological or pharmacological challenges (Herman et al., 2003). These structures thus have received primary attention in investigating the psychoneuroendocrinology of stress.

Not only does stress affect the brain function in response to stimuli, but also glucocorticoids can affect neuronal plasticity and electrophysiology of the areas where MRs and GRs are colocalized. In animals, it is well documented that prolonged exposure to glucocorticoids (either by pharmacological or psychological induction) leads to morphological changes in pyramidal neurons of layer CA3 in the hippocampus (Magarinos et al., 1996; McEwen and Magarinos, 1997; McEwen et al., 2002; Watanabe et al., 1992), increased apoptosis in dentate gyrus (McEwen et al., 2002; van der Beek et al., 2004) and altered neuronal excitability and Ca2+ influx in the subicular area CA1, which may affect plasticity of this region (Fuchs and Flugge, 1998; Joels et al., 2004). Furthermore, layers II and III in the medial prefrontal cortex seem to undergo morphological changes in terms of density and arborization of the dendritic processes (Cerqueira et al., 2007a; Cook and Wellman, 2004; Diorio et al., 1993; Patel et al., 2008; Radley et al., 2004; Wellman, 2001). Older studies reported on adverse effects of prolonged stress on organisms. New research is illustrating

that effects of glucocorticoid exposure on hippocampal and medial prefrontal cortex can also be passed on to offspring (Andrews et al., 2004; Catalani et al., 2002; Coe et al., 2003; Erdeljan et al., 2001; Meaney et al., 1996). For instance, the glucocorticoid-related morphological alterations caused by maternal behavior interact with gene expression in the hippocampus and set the stage for behavioral traits that will themselves determine the tonic and reactive aspects of HPA axis regulation (Meaney et al., 2000; Weaver et al., 2004). However, glucocorticoid-mediated changes in neural plasticity is increasingly considered as an adaptive mechanism that is determined by behavioral factors and complementary actions of the MR and GRs in order to optimize survival skills of the species (e.g. by processes of learning, or higher alertness) (Sousa et al., 2008).

In fact, the advent of neuroimaging has allowed to test the morphological link between the HPA axis function and the hippocampal or medial prefrontal cortex (Axelson et al., 1993; Bremner et al., 1995; Brown et al., 2004; Buss et al., 2007; Leverenz et al., 1999; Lupien et al., 1998; Lupien et al., 2007; Lupien et al., 2005b; Lyons et al., 2001; MacLullich et al., 2005; O'Brien et al., 1996; Ohl et al., 1999; Ohl et al., 2000; Pruessner et al., 2005; Pruessner et al., 2007; Starkman et al., 1992; Starkman et al., 1999; Vythilingam et al., 2004; Wolf et al., 2002; Yehuda, 1999). However, the emerging picture is not clear yet. For example, there is evidence that higher cortisol exposure in older adults is associated with smaller hippocampal volumes (Lupien et al., 1998) or reduced medial prefrontal gray matter (MacLullich et al., 2005); or that posttraumatic stress disorder (PTSD) is associated with smaller hippocampal volumes (Bremner et al., 1995; Lindauer et al., 2006; Wignall et al., 2004)—as measured from T1W MRI. However, the causality of this association is disputed both in

aging (Lupien et al., 2007; O'Brien et al., 1996) and in PTSD (Yehuda et al., 2007) and much research remains to be done.

Stress and Behavior

If glucocorticoids alter the neuronal properties of structures like hippocampus and medial prefrontal cortex—that are critical for regulation of behavior—then plausibly they alter behaviors that depend on those structures as well. Animal studies provide evidence that glucocorticoids can facilitate learning (Roozendaal et al., 2004; Sandi and Rose, 1994a; Sandi and Rose, 1994b) or cause cognitive deficiency (He et al., 2008; Roozendaal et al., 2003; Roozendaal et al., 2001). The phase, the time course, and the duration of exposure play a role in glucocorticoid-related modulation of the cognitive function. Moreover, genes, environment and experience seem to mediate the interactions of glucocorticoids and cognitive function (de Kloet et al., 2002; Meaney et al., 1991; Meaney et al., 1988). Of course, memory is only one aspect of behavior affected by glucocorticoids. For instance, GR knockout in the forebrain of mice has led to an increasingly popular transgenic model of depression (Chourbaji and Gass, 2008). On the other hand, increased MR expression reduces the anxious behavior and lowers the magnitude of the stress response (Rozeboom et al., 2007). Again, prenatal glucocorticoid exposure (Seckl and Meaney, 2004) and epigenetic factors (Meaney et al., 2007) are shown to play an important role in developing behavioral traits that influence the coping styles (and thus stress reactivity) during the course of life. These recent animal models corroborate the empirical human data showing an association between HPA axis dysregulation and stress related mental health problems such as posttraumatic stress disorder (Wessa et al., 2006), depression (Bhagwagar et al., 2005; Pruessner et al., 2003b) and chronic fatigue (Nater et al., 2008; Roberts et al., 2004).

Of course interindividual variations in HPA axis activity are not exclusive to clinical population. For instance, the baseline circadian patterns of cortisol, that emerge as early as 8 weeks after birth (Custodio et al., 2007), might vary depending on genetic or environmental factors in different individuals (Bartels et al., 2003; Wust et al., 2000a). Daily life stressors such as work load (Schlotz et al., 2004), bereavement (Meinlschmidt and Heim, 2005), financial hardship (Ranjit et al., 2005), socio-economic status (Bennett et al., 2004; Wright and Steptoe, 2005) and care giving (Wahbeh et al., 2008), as well as personality and psychological traits such as borderline personality (Lieb et al., 2004) and anxiety (Greaves-Lord et al., 2007; Kallen et al., 2008; Quirin et al., 2008) can lead to abnormal patterns of HPA axis activity.

Examining variations in reactive cortisol response to stressful situation can help uncover some of the underpinnings of interindividual differences in stress sensitivity. Recent studies in healthy subjects have been focusing on how cortisol response is modulated with traits such as motivation to preserve social self (Gruenewald et al., 2004; Tops et al., 2006), perfectionism (Wirtz et al., 2007), higher social hierarchy (Hellhammer et al., 1997), locus of control (Pruessner et al., 1997a), emotional intelligence (Mikolajczak et al., 2007); and states such as social evaluative threat (Andrews et al., 2007), social rejection (Blackhart et al., 2007) or social support (Wirtz et al., 2006). Cortisol reactivity traits can determine vulnerability to HPA axis dysregulation in populations like older adults (Kudielka et al., 1998), racial minorities (Richman and Jonassaint, 2008) and children growing up in adverse family environments (Hardie et al., 2002).

A major topic in stress research is thus to reduce the psychological correlates of

HPA axis response to a set of defined factors that can be tested in laboratory environments. Early work by Mason (Mason, 1968) surveyed reports of HPA axis activity in relation to acute stressors like flight experience, college examinations, hospital admission, dental anticipation, car racing, combat experience, and chronic stressors like caring for an ill child, as well as laboratory tests such as speech, monotonous tasks, film viewing, stressful interviews and enforced self analysis. In Mason's conclusion, factors such as novelty, uncontrollability, unpredictability and threat to ego are the common predictors of an HPA axis stress response.

Biondi and Picardi updated the state of knowledge by examining popular laboratory stressors such as mental arithmetic, public speech, interviews, Stroop tests and videogame playing as well as real life stressors such as bereavement, academic exams, anticipation of surgery, workload, and even parachute jumping. They concluded that the degree to which the HPA axis is stimulated is not related to the stressor per se, but to the perception of the situation as stressful (Biondi and Picardi, 1999). These authors listed evidence that personality, coping style, self esteem, social security modulate the neuroendocrine stress response; but they also caution that these variables alone would not necessarily predict stress; and that baseline physiological states were to be modeled in investigation of interindividual variability in neuroendocrine stress response—a conclusion reached by this current project as well, as will be discussed in the following chapters.

The most recent account of psychological substrates of HPA axis stress response is provided by Dickerson and Kemeny, who have meta-analyzed over 200 studies involving laboratory stress and cortisol sampling paradigms (Dickerson

and Kemeny, 2004). These authors have formulated a theory that the cortisol stress response is marked by the perception of "threat to the goal of self-preservation" (Dickerson and Kemeny, 2004). For instance, physical health is a self-preservation goal. If this goal is threatened by noxious stimuli, an HPA axis response follows. Similarly, if individuals are motivated to preserve their social self, by maintaining social status, esteem and acceptance, then they will elicit an HPA axis response if this goal is threatened. In fact, their analysis reveals that laboratory stress paradigms that incorporate social evaluative threat with uncontrollability are three times more effective in instigating a stress response than similar tasks without social evaluative threat. Or, they show that uncontrollability (which in both animals (Weinberg and Wong, 1986) and humans (Peters et al., 1998; Voigt et al., 1990)—but not all (Steptoe et al., 1993) causes a stress response) stimulates the HPA axis mostly if the participants are motivated to achieve a goal such as high performance scores. These theoretical models constitute the basis of the experimental paradigm used in this project.

Neuroimaging of Stress

Although the psychological substrates of neuroendocrine responses to stress in humans have been the topic of much research, little is known about the exact mechanisms by which the CNS modulates the HPA axis stress response. To test functional variations in brain response to psychological stress, or its structural association with HPA axis activity, in the context of a wealth of animal evidence could help identify objective measures for predicting interindividual variation in stress-sensitivity.

Neuroimaging studies of stress that examine neuroendocrine co-variations with brain activity are a few that involve mental arithmetic stress challenge in healthy

young adults (Dedovic et al., 2005; Ohira et al., 2008; Pruessner et al., 2008a; Wang et al., 2007; Wang et al., 2005); traumatic stimuli tested on normal and PTSD patients (Liberzon et al., 2007), and public speech tasks (Kern et al., 2008; Taylor et al., 2008). Despite methodological heterogeneity, these studies converge on identifying the medial prefrontal (MPFC), and the ventrolateral prefrontal (VLPFC) areas as important in the HPA axis stress response regulation. However, the reported results do not paint a unifying picture of the neural bases of stress control.

Wang et al have reported an increase in cerebral blood flow (CBF) in the right VLPFC in correlation with cortisol, concomitant with an increase in subjective rating of stress and anxiety (Wang et al., 2005); however in a later report they have shown that positive correlation in the rVLPFC is particular to men, and that a similar relation is present in the dorsal anterior cingulate cortex (dACC) in women (Wang et al., 2007). Interestingly, these workers report a sustained activity in the ACC and LPFC even during the rest periods, suggesting that the cortisol modulation of the PFC is related to state anxiety.

Similarly, Liberzon and colleagues have shown a positive co-variation between the activity of the rostral ACC (rACC) and the post scan ACTH levels, but only in PTSD patients (Liberzon et al., 2007). By contrast, the ACTH modulates the activity of dorsomedial prefrontal cortex (DMPFC) area in healthy combat exposed individuals. Moreover, they show that the neural activity in the rACC correlates with the prestimulus cortisol levels in all subjects, however the subgenual ACC (sACC) activations are particular to combat exposed PTSD individuals (Liberzon et al., 2007). These authors speculate that cortisol modulation of the sACC activity is linked to emotionality and sadness induced

in the PTSD subjects.

It is also not clear whether stress and cortisol modulate the neural activity positively or negatively. Our lab has reported a reduced blood-oxygen-level-dependent (BOLD) signal (i.e. deactivation) in the limbic system (especially the hippocampus) and ventromedial prefrontal area in response to a mental arithmetic challenge combined with psychosocial threat (Pruessner et al., 2008a). We have argued that because the hippocampus and the medial prefrontal cortex exert negative inhibitory feedback on the HPA axis (Herman et al., 2003; Herman et al., 2005), then deactivations marked a neural stress response, help disinhibit the HPA axis and increase cortisol availability in order to meet the metabolic demands of the stress challenge. However, mental arithmetic challenge in Wang et al (Wang et al., 2005) is associated with reduced CBF in the orbitofrontal and left VLPFC, as well as angular gyri and superior and middle temporal areas, concurrent with increase activation in the DMPFC and dACC, precuneus and left inferior temporal gyri.

The correlation between cortisol increase in relation to a public speech stressor and post stress glucose metabolism is reported in the DMPFC activity (negative correlation) and right LPFC activity (positive correlation) (Kern et al., 2008). Levels of stress cortisol in relation to a public speech stressor have been shown to modulate the right VLPFC activity in a task-independent threat detection paradigm (Taylor et al., 2008), perhaps suggesting a trait behavior in vigilance and anxiety. In fact, recent studies investigating the neural substrates of anxiety (Bishop et al., 2004; Engels et al., 2007; Holsen et al., 2008; Schunck et al., 2008; Straube et al., 2007) indicate that an interplay between different parts of the prefrontal cortex emerges in correlation with coping behavior, emotional and

conscious states, attentional processing and physiological arousal. Particularly, the VLPFC, together with rACC seem to be important in attentional control and behavioral adaptation in the presence of threatening stimuli (Bishop et al., 2004; Engels et al., 2007).

The exact interactions between subregions of the prefrontal cortex are also elusive. For instance, Bishop and colleagues have shown an inverse relation between state anxiety and rostral left DLPFC and left VLPFC (Bishop et al., 2004) activation in response to frequent threatening distractors. By contrast the left rACC seems to be mostly related to infrequent threat detection (Bishop et al., 2004). A lateralized prefrontal preference in responding to immediate threats (arousal anxiety) versus distant worries (apprehension anxiety)(Engels et al., 2007) is reported. Also, a dissociation between inhibitory response to threat (characterized by the right VLPFC activity) and sensitivity to threat (correlated with dACC and left PFC activity) is suggested (Taylor et al., 2008).

Moreover, social contexts and social cognition (which modulate the HPA response to social evaluative threat) also appear to impact upon the activity of the prefrontal regions (Fiddick et al., 2005; Fliessbach et al., 2007; Han et al., 2008; Lorberbaum et al., 2004; Rilling et al., 2008; Sander et al., 2005; Spitzer et al., 2007; Zink et al., 2008). Socially contextualized punishment modulates the activity of the lateral prefrontal cortex in a social interaction paradigm (Spitzer et al., 2007). Social cognition of hierarchy (Zink et al., 2008) and of trust (Rilling et al., 2008) are subserved by the DLPFC and DMPFC parts. However, much work remains to be done to integrate the neural correlates of anxiety and social evaluative processing with neuroendocrine correlates of 'stress'.

The Problem of Interindividual Variability

It is increasingly evident that individuals vary in behavioral adaptation. The role of factors such as personality (Canli et al., 2004; Engels et al., 2007; Gray et al., 2005; Kumari et al., 2004; Vrticka et al., 2008), motivation (Behrens et al., 2007; Bush et al., 2002; Hajcak and Foti, 2008; Pochon et al., 2002; Rushworth and Behrens, 2008; Rushworth et al., 2005; Rushworth et al., 2002; Rushworth et al., 2004) and even task-unrelated intrinsic states of neural activity (Clare Kelly et al., 2008; Raichle and Gusnard, 2005; Seeley et al., 2007; Yarkoni et al., 2005a; Yarkoni et al., 2005b; Zacks et al., 2001) play an important role in the ways that the brain processes the environmental stimuli and initiates behavioral adaptations. Similarly, but independently, stress researchers have also been long grappling with the problem of interindividual heterogeneity in HPA axis regulation in relation to genes (Wust et al., 2000a), personality (Bossert et al., 1988; Kirschbaum et al., 1995; Lai et al., 2005; Oswald et al., 2006; Pruessner et al., 2005; Pruessner et al., 1997a; Zorrilla et al., 1995), gender (Kirschbaum et al., 1999; Kudielka et al., 2004), and socioeconomic status (Carlsson et al., 2006; Hellhammer et al., 1997; Kunz-Ebrecht et al., 2004b; Ockenfels et al., 1995; Steptoe et al., 2003) to name a few. To tie these two fields of research together and to understand the patterns of brain activity that characterize neuroendocrine stress may thus be important in explaining the heterogeneity in studies that address:

- How stress affects cognitive function
- How stress contributes to interindividual differences in behavioral adaptation
- How stress might affect neural plasticity
- How stress increases vulnerability to developing mental or physical illness

Research Outline

The objectives of this research were motivated by our first study that showed a relation between deactivation of the mesolimbic system and increased cortisol response (Pruessner et al., 2008a). In the initial study, we found increased deactivation of the limbic system in a subgroup of individuals who showed an acute cortisol response after psychological stress. We hypothesized that the deactivation of the mesolimbic system was related to the "default mode" state of neural activity in these regions, that, as explained by Raichle, represented an intrinsic state of monitoring environment for signs of change and initiation adaptive behavioral responses (Raichle et al., 2001).

We considered a total of 112 neuroimaging and cortisol data sets (68 young and 44 old) that were collected in three independent studies designed to investigate the neural correlates of stress-adaptation in humans. Stress experiments were based on a novel technique, Montreal Imaging Stress Task (MIST), developed in our lab (Dedovic et al., 2005). This task stimulates a stress response by adding uncontrollability and social evaluative threat to a mental arithmetic challenge. We used cortisol measured during experimental condition as an independent variable to predict variations in blood oxygen level dependent (BOLD) response to experimental conditions (Chapters 2, 3 and 4). Furthermore, we used the cortisol awakening response, a 50-80% increase in salivary cortisol within half an hour after awakening (Pruessner et al., 1997b), as an index of basal HPA axis activity (Hellhammer et al., 2007), to predict morphological variations in the hippocampus and across the cortical mantle (Chapter 5).

Research presented in this thesis had two main objectives. First to use pre-existing data collected with the objective of examining neural correlates of stress

to examine whether neuroimaging can reveal a link between stress and brain areas such as the medial prefrontal cortex and hippocampus—target areas for glucocorticoid hormones and important for integration of HPA axis responses to psychological stressors. Second, it aimed to identify an objective index that could best characterize the variances in relation between the brain and the HPA axis activity. To avoid redundancy, theoretical background and rationale for each of individual analyses is outlined in the respective chapter. A general overview at the end will bind together the findings of these studies and will suggest future experimental designs for addressing research questions about the neural correlates of stress.

CHAPTER 2

THE ROLE OF THE HIPPOCAMPUS IN STRESS REGULATION

The hippocampus (HC) has a high number of both mineralocorticoid (MR) and glucocorticoid (GR) receptors, and plays a prominent role in the regulation of the HPA in the central nervous system (McEwen et al., 1986). The hippocampus is particularly known to exert a negative feedback on HPA axis activity after anticipatory stress (Herman et al., 2005). Previously, we have shown that the magnitude of hippocampal deactivation during a psychological stress task is correlated with cortisol response to stress (Pruessner et al., 2008a). Because deactivations represent deviation of neural resources from a baseline state of activity, we have hypothesized that a difference in the extent of hippocampal deactivation would be related to differences in hippocampal activity prior to stress induction. In the following manuscript we have put this hypothesis to test, by examining the patterns of HC activity in stress responders (i.e. those with increased cortisol response) and nonresponders in three independent cognitive tasks. In this experiment we have also examined the link between stress and impairment of cognitive function, which has been linked to

interactions of cortisol and GR-rich hippocampal neurons (Elzinga et al., 2005; Kirschbaum et al., 1996; Lupien et al., 2005a; Lupien and Lepage, 2001; Wolf et al., 2001b).

Manuscript 1: Hippocampal Activation During a Cognitive Task Is associated with Subsequent Neuroendocrine and Cognitive Responses to Psychological Stress

Authors:

Najmeh Khalili-Mahani[1,2]*, Katarina Dedovic [1], Veronika Engert[1,2], Marita Pruessner [1], Jens C Pruessner [1]

1. Douglas Mental Health University Institute, McGill University, Montreal, Canada
2. McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada

*Address correspondence to:

Najmeh Khalili-Mahani

Pavilion FBC 3, Douglas Mental Health Institute

6875 LaSalle Boulevard

Verdun, QC, H4H 1R3, Canada

Tel. (514) 398 8330

Fax (514) 888 4099

Email najma@bic.mni.mcgill.ca

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Contribution of co-authors

Marita Pruessner who studied correlation betweens hippocampal function, cortisol and memory initially inspired this study. Jens Pruessner designed the study and the computerized paradigms. Katarina Dedovic and Robert Renwick did subject recruitment and scanning. Katarina Dedovic and Jens Pruessner performed the manual hippocampal segmentation for an independent study. The research methodology, data processing and data analysis was fully conducted by Najmeh Khalili. Najmeh Khalili prepared the manuscript and interpreted the results. Veronika Engert proofread an earlier version of the manuscript. Jens Pruessner supervised all the above.

Abstract

Increased activation of the hypothalamus pituitary adrenal (HPA) axis, marked by increased secretion of cortisol, is a biological marker of psychological stress. It is well established that the hippocampus plays an important role in the regulation of HPA axis activity. The relationship between cortisol (stress-related elevation or exogenous administration) and the hippocampal related cognitive function is often examined. However, few human studies to date have examined the effect of stress on hippocampal activity and the interactions between stress-induced activation of the HPA axis and hippocampal function during different phases of cognitive function. Based on our previous work, we hypothesized that group differences in stress-sensitivity relate to differences in hippocampal-related stress-integration. To test this hypothesis, we conducted an fMRI study using tasks known to involve the hippocampal formation: novel-picture encoding, psychological stress, and paired-picture recognition. Based on their cortisol responses to stress, we divided subjects into stress-responders (increase in cortisol, n=9) and non-responders (decrease in cortisol, n=10). Responders showed higher hippocampal deactivation during the stress task and lower recognition scores due to a larger number of misses. Intriguingly, stress responders showed significant differences in hippocampal activation already prior to stress, with higher levels of hippocampal activity during the picture encoding. Although effects of both cortisol and hippocampal activation on recognition were present in responders, similar effects were absent in the nonresponder group. Our results indicate that hippocampus plays an important role in adaptive behavioral responses. We hypothesize that states of hippocampal activation prior to stress might reflect states of vigilance or anxiety, which might be important for determining interindividual differences in

subsequent stress response and cognitive performance.

Abbreviations: Area Under the Curve (AUC), Blood Oxygen Level Dependent signal (BOLD), Region of Interest (ROI), Cognitive Reserve (CR), Hippocampus (HC), Hypothalamus Pituitary Adrenal (HPA), Montreal Imaging Stress Task (MIST), Trier Social Stress Test (TSST)

Introduction

Activation of the Hypothalamic Pituitary Adrenal (HPA) axis, marked by increased cortisol secretion, is a biological marker of psychological stress. Cortisol is a glucocorticoid hormone that affects a wide range of physiological functions and mediates an adaptive response to metabolic demands of the stressed organism (Sapolsky et al., 2000). Within the central nervous system, the hippocampus has received particular attention in relation to HPA axis regulation. Attention to the hippocampus stems from early discovery of abundant distribution of glucocorticoid receptors in this structure (McEwen et al., 1968). Earlier animal studies have established that the hippocampus plays an important role in negative feedback inhibition of the HPA axis (Jacobson and Sapolsky, 1991; Sapolsky et al., 1991). More specifically, projections from the ventral subicular region of the hippocampus to the paraventricular neurons (PVN) of the hypothalamus are important for termination of the HPA axis response to anticipatory stressors (Herman et al., 2003).

In humans, the link between the hippocampus and the HPA axis function is often tested in relation to the effect of stress-related elevation of cortisol on memory (de Quervain et al., 2003; Elzinga et al., 2005; Lupien et al., 2002; Wolf et al., 2001a). Limited functional neuroimaging data confirms a cortisol-related

modulation of the neuronal activity in the medial temporal region that is associated with cognitive performance (de Leon et al., 1997; de Quervain et al., 2003; van Stegeren et al., 2007). However, the role of the hippocampus in initiation of the stress response in humans has received less attention.

Considering the animal evidence for particular importance of the hippocampus in controlling the HPA axis response to anticipatory stress (but not HPA axis response to physiological challenge; (Herman et al., 2003), it is possible that the effects of psychological stress response on hippocampal function (e.g. memory) are not entirely due to cortisol. Reciprocal and recursive signaling between the hypothalamus, limbic and cortical areas that are involved in perceptual processing of the stressor (Herman and Mueller, 2006) might play a more important role. In fact, emerging theories suggest that the hippocampus is a monitoring system that analyses any given stimuli in relation to a goal and optimizes behavioral approach by filtering unnecessary signals, weighing the outcomes of conflicting or competing approaches, and outputting appropriate signals to the rest of the brain in order to achieve that goal (Gray and McNaughton, 2003; McNaughton, 2006). This suggests that the hippocampus could play a critical role in processing psychological conditions that trigger an HPA axis response in humans: perception of novelty, uncontrollability, and anticipation of negative consequences (Mason, 1968), as well as threat to goals (Dickerson and Kemeny, 2004). If hippocampus is indeed involved in behavioral adaptation, then it is plausible that interindividual variations in HPA axis adaptive responses would be also associated with variations in hippocampal-related functions. Thus, examining interactions between cortisol stress response and hippocampal activation in vivo might further our understanding of the role of this structure in integration of stress responses.

To examine the neural activation during anticipatory stress, we developed the Montreal Imaging Stress Task (MIST) which induces psychological stress by adding uncontrollability and psychosocial evaluative threat to a mental arithmetic challenge (Dedovic et al., 2005). A meta-analytical review of stress literature has shown that uncontrollability and social evaluative threat are the most reliable source of experimentally induced cortisol stress response (Dickerson and Kemeny, 2004), albeit significant interindividual differences in responding to such threat are also noted (Biondi and Picardi, 1999; Dickerson and Kemeny, 2004). Indeed, our previous experiments with the MIST have revealed significant interindividual variations in cortisol stress response (e.g. (Pruessner et al., 2004a; Pruessner et al., 2008a). We have shown that while performing the MIST, stress responders (i.e. those with an elevated cortisol response following the MIST) show a significantly larger deactivation of the limbic system, including the hippocampus (Pruessner et al., 2008a). Considering the negative feedback inhibitory influence of the hippocampus on HPA axis activity (Herman and Mueller, 2006), and importance of the HPA axis in providing adaptive metabolic support (Sapolsky et al., 2000), we hypothesized that the hippocampal deactivation in response to experimental stress triggered the initial HPA axis activation. We thus attributed interindividual differences in the extent of limbic deactivation to intrinsic differences in perception of stress.

A question arising from our finding was whether individual differences in limbic system activity might also be present in the absence of stress. It is quite plausible that interindividual differences in hippocampal activity manifested during stress affect other, non-stressful aspects of cognitive processing. The current study aimed to investigate stressful and non-stressful hippocampal function, and the

interaction of both, by having two cognitive tasks interleaved with a stressful one, and investigating the relationship between the three. We used the MIST (Dedovic et al., 2005; Pruessner et al., 2008a), together with cognitive tasks (novel-picture encoding before stress and paired-picture recognition after stress) known to involve hippocampal activation (Stern et al., 1996). We investigated the cognitive performance as well as the state of hippocampal activation before, during and after stress in relation to differences in cortisol response of our participants. We tested the hypothesis that interindividual differences in cortisol response to stress were associated with differences in hippocampal activation and cognitive performance. We specifically predicted that greater cortisol response to stress would be associated with greater hippocampal deactivation during stress, and worse recognition performance after stress. We also investigated the relationship between cortisol, hippocampal activation and performance during cognitive tasks.

Materials And Methods

Subjects

Twenty-three young male college students (20-28 years, mean age = 22.5 ± 2 years) were recruited from McGill University (years of education = 15.9 ± 1.3). In accordance with the Research Ethics Board of the Montreal Neurological Institute, written informed consent was obtained from all participants prior to entry in the study. The subjects were interviewed to rule out the presence or history of psychiatric disease. Further exclusion criteria included previous surgery, metallic implants, current illness, and any history of endocrine or immune system disease. We only tested men in order to avoid the confounding effect of menstrual cycle hormonal variations on HPA axis activity in women. Four of the participants had to be excluded from the study because of

incomplete fMRI or cortisol data.

Experimental Design

The participants underwent about 20 minutes of anatomical MRI (aMRI) scanning, immediately followed by about one hour of functional MRI (fMRI) scanning (including encoding, MIST, and recognition). To measure levels of circulating cortisol, subjects provided saliva samples at given intervals. Details of these tasks are described below and schematically presented in Figure 1.

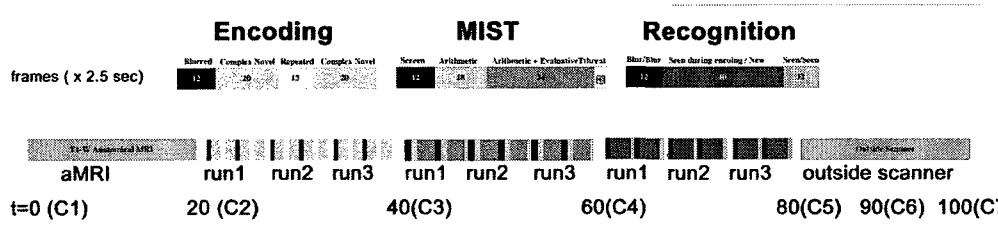

Figure 1: Schematic representation of the task design. Black bars represent the baseline; transparent bars represent the control; and solid colors represent the experimental condition. The time course of saliva sampling is indicated.

Novel-Picture Encoding

We used the picture-encoding paradigm described by Stern and colleagues (1996). This group has reported increased activation in the HC formation and parahippocampal region in response to the encoding of novel complex pictures. We selected 240 complex scenes of nonspecific daily urban life (120 to be shown during pictured-encoding and 120 to pair during picture recognition) from a database of 500 pictures (Art Explosion, Nova Development Corp. USA, 1996). The encoding paradigm consisted of 12 baseline, 12 control and 20 experimental stimuli, presented in block design with two repetitions per run for a total of three runs (each subject was asked to encode 120 novel pictures). Each picture was presented on the screen for 5 seconds. During the baseline condition, the

subjects were presented with a blurred, scrambled image with no recognizable item. Two such scrambled images were shown interchangeably and presented repeatedly throughout the baseline condition. The control condition consisted of two pictures from the database, which were shown interchangeably throughout the entire control session. Thus, the contrast between control and baseline shows the neural response to passive viewing of complex meaningless (baseline) versus complex meaningful (control) familiar stimuli. During the experimental condition, subjects were shown novel pictures of complex scenes from the database (one picture every 5 seconds) and were asked to memorize as many pictures as they could. We used the BOLD contrast between the experimental and the control condition as a measure of neural response to active encoding of complex novel scenes versus passive viewing of complex familiar scenes.

Montreal Imaging Stress Task (MIST)

The details of this task are described elsewhere (Dedovic et al., 2005). Briefly, during the baseline condition, the subject looks at the computer screen with no tasks presented. During the control condition, the subject has to perform timed arithmetic calculations without any feedback about his performance. During the experimental condition, the subject performs timed arithmetic similarly to the control session. In addition, the subject receives visual and verbal feedback about his performance. The subject is told to expect an 80-90% success rate. However, the task is programmed to control for inter-subject difference in arithmetic proficiency and to maintain a success rate between 40 and 50% of the subject's own performance. Each subject receives the same number of equations per run. The standardized success rate is achieved by adjusting the available response time and task difficulty. In this design, the control condition requires high attentional load without elements of social evaluation. In the experimental

condition, elements of social evaluation are added by means of individual performance indicators, average user performance indicators, and minimum performance requirements. In addition, computerized and verbal feedbacks by the investigator are added. This task is believed to induce psychosocial stress by implementing social evaluative threat, as previously identified as one of the major factors in inducing psychosocial stress (Dickerson and Kemeny, 2004). This task has been shown to produce significant increases in levels of salivary cortisol in about half of the tested subjects (Pruessner et al., 2004a; Pruessner et al., 2008a).

Here, the MIST was presented to the subjects in a block-design paradigm consisting of 12 baseline, 18 control and 34 experimental acquisitions, again in a block design with two repetitions per run. Each run of the MIST consisted of 128 frames. In each frame, a new random equation was presented to the subject in 5 seconds intervals. In order to increase power for the statistical analysis, each run of the MIST was repeated three times. After every run, additional negative verbal feedback was given to the participant in order to increase the level of social evaluative threat. The entire stress session lasted about 20 minutes (three runs plus feedback).

Paired-Picture Recognition

The recognition task was adapted from the original paradigm by (Stern et al., 1996). The block-design paradigm consisted of a total of 12 baseline, 12 control and 40 experimental acquisitions, with two repetitions of each block per run, for a total of three runs. During baseline, a pair of scrambled pictures was presented repeatedly. During control, a pair of complex pictures was presented repeatedly. The baseline and the control pair images were the same as shown during the baseline and control condition of encoding. During the presentation

of the baseline images, the subject was instructed to passively view the images. During the presentation of the control pair images, the subject had to click a mouse button to control for the brain activation associated with finger and hand movement. During the experimental condition, subjects were presented a familiar picture (shown during encoding) and a novel picture. They were then asked to choose the familiar picture by clicking the right or left mouse button, depending on the location of the recognized picture. Performance metric consisted of hits (correct recognitions), false alarms (a novel picture identified as familiar) and misses (failure to decide which picture was novel, within the given time).

Cortisol measurement and analysis

Cortisol was measured from saliva samples collected over a period of 90 minutes using salivettes (SARSTEDT, Quebec City, Canada). Seven samples were acquired at 10 minutes before the scan, immediately before the aMRI (t=0), after the aMRI and before memory encoding (t = 20), after encoding and before the MIST (t = 40), after MIST and before recognition (t=60), after recognition (t=80), and 10 minutes after the end of the scanning session (t=90) and after debriefing (t=100). To prevent the subject's head from moving, the investigator put the salivette into the subject's mouth wearing sterile gloves. The subject was instructed to refrain from chewing to minimize head movement. The salivettes were kept in the mouth for 2 minutes to saturate the cotton with saliva. The position of the subject in the head coil and the reference coordinates of each scan were kept unchanged with respect to the aMRI.

Saliva samples were analyzed for cortisol using a time-resolved fluorescence immunoassay. Intra- and inter-assay variability was less than 10% and 12%, respectively (Dressendorfer et al., 1992). We calculated the cortisol area under

the curve with respect to ground and increase over the course of the experiment (AUC_G and AUC_I, respectively; (Pruessner et al., 2003a).

Image acquisition and processing

Subjects were scanned on a 1.5-T Siemens Magnetom Vision Scanner (Siemens AG, Erlangen, Germany). Anatomical MRI scans were acquired using a T1-weighted ICBM (international consortium of brain mapping) protocol (3D SPGRE, TR/TE = 18/10, flip angle = 30°. 176 1-mm contiguous sagittal slices, FOV = 256 x 256 mm^2). The fMRI stimuli were presented in synchrony with an interleaved BOLD Mosaic 64 T2*-weighted (TR/TE=2500/50, flip angle 90°) echo-planar acquisition of each frame. Each fMRI frame consisted of twenty-eight 5-mm thick axial slices oriented along the long axis of the hippocampus (in-plane resolution 5 x 5 mm; field of view 256 mm). Each stimulus was presented three times (3x6 minutes per run). For each run 128 frames were produced.

fMRI analysis

The fMRI experiment consisted of a total of nine runs (3 encoding, 3 MIST and 3 recognition). The raw data was motion corrected to the third frame of each run to minimize the rigid head displacement between the 128 frames, as well as between the three sessions of each paradigm (Cox and Jesmanowicz, 1999). The motion-corrected data was then blurred with a 6-mm FWHM Gaussian kernel.

Analysis on individual fMRI data sets was performed using fmristat (Worsley et al., 2002). The first-level analysis involved computing the BOLD contrast of the experimental versus control condition for each of the three paradigms (encoding, MIST, recognition). The BOLD contrast here refers to the hemodynamic response to the experimental condition of the task compared to

the response to the control condition. A result of (experimental>control) is interpreted as activation, while (experimental < control) is interpreted as a deactivation in response to the experimental paradigm.

In the second-level, we combined the three runs of each paradigm by averaging the BOLD contrasts obtained from the first level analysis. This was achieved by combining the estimate of effect and standard deviation in a fixed effect analysis using multistat (Worsley et al., 2002)). The third-level analysis involved combining the between-subject data. To do so, individual aMRIs were linearly registered (Collins et al., 1994) to the MNI 152-average ICBM model. The transformation matrices were then used for spatial normalization of the second-level average maps. Having aligned all subjects' activation maps in standard space, a mixed effect analysis was performed on the entire sample by estimating the ratio of the variance of the random effects to the fixed effects. Regularization of this ratio was achieved by spatial smoothing with a Gaussian filter to yield 100 effective degrees of freedom (for details, see (Worsley, 2005b)).

ROI analysis of HC function

Because the linear alignment does not account for inter-individual variability in shape and stereotaxic location of the HC, we investigated HC activations within the manually segmented HC volume of each subject. The HC segmentation was performed using the interactive software package DISPLAY developed at the Brain Imaging Center of the Montreal Neurological Institute. Anatomical boundaries used for the hippocampus and a step-by-step segmentation protocol are described in detail elsewhere (Pruessner et al., 2000).

For each subject, we overlaid the HC mask on the second-level t-maps (i.e.

within subject average of each run). Within each subject's hippocampal mask, we located the highest peak (t-value of BOLD response to experimental versus control condition) and also estimated the extent of hemodynamic response by calculating the percentage of voxels that satisfied a threshold of $|t| = 2$ (fixed: df = 354, $p<0.05$; Worsley 2005) over the number of voxels in the HC mask for each hemisphere.

Performance metrics for recognition task

For each subject, we averaged over three runs the percentage of the number of hits (i.e. selecting the correct familiar picture from the presented pair), false alarms (i.e. collecting the novel picture as familiar) and misses (selecting neither of the paired pictures) over total recognition pairs (3x40). Because the neural responses to a correct recognition or false rejection are dissociable (Mathalon et al., 2003), these variables were not compounded into a single metric.

Results

Cortisol response

Table 1 shows the descriptive statistics of the cortisol samples for the entire group.

Table 1: Level of salivary cortisol (nmol/l) measured at different time points during the experiment

Saliva Sampling timeline	Min	Max	Mean	Std. Dev
Before MRI (t=0)	3.16	29.10	9.56	7.14
After anatomical (t=20)	3.11	33.44	8.86	7.15
After Encoding (t=40)	1.72	20.40	7.69	5.09
After MIST (t=60)	2.17	45.30	10.45	9.77
After Recall (t=80)	2.97	27.78	9.92	7.34
10 min after scan (t=90)	2.36	41.48	9.85	10.0
20 min after scan(t=100)	2.46	21.53	7.60	5.57
Valid N (listwise)	19			

Performing a one-way repeated measures (time) ANOVA with the seven cortisol levels as dependent variables resulted in a non-significant within-subject effect ($F(6,108)=0.87$, n.s.) suggesting that for the whole group cortisol levels did not change significantly over time. Because of the high between-subject variance in the cortisol reaction we used the AUC_I values to split the sample into nine responders (mean AUC_I = 301 ± 89) and ten nonresponders (mean AUC_I = -338 ± 113.55). As Figure 2 illustrates this grouping revealed a significant group difference in the profile of HPA axis cortisol response over time. A two way mixed design ANOVA (group * time) revealed significant effect of group by time interactions on cortisol responses over time ($F(6,102)=4.24$, $p<.001$). Cortisol levels changed significantly for the contrast prior to MIST (t=40) compared to after MIST (t=60), $F(1,17)=5.16$, $p<.04$). Also, cortisol changes from time of arrival (t= 0) to after anatomical scan (t=20) changed differently between responders and nonresponders ($F(1,17)=7.39$, $p<.02$.)

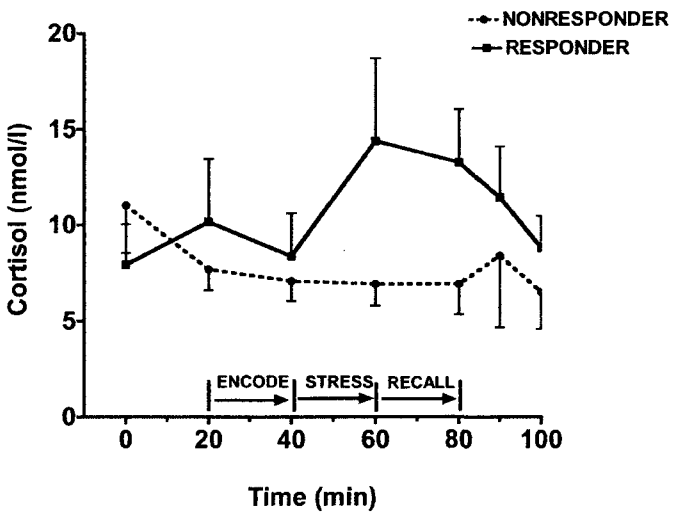

Figure 2: Cortisol profile (nmol/l) over the course of experiment for responders and nonresponders. Interactions between the time of arrival and after anatomical scan, and during the MIST were significant.

Overall brain activation

Figure 3 illustrates average task-induced activation and deactivations. Encoding novel pictures (versus looking at same familiar picture) bilaterally activated parahippocampal, occipitotemporal, fusiform and lingual gyri, and deactivated the right and the left (smaller in extent) angular gyrus and the parietal lobule. The MIST (i.e. performing mental arithmetic under psychosocial evaluative threat versus mental arithmetic under no threat) bilaterally deactivated the medial orbitofrontal gyri, nucleus accumbens, and anterior medial temporal area. Paired-picture recognition (versus looking at a repeated familiar picture) resulted in a broad activation of the parahippocampal, fusiform and lingual gyri as well as the middle cerebellum, thalamus, putamen and deactivation of the left angular, precentral and middle temporal gyri, right anterior cingulate, and inferior parietal lobule.

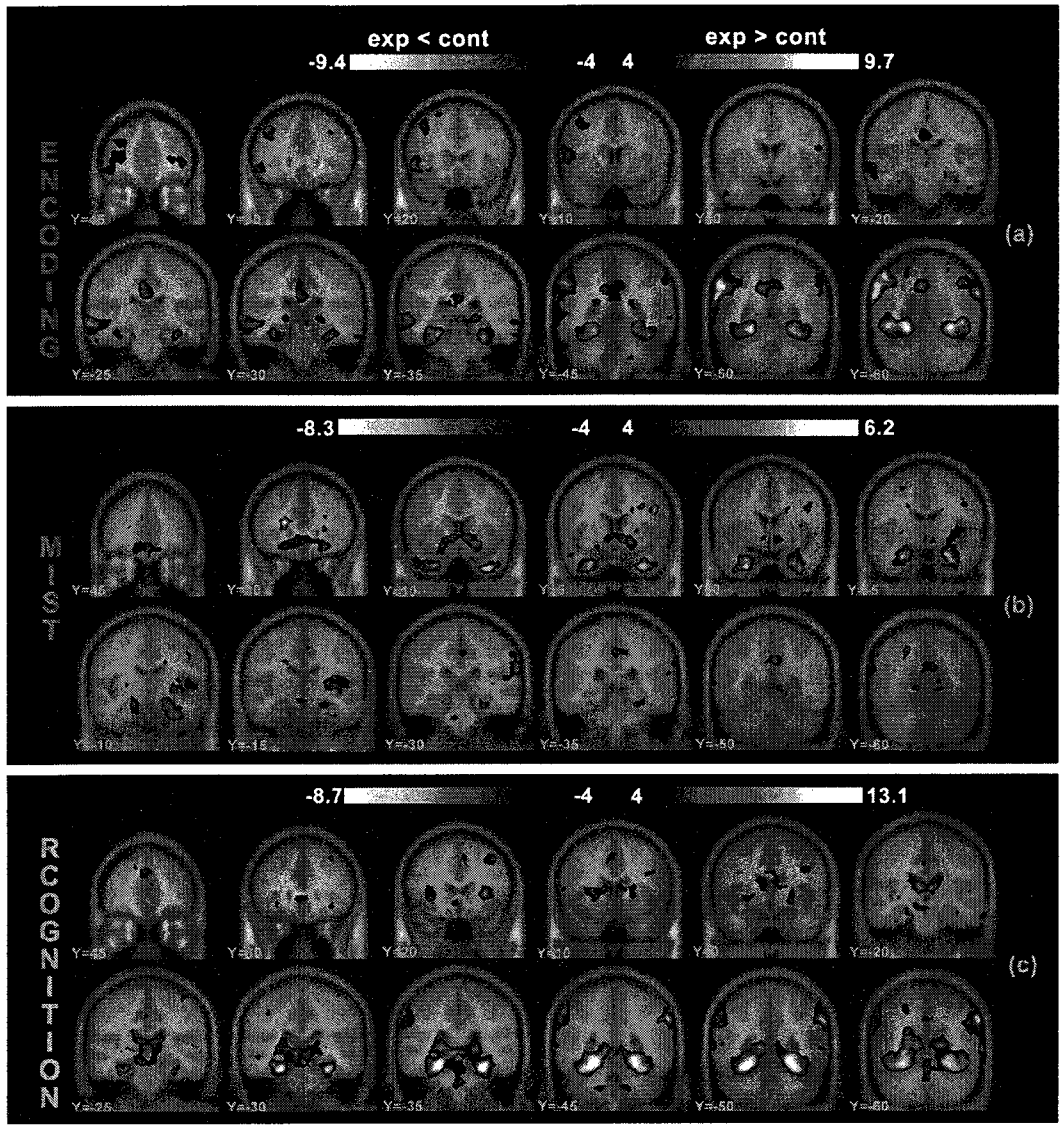

Figure 3: Average t-statistic maps presented at cluster threshold p<.01 (at least 100 contiguous voxels satisfying p<.0005 uncorrected at each voxel). Hot color scheme represents activation, i.e. BOLD: experimental > control. Cool color scheme represents deactivation i.e. BOLD: experimental < control. Medial temporal area was significantly activated by novel picture encoding and paired picture recognition tasks. The MIST deactivated the medial temporal area

Hippocampal activation

For the total sample, we observed a correlation between left HC deactivation during MIST and AUC_I ($r = .47$, $p < .04$). This observation validates or previous finding that cortisol stress response is modulated by hippocampal deactivation

(Pruessner et al., 2008). Other correlations between cortisol stress response and HC activation were not significant. However, as Figure 4 illustrates, group differences in the extent (but not magnitude) of hippocampal activation were obvious.

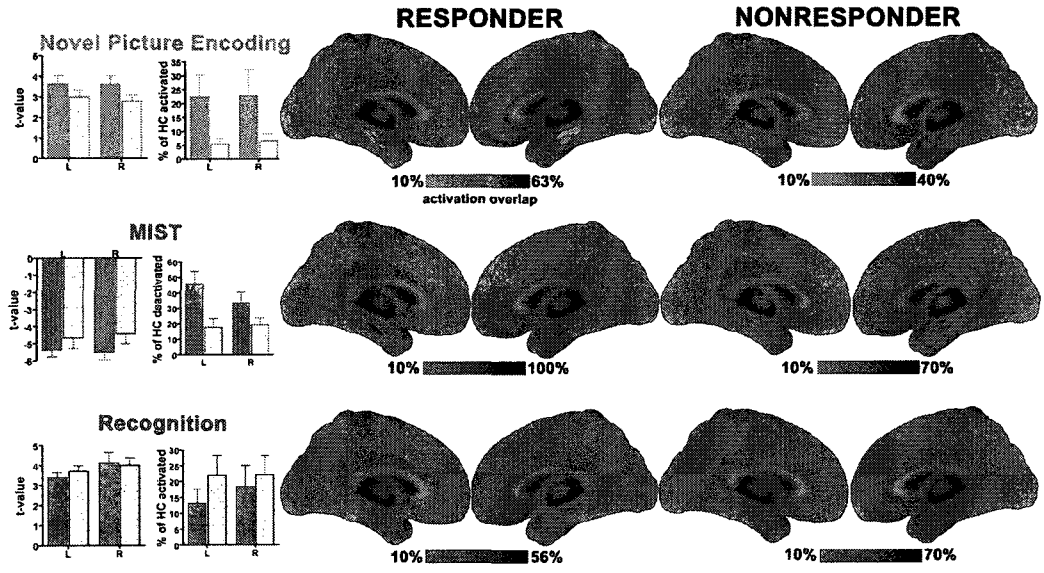

Figure 4: Group differences in task-related (experimental – control) hippocampal activation. Solid bars represent responders. Transparent bars represent nonresponders. Bar graphs illustrate group differences in (a) amplitude (i.e. highest t-value of the BOLD response within individual's hippocampal volume) and (b) extent (i.e. % of voxels that showed significant BOLD response with respect to total hippocampal volume of a subject) of hippocampal involvement. (c) The statistical parametric map of spatial overlap in hippocampal activation between subjects in each group. Colored surface areas indicate the extent of hippocampal activity. Highest percentages indicate greater between-subject similarity. Extent of hippocampal involvement in picture encoding and the MIST is significantly different between responders and nonresponder. Group differences in peak amplitudes are not significant. See Supplementary Tables 1 and 2 for comparison of the peak locations.

To investigate the effect of group by task interactions, we performed a three-way mixed design ANOVA (group*task*hemisphere) with group (responders and nonresponders) as the between factor and task (encoding, MIST, recognition) and hemisphere (left, right) as within-subject factors and the

percentage of HC activation (experiment > control) during cognitive or HC deactivation (experiment < control) during stress tasks as dependent variables. This test showed that responders and nonresponders significantly differed in extent of hippocampal involvement ($F(2,34)=4.55$, $p<.02$) during the three tasks; responders activated a larger percentage of HC during encoding ($F(1,51)=4.5$, $p<.04$) and deactivated a larger percentage of HC during the MIST ($F(1,51)=7.22$, $p<.01$), with no difference in recognition ($F(1,51)=.66$, $p>.40$). Further, neither the three-way interaction of group by task by hemisphere ($F(2,34)=1.36$, $p>.27$), nor the interaction of group by hemisphere, task by hemisphere, or the main effect of hemisphere were significant (all $Fs < 2$, $p > .40$). Similar analysis did not reveal any effect of group by task by hemisphere interaction on the magnitude of the peak BOLD response. This suggests that group differences in hippocampal activity were mostly related to spatial distribution of the hemodynamic response rather than a focal task-related modulation of the BOLD signal.

Because the hippocampus is functionally organized (Eichenbaum et al., 2007; Moser and Moser, 1998), we investigated the location of the peak HC response to each task within each subject's HC volume (See supplementary material for detail). This showed that in both groups, the peaks of hippocampal response to different tasks were separated by at least 8mm; with peaks of hippocampal deactivations in response to MIST distributed most rostrally and hippocampal activation during recognition distributed most dorsally along the long axis of the hippocampus. The stereotaxic location of the right hippocampal activation during recognition was more dorsocaudal in responders (mean [x,y,z]=[28, -31, -8]) compared to nonresponders ([27, -26, -14]; $p<.003$).

Picture recognition

Responders recognized the previously encoded pictures at a rate of 73±16%. The non-responders showed a trend for higher recognition rates (85 ±10%, $F(1,18)=3.94$, $p<.06$). Responders had a significantly higher rate of misses (12 ±16%) compared to non-responders (1.6 ±1.9%; $F(1,18)=4.83$, $p<.05$). The average rate of false alarms in the two groups was not significantly different (responders: 14 ± 11%; non-responders 13 ± 9%; $F(1,18)=.04$, $P>.90$). Figure 5 summarizes these results.

Linear multivariate regression analysis on the entire sample with percentage of hits as dependent, and percentage of activated HC volume per task as predictors did not reach significance ($F(3,15)=1.63$, $p<.23$). Effects of HC activation during encoding ($b=.03$) and during recognition on percentage of hits ($b=-.11$) were not significant ($p>.30$). The effect of HC deactivation during MIST on percentage of hits was marginally significant ($b=-.17$, $p<.06$). Hence we investigated linear relationships in each group.

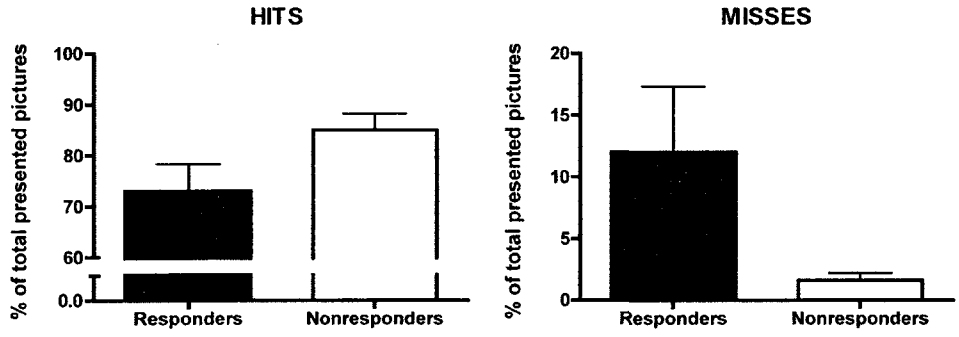

Figure 5: Differences in recognition performance between responders and nonresponders. A hit corresponds to a correct recognition. A miss corresponds to no answer or timeout. Responders have statistically significant higher number of misses.

In responders, number of hits correlated with the extent of HC (left + right) activation during recognition (r= -.69, p<.05, Figure 6 a) and with the levels of cortisol after stress (AUC_G(t=60:90 mins) r = .73, p<.04, Figure 6 b). No such correlation was present in nonresponders (Figure 6 c and d). A trend for positive correlation between number of hits and extent of HC activation during encoding was present in nonresponders (r = .53, p < .12) but not in responders (r = .29, p>.40).

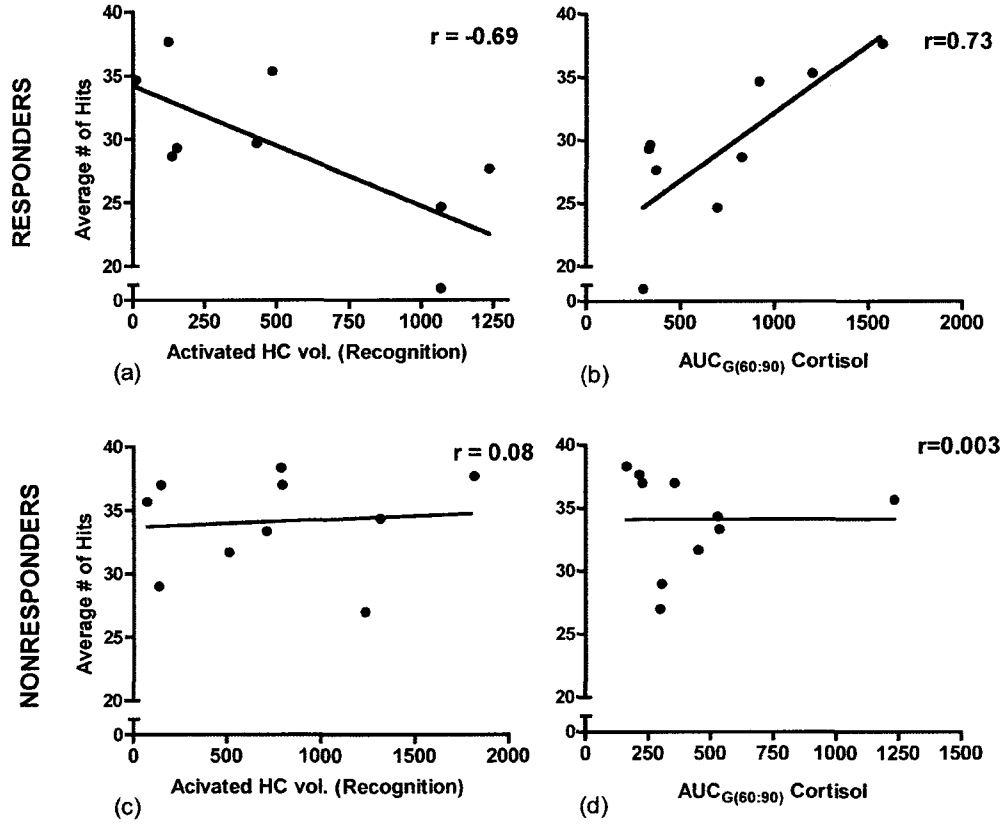

Figure 6: Group-differences in linear variation of performance with HC activity and cortisol levels. (a) Significant inverse correlation between HC activation during recognition and number of hits in responders (b) Significant correlation between total amounts of cortisol measured after the MIST (i.e. during recognition) and number of hits in responders. No significant correlations in the nonresponders (c,d).

Discussion

The current study was designed to investigate the interaction of stress and cognitive processing, HPA axis activation, and hippocampal function. To our knowledge, this is the first study to show that stress responders and nonresponders differ in the extent of hippocampal activation, not only during, but also prior to stress. In stress responders, larger hippocampal activation during encoding was followed by greater hippocampal deactivation during stress. The observation of reduced recognition memory in stress responders is consistent with previous findings that show a link between cortisol stress response and impairment of delayed retrieval of material learned prior to stress (de Quervain et al., 2003; Elzinga et al., 2005; Lupien et al., 2002; Wolf et al., 2001a). We also replicate our finding in a previous study (Pruessner et al., 2008a) by showing increased hippocampal deactivation (i.e. reduced BOLD signal during experimental stress condition compared to non-stressful control condition) in stress responders.

Observations made in the current study expand our previous findings by a critical aspect: While so far, we could show that more stress responsive subjects show differential hippocampal activation during stress, the current study suggests that hippocampal activation differences are also present in the absence of stress, during cognitive tasks that involve the hippocampus. Previously, we postulated that hippocampal deactivation was a stress response necessary to activate the HPA axis (Pruessner et al., 2008). Indeed, again we show that hippocampal deactivation accounts for 22% increase in AUC of cortisol during the experimental session. In our earlier paper, we interpreted this correlation in the context of the 'default mode network theory' (Raichle et al., 2001) according to which resting state activity in association areas represents the intrinsic states

of alertness and monitoring that ensure organism's readiness to initiate instantaneous adaptive responses to given stimuli. In this theoretical framework, deactivations (e.g. task-related reduced BOLD signal) are interpreted as a redirection of the available neuronal resources from these "monitoring" regions to other brain areas needed for task-specific processing. Now, we observe that not only responders and nonresponders deactivated the hippocampus differently during stress; but also they showed difference in hippocampal activation before stress (activation in responder > nonresponders) during picture encoding. While our measurements do not allow us to conclude a difference in resting state activation per se, we might still suggest that the "default" state of pre-stress hippocampal activity played a role in the subsequent stress response.

This "default mode" interpretation is also in line with the (Gray and McNaughton, 2003) theory that emphasizes the importance of hippocampus in monitoring competing or conflicting environmental stimuli. The septohippocampal theory of Gray and McNaughton originated from observations that alteration of ascending signals from noradrenergic system to the hippocampus (either due to septal lesions or due to anxiolytic drugs mimicking the septohippocampal lesion by reducing cholinergic signaling to the hippocampus) altered hippocampal theta rhythm (which is important for arousal) and diminished anxious behavior (Gray et al., 1977). In this theory, the hippocampus receives information about 'goals' and processes information such as novelty or familiarity in order to determine the optimal behavior to attain those goals (e.g. learning, (McNaughton, 2006). The neuronal activation in the hippocampus corresponds to detection of a goal and recursive integration of necessary information (from memory, stimulus cues or behavioral outcomes)

that produce neuronal outputs (especially from the subicular subfield) to brain regions needed to achieve that goal. In this view, whereas hippocampal hyperactivity results from conflicting goals or competing approaches that lead to anxiety, hippocampal hypoactivity corresponds to deficiency of behavioral optimization by learning or retrieving information that lead to amnesia (McNaughton, 2006). Our stress responders engage the hippocampus more extensively during picture encoding (more activation) and during experimental stress (more deactivation)—but not significantly so during recognition. It is arguable to suggest that compared to recognition, the encoding task is more susceptible to individual differences in attention and arousal (Kensinger et al., 2003; Uncapher and Rugg, 2005). It has been suggested that hyperactivity of the hippocampus reflects increased anxiety in the presence of, or due to the perception of, conflicting stimuli (McNaughton, 1997). If we assume that in the responders, higher hippocampal activation is associated with higher anticipation of outcome, then this could also help explain the stronger hippocampal deactivation after stress in this group. If prior to stress the hippocampus is in a state of increased arousal, anticipating and processing the demands of the external stimuli against the internal resources of the individual, once the event occurs, the metabolic resources of the hippocampus are directed to other regions necessary for execution of task-specific responses. Thus, we might speculate that while responders engaged the hippocampus to be more vigilant (or anxious) about encoding the novel stimuli, or in anticipation of upcoming tasks, once faced with the stress challenge they had to shift their focus to performing metal arithmetic under time pressure and social evaluative threat. We caution that we have not directly compared the hemodynamic fluctuations during these tasks, but only have examined interindividual variations in task-related hemodynamic response to experimental versus control condition within

each task. Nevertheless, given that the hippocampal control of the HPA axis is stimulus-dependent and particular to anticipatory stressors (Herman et al., 2003), showing group differences in hippocampal function might still reflect differences in everyday adaptations. In fact, the link between personality trait, coping strategies and HPA axis stress response is well established (Lai et al., 2005; Lazarus, 1974; Oswald et al., 2006; Pruessner et al., 2005). Inter-individual variations in hippocampal activity might relate to innate differences in intelligence or life experience (Scarmeas and Stern, 2003) and personality (Gray et al., 2005; Kumari et al., 2004) that variably recruit neural networks involved in task processing and behavioral control (Stern et al., 2005). Without data on our participants' subjective assessment of emotional and cognitive experience during these tasks, we cannot conclude what caused group differences in hippocampal activity. However, with cortisol as a biomarker of different anticipatory stress response, these results support the theory that the hippocampus plays a role in integration of psychological stimuli. To consider the role of hippocampus in daily adaptation encourages future studies to examine relation between resting state hippocampal activity and neuroendocrine stress sensitivity.

Considering the interference of the pre-stress state of hippocampal activity with HPA axis stress response may also be important for better understanding the relation between stress hormones and memory—which is far from simple (Het et al., 2005). Increasingly, it is becoming evident that the current states of brain activity play an important role in the brain's response to subsequent tasks and the eventual behavioral (cognitive or emotional) outcomes (Bellec et al., 2006; Dosenbach et al., 2007; Hasson et al., 2007; Raichle and Gusnard, 2005; Seeley et al., 2007). It is thus plausible that interindividual differences in physiological

(e.g. HPA axis response) or psychological (i.e. states of vigilance and attention) states modulate the way stress interacts with cognitive performance. For instance, our observation that despite higher hippocampal activation during encoding, the responders have lower performance scores contradicts the evidence that higher hippocampal activity during encoding predicts higher rate of delayed recognition (Davachi et al., 2003; Ranganath et al., 2004). But it is important to consider that here a stress session takes place between encoding and recognition sessions. In responders, cortisol elevation and reduced hippocampal activation after encoding (i.e. due to MIST) might interfere with the electrophysiology of learning process in this group—as previously suggested by (de Quervain et al., 2003). Moreover, the lower performance in responders is characterized by higher number of misses, which might have been caused by cortisol-related increases in recognition latency (Sandi et al., 2004) and thus a timeout. Interestingly, in the nonresponders a trend for positive correlation between HC activation during encoding and number of hits exists ($r = .52$). Accurate recording of the recognition scores (e.g. reaction time) or collecting data on the subjective experience of cognitive tasks should be considered in designing future experiments.

Although we are currently unable to comment on behavioral determinants of cognitive variations, group differences in correlation between performance and hippocampal activation highlight the complexity of relation between psychological stress and hippocampal related memory. To observe a lower memory performance in stress-responders is consistent with previous studies (Kirschbaum et al., 1996; Kuhlmann et al., 2005; Pruessner et al., 2007; Wolf et al., 2001b) that have shown brief exposure to stress elevates cortisol and lowers recognition scores. The inverse relationship between cortisol levels and memory

is attributed to glucocorticoid suppression of neuronal excitability in the hippocampal structures that cause cognitive impairment (Sapolsky et al., 2000). However, notwithstanding the small sample size in each group, the picture emerging for the responders after stress is contradictory: Here, cortisol levels during recognition explain over 53% of variation in increased rate of hits, suggesting an enhancing effect of cortisol on recognition in this group. This means that in groups who are perhaps hyper-vigilant and perceive a situation as more stressful, a greater cortisol response is beneficial for memory function. This hypothesis is in line with recent evidence that stress might have memory enhancing effects, in both animal (Roozendaal et al., 2006) and human studies (Schwabe et al., 2008). Furthermore, hippocampal activation in the responders during recognition explains over 47% of variation in rate of hits, with greater activation being related to fewer hits. Thus, the question arises why less hippocampal activation during recognition in the stress responders is beneficial for their ongoing cognitive performance. The answer might be that this correlation stems from cognitive strategies adopted during encoding. Several studies have shown that the strength of object familiarity can reduce the neuronal activity in the medial temporal lobe during recognition (Brown and Aggleton, 2001; Gonsalves et al., 2005; Henson and Rugg, 2003). A speculative scenario would be that although stress impaired the recognition performance of the non-responders, their higher arousal (inferred from increased hippocampal activity) during encoding helped strengthen the degree of familiarity of the pictures that they would correctly recognize during recognition task. Considering that the effect of familiarity on picture recognition seems to be right-lateralized (Rombouts et al., 2001), the right-specific group differences in location of the peak of recognition (see supplemental material) might relate to recognition effort in the responders. We also point out that right hippocampal

activation (both during encoding and recognition) explained greater variance in performance (in nonresponders and responders, respectively. See Supplemental table 3). Therefore, Examining the interactions between emotional and attentional experiences and hippocampal function are important for understanding effects of neuroendocrine stress responses on cognition.

We caution that our report lacks a discussion of the role of other 'default mode network' brain areas such as medial prefrontal and precuneal areas that are known to regulate cognitive and emotional interactions and show a significant BOLD response to our experimental condition. Of course, the hippocampus does not operate in isolation from the rest of the brain. It is an anatomically heterogeneous structures that is topographically connected to the hypothalamus (Swanson and Cowan, 1975) and the cerebral cortex (Cenquizca and Swanson, 2007b); with regionally different metabolic susceptibilities (Sloviter, 1994) that might delineate its functionally specialized regions (Eichenbaum et al., 2007; Kohler et al., 2002; Rombouts et al., 2001; Small et al., 2001). Indeed, our data indicates that the peaks of hippocampal BOLD response to encoding, MIST and recognition are not overlapping. Notwithstanding the low resolution of the echo planar image and the blurred hemodynamic response, this suggests that each task involves a different subregion of the hippocampus. It is noteworthy that in this study most of the variance in hippocampal activation is in terms of spatial distribution of the BOLD signal and not the amplitude of the peaks. It has been shown that attentional demands can increase the extent of hemodynamic response (to somatosensory stimulation) without affecting the amplitude of the electrophysiological response (Arthurs et al., 2004). We speculate that spatial variance in BOLD signal in our study reflects variation in functional connectivity between the hippocampus and other brain areas

recruited depending on an individual's cognitive strategy. As a full treatment of the role of other brain networks is beyond the scope of this manuscript, we will report them independently (Khalili-Mahani et al., in preparation).

In summary, our findings suggest that the states of hippocampal activity during non-stressful cognitive tasks might influence later responses to stress. We suggest that in studying the link between hippocampus, neuroendocrine effects of stress and memory, behavioral traits and baseline neural activations need to be carefully considered.

Supplementary Analysis: Functional Localization of The Hippocampus

The hippocampus is functionally organized (Kohler et al., 2002; Rombouts et al., 2001; Small et al., 2001) and is topographically connected to the hypothalamus (Swanson and Cowan, 1975). We examined whether group differences in performance were related to differences in stereotaxic location of peak BOLD responses to each experimental condition.

To examine group differences in spatial distribution of BOLD response to each task, we averaged (across subjects in each group) the stereotaxic coordinates of the peak activation (or deactivation) within each subject's hippocampus (Supplementary Table S1). The largest difference was detected along the z-axis in the right HC, suggesting that on average the responders involved the more dorsocaudal region of the hippocampus.

To determine proximity between peaks of hippocampal response to each task within each subject; for every subject we calculated the distance between the peaks of hippocampal response to different tasks (distance $_{\text{peak1 vs. peak2}}$ = $\text{sqrt}(x_{peak1}-x_{peak2})^2 + (y_{peak1}-y_{peak2})^2 + (z_{peak1}-z_{peak2})^2)$, where x, y and z are the stereotaxic locations of peaks (i.e., distances between peak of HC activation during encoding and peak of HC deactivation during the MIST; peak of HC activation during recognition and peak of HC deactivation during the MIST; and peak of HC activation during encoding and peak of HC activation during Recognition. We then compared these distance variables between groups to determine whether responders and nonresponders differed in involving

different subregions of the hippocampus. Supplementary Tables 1 and 3 summarize these results. Interestingly, the most significant group difference emerged in the right hippocampus, whose activation during encoding and recognition explained between 40-60% of variations in recognition hits. Spearman correlation analysis showed a right lateralize correlation between rate of hits and hippocampal activation during encoding in nonresponders (ρ=.63, p<.02), and during recognition in responders (ρ=-.77, p<.02) (Supplementary Table 3). This observation might further corroborate the hypothesis that behavioral difference between responders and nonresponders were manifested in hippocampal activity.

Table S1: Average stereotaxic location and average amplitude of peaks detected within the hippocampus of each subject ± standard error of mean

	Left				*Right*			
	t-valye	Coordinate ± mm			t-valye	Coordinate ± mm		
		X	Y	Z		X	Y	Z
Encoding								
Responder	3.6 ± 0.4	-26 ± 1.6	-24 ± 2.9	-14 ± 2.1	3.6 ± 0.4	27 ± 2.0	-24 ± 2.7	-14 ± 1.6
Nonresponder	3.0 ± 0.3	-27 ± 2.1	-26 ± 2.8	-13 ± 1.6	2.8 ± 0.3	28 ± 2.1	-21 ± 2.9	-15 ± 1.6
MIST								
Responder	-5.4 ±0.4	-24 ± 1.4	-15 ± 1.4	-20 ± 1.6	-5.5 ± 0.5	20 ± 1.4	-12 ± 1.7	-19 ± 1.6
Nonresponder	-4.7 ±0.6	-26 ± 1.3	-17 ± 3.2	-18 ± 2.5	-4.4 ± 0.6	21 ± 1.3	-15 ± 2.0	-19 ± 2.1
Recognition								
Responder	3.4 ± 0.3	-27 ± 1.8	-29 ± 2.5	-14 ± 1.7	4.1 ± 0.5	28 ± 1.6	-31 ± 2.9	-8 ± 1.4
Nonresponder	3.73± 0.3	-26 ± 2.1	-27 ± 2.4	-12 ± 2.1	4.0 ± 0.4	27 ± 1.2	-26 ± 2.0	-14± 0. 9

Table S2: Average distance ± SEM (mm) between peaks of hippocampal activity per each task

	Left			*Right*		
	Responder	Nonresponder	t (p-value)	Responder	Nonresponder	T (p-value)
MIST and Encoding						
	13.13± 2.98	18.30± 3.16	-1.18 (n.s.)	15.20± 3.25	19.02 ± 2.96	-0.87 (n.s.)
MIST and Recognition						
	17.01± 2.97	22.47± 3.44	-1.19 (n.s.)	23.78± 2.14	17.34 ± 2.78	1.80 (p=.07)
Recognition and Encoding						
	11.13± 3.16	15.86± 1.95	-1.30 (n.s.)	14.81± 2.74	8.53 ± 1.86	1.93 (p=.09)

Table S3: Correlation coefficients *(Spearman ρ)* for performance correlation with extent of HC activity and AUC of cortisol in different tasks.

		Responder			**Nonresponder**		
		Hit	Miss	False alarm	Hit	Miss	False alarm
Encoding	**LHC**	.47	.03	-.35	.60†	-.23	-.62†
	RHC	.00	.13	-.117	.63*	-.14	-.66*
	AUC (20:40)	.2	-.46	-.07	-.42	-.11	.38
MIST	**LHC**	-.43	.30	.35	-.37	.15	.25
	RHC	-.10	.025	.42	-.12	-.31	.08
	AUC (40:60)	.43	-.56	-.05	-.42	-.11	.38
Recognition	**LHC**	-.317	.269	-.08	,02	0.02	-.00
	RHC	-.77*	.40	.22	,29	.03	-.25
	AUC (60:90)	.73*	-.28	-.62	-.43	-.13	.39

LHC: Left hippocampus; RHC: Right hippocampus; AUC: Area under the curve with respect to ground. *significance at p<.05; † significance at p<.10.

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CHAPTER 3

OTHER BRAIN AREAS LINKED TO STRESS RESPONSE

In the previous chapter, we demonstrated group differences in cognitive and neuroendocrine stress responses to be associated with differences in hippocampal function. The role of the hippocampus in down regulation of HPA axis responses after psychological stress is well documented (Herman et al., 2003). However, we showed that the group differences in hippocampal activity were present not only during the ongoing stress, but also prior to the onset of stressing task. Based on emerging theories emphasizing the role of the hippocampus in vigilance and behavioral planning (Gray and McNaughton, 2003), we hypothesize that group differences in hippocampal function were related to state or trait differences in cognitive processing or behavioral adaptation that made one group less sensitive to stress stimuli than the other. Extensive data establish that cognitive and emotional appraisal play a significant role in modulating the HPA axis response to a given stressor (Biondi and Picardi, 1999; Dickerson and Kemeny, 2004; Lazarus, 1993). If so, we asked whether group differences we observed in the extent of hippocampal activation

represented recruitment of different brain networks during cognitive and emotional processing of the stress stimuli? Because in our previous studies we have noted significant association between cortisol stress response and deactivation of the mesolimbic system, we have focused on the Default Mode Network theory that postulates task-dependent deactivation of brain areas, such as medial prefrontal, posterior cingulate and precuneal areas, depends on an interplay between the cognitive or emotional aspects of goal-directed actions (Raichle et al., 2001) and linked to intrinsic differences in behavioral adaptation (Raichle, 2005). In this chapter, we re-examine the data presented in previous chapter to explore group differences in the involvement of the DMN during different tasks.

Manuscript 2: Interindividual Differences in Stress Response Are Reflected in Different Activation Patterns of the "Default Mode Network"

Authors:

Najmeh Khalili-Mahani[1,2]*, Vincent Corbo[1], Alan C Evans [2], Jens C Pruessner[1]

1. Douglas Mental Health University Institute, McGill University, Montreal, Canada
2. McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Canada

*Address correspondence to:

Najmeh Khalili-Mahani

Pavilion FBC 3, Douglas Mental Health Institute

6875 LaSalle Boulevard

Verdun, QC, H4H 1R3, Canada

Tel. (514) 398 8330

Fax (514) 888 4099

Email najma@bic.mni.mcgill.ca

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Contribution of co-authors

This manuscript is prepared based on the same data presented in the previous chapter. Please see contributions of co-authors in Chapter 3 to the study design and data collection. Additionally, Vincent Corbo proofread the manuscript and Dr Evans provided critical advice on this manuscript.

Abstract

Previously, we have shown that the hippocampus—a region important for stress regulation and cognitive function—manifests different activation characteristics in stress responders and non-responders during different cognitive tasks. We linked this observation to perceptual and behavioral differences that predicted individual stress sensitivities. In our observation, group differences were measured in the extent and spatial distribution of the BOLD response (not its peak amplitude). If we consider the hippocampus as the integrator of stress and response signals, then it becomes plausible that the extent of hippocampal activation be related to variations in the topography of overall brain activation. In the current analysis, we tested this hypothesis by first, examining the main effect of each of the tasks on neural activity of the responder and non-responder groups; and next, performing a voxel-wise group comparison of the task-related BOLD signal to determine brain areas that characterized group differences. These differences emerged in neural activity of regions that constitute the so-called "default mode network" (DMN). During novel-picture encoding (ENC) non-responders deactivated large clusters in the prefrontal, parietal and medial temporal regions, while the responders only deactivated a much smaller clusters in the precuneus. During the Montreal Imaging stress Task (MIST), responders showed more extensive deactivation of the medial prefrontal and mesolimbic areas compared to non-responders. Similarly, during paired picture recognition (REC) deactivations of prefrontal, precuneal and medial temporal regions were more extensive in responders compared to non-responders. Voxel-wise independent t-test also revealed a consistent difference in the DMN regions: namely superior temporal, precuneal, medial temporal and prefrontal areas. Most notably, in the precuneus and the anterior cingulate cortex (ACC),

omnipresent parts of the DMN involved in self-referential processing and emotional and cognitive integration, we observed dichotomous patterns of activity. Significant inter-task correlation in the magnitude of the BOLD response in these DMN regions might suggest an intrinsic task-independent difference in the neural correlates of behavioral responses in our groups. An exploratory post-hoc analysis provided preliminary evidence for this hypothesis. We noted a significant association between self-esteem and a factor explaining largest portion of common variance in the networks detected in three tasks. Future examination of the covariations of the DMN and HPA axis activities might provide quantifiable characterization of interindividual differences in stress sensitivity.

Introduction

Over the past half-century, numerous studies have established that psychological stress can invoke adaptive physiological responses similar to physiological stressors (Mason, 1968; Selye, 1956). Increased secretion of cortisol due to increased activation of the hypothalamic-pituitary-adrenal (HPA) axis is a common marker of a stress experience in humans (Mason, 1968). Cortisol—a corticosteroid hormone that is synthesized and released from the adrenal cortex into the blood stream—targets glucocorticoid receptors, especially in the limbic system, where it provides energy resources for motivation and arousal necessary for physiological and behavioral adaptations (De Kloet et al., 1998; Sapolsky et al., 2000).

Individuals greatly vary in terms of reaction to a given psychological stressor. Early work by Lazarus and colleagues showed that cognitive and emotional appraisal would modulate motivational states and coping behaviors, thus

leading to great interindividual heterogeneity in neuroendocrine stress reactivity (Lazarus, 1993). Corroborating Lazarus' theoretical view, a more recent meta-analysis of 208 laboratory psychological stress studies has shown that lack of control, unpredictability of an outcome and perception of threat to one's goals and social self are most consistently correlated with an HPA stress response (Dickerson and Kemeny, 2004). Based on these findings, Dickerson and Kemeny have put forth a theoretical model according to which "the cortisol system is activated in goal-relevant situations (motivated performance tasks), when a central goal is saliently threatened (social evaluative threat) and the process for attaining this goal is impeded (uncontrollability)" (Dickerson and Kemeny, 2004). The underlying mechanisms of interindividual differences, thus, may depend on differences in effort and engagement, or differences in appraisal of self-relevancy or self-threatening aspects of the stressor.

To examine the neural mechanisms of stress reactivity, we developed the Montreal Imaging Stress Task (Dedovic et al., 2005) that, based on Dickerson and Kemeny's theoretical framework, induces psychological stress by adding uncontrollability and social evaluative threat to a mental arithmetic challenge. During the control condition, subjects solve arithmetic equations, provide the answer, and receive feedback on whether they have answered correctly, wrongly, or have run out of time. During the experimental condition, in addition to doing the arithmetic task of control condition, subjects are 'stressed'. They are told that 1) the examiner will be monitoring their ongoing performance; 2) their performance is compared to the average performance of their peers and 3) the success of the experiment depends on their average performance—a challenge they cannot achieve because the task is automated to ensure that the level of task difficulty exceeds the subject's capabilities. In this way, the experimental

condition of the MIST challenges the goal of social self-preservation. It sets the goal of "perform up to an average standard in order for your participation to be worthwhile". However, it makes the task uncontrollable by limiting the response time, and diminishing it if they do well. Plus it adds social evaluative threat by presenting a visual score that evaluates their performance compared to "their peer group", while under the examiner's watch. Then, if a subject 'cares' to attain the set goal, then perception of a threat to achieving it combined with desire for social self-preservation (acceptability) will lead to an increased cortisol response.

Our recent experiments with this task have revealed a great degree of interindividual variability in cortisol stress response (e.g. Pruessner et al., 2004a) and Pruessner et al., 2008a). Interestingly, we have shown that while performing the stress task, stress responders (i.e. those with an elevated cortisol response after the task) show a significantly larger deactivation of the limbic system, especially in the hippocampus (Pruessner et al., 2008a). Our earlier findings have raised the question of whether baseline states of neural activity are representative of intrinsic behavioral differences that predict interindividual variations in stress sensitivity. We have recently explored this question by investigating the interindividual differences in pre-stress states of hippocampal activity, and have shown that indeed stress responders and non-responders differ in hippocampal activation even during a non-stressful picture-encoding task (Khalili-Mahani et al., 2009). Because the hippocampus is a key coordinator of the HPA axis response to anticipatory stress (Herman et al, 2003), we postulated that the group difference in states of hippocampal activity related to stress monitoring and behavioral adaptation (Khalili-Mahani et al., 2009). We also emphasized that pre-stress states of hippocampal activity were

important in priming the subsequent responses to stress and cognitive tasks.

In our previous interpretation, we emphasized the role of the hippocampus in the baseline or "default" states of vigilance and behavioral optimization. Because most of the group variance in hippocampal activation was in spatial distribution of the BOLD signal (and not the amplitude of the peaks) we speculated that differences between responders and non-responders were linked to differences in functional topography of brain areas recruited depending on an individual's behavioral strategy. In recent years, behavioral adaptation is linked to intrinsic (or "default") characteristics of neural activity in a network of brain regions that show high metabolic rates during resting conditions and consistently become deactivated during goal-directed cognitive actions (Fox et al., 2006; Gusnard and Raichle, 2001; Fox et al, 2005; Raichle et al., 2001). This intrinsic activity in this so called "Default Mode Network" (DMN) seems to reflect the state of alertness and monitoring of the brain even in the absence of extrinsic stimuli (Fox et al., 2007). The topography of the DMN activation is altered by self-referential thought (Cavanna, 2007; Gusnard et al., 2001), attentional (Drummond et al., 2005; Kelly et al., 2008), executive (Fox et al., 2007) and cognitive (Greicius et al., 2003) controls, and even social cognition (Rilling et al., 2008; Schilbach et al., 2008). Because in our previous observations most significant differences between stress responders and non-responders have been observed in deactivation of the mesolimbic areas, we have focused on the DMN theory to postulate that differences in deactivation related to differences in goal-perception and the subsequent behavioral adaptation. Therefore, in the current analysis we aimed to address the following questions: 1) Do differences in stress-reactivity manifest differences in the topography of the DMN activity during non-stressful tasks? 2) Is there a significant inter-task

correlation in the BOLD signal of the brain areas whose activity differs between responders and non-responders? 3) Are differences in neural activity in responders and non-responders linked to intrinsic traits?

Materials and Methods

Subjects

Nineteen young male college students between the ages of 20-28 years (Mean age = 22.5± 2 years) were recruited from McGill University (Education = 15.9 ± 1.3 years) and scanned in accordance with the Research Ethics Board of Montreal Neurological Institute. Subjects were telephone screened to exclude subjects with a history of depression, drug abuse, brain injury, or chronic illness.

Experimental design

The fMRI experiment consisted of a total of nine runs. The first three runs consisted of a novel-picture encoding task (Stern et al., 1996), followed by three runs of the Montreal Imaging stress Task (MIST, an arithmetic challenge performed under psychological pressure (Dedovic et al., 2005)), and finally three runs of a paired-picture recognition (Stern et al., 1996). All three tasks were performed using a block design. Details of the experimental design are described previously (Chapter 3). Briefly:

Encoding (ENC): During control blocks, subjects viewed complex but familiar (repeated) scenes (2 blocks of 6 pictures per run, for a total of 36 pictures). During experimental blocks, the subjects were asked to encode complex novel scenes. For each run, novel pictures were presented in 4 blocks, 10 pictures each, for a total of 120 pictures in total.

The Montreal Imaging Stress Task (MIST): During control condition, subjects solved arithmetic equations (2 blocks of 9 equations per run, for a total of 54 equations). During stress condition, they solved similar equations (2 blocks of 17 equations per run, for a total of 102 equations) but they were put under the impression that their performance was being monitored and the usefulness of their participation was judged based on compatibility of their performance with their peers. Moreover, they were repeatedly and assertively asked to try harder to bring their performance up to the acceptable average level, while time limits were reduced to ensure they did not succeed. Due to technical difficulties, we did not measure reaction time, however 'correct', 'incorrect' and 'timeout' scores were recorded.

Recognition (REC): During control blocks, the subjects clicked the mouse while looking at a pair of familiar complex pictures (2 blocks of 6 familiar pairs per run, for a total of 36 pairs). During experimental condition, they clicked the mouse on the familiar picture recognized from the encoding task (2 blocks of 20 paired pictures per run, for a total of 120 paired recognition). A 'hit' represented the correct recognition of a previously seen image. A 'false alarm' represented the incorrect recognition of novel picture as one that was previously seen. A 'miss' represented the absence of a response due to inability (either due to slow response time or indecision) to recognize which of the paired images was seen before.

fMRI acquisition

Subjects were scanned on a 1.5-T Siemens Magnetom Vision Scanner (Siemens AG, Erlangen, Germany). Anatomical MRIs were acquired using a T1-weighted ICBM (international consortium of brain mapping) protocol (3D SPGRE, TR/TE = 18/10, flip angle = 30°. 176 1-mm contiguous sagittal slices, FOV =

256 x 256 mm^2). The fMRI acquisition was done using an interleaved T2*-weighted (TR/TE=2500/50, flip angle 90) echo-planar acquisition. Each frame consisted of twenty-eight 5 mm thick slices positioned along the long axis of the hippocampus (in-plane resolution 5 x 5 mm; field of view 256 mm). Each run of the fMRI acquisition consisted of 128 frames. The cognitive stimuli were presented at 5 seconds intervals.

Cortisol measurement and assessment of stress sensitivity

Cortisol was measured from saliva samples collected over a period of 90 minutes using salivettes (SARSTEDT, Quebec City, Canada). Seven samples were acquired at the following time points: 10 minutes before the scan, immediately before the anatomical scan (t=0), after the anatomical scan and before ENC (t = 20), after encoding and before the MIST (t = 40), after MIST and before REC (t=60), after REC (t=80), and 10 minutes after the end of the scanning session (t=90) and after debriefing (t=100). To prevent the subject's head from moving, the investigator put the salivette into the subject's mouth wearing sterile gloves. The subject was instructed to refrain from chewing to minimize head movement. The salivettes were kept in the mouth for 2 minutes to saturate the cotton with saliva.

Saliva samples were analyzed for cortisol using a time-resolved fluorescence immunoassay. Intra- and inter-assay variability was less than 10% and 12%, respectively (Dressendorfer et al., 1992).

In order to determine stress sensitivity we computed the increase in the area under the curve (AUC_i) of cortisol levels measured from the seven saliva samples obtained during 100 minutes course of the experiment (Pruessner et al., 2003a). The reason for including all saliva samples (as opposed to

measurements related to each task) is that cortisol stress response is a heterogeneous delayed adaptive response. By incorporating all the data into a combined variable we aimed to minimize the effect of variability in HPA axis activation delays. For the same reason, we further reduced the dimension of the data by classifying subjects based on the AUC_i. Subjects who had a positive AUC_i were considered as responders, and those with a zero or negative AUC_i were classified as non-responders.

Behavioral data

We also administered the locus of control measure (G. Krampen, Competence and Control Questionnaire, Göttingen, Hogrefe, 1991) previously shown to be predictive of cortisol response (Pruessner et al., 2005; Pruessner et al., 1997a; Pruessner et al., 1999; Pruessner et al., 2004b). This measure includes subscales for self-esteem (general feeling of self worth, e.g. "I think I am creative"), internality (e.g., "I can determine many things that are happening in my life"), chance (e.g., "Many events in my life happen by chance"), and perception of other's control (e.g., "Other people often prevent the fulfillment of my plans"). Only 16 out of 19 subjects provided completed questionnaires.

fMRI analysis

The fMRI design resulted in a total of nine runs per subject, three for each task (ENC, MIST, REC). The raw data was motion corrected to the third frame of each run to minimize the rigid head displacement between the 128 frames, as well as between the three sessions of each paradigm (Cox and Jesmanowicz, 1999). The motion-corrected data was then blurred with a 6-mm FWHM Gaussian kernel in order to increase the signal to noise ratio (SNR). In order to normalize the brains to stereotaxic space, we used the anatomical MRIs to register (Collins et al., 1994) each individual brain to the Montreal

Neuroimaging Institute template (MNI152, Mazziotta et al., 2001).

First level, and higher level analyses were performed using *fmristat* and *multistats,* respectively (Worsley et al., 2002) as described below:

First level: Estimation of the Blood Oxygen Level Dependent (BOLD) contrast by fitting the hemodynamic response function (standard gamma function used in *fmristat*). For each subject, and for each run of each task, the first level analysis produced two brain maps. In these maps each voxel represented the effect size and standard deviation of the fitted BOLD signal (experimental – control) at that location.

Second level: Combining three runs of each task per each subject, using a fixed effect analysis as described in *multistat.* This analysis produced an effect size map and a standard deviation map, where each voxel represented the average BOLD signal and between run standard deviations for each subject.

Third level: Obtaining group averages by first standardizing individual's activation maps (from second level analysis) to MNI152 template to reduce gross inter-subject variability and then combining results of second level analysis for each task. This analysis produced a single t-map for each task, where each voxel represents the ratio of the estimated average effect size to the estimated standard deviation across all subjects.

Group comparison: Voxel-wise generalized linear modeling (GLM), with the BOLD signal estimated in second level as independent variables and stress-response groups as the dependent factor. This analysis produced a single map

for each task, where each voxel represents the t-statistics of the between-group activation difference at that stereotaxic location.

Statistical analysis

Statistical significance of the group average t-maps was determined in terms of cluster size. Cluster thresholds were set to ensure $p<.05$, corrected for multiple comparison according to random field theory (Worsley, 2005a). Clusters larger than 200 contiguously activated or deactivated voxels were considered significant.

For the between-group comparison maps, cluster thresholds were set at $p<.01$. corrected for multiple comparison. Clusters with more than 500 continuous voxels at p-value $<.001$ were considered significant and selected for further analysis. Within these clusters, we obtained the stereotaxic location and the t-value of the local minima or maxima.

To determine whether brain networks that differentiated responders and non-responders were related across subsequent tasks, we performed Pearson correlation analysis on the cluster peaks (which represent the highest average activity within a spherical radius of 6mms). A principle component analysis was then performed to reduce the data dimension and to obtain factors that explained the largest portions of variations in 'difference' networks. It has to be noted that our sample size was smaller than traditionally used in factor analysis. However, since these voxels corresponded to an effect observed with at least 100 degrees of freedom (a condition ensured in random field correction of statistical significance, Worsley, 2005a) we deemed this approach suitable for exploratory data reduction. These factors were then used in multivariate regression analysis with behavioral data (MIST performance, REC performance and locus of

control data) to determine the variables that predicted the variations of that factor. These analyses were performed using SPSS11 for Mac OS X (SPSS, Chicago, IL).

Results

Cortisol measurement and analysis

The compound cortisol measure AUC_i (Pruessner et al., 2003a) was used to split the sample into responder (N=9, AUC_i = 301 ± 89) and non-responders (N=10, AUC_i = -338 ± 113.55). The cortisol profile of these groups is presented in Figure 1. A two way mixed design ANOVA (group * time) revealed significant effect of group by time interactions on cortisol responses over time ($F(6,102)=4.24$, $p<.001$). Cortisol levels changed significantly for the contrast prior to MIST (t=40) compared to after MIST (t=60), $F(1,17)=5.16$, $p<.04$). Also, cortisol changes from time of arrival (t= 0) to after anatomical scan (t=20) changed differently between responders and nonresponders ($F(1,17)=7.39$, $p<.02$).

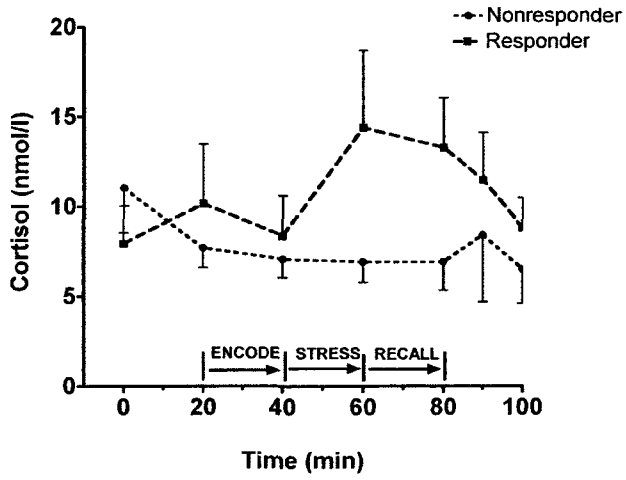

Figure 1: Profile of cortisol response at experimental intervals. The main effect of time was only significant in responders who increased cortisol levels after the MIST.

Brain activation results

For each task, we averaged the BOLD contrast (experimental-control) in each group and thresholded the activation maps at minimum cluster size of 200 contiguous voxels at $|t|>4.3$. Details of the cluster sizes and regions are available in supplementary Tables S1, S2 and S3. Patterns of deactivation were significantly different in structure of the DMN in responders and non-responders.

ENC

Figure 2 depicts significant differences in deactivation of prefrontal and posterior parietal areas between groups. Details of the cluster sizes and regions are available in supplementary Table S-1. The non-responders showed extensive deactivation in the lateral prefrontal, precuneal, superior temporal and medial prefrontal areas. Deactivations in the responder group were smaller and significant only in precuneus and the right superior temporal area. Both responders and non-responders bilaterally activated the middle occipital and cuneus, fusiform and parahippocampal regions.

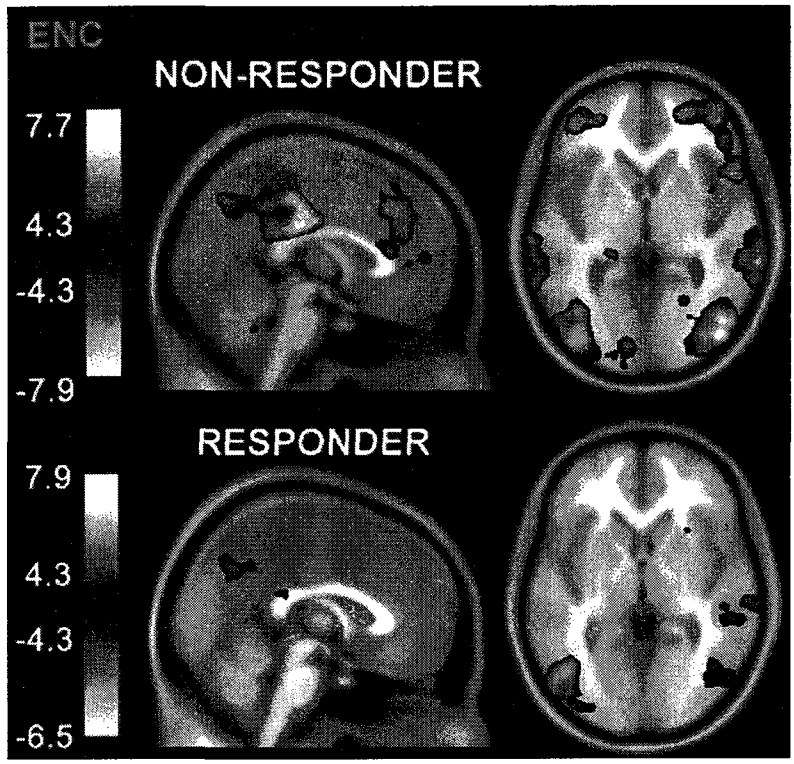

Figure 2: t-statistic maps of group activation (hot) and deactivation (cold) during novel-picture encoding. T-maps are set at cluster threshold of $p<.05$, corrected; and superimposed on the MNI152 template. Significant differences are present in deactivation of medial frontal and precuneal areas. See Table S1 for more details.

MIST

Figure 3 depicts significant differences in deactivation of the medial temporal, precuneal and medial frontal regions. Although small clusters of deactivation in the ventromedial prefrontal and right hippocampus were present in the non-responders, the anteromedial deactivations in responders were significantly more extensive and they also included insula, the head of caudate and the putamen. Details of the cluster sizes and peak regions are available in supplementary Table S2.

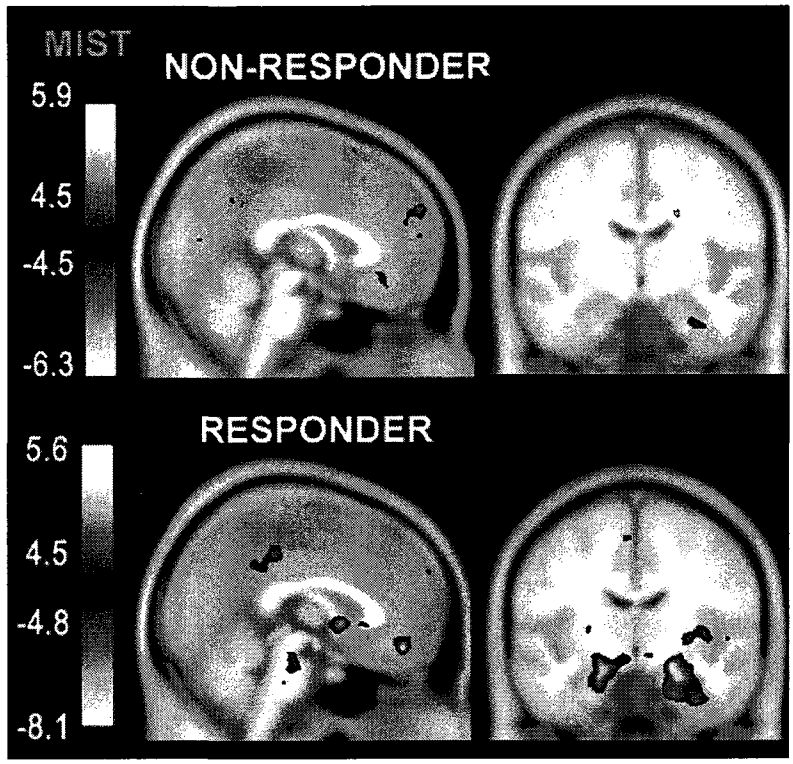

Figure 3: t-statistic maps of group activation (hot) and deactivation (cold) during MIST. T-maps are set at cluster threshold of p<.05, corrected; and superimposed on the MNI152 template. Significant differences are present in deactivation of medial frontal, medial temporal and precuneal areas. See Table S2 for more details.

REC

Figure 4 depicts group differences in deactivation of the precuneal and medial frontal regions. Details of the cluster sizes and regions are available in supplementary Tables S3. In both groups, cerebellum, occipitotemporal regions, insular and posterior parahippocampal region, as well as thalamus and the head of caudate were bilaterally activated. Common deactivations were in the supramarginal and precuneal areas. However, the extents of activations were larger in the non-responder group. By contrast the responders deactivated the medial prefrontal and precuneal areas.

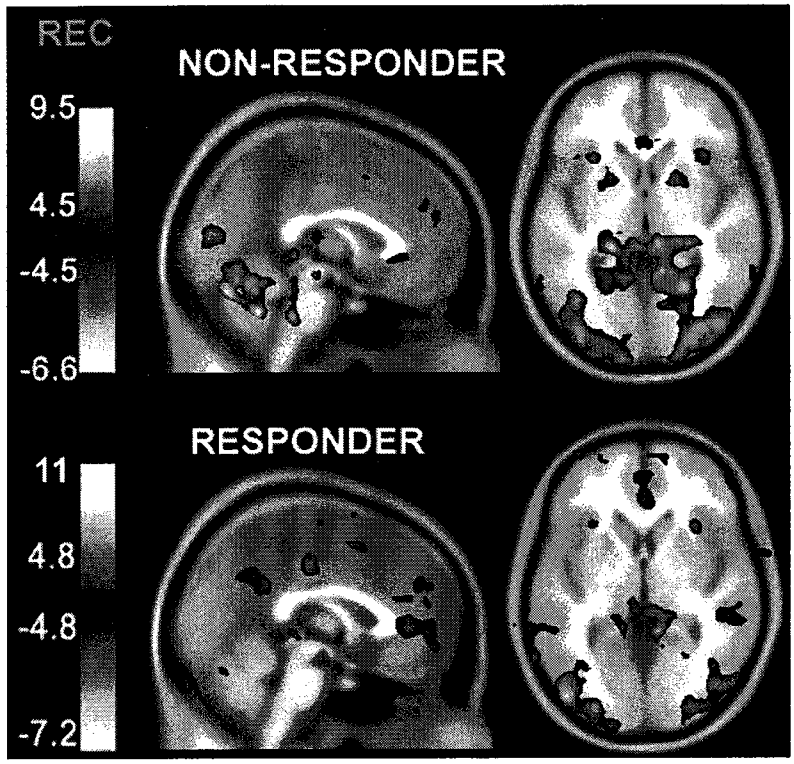

Figure 4: t-statistic maps of group activation (hot) and deactivation (cold) during paired-picture recognition. T-maps are set at cluster threshold of $p<.05$, corrected; and superimposed on the MNI152 template. Significant differences are present in deactivation of medial frontal, medial temporal and precuneal areas, as well as in the extent of activation in the medial occipital and parahippocampal regions. See Table S3 for more details.

Group difference in brain activation

The t-map of the voxelwise comparison of the responders and nonresponders was thresholded at minimum cluster size of 500 contiguous voxels at $|t|>3$ (Table 1).

Table 1: Talairach location of the most significant difference in brain activation obtained from voxel-wise comparison of BOLD contrast in responders versus non-responders (minimum cluster size=500 voxels)

	Area	t-peak	X	Y	Z	Cluster (voxels)	p-value
ENC	R rACC B24	4.93	3	34	12	4885	<.001
	R dACC B24	4.83	1	-12	34	3237	<.001
	L sTMP B42	4.44	-59	-29	12	949	<.001
	R sTMP B42	4.05	58	-14	10	856	<.001
	L precun B40	5.07	-64	-38	29	616	.003
	L lPFC B9	4.11	-23	39	37	597	.003
	L dACC B24	4.58	-6	1	26	580	.004
MIST	R mTMP B21	-4.87	42	-.01	-29	607	.003
	R lPFC B10	-4.52	21	68	14	569	.005
	R rACC B32	-4.12	6	53	-1	519	.008
REC	R rACC B32	-4.27	6	56	-3	1366	<.001
	L lPFC B10	-5.33	-25	64	0	995	<.001

dACC:dorsocaudal anterior cingulate,**rACC**:rostral anterior cingulate, **mTMP**:middle temporal gyrus, s**TMP**:superior temporal gyrus; **precun**:precuneus, **lPFC** lateral prefrontal.

The amplitude of the local minima and maxima within these clusters are illustrated in Figure 5. A dichotomous pattern of BOLD response was detected in these regions, although the average amplitude of the BOLD response for each group was moderate and the peak values did not reach statistical significance.

During ENC, the non-responders showed a positive BOLD response in the DMN regions such as precuneus, and anterior cingulate cortex; but these regions were relatively inactive in the responders. During MIST, however, the responders showed a notable deactivation of the temporal and frontal regions, while non-responders did not significantly activate or deactivate them. During the REC, the responders again deactivated the medial frontal and temporal areas, while non-responders moderately activated them. Also, the non-responders deactivated the inferior parietal lobule, while it remained inactive in responders. Most notably, a significant group difference in the right anterior cingulate cortex (ACC) activity was observed in each of the tasks (Figure 5).

Whereas the responders showed increased activity in the dorsocaudal part of the medial prefrontal area during the ENC (prior to stress), they showed decreased activity in the rostral part of the medial prefrontal area during the MIST and the REC (Figure 5).

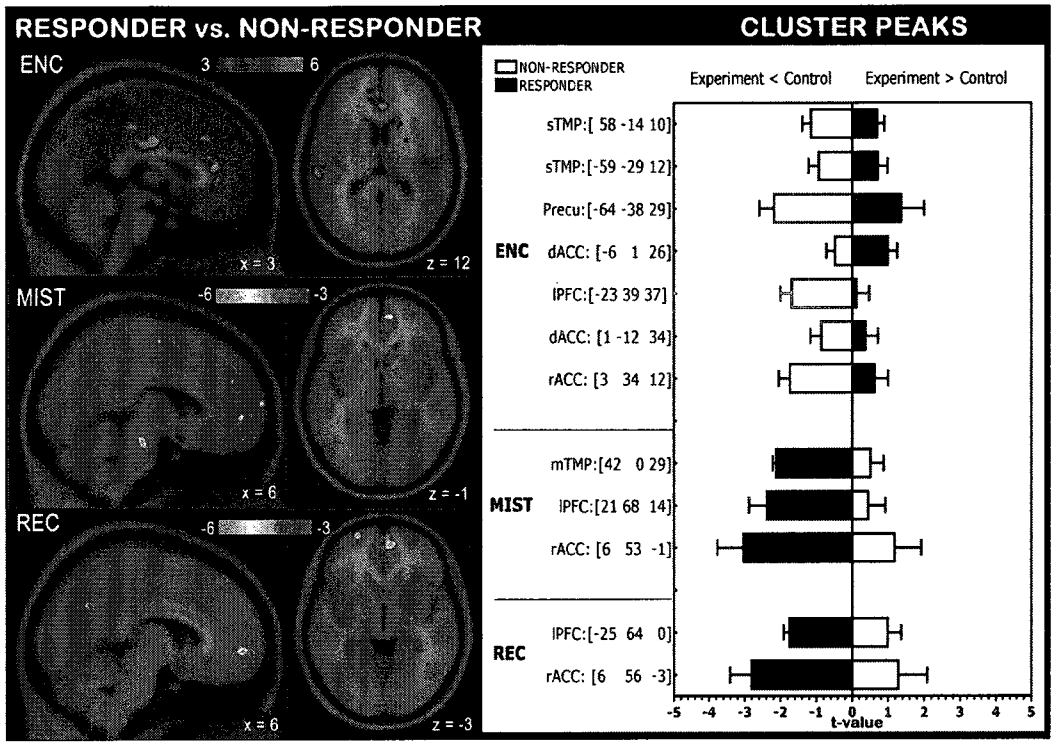

Figure 5: BOLD response differences between responders and non-responders (p-value cluster peak <.001, uncorrected; cluster threshold> 500 voxels). In the left panel, significant group differences in the medial prefrontal area are shown. Note the significance of difference in the right rACC. In the right panel, group (mean ± sd) of the local minima (t<0) and maxima (t>0) of significant clusters are shown.

Exploratory principle component analysis

A high degree of between-task correlations were present in the ROIs where differences in brain activity of responders and non-responders were significant. The matrix of correlation coefficients is presented in Table 2.

Table 2: Pearson correlation coefficients of between- and within-task regional covariaions

Loci of different activity	ENC							MIST			REC	
	1 rACC	2 dACC	3 sTMP	4 sTMP	5 precu	6 lPFC	7 dACC	8 mTMP	9 lPFC	10 rACC	11 mPFC	12 lPFC
1 **rACC** B24 [3 34 12]	1.000											
2 **dACC** B24 [1 -12 34]	.60**	1.000										
3 **sTMP** B42 [-59 -29 12]	.69**	.40*	1.000									
4 **sTMP** B42 [58 -14 10]	.49*	n.s.	.76***	1.000								
5 **prec** B40 [-64 -38 29]	.68**	.56**	.70***	.59**	1.000							
6 **lPFC** B9 [-23 39 37]	.70***	.49*	.79***	.481*	.62**	1.000						
7 **dACC** B24 [-6 1 26]	.54**	n.s.	.68**	.73***	.50*	.69**	1.000					
8 **mTMP** B21 [42 0 -29]	-.74***	-.61**	-.70***	-.60**	-.54**	-.62**	-.55**	1.000				
9 **LPFC** B10 [21 68 14]	-.46*	-.54**	-.68**	-.69**	-.71***	-.50**	-.40*	.73***	1.000			
10 **rACC** B32 [6 53 -1]	-.48*	-.42*	-.66**	-.59**	-.52*	-.70***	**-.79*** **	.69**	.56**	1.000		
11 **mPFC** B10 [6 56 -3]	-.45*	n.s.	-.61**	-.71***	-.62**	-.49*	**-.80*** **	.48*	.58**	.68**	1.000	
12 **lPFC** B10 [-25 64 0]	-.61**	n.s.	-.71***	-.77***	-.66**	-.41*	-.66**	.63**	.67**	.56**	.82***	1.000
AUCI	**.62****	**n.s.**	**.59****	**.61****	**.59****	**.44***	**.57****	**-.56****	**-.41***	**n.s.**	**-.61****	**-.73*****

*p<.05; ** p<.01; *** p<.001*

dACC:dorsocaudal anterior cingulate,**rACC**:rostral anterior cingulate, **mTMP**:middle temporal gyrus, **sTMP**:superior temporal gyrus; **precun**:precuneus, **lPFC** lateral prefrontal.

To reduce these correlated data into factors that explain the common variance in these ROIs, we performed an exploratory principal component analysis (PCA) on the peak of BOLD response (seeTable 1).[3] We set several constraints to reduce errors associated with small sample size. The Kaiser-Meyer-Olkin test ensured sampling adequacy (MKO = 0.76, p<.001). Using Kaiser's criterion (eigenvalues of the unrotated solution >1) and a Scree plot we determined two factors that explained 74% of cumulative variance. Factor 1 explained 63% of variance in the network (reduced to 40.5% after rotation); and Factor 2 explained 10.7% of variance in the network (increased to 33.4% after rotation). Factor loadings below absolute value 0.40 were suppressed. The rotated component matrix is presented in Table 3.

[3] We caution that our sample size is smaller than the suggested "rules of thumb" for PCA analysis as reviewed by Osborn and Costello (2004). However, it must also be noted that our PCA analysis is performed on peaks of neural activity estimated from signals at a large number of timepoints. The factors we have identified here correspond to eigenvalues that describe most of the orthogonalized linear structure in the data.

Table 3: Rotated factor loads of regions that differentiate responders and non-responders

Task	Stereotaxic [x y z]	Region	Factor1	Factor2
ENC	[3 34 12]	rACC B24		.777
	[1 -12 34]	dACC B24		.888
	[-23 39 37]	lPFC B9	.463	.668
	[-64 –38 29]	Prec B40	.487	.668
	[-59 –29 12]	sTMP B42	.676	.576
	[-6 1 26]	dACC B24	.852	
	[58 –14 10]	sTMP B42	.865	
MIST	[6 53 –1]	rACC B32	-.691	-.432
	[21 68 14]	lPFC B10	-.519	-.607
	[42 0 29]	mTMP B21	-.451	-.734
REC	[6 56 –3]	rACC B32	-.884	
	[-25 64 0]	LPFC B10	-.787	

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

Behavioral correlations

Having reduced the multivariate data to two factors, we looked at correlations between behavioral variables and factor scores. Table 4 represents tested models. The regression score of factor one was significantly correlated with self esteem and with cortisol increase due to stress task. Other components of the control and competence questionnaire were not correlated with factor scores. There were no correlations between factor scores and performance in the arithmetic or performance in memory tasks.

Table 4 : Regression analysis (principle components of the DMN versus behavioral factors)

Behavioral Data	Multivariate independent variables	Correlation coefficient	
		Factor 1	Factor 2
Personality (n=16)	**Self Esteem**	**-.51***	-.38
	Internality	-.19	-.32
	Perception of other's control	.38	-.004
	Belief in chance outcome	.248	-.246
Stress Reaction (n=19)	**Cortisol increase after MIST**	**.633****	.273
Arithmetic Performance (n=19)	Correct	.084	.303
	Incorrect	-.170	-.291
	Timeout	.008	-.173
Recognition Performance (n=19)	Hits	-.121	-.394†
	False alarms	-.050	.223
	Misses	.183	.283

* *p<.05; ** p<.01,* † *p<.1*

Discussion

The current analysis aimed to extend the ROI from the hippocampus and investigate whether differences in stress-reactivity were linked to the topography of the DMN activity; and whether neural signals characterizing group differences in stress sensitivity were linked to intrinsic and task-independent factors. Our observations here confirm our hypothesis that group differences in stress reactivity are linked to neural activity in brain regions important for perceptual integration and behavioral adaptation. Here, we confirm our previous finding that stress responders and non-responders differ in deactivation of the mesolimbic system (Pruessner et al., 2008a), and expand our findings to show that groups also differ in the DMN deactivation (especially in the posterior parietal and the medial prefrontal regions of the network) during non-stressful tasks.

Differences in the topography of the DMN deactivation prior to stress (i.e.

during picture encoding) are especially important because they corroborate our previous hypothesis (Khalili-Mahani et al., 2009) that linked increased hippocampal activity in stress responders to heightened vigilance and anxiety. Here, while non-responders deactivate the precuneus--an area involved in self-referential thought (Cavanna and Trimble, 2006) or the rostral ACC (an area involved in emotional processing (Bush et al., 2000), the responders do not show any neural response in these regions. One might speculate that in responders, the absence of deactivation in the self-processing areas (Gusnard, 2005; Gusnard et al., 2001), relates to a trait of self-focused attention that often correlates with social anxiety (Spurr and Stopa, 2002). The observation of difference in the pattern of DMN deactivation prior to stress (in the ENC task) also corroborates the notion that the baseline state of the DMN is likely to influence the brain's response to later stimulation and the subsequent behavioral (cognitive or emotional) outcome (Bellec et al., 2006; Dosenbach et al., 2007; Hasson et al., 2007; Raichle and Gusnard, 2005; Seeley et al., 2007). Interestingly, differences in deactivation of the ACC and precuneal areas are also present after stress (in the REC task). Here, the responders show significant deactivation of the rACC and precuneus. This might reflect the "disengagement" of the emotional (rACC) and self-referential (precuneus) regions in response to increased "cognitive" demand of picture recognition. The absence of similar deactivation patterns in the non-responders could suggest that during the baseline of the given task, they were not using the emotional processing resources of the brain. Therefore, the activity in these regions were unaffected by the experimental condition. It might be inferred then that the responders and non-responders approached the task with different perceptual traits or coping strategies that were consistent across tasks. The observation of significant correlations in the signal of the brain areas that distinguish

responders and non-responders supports this claim that intrinsic neural activity and behavioral adaptation are linked. In fact, our exploratory analysis indicates that a trait factor such as self-esteem—a personality trait that determines individual stress-sensitivities (Pruessner et al., 2005; Pruessner et al., 1997a; Pruessner et al., 2004c) is linked to a factor that describes most of the common variance in brain activity during different tasks.

We should caution that due to methodological limitations, we are limited in definitive interpretation of the intrinsic characteristics that predict stress-sensitivity. Often, the intrinsic fluctuations of the brain activity are estimated from examining the resting state fMRI data that allows examining correlated covariations of the neural signals across the brain. Nevertheless, our analysis yields two significant findings with potentially important implications for designing future experiments.

First, we report a prominent presence of the prefrontal (especially the anterior cingulate) areas in distinguishing stress responders and non-responders. Our findings are in line with recent reports on the neural correlates of the HPA axis response to arithmetic (Ohira et al., 2008; Wang et al., 2007; Wang et al., 2005); traumatic (Liberzon et al., 2007), and a public speech (Kern et al., 2008; Taylor et al., 2008) stressors. These studies report a robust involvement of dorsomedial, ventrolateral and orbital prefrontal regions in regulation of stress response. We observed a dichotomous pattern of activity in the rostral ACC that falls in line with previous findings. Here, during ENC, we note a negative rACC BOLD response in non-responders but a negligible positive effect in responders. By contrast, during the MIST and REC, stress responders show a negative BOLD response in the rACC but non-responders show a small positive BOLD effect.

There is evidence that the cerebral blood flow in the rACC (prior to stress stimulation) is positively correlated with cortisol response to the subsequent stress stimuli (Liberzon et al., 2007). Also, more recently an inverse correlation between stress induced cortisol levels and subsequent rate of glucose metabolism in the rostromedial prefrontal cortex has been reported (Kern et al., 2008). Behaviorally, the rACC activity in response to threat regulation and stress sensitivity (i.e. cortisol stress response to a psychological stressor applied in a different session) has also been reported (Taylor et al., 2008). Interestingly, reduced activity of the rACC is also linked to attentional control and behavioral adaptation in state anxiety (Bishop et al., 2004; Engels et al., 2007). Although variations in experimental set up of these studies preclude a direct comparison of the results, we might infer that effects observed in the rACC reflect its role in behavioral modulation of HPA axis adaptations. Earlier, we hypothesized that the responders experienced heightened states of vigilance and or anxiety, which predicted their stress response (in terms of cortisol and impaired recognition) to subsequent cognitive tasks (Khalili-Mahani et al., 2009). Here, we demonstrate significant group differences in neural response of a region that is linked to behavioral adaptation and HPA axis activity. Of course, in the absence of subjective ratings on individual's emotional experience or cognitive effort during tasks, we cannot interpret behavioral causes of the differences observed in stress responders and non-responders. Nevertheless, our findings emphasize that the ACC's function is related to individual's variations in HPA axis sensitivity to psychological stress.

Secondly, the inter-task correlations in the activity of different ACC subregions (e.g. inverse correlation between dACC activation during ENC and rACC deactivation during MIST, Table 2) might help construct more complex models

of cognitive and emotional interactions with HPA axis stress responses. The ACC function is important for integrating visceral, attentional, emotional and cognitive signals (Botvinick et al., 2004; Critchley, 2005)—perhaps to optimize goal-directed action planning (Rushworth et al., 2004; Walton et al., 2004; Yarkoni et al., 2005a). As several studies have suggested (Bush et al., 2000; Critchley, 2005; Simpson et al., 2001a; Simpson et al., 2001b), the dichotomous pattern of dorsal (in our case: responder > non-responder) versus rostral (responder < non-responder) ACC activity might relate to an interplay of cognitive and emotional traits that are stable across tasks, but different between groups.

The finding of increased deactivation in the medial prefrontal area during and after stress might corroborate animal studies that have shown that the medial prefrontal cortex and the hippocampus are critical for negative feedback inhibition of the HPA axis activity (Herman et al., 2005; McEwen, 2007). Not only does the medial prefrontal (especially the ACC) have a high concentration of glucocorticoid receptors—by which it assists fast-feedback inhibition of the hypothalamic nuclei (Diorio et al., 1993), but also it has bidirectional afferents to the hypothalamus (Feldman et al., 1995) that act on the GABAergic neurons of the hypothalamic projections (Herman et al., 2003; Herman et al., 2005). The precise mechanisms by which the mesolimbic structures regulate the HPA axis response to psychological stressors are not well established yet. For instance, recently, an excitatory role of the mesolimbic structures on the HPA axis has also been suggested (Herman and Mueller, 2006). We must caution that significant differences obtained from voxel-wise comparison of responders and non-responders are not associated with strong peak BOLD response in these regions (Figure 3). Further analysis is needed to investigate whether these

subtle differences in BOLD amplitude are linked to fluctuations of these regions, or to their metabolic responses. Nevertheless, our findings demonstrate that the ACC itself is a functionally heterogeneous area and that care must be taken when investigating it morphological or functional correlations with the HPA axis regulation. Functional dissociation of various ACC subregions might help resolve the ambiguity related to the role of limbic system in modulation of the HPA axis stress response.

Finally, although we caution that the results of our PCA factor analysis are exploratory, we emphasize that such analysis provides a useful methodology for reducing the large number of neuroimaging variables to a few factors that can be regressed against behavioral or physiological variables of choice. One limitation of our analysis here is that we have employed a standard factor analyses tool from SPSS to reduce the correlation matrix that describes the covariance of brain areas that differentiate responders and non-responders. In such standard factor analysis, factor reliability is often a function of sample size (Osborn and Costello, 2004). Clearly, our sample size to variables size ratio does not meet some of the suggested criteria (Osborn and Costello, 2004). However, the variables used in this factor analysis represent data that is averaged over hundreds of samples collected during the time course of the fMRI experiment. To increase factor reliability, we have ensured KMO > 0.76; have suppressed factors with less than 40% commonality and report factors with highest loading. However, presently we have no estimate of the effect of subject numbers on reliability of factors describing inter-regional correlations of the BOLD signal. Recent neuroimaging methodologies are developed to allow model-free independent component or principle component analyses on the entire neuroimaging data (e.g. Beckmann et al., 2005, McIntosh and Lobaugh,

2004). It would be interesting to see if such image-based factor analyses would reveal similar networks and similar associations with self-esteem and cortisol response as we have found.

Despite limitations, the central findings of this study suggest that brain regions involved in perceptual processing and environmental monitoring are differently activated in stress responders and non-responders, and that these differences might be related to intrinsic and task-independent differences in behavioral adaptation.

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Supplementary Materials

Abbreviations

ang angular gyrus
dACC:dorsocaudal anterior cingulate
InfF Inferior Frontal
Ins Insula
lPFC lateral prefrontal
InfpL Inferior parietal lobule
L, left
mFC, medial frontal cortex
midTmp middle temporal gyrus
mOccip middle occipital;
mPFC medial prefrontal cortex
mTMP medial temporal are,
OFC orbitofrontal cortex
parH Parahippocampal
PCC posterior cingulate cortex
pL parietal lobule
precun precuneus
R right
rACC rostral anterior cingulate,
sTMP superior temporal gyrus;
supF suprior frontal gyrus
supTmp superior temporal gyrus

Activation tables

ENC

Table S1: Talairach location of the peaks at the center of significant clusters of brain activation and deactivation during ENC.

BOLD(EXPERIMENT – CONTROL)>0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
R mOccip B19,30	23	-58	-14	7.77	36921****	R mOccipB19	46	-83	5	7.71	40228****
L mOccip B19,30	-47	-72	-5	7.92	31150****	L mOccipB19	-42	-88	2	7.08	35514****
L parH B35	-21	-20	-12	5.05	290 *						
L occip B17	-9	-86	7	4.72	263 *						
L dACC B24	0	-1	32	4.6	215 *						

BOLD(EXPERIMENT – CONTROL)<0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
R PLB40	60	-55	33	-6.53	4192****	R pL B40	61	-50	43	7.88	21335****
R PrecunB7	2	-72	44	-5.34	865 ****	R PCC B31	2	-29	47	7.52	9377****
R PrecunB7	12	-47	35	-4.77	795 ****	L pL, B40	-58	-61	30	6.64	9022 ****
						R supF B10	25	52	9	5.81	8117 ****
						R mPFC B32	1	34	38	7.62	6452 ****
						L infF B10	-42	48	1	5.72	6062 ****
						R mFC B8	41	29	40	6.68	5456 ****
						R InfF B47	50	21	-6	5.49	3087 ****
						LmidTmpB21	-69	-50	2	5.37	1121 ****
						L mFC B6	-43	11	49	5	727 ****

Cluster significance * p<.05; ** p<.01; *** p<.001; ****p<.0001

Table S2: Talairach location of the peaks at the center of significant clusters of brain activation and deactivation during MIST

BOLD(EXPERIMENT – CONTROL)>0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
R Precun B7	24	-64	39	5.65	594***	L InfF B6	-44	2	30	5.25	1090****
L Precun B7	-26	-73	45	4.9	266 *	R ACC B32	22	31	15	5.01	392 **
						RmPFCB11	-19	20	19	5.97	355 **
						L ACC B24	-20	-10	33	5	240 *

BOLD(EXPERIMENT – CONTROL)<0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
R Hypothalamus, basal ganglia, mesolimbic MPFC	26	-2	-27	-8.14	33741****	LprecenB9	-34	10	-38	-6.73	2161****
L insula, basal ganglia, mesolimbic	-35	-22	13	-7.25	19682****	L OFC B47	-25	12	-13	-5.84	1680****
L precun B7	-4	-47	37	-5.41	1511****	L supF B9	-7	58	32	-5.59	1254****
R supF B8	12	51	46	-5.07	507***	L pL 40	-67	-28	22	-5.68	889****
L OFC B47	-26	28	-12	-4.75	506***	R OFC B47	23	24	-13	-4.94	859 ****
R ang B39	51	-68	47	-5.22	482***	R uncusB20	26	1	-38	-5.47	801****
L supF B10	-29	67	6	-4.51	323 *	L supF B8	-20	35	51	-5.12	339 **
L supF B10	-16	67	20	-4.69	286 *						
R PL B40	65	-27	36	-5.19	246 *						

Cluster significance * p<.05; ** p<.01; *** p<.001; ****p<.0001

Table S3: Talairach location of the peaks at the center of significant clusters of brain activation and deactivation during Recognition.

BOLD(EXPERIMENT – CONTROL)>0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
R ling B19	23	-41	-13	11.05	50654****	L ling B19	-29	-52	-18	9.53	145410***
L ling B19	-27	-52	-15	9.25	48310 ****	R putamen	20	6	-5	6.51	5996****
L PCC B30	-16	-57	8	5.72	1479 ****	R cerebell	25	-43	-44	6.49	1682****
R Ins B13	31	23	4	6.12	1047 ****	L Ins B13	-31	20	4	7.68	1047****
R supF B6	2	10	50	5.37	932 ****	R OFC B47	35	24	-1	5.54	807****
R cerebell	3	-71	-25	5.57	772 ****	R parHB28	22	-5	-16	5.43	486**
L caudate	-17	-15	25	5.28	585 ***	L infF B9	-41	3	31	4.88	457**
L parH B30	-18	-36	4	5.14	468**	RinfPLB40	39	-31	45	5.4	412**
R thalamus	8	-22	13	5.37	466**	L putamen	-23	9	12	4.76	327*
R caudate	16	-10	24	5.62	429**	L cun B17	-12	-87	9	4.6	245*
L Ins B13	-29	24	4	4.98	276 *						
L thalamus	-9	-21	12	5.37	243 *						
L infF B9	-47	4	31	5.13	214 *						

BOLD (EXPERIMENT – CONTROL)<0

Responders						Non-responders					
Structure	Cluster					Structure	Cluster				
	X	Y	Z	t-peak	#voxels		X	Y	Z	t-peak	#voxels
LsupTmpB22	-69	-59	18	-7.15	5876 ****	R pL B40	53	-40	58	-6.02	3259 ****
R PL B40	58	-55	32	-5.49	3357 ****	L PL B40	-61	-57	37	-6.28	1667 ****
R precun B7	8	-58	36	-5.05	1238 ****	L mFC B6	-21	13	61	-4.75	258 *
R pL B40	54	-47	43	-4.54	317 **	RACC B32	8	22	-9	-5.29	254 *
R supF B8	11	39	56	-4.98	272 *						
L mFC B8	-37	22	43	-4.79	199 *						
R midTmpB21	51	3	-37	-5.14	197 *						

Cluster significance * p<.05; ** p<.01; *** p<.001; ****p<.0001

CHAPTER 4

NEURAL CORRELATES OF THE HPA AXIS RESPONSE

In the previous chapters, we showed that variations in HPA axis stress response predict group differences in the neural activity of areas such as the ACC and precuneus that are considered important 'self' monitoring and regulating' centers, whose activation or deactivation depends on the cognitive and emotional interactions during goal-oriented functioning (Gusnard et al., 2001; Raichle and Gusnard, 2005; Raichle et al., 2001). Findings in the previous chapters are consistent with our earlier hypotheses that 1) increased HPA axis stress response is associated with increased deactivation of the limbic structures involved in negative feedback inhibition of the stress response (Pruessner et al., 2008a) and 2) self-esteem is an important factor in determining stress sensitivity (Pruessner et al., 2005; Pruessner et al., 1997a). In the following studies, we have followed up on these hypotheses by investigating the following questions:

1. Is MIST able to elicit the same pattern of neuroendocrine activity in the old as it does in the young?
2. Is there a modulatory effect of cortisol on the activity of the hippocampus and other "default mode network" regions?

This is the first study to investigate the neural correlates of endogenous HPA axis stress response in an aging population.

Manuscript 3: Whose Brain Is Stressed? Evidence for cortisol-modulation of the BOLD signal in the "Default Mode Network"

Authors

Najmeh Khalili-Mahani[1,2]; Claudia Buss[3], Mehereen Wadiwalla[1], Jürgen Germann[1], Carole Scherling[1], Julie Andrews[1], Jens Pruessner[1]

1. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
2. Douglas Mental Health Institute, Verdun, Quebec, Canada
3. University of California, Irvine, CA, USA

Corresponding Author

Najmeh Khalili-Mahani

Pavilion FBC 3, Douglas Mental Health Institute

6875 LaSalle Boulevard

Verdun, QC, H4H 1R3, Canada

Tel. (514) 398 8330

Fax (514) 888 4099

Email najma@bic.mni.mcgill.ca

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Contribution of co-authors

Claudia Buss (PhD, university of Trier, Germany) coordinated the overall study and offered comments on the manuscript. Jürgen Germann (MSc, PhD Candidate, McGill University) helped with anatomical labeling and offered comments on the manuscript. Mehereen Wadiwalla (MSc, McGill University), Carole Scherling (MSc, McGill University) and Julie Andrews (PhD Candidate, McGill University) were involved in different aspects of data collection and helped with scanning. Najmeh Khalili coordinated and supervised the scanning of the older subjects. The neuroimaging data processing, analytical approach, statistical analysis, manuscript preparation, and interpretation of the findings were fully conducted by Najmeh Khalili. Alan Evans provided the computational resources for the analysis. Jens Pruessner supervised all the above.

Abstract

Previously, we have shown an association between cortisol stress response to Montreal Imaging Stress Task (MIST) and deactivation of the medial prefrontal and hippocampal areas that contribute to negative feedback inhibition of the hypothalamic-pituitary-adrenal axis response to psychological stress. We also have shown that variations in stress response are linked to intrinsic variations in a network of regions known as the default mode network (DMN)—potentially reflecting variations in resources available for behavioral adaptations. In the current study, we extend the population under study to older healthy adults to first investigate the effectiveness of the MIST in inducing a stress response in this demographic group; and to examine whether activity of the DMN is modulated by cortisol stress response. In two independent studies, we looked at 49 young (23.3 ±3 years) and 40 old (66.6 ± 5 years) healthy subjects while performing the MIST; and measured salivary cortisol at given intervals. Overall, the MIST produced a significant stress response in the Young, but not in the Old. The MIST led to extensive mesolimbic deactivation in the Young, but more limited mesolimbic deactivations in the Old. Instead, the Old deactivated large parts of the posterior parietal cortex and cerebellar areas, perhaps indicative of decrease in visual attention and motor activity during stress compared to control condition (arithmetic without stress). As expected, a significant modulatory effect of cortisol on the neural activity of the DMN was present. In both age groups total cortisol and cortisol increase during the MIST were correlated with the BOLD response of the medial temporal and medial prefrontal areas. Posthoc analysis revealed significant role of the right rostral ACC and orbitofrontal cortex in predicting distinct patterns of the HPA axis adaptive responses to experimental conditions. Overall, these findings confirm

the inhibitory role of the medial prefrontal cortex on HPA axis activity. However, they also demonstrate that the pre-stress states of HPA axis activity play a role in task related modulation of the DMN activity.

Introduction

Stress is an adaptive mechanism that by triggering myriad physiological and behavioral responses ensures that species survive the physical and psychological challenges of life (Chrousos, 2000). The hypothalamus pituitary adrenal (HPA) axis is an important part of the stress system. It has complex connections to association and limbic brain regions that "perceive" a threat (physical or psychological). These networks stimulate a rapid cascade of neuroendocrine signaling between the hypothalamus, the pituitary and the adrenal cortex, where glucocorticoid hormone (cortisol in human) is synthesized and released into the circulation. The initial neuroendocrine stress response is then attenuated by a fast feedback down regulation of the HPA axis activity—mediated by glucocorticoids—and also by neuronal inputs from limbic structures like the hippocampus and the medial prefrontal cortex that play an important role in HPA axis response to anticipatory stressors (Herman et al., 2003). Although stress is important for adaptation, if the stress system is chronically activated, or if it is not able to down-regulate itself efficiently, it can lead to both physical and mental disorders (McEwen, 2007). For this reason, researchers are particularly interested in understanding the interactions between the psychological substrates of stress and the neural mechanisms of HPA axis stress regulation.

In the past decade, advances in functional neuroimaging have provided researchers the opportunity to examine the neural substrates of mind, self and emotion. Awareness is growing that behavioral adaptation is linked to intrinsic

characteristics of neural activity in what is referred to as the "default mode network" (DMN, Fox et al, 2007; Raichle et al, 2005). The DMN consists of the medial prefrontal, posterior cingulate and precuneus and angular areas that consistently deactivates in response to goal-directed behavior. Baseline activity of these regions is linked to the net availability of neural resources that determine states of alertness and action planning (Dosenbach et al., 2007; Fox et al., 2007; Raichle and Gusnard, 2005). Although the contribution of the HPA axis to behavioral adaptation has been long established, it is only recently that researchers have begun to investigate the link between brain activity and the HPA axis. So far, the existing neuroimaging data confirm that brain activity in regions like the medial and the ventral prefrontal cortex (the most important parts of the DMN and critical for attention and action monitoring) correlate with both subjective rating of stress and stress cortisol response (Wang et al., 2007; Wang et al., 2005). The correlation between cortisol and medial prefrontal activity is present not only during stress stimulation (Pruessner et al., 2008a), but also during baseline conditions (Wang et al., 2007; Wang et al., 2005) or even during preceding cognitive tasks (Chapters 3 and 4). Also, pre-stimulus cortisol levels (Liberzon et al., 2007) or pre-stimulus stress sensitivity (Taylor et al., 2008) seem to modulate the prefrontal activity while processing threat-related stimuli.

Recently, we developed the Montreal Imaging Stress Task (MIST) that combines mental arithmetic challenge with negative psychosocial evaluation (Dedovic et al., 2005). This task differs from other arithmetic challenge tasks (Wang et al., 2005) as it explicitly challenges the objective of social self-preservation—which seems to explain the largest portion of variation in cortisol response to laboratory stressors (Dickerson and Kemeny, 2004). During the

stress condition of the MIST, participants are pressed for time, and are made aware of their performance with reference to the average performance of their peers. In addition, the participants are told that the success of experiment depends on their above-average performance. Previously, we have shown a great degree of interindividual heterogeneity in stress cortisol response linked to limbic system deactivation during performance of the MIST (Pruessner et al., 2008a). We have interpreted our findings in relation to the DMN theory. We have hypothesized that the MIST-induced deactivation of the limbic and paralimbic areas reflects an adaptive stress response aimed at activating the HPA axis (by reducing the inhibitory influence of the hippocampal and medial prefrontal areas to GABAergic interneurons of the paraventricular nucleus (PVN) of the hypothalamus (Herman et al., 2005)). Because the stress condition of the MIST adds psychosocial evaluative threat to mental arithmetic, we have suggested that interindividual heterogeneity in limbic system deactivation is related to perceptual differences that modulate individual's stress sensitivity. This hypothesis raises the question of whether this particular social evaluative threat has a similar effect on the neural and endocrine responses of a different age group. Furthermore, it encourages searching for a common mechanism that—irrespective of demographics, psychological traits or situational conditions—can be used to predict or modulate a physiological stress response.

In the current study we have aimed to address these questions by studying the neural and endocrine responses in an old group of healthy subjects who perform the MIST. The young group studied here is different from the population reported in Chapter 2. We have added new young subjects to the data that was previously reported (Pruessner et al., 2008a); and in addition have performed

voxel-wise correlation analysis on each age group to investigate which brain areas are modulated by total amount of plasma cortisol, or cortisol variations in response to the MIST.

Materials and Methods

Subjects

The data reported here were obtained from two related studies that aimed to investigate several aspects of cortisol regulation, cognition, and personality in relation to psychological stress. In the first study 54 young adults (20-30 years, hereafter referred to as the Young) were recruited by distributing fliers in the local college community. Some of these data were presented in our previous study (Pruessner et al., 2008a). In the second study, 52 older adults (60-75 years, hereafter referred to as the Old) were recruited by ads in the local newspapers. In accordance with the Research Ethics Board of the Montreal Neurological Institute, written informed consent was obtained from all participants prior to entry in the study. The subjects were screened on the telephone using questionnaires to rule out the presence or history of psychiatric disease. Further exclusion criteria included previous surgery, metallic implants, current illness, and any history of endocrine or immune system disease. The Old and the Young were examined under different experimental conditions, but the MIST sessions were almost identical.

The Young arrived at the MRI testing unit 30 minutes prior to entering the scanner. Upon arrival, they received explanation about the performance requirements of the fMRI protocol and completed two autobiographical memory tests (AMT) before entering the scanner. After the MIST, they were taken out of the scanner, and were given three implicit self-esteem tasks (lexical

decision, dot probe and implicit association test, IAT).

The Old had made two visits to the laboratory. In the first visit, they performed the AMT and the implicit self-esteem tasks. In addition, they performed the Trier Social Stress Task—involving similar elements of psychosocial evaluative threat as MIST. The scanning visit took place at least two weeks after the first one. In the second visit, the Old arrived at the MRI testing unit 10 minutes prior to starting the MRI session. They were explained the fMRI experiment before entering the scanner unit. Between each run of the MIST, they received verbal instructions and pressed to do better on the arithmetic task. After the MIST, they were asked to remain in the scanner for 21 minutes of DTI acquisition. They were told to relax and close their eyes. Debriefing about the 'deceptive' component of the MIST (i.e. impression that their performance was being monitored and compared to their peers) was done at the end of experimental session.

While differences in experimental setting are highly likely to alter the perceptual and coping components of the experimental stress task, we were interested in testing the robustness of the MIST to variations in other experimental conditions. The neuroimaging protocols tested on the Old were identical, with the exception of using larger fonts on the MIST interface to compensate for natural age-related visual impairment. When necessary, both groups were given MRI-safe glasses according to their optometric prescription.

Complete neuroimaging and cortisol data was obtained in 49 of the Young (24 male, age = 23.3 ±3; 25 female age = 23 ± 3.1) and 40 of the Old (20 male, age = 66.6 ± 5.3 years; 20 female, age = 66.5 ± 4.9).

fMRI experiment: Montreal Imaging Stress Task (MIST)

Perceiving social evaluative threat is known to reliably stimulate the HPA axis and lead to a stress response (Dickerson and Kemeny, 2004). The Montreal Imaging Stress Task (MIST, Dedovic et al., 2005) combines mental arithmetic challenge with psychosocial stress. We have previously shown that the MIST can produce significant increases in levels of salivary cortisol in about 50% of the tested subjects (Pruessner et al., 2004a; Pruessner et al., 2008a).

We presented the MIST in a block-design ABCABCABC fashion, where A = 14 baseline, B=22 Control and C = 40 experimental frames. The baseline condition is passive viewing of the task screen; control condition involves performing timed arithmetic calculations; and experimental condition adds to the control a time-bar, a performance bar and a distraction box (with a set of 40 words appearing at each stimulus interval) to augment the mental challenge. The subject is led to believe that the performance bar reflects his performance in comparison to the average performance of his age group. With every mistake, or time-out, the subject's score lowers. The task is automated to maintain a success rate of between 40-50% of the subject's own performance. Adding operands to the equation, and slightly reducing the available response time achieve this. The goal is to exert time pressure, to reduce control, and to manipulate the subject's perception of his rate of failure to emulate some of the conditions necessary for a psychological stress response (Kirschbaum et al., 1993; Mason, 1968). The stress response is measured by subtracting the control from the experimental condition, where the control condition requires high attentional load and experimental condition adds to attentional load elements of uncontrollability (time limited response opportunity), and social evaluative

threat (performance comparison indicator and verbal negative feedback by the investigator). To increase power, the MIST is presented in two runs, between which the subject is verbally instructed to improve his performance. Each run consists of 228 frames and takes about 10 minutes.

MRI acquisition

Subjects were scanned on a 1.5-T Siemens Magnetom Vision Scanner (Siemens AG, Erlangen, Germany). Anatomical MRIs were acquired using a T1-weighted ICBM (international consortium of brain mapping) protocol (3D SPGRE, TR/TE = 18/10, flip angle = 30°, 176 1-mm contiguous sagittal slices, FOV = 256 x 256 mm^2). Functional scans were acquired using an interleaved BOLD Mosaic 64 T2*-weighted (TR/TE=2500/50) echo-planar acquisition. Twenty-eight axial slices 4 mm thick were acquired at in-plane resolution 4 x 4 mm; field of view 256 mm, TR/TE 2500/50, and flip angle 90.

Cortisol measurement and analysis

Cortisol was measured from nine saliva samples collected using salivettes (SARSTEDT, Quebec City, Canada). Saliva samples were analyzed for cortisol using a time-resolved fluorescence immunoassay. Intra- and inter-assay variability was less than 10% and 12%, respectively (Dressendorfer et al., 1992).

The Old and the Young underwent different experimental conditions prior to and after the MIST. Therefore, we calculated the total area under the curve (AUC_{total}) and the increased AUC with respect to baseline ($AUC_{increase}$) (Pruessner et al., 2003a), at time point t=0, +10 and +20 minutes with respect to the MIST onset. The experimental conditions at these time points were

identical between age groups.

Statistics analysis

fMRI analysis

Data were preprocessed to correct motion (Cox and Jesmanowicz, 1999) and were spatially normalized by Gaussian blurring with a 6-mm FWHM kernel to increase the signal to noise ratio.

First-level analysis was performed using *fmristat* (Worsley et al., 2002), where a standard model of hemodynamic response was fit to the BOLD contrast of the control minus baseline, and experimental minus control condition.

In the second-level analysis, *multistat* (Worsley et al., 2002) was used to average the effect of the two runs by combining the estimate of effect and standard deviation from first-level analysis.

The third-level analysis involved combining the between-subject data. To do so, individual anatomical MRIs were linearly registered to the MNI 152-average ICBM model (Collins et al., 1994). The transformation matrices were then used for spatial normalization of the second-level average maps. Having aligned all subjects' activation maps in a standard space, a mixed effect analysis was performed on all subjects in a group, estimating the ratio of the variance of the random effects to the fixed effects. Regularization of this ratio was achieved by spatial smoothing with a Gaussian filter to yield 100 effective degrees of freedom.

Group activation maps were set to ensure cluster threshold of $p<.001$

(corrected), consisting of at least 100 contiguous voxels at the peak threshold of $|t| > 5.7$ (peak $p < 0.001$, corrected). Because anatomical variability is greater in older adults, the activation peaks are less likely to overlap. Therefore, in the Old we relaxed the cluster threshold to ($p<.01$, within the ROI determined from the Young activations) by looking for 100 contiguous voxels at peaks $|t| > 3.8$ (peak $p<0.01$, corrected).

Regression analysis of variations in brain activation with cortisol

To investigate the linear association between cortisol concentrations and the BOLD signal, a voxel-wise regression analysis was performed with BOLD as the dependent and the AUC_{total} and $AUC_{increase}$ as independent variables, in two separate models.

Considering the absence of an overall MIST response in the Old, plus the experimental heterogeneity, this analysis is more suitable than splitting the sample to responders and non-responders as it captures the effect of between-subject variances in HPA axis activity on neural activity.[4] The $AUC_{increase}$ is calculated in reference to each subject's HPA-axis baseline with respect to the onset of the MIST. Therefore, it arguably represents the degree of task-induced variation in cortisol levels for each subject, thus reflecting dynamics of HPA axis activity. By contrast the AUC_{total} reflects total amount of cortisol circulating during the course of the experiment, thus incorporating notonly task-related dynamics of HPA axis activity with individual's baseline cortisol levels. Because cortisol plays an important role in metabolic adaptation, we used AUC_{total} to examining whether it modulates the BOLD signal across the brain.

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[4] For analyses similar to previous chapters, see Supplementary Analysis 1.

Significant correlations were determined at cluster thresholds of $p < .05$. Correction for multiple comparison was performed according to random-field theory criteria that account for the similar pattern of correlations in at least 100 adjacent voxels of $|t|>3.1$ (peak $p<.001$, uncorrected) in the Young; and $|t|>2.3$ (peak $p<.01$, uncorrected) in the Old (for details, see Worsley, 2005a).

Results

Cortisol profile during the MIST

Figure 1 illustrates the cortisol profile during the MIST for each age group. One-way repeated measure (time) ANOVA revealed significant effect of MIST on HPA activation in the Young ($F(3,147)=4.93$, $p<.003$) but did not reach statistical significance in the Old ($F(3,117)=2.38$, $p<.08$). Tukey's post-hoc analysis in the Young showed that the level of cortisol at t= +20 minutes after start of the MIST was significantly larger than t=+10 (95% CI: 2.42-0.2) and t=0 (95% CI: 2.5-0.31).

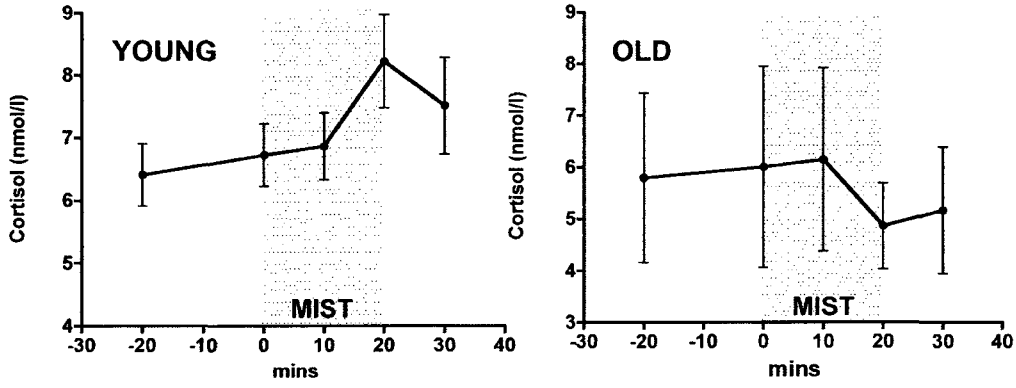

Figure 1: Cortisol Profile measured during scanning session. (a) In the young, cortisol level has significantly increased 20 minutes after the MIST onset. (b) In the old, large variance in cortisol values was present across sampling time points. The AUCs are calculated over times 0-20 minutes.

Table 1 summarizes the statistics of the compound cortisol variables.

Table 1: Statistics of the compound cortisol variables

AGE	AUC	Minimum	Maximum	Mean	Std. Deviation
Old (N=40)	Total	22.15	388.40	130.0	83.0
	Increase	-60.45	156.20	3.95	38.25
Young (N=49)	Total	23.80	500.00	180.37	103.58
	Increase	-51.10	232.70	19.73	52.80

Note that the saliva samples in the old and t he young group were analyzed with *independent assays; therefore the values between the old and the young may not be* comparable.

Brain activation patterns in the old and the young groups

Mental arithmetic without stress

As Figure 2 illustrates, both age groups had the same pattern of activation and deactivation in response to mental arithmetic compared to baseline (looking at screen without doing anything). Mental arithmetic resulted in deactivation of the anterior cingulate, posterior cingulate and the precuneal area—which is characteristic of DMN response to cognitive tasks. Activations were present in the cerebellum, basal ganglia and the post central area.

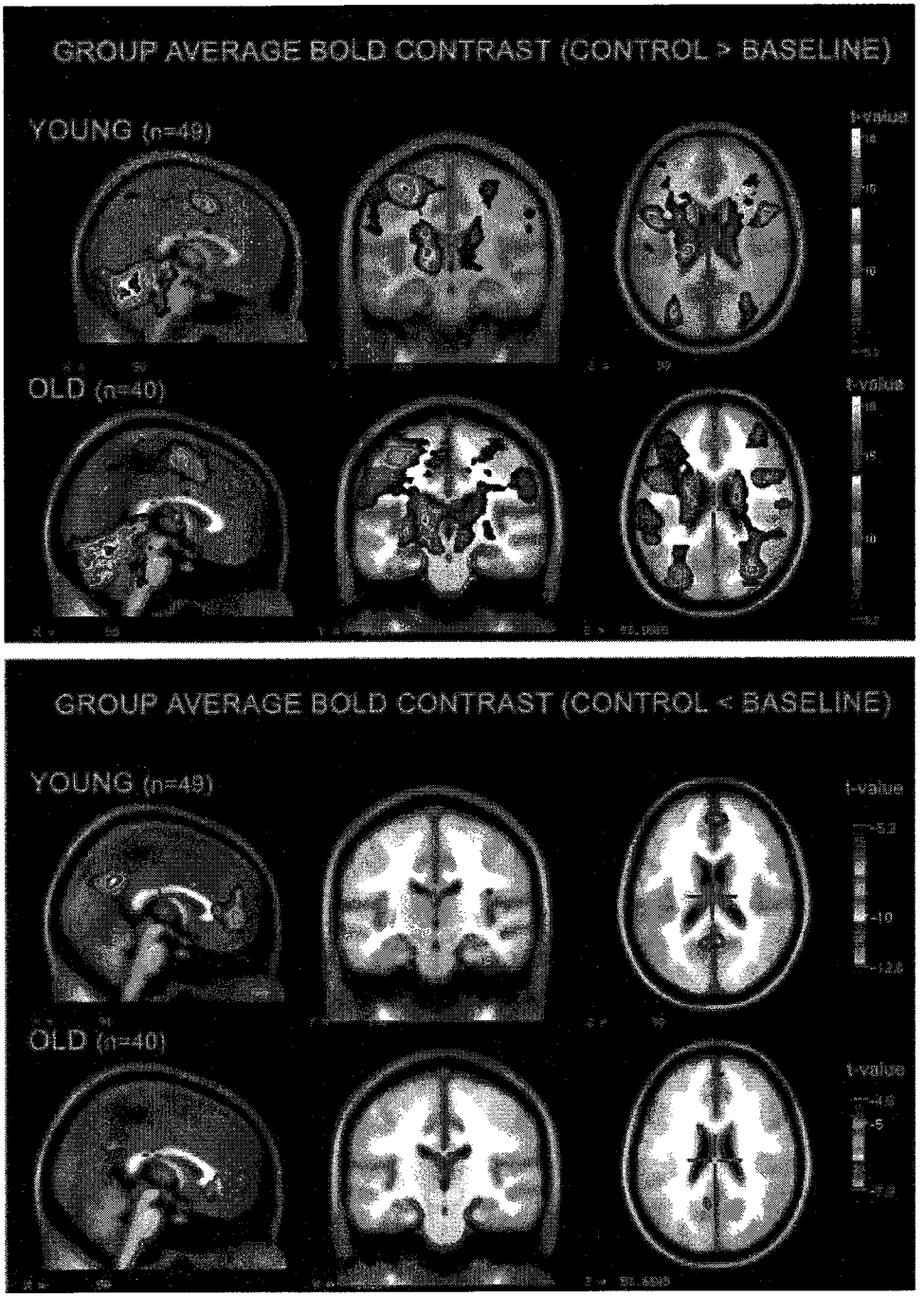

Figure 2: Group average activation maps Control (arithmetic) minus Baseline (just passive viewing of the MIST screen). Activation (top panel) and deactivation (bottom panel) t-maps are set at cluster threshold p<.001 (young) and p<.01 (old), corrected.

Uncontrollability and social evaluative stress

Patterns of brain activation in stress condition were largely different between the age groups. Figure 3 and tables 2 and 3 summarize the group average activation maps (stress versus control) for the Young and the Old, respectively.

In the Young, the BOLD signal in the medial frontal and medial temporal structures was significantly lower during stress (i.e. mental arithmetic under psychological pressure) compared to control (mental arithmetic without psychological pressure). On average, the Young activated the thalamus, insula (BA 13), precuneus (BA 7), occipitotemporal (BAs 37, 39) and precentral (BAs 4, 6) areas; and deactivated a large part of rostral anterior cingulate (BA 24), operculum (BA 38) and parietal lobule (BA 40).

In the Old, the largest cluster of activations was in the medial occipital and medial parietal areas (including BAs 18), as well as in the mid dorsolateral prefrontal areas (BA 9, 46). The largest cluster of deactivation was present in the middle occipital and medial parietal are (BAs, 19, 40, 30) and precentral area (BA 4). Similar to the Young, the Old deactivated the anterior cingulate (BA 24) and superior temporal region (BA 22). Unlike the Young, the Old deactivated the thalamus, insula and the putamen.

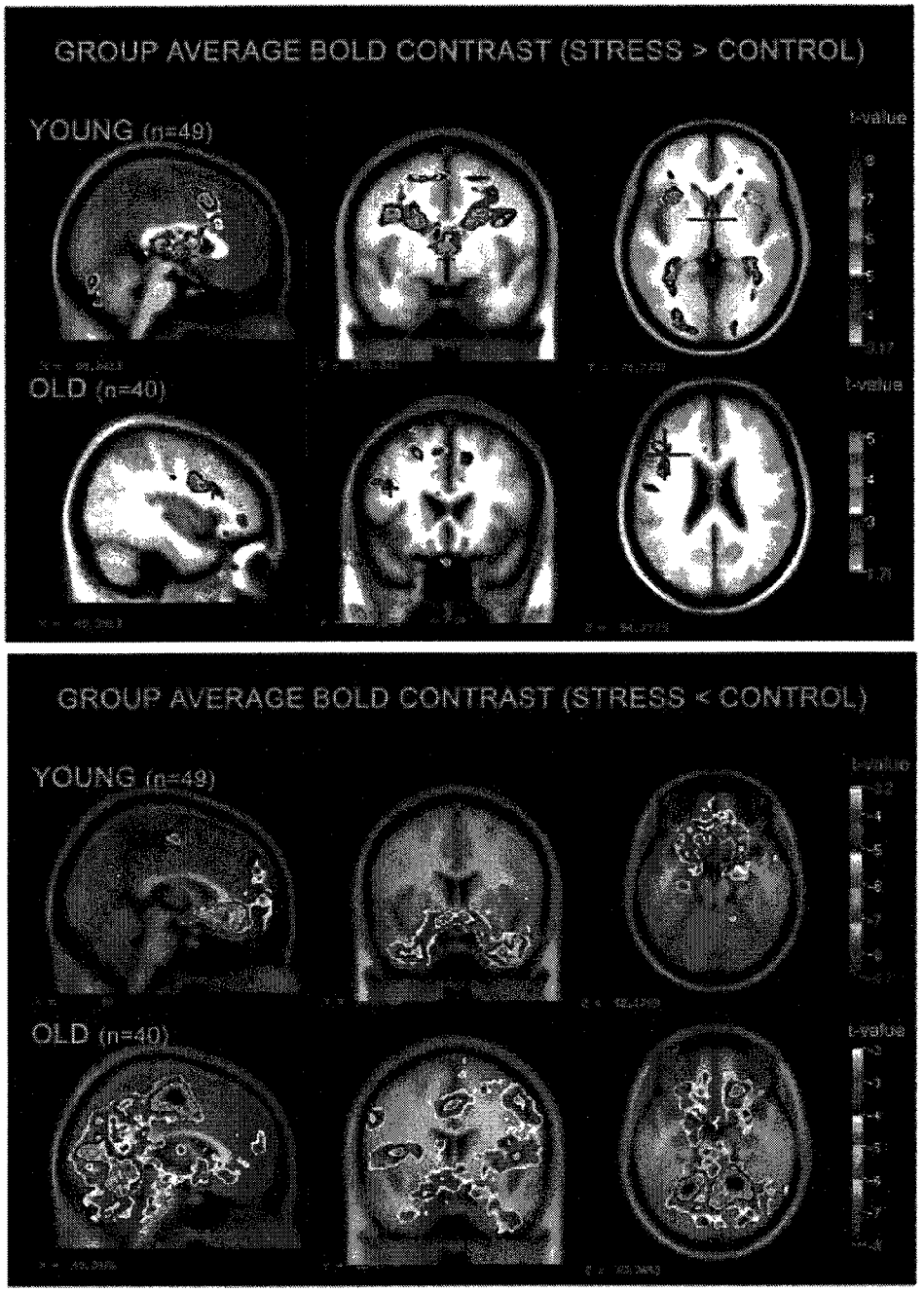

Figure 3: Group average activation maps Stress (arithmetic plus social evaluative threat) versus Control (arithmetic). Activation (top panel) and deactivation (bottom panel) t-maps are set at cluster threshold p<.001 (young) and p<.01 (old), corrected. Both groups deactivate the medial prefrontal areas; but the medial prefrontal deactivation in the young subjects is stronger in magnitude and broader in extent.

Table 2: Local minima and maxima within the clusters that are significantly activated or deactivated (cluster p<.001, corrected) in the young group.

Activation (Stress > Control)							
Structure	BA	Hemisphere	X	Y	Z	t-value	Cluster volume ml^3
Caudate		R	32	-40	4	7.5	1.821
Thalamus		L	0	-4	5	8.4	1.137
-		R	1	-22	9	6	0.25
Fusiform Gyrus	37	L	-48	-58	-14	7.3	1.306
Insula	13	L	-33	18	2	7.9	1.093
Precuneus	7	L	-24	-67	39	6.3	1.02
-	-	R	19	-56	51	7.4	0.75
Precentral Gyrus	4	L	-46	-15	39	6.4	0.635
Angular Gyrus	39	R	31	-64	33	6	0.176
Medial Frontal Gyrus	6	L	-18	2	57	6.4	0.228
Deactivation (Stress < Control)							
Structure	BA	Hemisphere	X	Y	Z	t-value	Cluster volume ml^3
Anterior Cingulate	24	L	-6	28	-4	-8.7	21.585
Superior Temporal Gyrus	38	R	40	13	-35	-7.0	3.522
-	-	L	-30	6	-38	-6.2	0.807
Parietal Lobule	40	L	-49	-33	56	-6.7	1.257
-	-	R	60	-23	28	-6.3	0.282
Angular Gyrus	39	L	-52	-75	33	-6.5	0.635

Table 3: Local minima and maxima within the clusters that are significantly activated or deactivated (cluster p<.01, corrected) in the old group.

Activation (Stress > Control)							
	BA	Hemisphere	x	y	z	t-value	Cluster volume ml^3
Middle Occipital area	18	L	-18	-100	1	5.1	1.102
Inferior Frontal Gyrus	9, 46	L	-43	3	30	4.2	0.792
Middle Temporal Gyrus	21	R	70	4	-9	3.8	0.201
Deactivation (Stress < Control)							
	BA	Hemisphere	X	Y	Z	t-value	Cluster volume ml^3
Middle parietal area	40	L	-44	-31	47	-10.4	23.503
-	-	R	57	-48	35	-10.6	16.967
Middle Occipital Gyrus	19	L	-51	-71	6	-10	2.811
-	-	R	57	-68	-9	-6.0	0.119
Precentral Gyrus	4	R	41	-14	60	-9.1	1.027
Cingulate Gyrus	24	L	-5	-9	51	-8.7	8.18
Superior Temporal Gyrus	22	L	-62	-56	14	-6.2	0.206
Insula	13	L	-41	-3	14	-7.3	0.362
Cuneus	30	L	-9	-71	5	-7.7	0.352
Putamen		L	-30	-6	8	-6.3	0.391
Thalamus		L	-16	-23	3	-7.0	0.452

Correlation of brain activation with cortisol

Correlation of brain activation with AUC_{total}

Figure 4 and Table 4 summarize the BOLD and AUC_{total} correlations within the ROIs (including insular and prefrontal cortices, as well as the medial and superior temporal regions) previously reported in association with the HPA response to stress (Liberzon et al., 2007; Pruessner et al., 2008a; Wang et al., 2003; Wang et al., 2005).

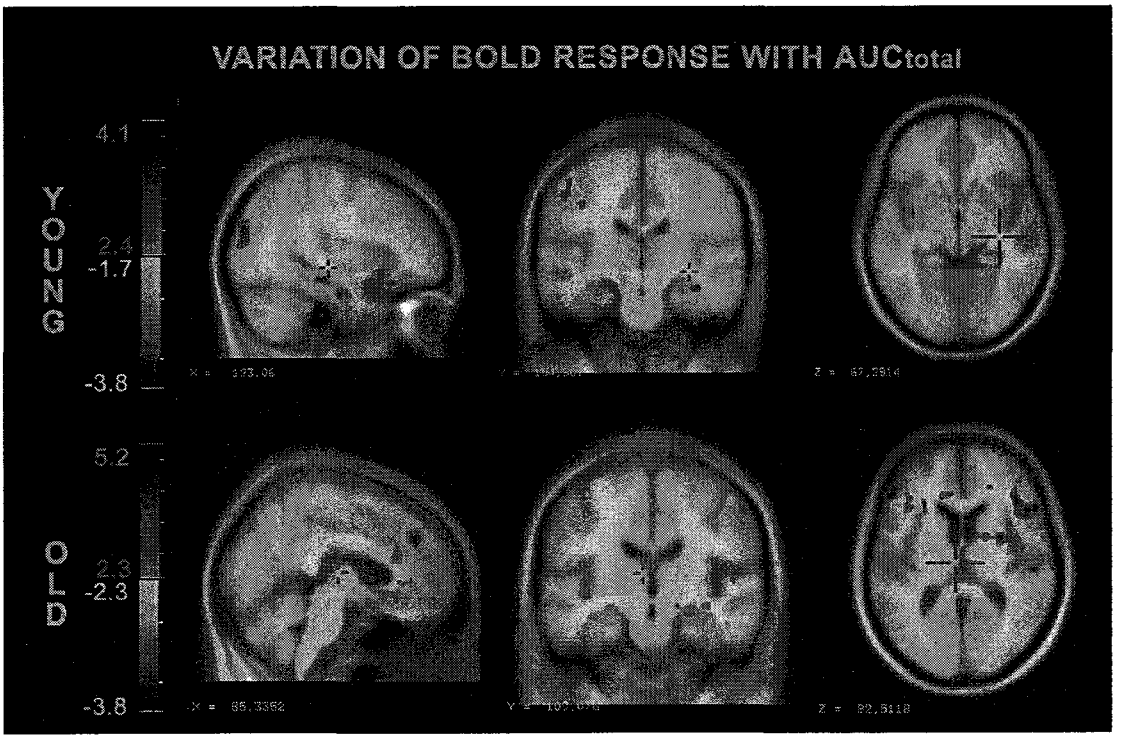

Figure 4: t-statistic maps of voxel-wise regression analysis of BOLD response to stress in relation to total cortisol present during the MIST. Positive (red) and negative (green) correlations are presented at cluster thresholds of at least 100 contiguous voxels at statistic significance of $p<.01$ (young and old).

Table 4: Location, cluster size, and cluster peaks of brain areas where the AUCtotal modulated the BOLD response.

		YONG N=49								OLD N=40					
		BA	Voxels	X	y	z	t			BA	Voxels	x	y	Z	t
Frontal															
L	ACC	32	921***	-13	39	-3	-3.6	L	ACC	24	573**	-12	13	24	3.8
L	DmPF	8	235**	-12	37	40	4.1	L	ACC	24	1080***	-6	26	22	3.4
								L	ACC	32	317*	-14	35	9	3.2
								R	ACC	32	315*	21	40	12	3.2
								L	DmPF	6	3023**	-7	41	35	4.5
								R	DmPF	6	2736**	13	12	59	3.7
R	OFC	11	211**	5	49	-28	3.1	R	LPFC	44	13806** *	58	9	18	4.7
R	OFC	47	149*	34	27	-16	3.6	L	LPFC	46	12037** *	-54	34	6	5.2
								L	Ins	13	1467***	-45	-28	19	3.9
Pareital															
R	Prec	31	248**	19	-68	21	3.4	R	Precu	7	215*	27	-65	28	-3
R	PCC	23	142*	3	-62	15	3	R	iPL	40	229*	53	-59	44	2.7
L	IPL	7	116*	-26	-55	62	-2.9	L	PCC	30	994***	-30	-71	7	-4.1
								L	PCC	23	109*	-10	-34	20	-3.4
Temporal															
R	ParH	36	1119***	37	-25	-16	4	R	Uncu	20	228**	21	-5	-40	-3.4
R	Uncu	38	249**	21	0	-41	3.5	R	sTMP	42	1156***	70	-21	9	4.7
R	Hipp		77*	29	-39	5	-2.8	L	Angul	39	358*	-48	-59	26	3.6
L	Uncu	38	1881** *	-46	20	-25	4	R	Oper	22	340*	56	6	0	3
Subcortical															
R	Puta		106*	29	-10	4	2.7	R	Puta		234*	26	5	2	3.4
R	Caud		106*	18	-27	29	-3	R	Caud		270*	12	12	2	3.5
								R	Thal		261*	13	-23	21	3.4
								L	Thal		233*	-8	-7	19	3.4

*** p<.05; ** p<.005; *** p<.0001;** Corrected according to random field theory (Worsley et al, 2005)
ACC, anterior cingulate cortex; **Angul,** angular gyrus; **Caud**, caudate; **dmPF,** dorsomedial prefrontal cortex; **Hipp**, Hippocampus; **Ins,** insula; **iPL,** Inferior parietal lobule; **LPFC,** lateral prefrontal cortex; **OFC,** orbitofrontal cortex; **Oper,** operculum; **parH,** parahippocampal area; **PCC,** posterior cingulate cortex; **Precu,** precuneus; **Puta**, putamen; **sTMP,** superior temporal gyrus; **tempP,** temporal pole; **Thal;** thalamus; **Unc,** uncus

In the Young, significant correlations between cortisol concentration (AUC_{total}) and the BOLD response were present in the temporal lobe (BAs 20, 36, 38) and medial occipital (BA 17, 18, 19) regions. Small clusters of significant contiguous correlations were present in the dorsal (BA 10) and orbital (BAs 11, 47) frontal regions, as well as in the right putamen. In the Young, the BOLD signal in the medial frontal (BA 10, 11), anterior cingulate (B32) and the hippocampus was negatively correlated with AUC_{total}.

By contrast, the Old had large clusters of positive correlation between AUC_{total} and the BOLD in the lateral prefrontal (BA 44, 46), the medial prefrontal (BAs

6, 8, 9, 10), the anterior cingulate (BAs 32, 24), the Insular (BA 13) and superior temporal (BAs 39, 22) cortices; as well as in the nuclei putamen, caudate and thalamus. Negative correlation between AUC_{total} and the BOLD in the Old was present in the inferior temporal (BA 20) as well as precuneus (BA 7).

Correlation of brain activation with $AUC_{increase}$

Figure 5 shows the ROIs that were significantly correlated with $AUC_{increase}$ Table 5 summarizes the most significant clusters of BOLD and cortisol correlation within the ROI.

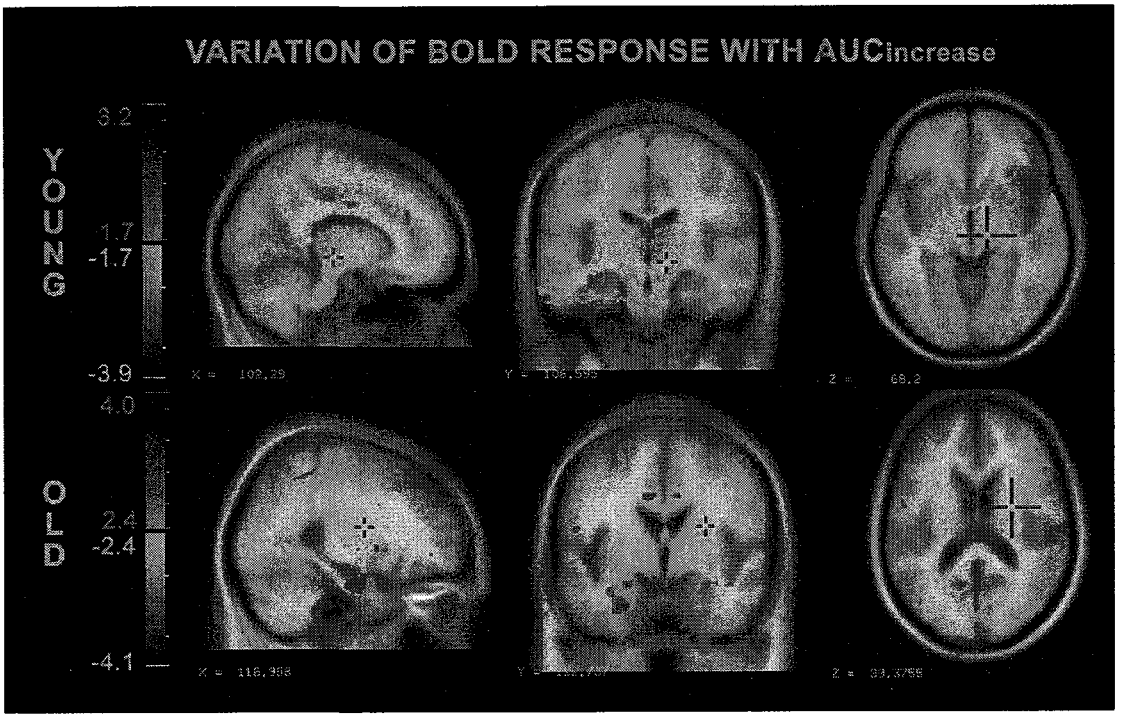

Figure 5: t-statistic maps of voxel-wise regression analysis of BOLD response to stress in relation to cortisol increase after MIST. Positive (red) and negative (green) correlations are presented at cluster thresholds of at least 100 contiguous voxels at peak significance of $p<.05$ (young) and $p<.01$ (old), uncorrected.

Table 5: Location, cluster size, and cluster peaks of brain areas where the AUCincrease modulated the BOLD response

		YONG N=49							OLD N=40					
		BA	voxels	X	Y	Z	t		BA	voxels	X	Y	Z	T
Frontal														
R	ACC	24	288**	15	-5	38	-4	R ACC	24	453**	9	1	37	3.8
R	OFC	47	309***	29	27	-4	-3	R ACC	24	446**	14	-7	33	3.6
								L ACC	24	403*	-13	1	37	3.3
								L ACC	24	216*	-10	8	30	3.2
								R Ins	13	493**	45	-8	-1	2.9
								R LPFC	45	235*	55	25	13	3.2
								R OFC	11	504**	2	46	-24	3.3
Pareital														
L	IPL	40	295**	-54	-32	38	-3							
L	IPL	40	378**	-37	-46	46	-3							
Temporal														
L	sTMP	22	132*	-63	-4	3	-3	R parH	35	2463***	24	-14	-23	3.5
R	sTMP	22	210**	64	3	-2	-3	L Unc	20	1064***	-27	-6	-36	3.8
								L tempP	38	309*	-27	17	-35	2.9
								L Oper	22	1898***	-47	-8	4	3.7

*** p<.05; ** p<.005; *** p<.0001;** Corrected according to random field theory (Worsley et al, 2005)
ACC, anterior cingulate cortex; **Ins,** insula; **iPL,** Inferior parietal lobule; **LPFC,** lateral prefrontal cortex; **OFC,** orbitofrontal cortex; **Oper,** operculum; **parH,** parahippocampal area; **sTMP,** superior temporal gyrus; **tempP,** temporal pole; **Unc,** uncus

In the Young, a small cluster in the medial frontal area (BA 9) was positively correlated with the $AUC_{increase}$. However, negative correlations were present in the ventromedial (BAs 11, 24), orbitofrontal (BA47), medial occipital (BAs 18, 19) precuneal (BAs 40, 7) and the fusiform areas (BA 20).

By contrast, the Old had large clusters of positive correlation between the BOLD and the $AUC_{increase}$ in the medial temporal (hippocampus, uncus), superior temporal (BAs 22, 38), anterior cingulate (BA 24) and medial frontal (BAs 6, 9, 10, 11) areas. The negative correlations were in the medial occipital (BAs 18, 19) and posterior cingulate region (BAs 23, 30).

Posthoc analysis

Conjunction analysis of correlation maps to identify similar correlations in the Old and the Young

Because the morphological differences in the two age groups were not resolved by an affine transformation, also because the plane of image acquisition was set

differently in each group, we could not pool the imaging data to look at co-variation of the cortisol measures across the whole population (n=89). Instead, we looked at the conjunction of voxel-wise correlation maps (set at threshold of $p<0.05$, uncorrected) to identify collinear peaks. For each age group, and for each cortisol variable (AUC_{total} and $AUC_{increase}$), the correlation maps described above were overlaid. Within the overlapping clusters for each age group, we identified the highest peak. In this way, we identified a peak in the right orbitofrontal cortex (OFC, BA11, Young: t-peak [12, 45, -16] = -1.8, $p<.05$, uncorrected; and Old: t-peak [2, 47, -24] = -1.7, $p<.05$, uncorrected) that explained over 11% of variations in AUC_{total} ($r=-.34$, $p<.001$, $N=89$). Similarly, we identified right rostral anterior cingulate cortex (rACC, BA32, Young: t-peak [16, 53, -2] = -2.35, $p<.05$, uncorrected ; Old: t-peak [13, 58, -7]= -2.57, $p<.05$, uncorrected) that explained over 13% of variations in the AUC of increased cortisol($r=-.36$, $p<.001$, $N=89$). The BOLD signals in these peaks were not correlated ($p>.4$); therefore despite proximity of the regions, we considered them separately.

Predicting interindividual differences in HPA axis response from the heterogeneity of the mPFC activity

We observed a significant degree of heterogeneity in the BOLD signal of the rACC and OFC peaks obtained above. As Figure 6 illustrates, the rACC and OFC were either activated or deactivated. Several studies have shown that interchanging patterns of activation and deactivation within the medial prefrontal cortex represent an adaptive interplay between cognitive and emotional components of tasks (for example see Bush et al., 2000; Drevets and Raichle, 1998; Gusnard et al., 2001; Walton et al., 2004). Therefore, we hypothesized that by examining different patterns of activation within the OFC and rACC region, we might be able to identify interindividual differences in the

time-course of adaptive HPA axis responses, not only during stress, but perhaps also before and after stress.

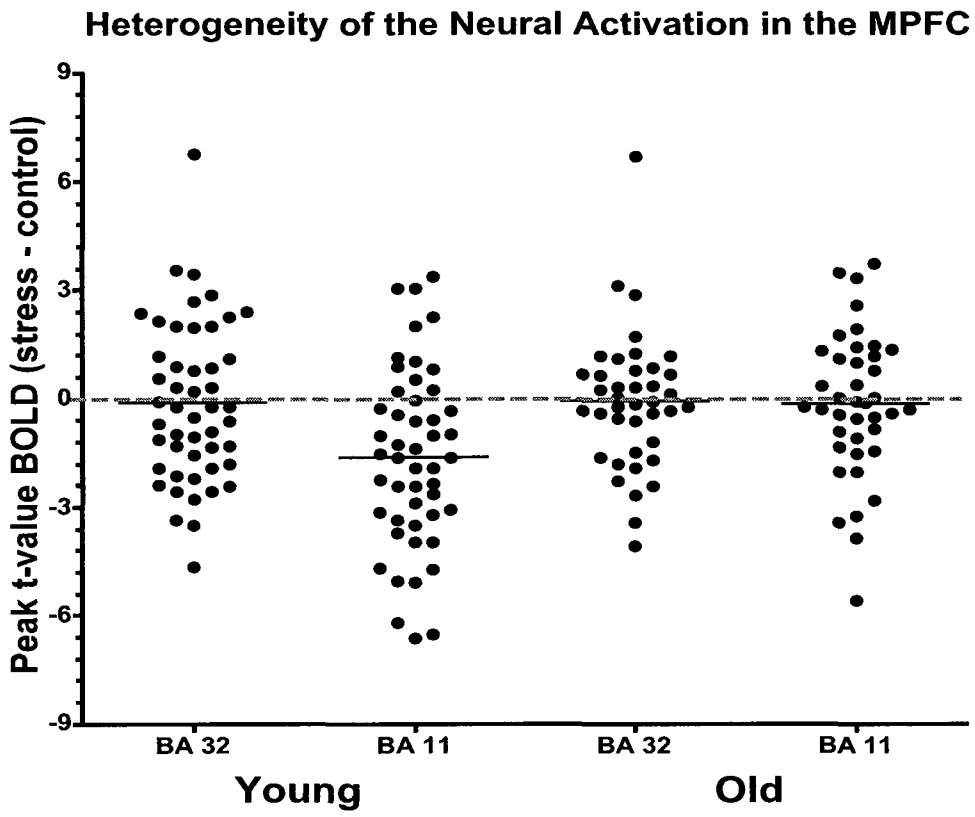

Figure 6: Peak-value (averaged over a spherical radius of 1-mm) of the BOLD response extracted in the right rostral ACC (young:[16, 53, -2]; old:[13, 58, -7]) where higher $AUC_{increase}$ is associated with lower BOLD response (r=-.36, p<.001, N=89); and in right OFC (young: [12, 45, -16] and Old: [2, 47, -24]) where higher AUC_{total} is associated with lower BOLD response (r=-.34, p<.001, N=89).

We identified four groups as summarized in Table 6. A one-way ANOVA ensured that means of activations in groups were significantly different (Old: F's(3, 39) >11, p's<.000l; Young: Fs(3, 48)>18, p's<.0001).

Table 6: Statistic summary of the peak BOLD response and Cortisol for groups classified based on rACC and OFC activation

	Group	N		BOLD (Mean ±Sd)		AUC (t=0-30 mins)	
				OFC	ACC	Total	Increase
Young	1	15	-/+	-2.72±2.01	1.87±1.68	149.0±93.4	7.2±34.4
	2	6	+/+	1.64±1.24	2.11±1.06	132.0±124.1	-17.5±15.3
	3	22	-/-	-2.61±1.71	-1.74±1.17	213.5±102.7	43.7±62.9
	4	6	+/-	1.47±1.16	-1.31±.89	185.7±91.2	0.3±38.6
Old	1	11	-/+	-1.74±1.80	1.43±1.91	137.2 ± 87.2	-19.2±19.3
	2	8	+/+	1.52±1.01	1.06±.97	82.0± 33.3	3.4±12.8
	3	11	-/-	-1.25±1.05	1.43±1.07	169.6±111.7	23.1±59.6
	4	10	+/-	1.51±1.26	-1.21±1.30	116.9±49.4	8.8±26.9

Stereotaxic location of peaks: ACC (young:[16, 53, -2]; old:[13, 58, -7]) ; OFC (young: [12, 45, -16] and Old: [2, 47, -24])

We performed a two way mixed design ANOVA (group x time) with activation group as a between subject and nine cortisol measures as a within subject factor. Figure 7 illustrates the group differences in cortisol profile. In both age groups the group by time interaction approached statistical significance (Young: $F(24,360)=1.52$ $p=.059$; Old: $F(24,280)=1.6$ $p=.054$). Given the exploratory nature of this post-hoc analysis we considered that a less than 6% rate of type one error to be significant enough to warrant decomposition of main effects and Tukey's post-hoc analysis.

In the young group, cortisol levels before (t= -50, -40 min) and after (t=+20, +40 min) the onset of MIST were different among groups (F's (3,405) > 2.93, p's<.04). Tukey's post hoc test showed that baseline levels of cortisol (t=-50) were significantly lower in Group 2 (OFC/ACC +/+) compared to Group 4 (OFC/ACC +/-). In the Old, cortisol levels measured at the onset of and after MIST (t=0, +10 and +20, +40 min) were different among groups (F's(3,315) > 2.7, p's<.05). In both age groups, Tukey's post-hoc test indicated that Group 3 (OFC/ACC -/-) had a significantly higher cortisol after MIST, compared to Group 2 (OFC/ACC +/+).

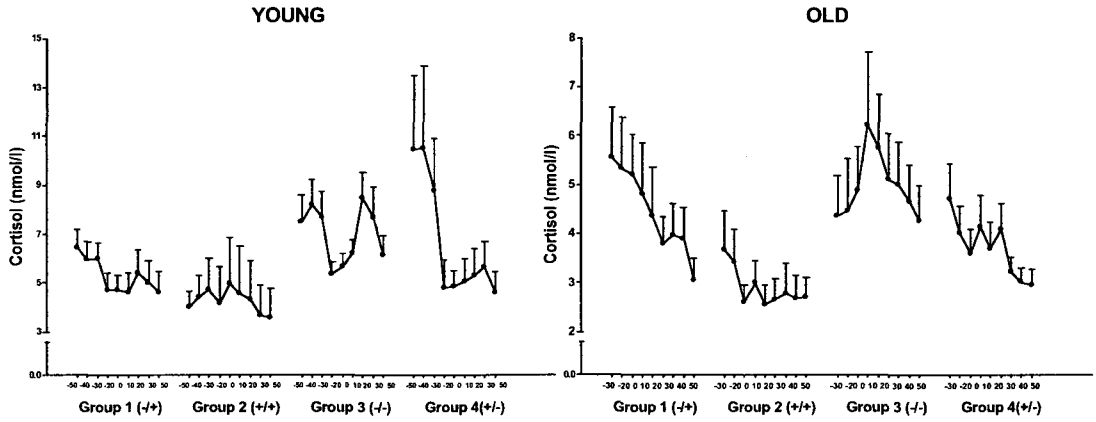

Figure 7: Group differences in cortisol profile. Group 3 (45% of the young and 27.5% of the old) deactivating both rACC and OFC) show a significant cortisol stress response compared to Group 2 (12% of the young and 20% of the old, activating both rACC and OFC). In Group 4 (12% of the young and 25% of the old) pre-stress cortisol levels has a significant effect on the time-course of the cortisol response.

The simple main effect of time in the Young was significant in groups 1 (OFC/ACC -/+; $F(8,360)=2.24$, $p<.03$), 3 (OFC/ACC -/-; $F(8,360)=2.34$, $p<.02$) and 4 (OFC/ACC +/-; $F(8,360)=9.9$ $p<.0001$). With the exception of Group 2 (OFC/ACC -/-), baseline (t=-50) cortisol level was significantly higher compared to the time of MIST onset (t=0). By contrast, in the Old, the effect of times was significant only in Group 1 ($F(8,280)=3.12$, $p<.003$)) who (similarly to the young Group 1) had significantly higher cortisol levels prior to MIST (t=-30, -20) compared to the end point (t=+60).

Discussion

We performed the MIST in a large group of young and older healthy participants. Unlike in the young subjects, the MIST did not result in a significant stress response in the old participants. Nevertheless, cortisol variables (AUC_{total} and $AUC_{increase}$) modulated the Blood Oxygen Level

Dependent (BOLD) response in the medial prefrontal, insular, precuneal and medial temporal regions. Particularly, the BOLD response in the right rostral ACC and right OFC characterized a trend for interindividual differences in HPA axis responses to experimental conditions.

Differences in neural and endocrine response to the MIST in the Old and the Young

The observation of different patterns of neural response to MIST in the older subjects underlines several design factors that should be addressed in future experiments. To get stressed, one would have to anticipate novelty or an uncertain negative outcome (Mason, 1968); to perceive a social evaluative threat in terms of other's judgment; or to experience lack of control to achieve a goal (Dickerson and Kemeny, 2004). Of course, coping strategies would play an important role in modulation of stress response (Lazarus, 1993). Recent studies are emphasizing that the motivation to achieve a goal is the main determinant of whether stressors such as social evaluative threat or uncontrollability induce a cortisol stress response (Dickerson and Kemeny, 2004; Tops et al., 2006). Here, we have presented the MIST to two groups of healthy men and women (in the average age categories of 23 and 65). Although the MIST was presented in two almost identical block-design protocols, each group demonstrated vastly different patterns of brain activity. Several experimental details could explain this variance. One is the pre-stress experience. The Young arrived at the hospital approximately 50 minutes prior to the MIST. In that period, they filled out some questionnaires and performed an autobiographic memory test (ATM). During this time, they had high levels of cortisol, which sharply declined after they were put in the scanner, and sharply increased after the second run of the MIST (See supplementary Figure S1-1). In contrast, the older subjects arrived at the hospital about 20 minutes prior to the MIST. In the 10-minute period

between arrival and onset of anatomical scanning, they were only given instructions about the MIST. For many of these older subjects this was their first exposure to an MRI scanner. However, this was not their first exposure to experimental stress, as they had been administered the Trier Social Stress Task a few weeks prior to the MIST. The overall cortisol pattern in this group was highly variable at each time point, and flat on average compared with the Young. The absence of time effect on the cortisol profile of the old subjects might be interpreted as lack of cortisol stress response, either because their HPA axis activity was at its highest peak throughout the experiment, or because they did not perceive the MIST as stressful as the Young did.

It is highly plausible that the Old perceived the stressful stimuli differently than the Young did. Motivational factors might explain this difference. In this case, the older subjects were recruited to participate in a "Memory and Aging" study. Presence of the word "distractor" (random words appearing on the MIST interface) might have diverted their "motivation" and "attention" from performing arithmetic (which many found too hard to care about) to memorizing random words (which they thought they would be tested on; and which was perhaps more relevant to their motivation for participating in the study). It is also likely that the Old experienced a different kind of stress. For instance, the novelty of the MRI scanner environment, or uneasiness with manipulating the response buttons (a task that is relatively easy for young adults), could have been more stressful than the psychosocial evaluative stressor of the MIST. Having experienced the experimental social evaluative stress of the (Trier Social Stress Task) TSST, the Old had become familiar with the "dramatization" of the social evaluative threat. Hence, it is plausible that they did not perceive the same level of threat to ego while undergoing the

experimental condition of the MIST.

In fact, our neuroimaging results offer clues that the Old and the Young engaged in different coping behavior. Our data show that the Young increased activation of the preemptor, thalamus and basal ganglia; and concurrently decreased activation of the ventromedial frontal and medial temporal regions—perhaps due to increased task demands (McKiernan et al., 2003; Menon et al., 2000). Conversely, the Old did not show increased activity in the motor area, rather in the lateral and medial prefrontal areas—perhaps reflecting an age-related increase in recruitment of the frontal attentional network (Solbakk et al., 2008). Furthermore, the Old deactivated the medial posterior parietal and cerebellar regions, which are important for motor coordination during visual attention and hand movement (Ramnani et al., 2001) while performing the MIST. Here, the stress condition of the MIST asked subjects to perform up to a minimum standard (presumably that of their peer's average) under examiner's watch (threatening ego) and time pressure (uncontrollable). It seems that whereas the Young tried harder, the Old gave up trying as the task became more and more uncontrollable. As mentioned before, it is plausible that different motivations led them to adopt very different coping strategies (i.e. instead of "stressing" over performing a difficult cognitive task requiring motor skills, they chose the memory encoding—as perhaps a "good memory" was more relevant to their goal of "self' preservation in later years of life. A subjective rating of the pre- and peri-stress experience in these individuals would have been essential to untangle the discrepancies observed between the age groups' responses. At present, our inferences are made speculatively and based on the evident patterns of brain activation and the pattern of cortisol response. However, these observations underline the highly subjective nature of

the MIST (or any stress paradigm) that depends on myriad factors, from the subject's motivations or mental states, to his or her interactions with the examiners and the experimental environment.

Interindividual variations in cortisol modulation of the default mode network

An important finding of this study is to observe a modulatory effect of cortisol on the BOLD response in areas like the medial prefrontal and medial temporal regions (figures 4 &5; and tables 4 & 5). Notwithstanding the differences in the direction of correlations (which might depend on group differences in pre-stress experimental conditions (Chapters 2 and 3), the importance of this observation rests in providing the largest *in vivo* human evidence for an association between different aspects of the HPA axis function (i.e. total amount of free cortisol; and dynamic of cortisol increase with respect to an experimental condition) and neural activity in brain regions like medial prefrontal area and the hippocampus. These areas have a large concentration of glucocorticoid receptors (Diorio et al., 1993; McEwen et al., 1968), and play an important role in regulation of the HPA axis responses to psychological stress (Herman et al., 2003; Herman et al., 2005). Our analysis also show areas outside the traditional ROIs of stress research, regions such as the precuneus (involved in self-referential thought (Cavanna and Trimble, 2006)) and insula (involved in visceral awareness (Critchley et al., 2004)) that can modulate the perceptual processing of stress, and might be important for HPA axis regulation.

Brain areas such as the medial prefrontal cortex and the precuneus are omnipresent parts of the default mode network (DMN), characterized by high metabolic rates during resting conditions (Raichle et al., 2001) and consistent deactivation in goal-directed cognitive functions. The resting state activation of

the DMN presumably reflects the states of consciousness and monitoring of the internal and external environment for signs of change. There function is thus to initiate optimized adaptive responses that ensure the stability of the organism (physiological and behavioral) in presence of internal and external signals (Raichle et al., 2001). Precisely, this function has long been attributed to the HPA axis (Chrousos, 1998). To observe a correlation between cortisol—a biomarker for adaptive homeostatic control (Sapolsky et al., 2000)—and different parts of the DMN adds evidence to growing body of literature that seek the 'intrinsic' determinants of motivation and behavior (Fox et al., 2007; Raichle and Gusnard, 2005) or cognitive reserves (Stern et al., 2005) in the DMN fluctuations. In this sense, the significance of this finding goes beyond determining the neural correlates of stress *per se*, as it offers another objective measure (cortisol) for examining the interindividual differences of adaptive behavior in humans.

Implications of findings in aging studies

An example of utility of using cortisol and neural activity together, as a biomarker for interindividual differences, is in studies that seek the behavioral and physiological substrates of 'successful aging' (Rowe and Kahn, 1997). Traditionally, (and based on animal models,) the aging of brain structures such as hippocampus has been considered a causal factor in HPA axis dysregulation (Sapolsky et al., 1986) as aging would reduce the efficiency of the neural control of the HPA axis and subsequently lead to dysregulation of the neuroendocrine system. The HPA axis dysregulation would create a cascade of further brain damage (especially in the hippocampus) due to glucocorticoid toxicity, thus exacerbating the aging symptoms (McEwen and Magarinos, 1997). However, the glucocorticoid cascade hypothesis of aging is increasingly questioned (Lupien et al., 2007), and laboratory examinations of the HPA axis response to

stress in older humans reveal a great degree of heterogeneity in age-related reduction of the HPA axis agility in stress regulation (Kudielka et al., 2000; Seeman and Robbins, 1994). Age-related cognitive decline (in hippocampal and prefrontal memory functions) in relation to HPA axis function is also largely heterogeneous (Lupien et al., 2005a; Lupien et al., 2005b; Pruessner et al., 2004c). As evidence grows that aging does not affect all in the same way (for a recent review see Grady, 2008), researchers become increasingly interested in uncovering the factors that modulate the aging process.

Here, we are reporting a prominent correlation between cortisol and the hemodynamic responses in the hippocampal regions and the medial prefrontal area (Figure 4) that are widely studied in aging. To our knowledge, this is the first time that a modulatory effect of endogenous cortisol on prefrontal and hippocampal activity is reported in healthy older adults. If cortisol explains the heterogeneity of the neural activity in regions that are involved in cognitive processing, then it is plausible to include it as a control variable while studying age-related differences in cognitive performance.

It has to be noted that we observe a positive correlation between the ventromedial part of the hippocampus (and perhaps basolateral amygdala) and the $AUC_{increase}$ in the older group. This is against the common view that the hippocampus exerts and inhibitory influence on the HPA axis activity. However, recent evidence suggest that the ventral subiculum is particularly important for integrations of the HPA axis stress response, exerting inhibitory or excitatory influence depending on the circumstance or interindividual differences in presence of a given stressor (Herman and Mueller, 2006). In fact, we have recently shown that states of hippocampal activity prior to stress might predict

the later neuroendocrine response to stress (Khalili-Mahani et al., 2009). Irrespective of such considerations, however, our findings offer incentive to consider the profile of HPA axis function while examining the interindividual differences in emotional and cognitive tasks that depend on these limbic structures.

A region of interest to predict interindividual differences in stress sensitivity

We performed an exploratory analysis on the correlation maps of the Young and the Old to find that the activity of the right rACC and OFC is similarly modulated by cortisol in both age groups. Notwithstanding the small effect sizes observed in these locations, it is highly interesting that a data-driven analysis would reveal a location similar to the area we previously detected in characterization of stress responders and non-responders.[5] Here too, a significant amount of heterogeneity in the BOLD response (Figure 5) was present, allowing us to split the sample into four groups (Table 4). This methodology yielded a similar result to our earlier observation: in both age groups deactivation of both OFC and rACC was associated with a sharp increase in cortisol response after the MIST (Figure 6), consistent with our hypothesis that medial prefrontal deactivations marked a stress response (Pruessner et al., 2008a). The right laterality of this observation is interesting as animal studies have shown that that stress-related prefrontal control of the HPA axis is mostly right dominant (Sullivan and Gratton, 2002). The asymmetric nature of neural stress response deserves further examination in relation to behavioral variables, especially because the rACC seems to be particularly important in regulation of anxious states (e.g. Taylor et al., 2008 and Liberzon

[5] In Chapter 4, we showed a dichotomous patterns of rACC activity at stereotaxic locations [6, 53, -1] during the MIST and [6, 56, -3] after MIST.

et al., 2007).

Our results also underline the importance of considering the functional subdivisions of the prefrontal area in relation to HPA axis control. It is increasingly evident that differences in context or motivation can alter the ways in which the subregions of the medial prefrontal cortex interact to process and respond to cognitive or emotional stimuli (Hajcak and Foti, 2008; Rushworth et al., 2007). For instance, it has been shown that functional dissociation in different parts of the medial prefrontal cortex (namely OFC and ACC) is modulated by instantaneous adaptations according to on ongoing cognitive processes (Rushworth et al., 2007). Here, in almost 30% of subjects (Group 1 who concurrently deactivated the OFC and activated rACC), the cortisol levels were highest prior to stress induction. The interactions of the OFC and rACC might be specific to how these subregions coordinate the adaptive responses of the HPA axis to pre-stress states.

The significance of our observations rests in identifying the rACC—a region that is important for behavioral control of anxiety (Bishop et al., 2004)—and the OFC—a region involved in visceral regulation (Critchley, 2005)—from a data-driven analysis based on two objective measures (cortisol and neural activation). Our study lacks behavioral indices of the effect of interactions between the rACC and the OFC and HPA axis responses in each of the four groups. Nonetheless, our findings are in line with studies that have shown a link between the neural activity of these regions and cortisol (Liberzon et al., 2007; Wang et al., 2007; Wang et al., 2005). Our findings are also plausible in the context of theories that consider the ACC and OFC important for adaptive behavioral motivation (Hajcak and Foti, 2008; Rushworth et al., 2007) and

integration of emotional, physiological and cognitive stimuli (Bush et al., 2000; Critchley, 2005). These results may have implications for studies that aim to modulate the activity of these regions of interest (for instance by processes such as preparatory anticipation (Ursu et al., 2008), error monitoring (Hajcak et al., 2003), or reward processing (Taylor et al., 2006)) to test different models of stress regulation in humans.

Limitations and future work

The primary objective of this study was to examine the neural correlates of HPA axis response to a psychological stressor, with the hope to identify a common factor that could explain the interindividual differences in stress response objectively. For this reason, our report is omitting a perspective on sex differences that have been shown to interact with neural (Wang et al., 2007) and endocrine (Kirschbaum et al., 1993; Kudielka et al., 2004) stress responses. We have also not considered interactions of personality variables such as self-esteem and locus of control, previously shown to modulate cortisol stress response (Dandeneau et al., 2007; Pruessner et al., 2005). These topics deserve a comprehensive treatment, which will enhance the current understanding of the stress process. (See Supplementary Analysis 2 for preliminary data.)

Despite these limitations, this is the largest neuroimaging study (in terms of sample size and demographics) to date to provide human evidence for a link between medial prefrontal and medial temporal areas and the HPA axis function. To find correlations between the HPA axis and the DMN is highly relevant, considering the theoretical underpinning of the DMN. The HPA axis is an important system for coordinating adaptive response to physical and psychological stressors (Chrousos, 1998; Sapolsky et al., 2000). The DMN represents the intrinsic states of alertness and preparation for adaptive

responses to internal and external signals (Raichle and Gusnard, 2005; Raichle et al., 2001). Our study suggests that modeling these two systems together might help better characterize the physiological substrates of variations in behavioral adaptation. Such an approach might provide tremendous opportunities for diagnosis of individuals at higher risk for developing stress-related mental or health disorders.

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Supplementary Analysis 1: Role of Experimental Heterogeneity in the Observed Age-related Differences

The aim of this analysis was to provide an analysis method identical to the one used in Chapter 3; to uncover possible neural and endocrine differences caused by different experimental protocols.

Different age groups experienced a different sequence of experimental conditions, which reflected in different HPA axis activity profiles (Figure S1-1). Considering the entire sample, a repeated measure ANOVA revealed significant effect of time on cortisol profile in the young ($F(8,384)=6.9$, $p<.001$), but not in the old ($F(8,312)=0.7$, $p>.60$), suggesting that tasks induced a significant HPA axis response in the Young.

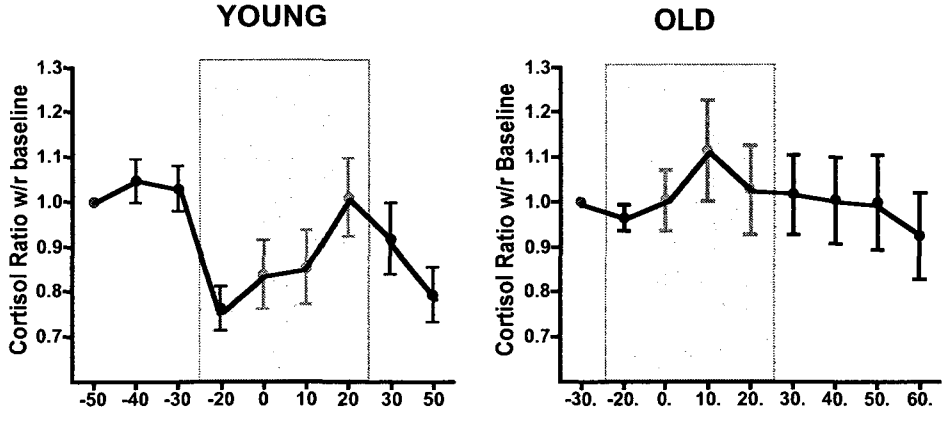

Figure S1-1: Cortisol profile during the entire course of experiment. Cortisol values are normalized to the first sample taken from each individual. The red error bars correspond to cortisol samples before MIST, after fist run and after second run. Whereas the cortisol stress response increased after the first run in the Young, it declined in the Old.

Because the pre- and post-MIST tasks induced a significant response in the Young, we used the $AUC_{increase}$ of cortisol in the period –20 to +20 after the onset of MIST to split groups to responders ($AUC_{increase}>0$) and non-responders ($AUC_{increase}<0$). Table S1-1 summarizes results. Figure S1-2 illustrates the cortisol profile for each group. A mixed design ANOVA revealed significant group x time interaction in the Young ($F(3,141)=48.5$, $P<.0001$) and in the Old ($F(3,114)=40.96$, $p<.0001$).

Table S1-1: Mean ± Sd of AUCincrease calculated between times –20 to +20 with respect to MIST onset

	YOUNG		OLD	
	N	$AUC_{increase}$	N	$AUC_{increase}$
Responder	23	27.5±4.36	18	29.7±8.3
Non-responder	26	-8.8±1.5	22	-16.7±3.2

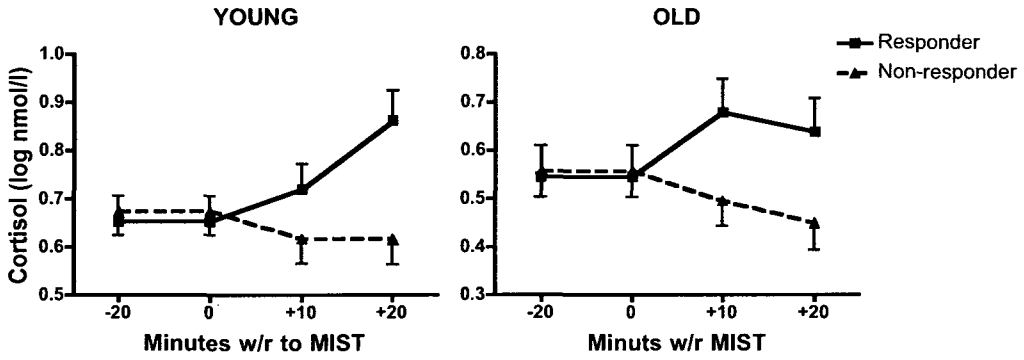

Figure S1-2: Stress responders and non-responders split based on $AUC_{increase}$(calculated between times -20:20 minutes).

Figure S1-3 illustrates the BOLD response in responders and nonresponders of each age group, showing group differences in the extent of medial prefrontal and precuneal deactivations. In order to better account for between-subject variance in HPA axis activity, in Chapter 4 we have adopted a voxel-wise

regression analysis with $AUC_{increase}$(representing between subject variation in the dynamics of HPA axis function within an individual) and AUC_{total} (representing between subjects variations in total amounts of free cortisol) as independent and the BOLD signal as dependent factor. This methodology enables us to predict BOLD variations in the DMN without presumptions about task-related HPA axis activity. The group comparisons illustrated in this supplementary analysis also confirm that stress responders and nonresponders exhibit significant differences in the patterns of DMN deactivation. Interestingly, these differences are also present in control vs. baseline BOLD contrast, which strengthens the hypothesis that stress responders and non-responders differ in baseline states of neural processing.

It is noteworthy that the patterns of brain activation in control minus baseline contrast (i.e. BOLD response to simple math) were similar. The significant group by age interactions were mostly in the extent and the topography of deactivations. Especially, activation clusters were not statistically significant—with the exception of the basal ganglia in young responders of the second study (data in Chapter 4)—whereas group averages illustrated significant deactivated clusters in the experimental versus control contrast. As will be discussed in Chapter 6, this observation calls for closer examination of the neurovascular response and metabolic rates in these corticosteroid-rich regions.

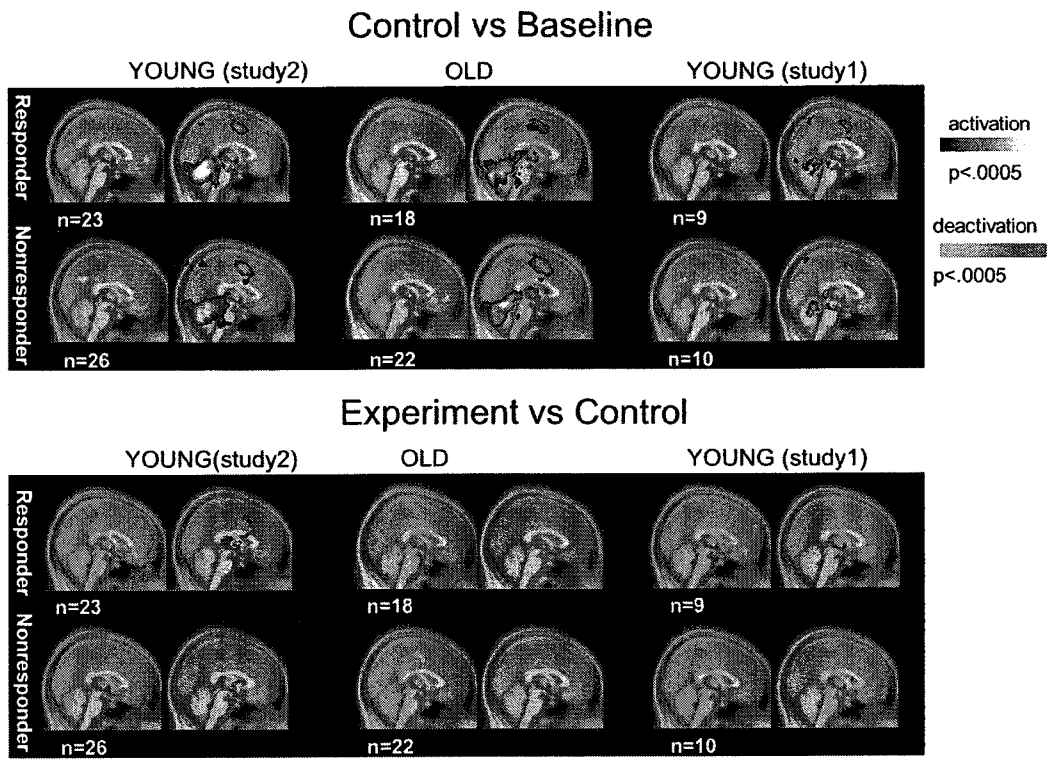

Figure S1-3: Responder and non-responder differences in activation and deactivation. t-maps are thresholded at peak p<.0005, uncorrected.

Supplementary Analysis 2: Behavioral Modulation of the BOLD Signal in Response to Stress

To further examine the role of behavioral factors in modulation of neural responses to the MIST, we considered the the locus of control measure (G. Krampen, Competence and Control Questionnaire, Göttingen, Hogrefe, 1991) that had been predictive of cortisol response in our previous studies (Pruessner et al., 2005; Pruessner et al., 1997a; Pruessner et al., 1999; Pruessner et al., 2004b). This measure includes subscales for self-esteem (general feeling of self worth, e.g. "I think I am creative"), internality (e.g., "I can determine many things that are happening in my life"), chance (e.g., "Many events in my life happen by chance"), and perception of other's control (e.g., "Other people often prevent the fulfillment of my plans"). We had complete QCC data in 40/41 old and 43/49 young subjects.

Voxel-wise regression analysis with QCC factors as independent and the BOLD response (experiment vs control) as dependent revealed significant positive correlation between 'self-esteem' and neural activity in the anterior cingulate cortex (Figure S2-1a). By contrast, a positive correlation between the 'perception of others control' and neural activity in the posterior cingulate and precuneal area was observed in the Old (Figure S2-1b). Whereas in the Old self-esteem and perception of other's control were negatively correlated ($r=-.51$, $p < .001$); this correlation in the Young was not present ($r=-.2$, $p > .2$).

Mixed design ANOVA with age as between and personality scores as within subject variable revealed a significant age effect ($F(1,79)=8.7$, $p<.005$). The old subjects had higher self-esteem and lower perception of other's control (Figure

S2-2).

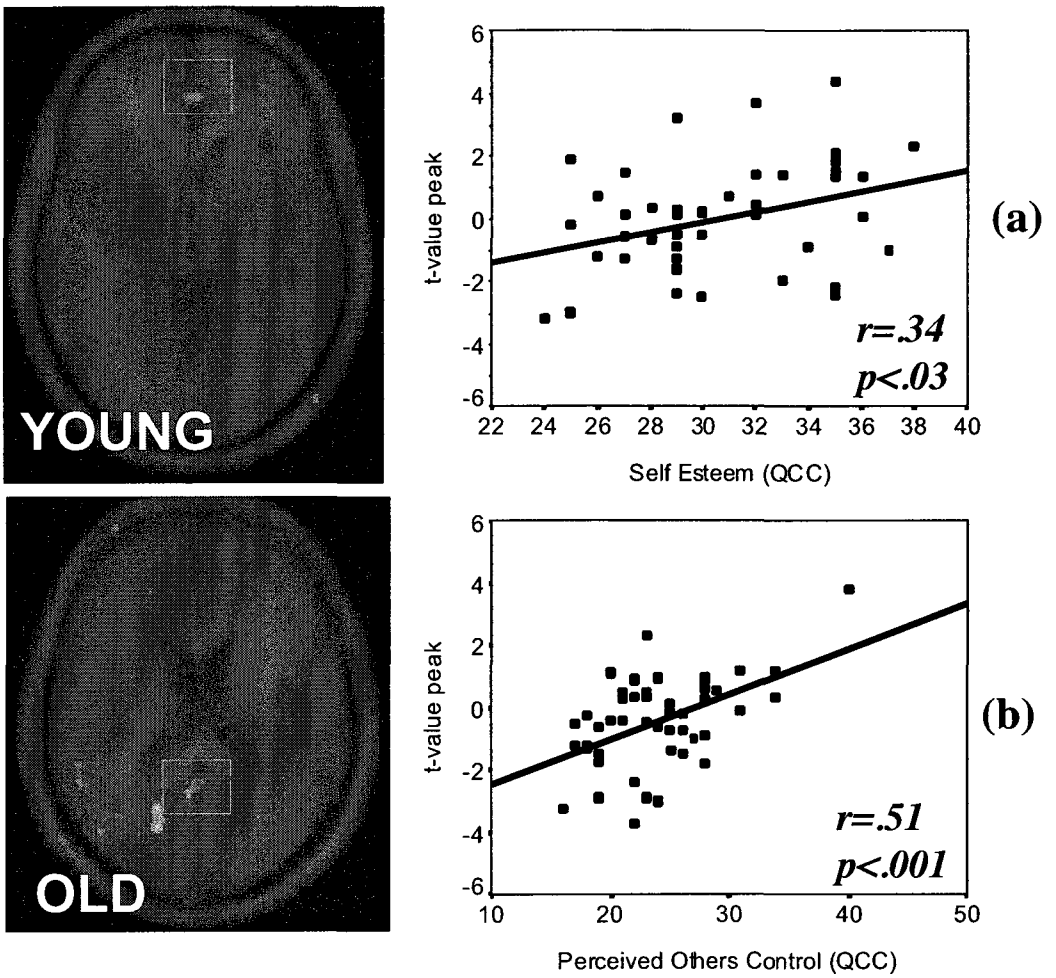

Figure S2-1: Voxel-wise regression analysis of BOLD variations with personality factors. (a) Self-esteem is positively correlated with the BOLD signal in the ACC (cluster size 675, p < .01 corrected); (b) Perceived other control is positively correlated with BOLD signal in the precuneus and posterior cingulate cortex (cluster size 1162, p < .01, corrected).

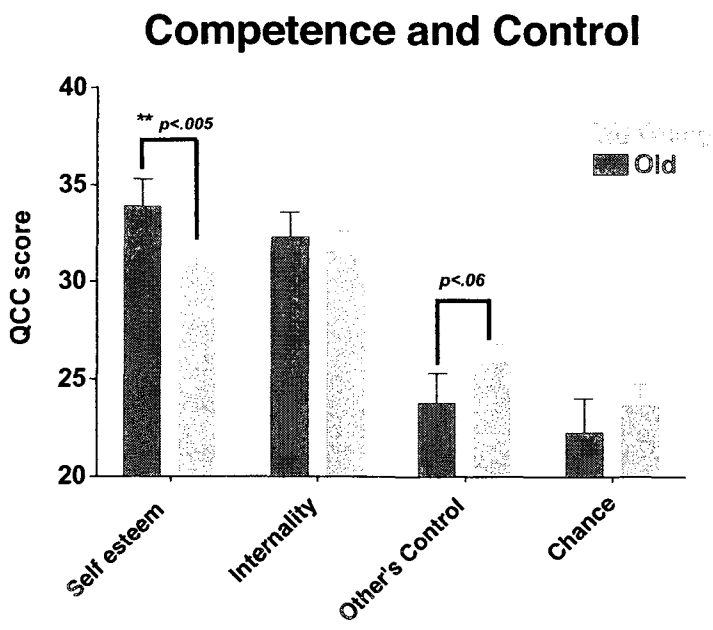

Figure S2-1: Age-group differences in personality variable.

These preliminary observations are significant because the personality factors that were significantly different between the two age groups predicted BOLD variations in regions of the DMN deactivated in response to the MIST's experimental condition. In the Young, we see a positive correlation between self-esteem and the BOLD response to stress. This is consistent with the observation in Figure S1-3 that the young stress responders deactivate the MPFC more than the young non-responders do. By contrast, in the Old, perception of other's control is associated with higher BOLD response in the precuneal region, which is also consistent with Figure S1-3. In the case of older subjects, the stress nonresponders deactivate the precuneal area, but responders do not. These observations indicate that brain areas known for intrinsic default mode activity relate to psychological predictors of stress (e.g. personality). This emphasizes that personality is a trait variable that might explain significant variations in neural correlates of stress.

CHAPTER 5

MORPHOLOGICAL CORRELATES OF THE HPA AXIS FUNCTION

Previous experiments in Chapters 4 and 5, plus other studies to have looked at neural correlates of cortisol response (Kern et al., 2008; Liberzon et al., 2007; Wang et al., 2007; Wang et al., 2005) or anxiety (Bishop et al., 2004; Taylor et al., 2008) confirm animal findings that show prominent involvement of mesolimbic and paralimbic systems in regulation of the HPA axis stress response. In this final chapter, we tested the hypothesis that interindividual variation in HPA axis regulation would also be associated with morphological co-variations in these areas. Our hypotheses is based on a wealth of literature showing that chronic exposure to glucocorticoids leads to reconfiguration of neuronal morphology, especially in the hippocampus (He et al., 2008; Joels et al., 2004; McEwen, 2007) and medial prefrontal areas (Cerqueira et al., 2005; Cook and Wellman, 2004; Radley et al., 2004; Wellman, 2001). To test this hypothesis, we have used the cortisol awakening response (CAR) a seemingly stable trait variable of the HPA axis regulation (Edwards et al., 2001; Hellhammer et al., 2007; Pruessner et al., 1997b) which is often studied to underpin the interindividual susceptibilities to stress-related maladaptation

(Huber et al., 2006; Pruessner et al., 2003b; Young et al., 2004) or interindividual heterogeneity in successful aging (Kudielka et al., 2000; Pruessner et al., 2005; Wright and Steptoe, 2005).

Manuscript 4: Awakening Cortisol Levels Predict Dissimilar Morphological Variations in the Anterior Cingulate Cortex and the Hippocampus

Authors: Najmeh Khalili-Mahani [1, 2], Claudia Buss[3], Veronika Engert [1,2], Jason Lerch[4], Claude Lepage[1], Alan C. Evans [1], Jens C. Pruessner [2]

1. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada
2. Douglas Mental Health University Institute, Verdun, Quebec, Canada
3. University of California, Irvine, CA, USA
4. University of Toronto, Toronto, Ontario, Canada

Corresponding author

Najmeh Khalili-Mahani

Pavilion FBC 3, Douglas Mental Health Institute

6875 LaSalle Boulevard

Verdun, QC, H4H 1R3, Canada

Tel. (514) 398 8330

Fax (514) 888 4099

Email najma@bic.mni.mcgill.ca

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Contribution of co-authors

This data is selected from the same study presented in Chapter 5. Please see contributions listed before. Additionally, Veronika Engert did the manual hippocampal segmentation used in this study. Jason Lerch assisted with cortical thickness statistical analysis and provided comment on an early version of the manuscript. Claude Lepage helped with improvement of the data processing pipeline. Oliver Lyttelton helped with painting the ROI. Najmeh Khalili conducted data processing, quality control, statistical analysis, manuscript preparation and interpretation of results.

Abstract

Animal studies have established that corticosteroids (cortisol in humans) resulting from the hypothalamus pituitary adrenal (HPA) axis activity affect the neuronal plasticity of the hippocampus and medial prefrontal cortex that have high receptor capacity for these hormones. This study aimed to examine morphological variations in the hippocampal (HC) volume and cortical thickness related to baseline HPA axis activity. We estimated baseline HPA axis activity from the cortisol awakening response (CAR) measured at times 0, +30 and +60 minutes after awakening. In 44 older healthy adults (age= 67± 5 years, 23 women) we showed significant within-subject reliability of the area under the curve of the CAR with respect to ground (AUC_G). The AUC_G predicted an increase in the HC volume in men only, after controlling for the effects of age. In women, the AUC_G and the HC volume were not correlated, but an age related reduction of the HC volume was observed. Vertex-based statistical analysis across the entire cortical mantle revealed a focal unilateral reduction of cortical thickness in the left pregenual anterior cingulate cortex (ACC). Variation in the ACC thickness was independent of age, sex and hippocampal volume. These findings corroborate the animal evidence that over-exposure to corticosteroids leads to left-lateralized reduction of dendritic processes in the ACC. However, effects of the CAR on the HC volume seem to be mediated by more complex models. Inferring from animal studies, we speculate that differences in neuronal plasticity of the HC and the ACC might be explained by differences in distribution of corticosteroid receptor subtypes in these areas.

Keywords: Anterior Cingulate Cortex (ACC), Hippocampus (HC), Cortisol

Awakening Response (CAR), Cortical Thickness, Age, Sex differences, Hypothalamic-Pituitary-Adrenal (HPA) axis

Introduction

The HPA axis plays a vital role in adaptive restoration of homeostasis disturbed by stress (Chrousos, 1998; Sapolsky et al., 2000). The HPA axis activation is marked by release of glucocorticoids (cortisol in humans) from the adrenal cortex. Glucocorticoids play a complex role (both stimulatory and suppressive) in adaptive physiological responses of an organism (Sapolsky et al., 2000). The HPA axis closely interacts with the limbic system, via 1) bidirectional projections to the paraventricular nucleus (PVN) of the hypothalamus that modulate its response to psychological stress (Herman et al., 2003) and 2) corticosteroid receptors that are abundant in the limbic system—especially the hippocampus (Chao et al., 1989) and regulate both basal and transient adaptive responses of the HPA axis throughout life (De Kloet et al., 1998). Depending on dose and time- course of exposure (e.g. during developmental process or learning), corticosteroids influence neurogenesis, proliferation and plasticity of the hippocampal neurons in a site-specific manner; as reviewed elsewhere (Joels, 2008; McEwen, 2007; Sousa and Almeida, 2002). Stress- or corticosteroid-related morphological reconfiguration also occurs in other limbic regions such as the medial prefrontal cortex (Cerqueira et al., 2005; Cerqueira et al., 2007b; Cook and Wellman, 2004; Perez-Cruz et al., 2007; Radley et al., 2004; Wellman, 2001) and amygdala (Vyas et al., 2006). Because these brain regions are important for emotional and cognitive regulation, effects of HPA axis activity on their plasticity is suggested to determine variations in behavioral adaptation, aging or vulnerability to mood disorders (McEwen, 2007).

In humans, the morphological link between the HPA axis function and the hippocampus is reported in studies of aging (Gianaros et al., 2007b; Lupien et al., 1998; Lupien et al., 2005b; O'Brien et al., 2004; Pruessner et al., 2005; Wolf et al., 2002) and stress-related disorders (Bremner et al., 1995; Pavic et al., 2007; Yamasue et al., 2007). Similarly, variations in the morphology of the medial prefrontal cortex are linked to the HPA axis function in aging (MacLullich et al., 2005; MacLullich et al., 2006; Wolf et al., 2002) and post traumatic stress disorders (Kasai et al., 2008; Kitayama et al., 2006; Woodward et al., 2006; Yamasue et al., 2003). Majority of these studies have shown a volume reduction in association with hyperactivity of the HPA axis or stress-related symptoms. However, age-related hippocampal atrophy is not solely explained by HPA axis dysregulation (O'Brien et al., 2004); abnormally high diurnal cortisol levels in first episode schizophrenic subjects don't seem to predict hippocampal atrophy (Gunduz-Bruce et al., 2007), and HPA axis-related variations are not necessarily accompanied by mediotemporal or mediofrontal atrophy in aging (MacLullich et al., 2005). Even a positive correlation between the hippocampal volume and cortisol responses to stress and awakening is reported (Pruessner et al., 2007), challenging the notion that higher glucocorticoids lead to hippocampal atrophy. Overall, our current understanding of the morphological correlates of HPA axis function in humans is limited.

In humans, the HPA axis function might be assessed from salivary cortisol (Kirschbaum and Hellhammer, 1989; Peters et al., 1982). Diurnal cortisol, cortisol stress response or responses (endocrinological or behavioral) to pharmacological HPA axis manipulation are often used to quantify the resilience of the HPA axis function in aging (Ferrari et al., 2001; Seeman and Robbins, 1994; Van Cauter et al., 1996) or in stress-related disorders (de Kloet

et al., 2006). These variables are highly state-dependent and, unless measured longitudinally, would not provide a representative measure of HPA axis activity traits. Therefore, a limiting factor in examining morphological variations related to HPA axis function is that controlled modulation of cortisol secretion in humans is virtually impossible. In recent years, the cortisol awakening response (CAR, a 50-75% increase in cortisol levels within 30-45 minutes after awakening) has emerged as a relatively stable measure of the HPA axis activity (Edwards et al., 2001; Hellhammer et al., 2007; Pruessner et al., 1997b; Wilhelm et al., 2007; Wust et al., 2000b). Studies in twin children (Bartels et al., 2003) or twin adults (Kupper et al., 2005) suggest a significant heritability that is particular to awakening cortisol measures. A recent study suggests that the awakening cortisol response represent a reliable trait measure of the awakening cortisol when measured on at least two occasions (Hellhammer et al., 2007). Also, it has been shown that the total amount of the awakening cortisol accounts for most variation in the diurnal cortisol levels (Edwards et al., 2001). Thus it is plausible to consider the CAR as a standardized index of daily exposure to cortisol, and to use it in models that test the link between the basal HPA axis and brain morphology.

An added incentive to investigate correlation between the CAR and brain morphology comes from emerging evidence that psychopathologies such as depression (Bhagwagar et al., 2005; Pruessner et al., 2003b), psychosis (Pruessner et al., 2008b), burnout (Grossi et al., 2005; Pruessner et al., 1999), chronic fatigue (Roberts et al., 2004), and posttraumatic stress disorder (Wessa et al., 2006)—many of which are marked by abnormalities of the corticolimbic areas—can alter the CAR. Correlations between the CAR and bereavement (Meinlschmidt and Heim, 2005), socioeconomic status (Wright and Steptoe,

2005), attachment anxiety (Quirin et al., 2008), employment (Kunz-Ebrecht et al., 2004a), work overload (Schlotz et al., 2004) are some examples that link the CAR to chronic stress. In fact, the CAR seems to relate to variations in the hippocampal volume in different healthy populations (Pruessner et al., 2005; Pruessner et al., 2007; Wolf et al., 2002). Blunted CAR is also linked to hippocampal atrophy in a clinical population (Bruehl et al., 2009). A unique study of patients with brain lesions has shown that the CAR is almost completely abolished in subjects with hippocampal lesion (Buchanan et al., 2004). This finding emphasizes the role of hippocampus in controlling this specific component of the HPA function. However, it leaves open the question of whether morphological variations associated with the CAR occur in other parts of the brain as well.

In fact, the second limitation of most of existing human studies is that they often rely on volumetric assessment of the hippocampus (and very occasionally the medial prefrontal cortex) in isolation from the rest of the brain. The advantage of volumetric methods is that raters can trace the boundaries of structures like hippocampus irrespective of variations in shape and stereotaxic location of this structure (e.g. (McHugh et al., 2007; Pruessner et al., 2000). However, manual segmentation of cortical areas is not ideal due to costs and the time involved. Recently, we have introduced an automated corticometry method (vertex-base measurement of the cortical thickness; for details see (Kim et al., 2005; Lerch and Evans, 2005) that allows predicting regional variations in cortex in relation to biological or psychological variables. Compared to manual parcellation methods, automated corticometry is not subject to inter-rater variability and is sensitive to focal variations in cortical thickness. For example, this method has been successfully used to reveal the cortical changes in relation to the APOE4

gene (Shaw et al., 2007), brain development (Lenroot et al., 2007; Shaw et al., 2006) and Alzheimer's disease (Lerch et al., 2008; Lerch et al., 2005). Automated corticometry has also revealed cortical changes associated with cognitive impairment in multiple sclerosis patients (Charil et al., 2007). Hence, this method might reveal a better picture of regional variations of brain morphology with HPA axis activity.

In the current study, we have shown that the area under the curve with respect to ground of the CAR (AUC_G) shows significant within-subject stability across days. We have hence used the two-day average of the AUC_G to predict variations in hippocampal volume and the cortical thickness in healthy older adults.

Materials and Methods

Subjects

One hundred subjects (between 60-75 years of age) were recruited from the local community for participation in a study of aging. The participants were screened for neurological disease, brain trauma, cardiovascular disease, hypertension, diabetes and depression and excluded if any of these conditions were present. MRI was performed on 49 of these participants that met the inclusion criteria. We excluded 5 data sets due to detection of a lesion (1 subject), poor image quality dental artifact (1 subject), and incomplete awakening cortisol samples (3 subjects). In total, 44 healthy subjects (mean age = 67± 5 years; 21 men and 23 women) with complete neuroimaging data and saliva samples were included. Written informed consent was obtained from each subject prior to entering the study, in accordance with the Ethics board regulation at the Douglas Hospital Research Center, and the Montreal

Neurological Institute.

Saliva sampling and cortisol analysis

Saliva sampling was performed using salivettes (SARSTEDT, Quebec City, Canada), cotton-swab sampling devices. The CAR[6] was measured on two consecutive days with reference to time of awakening at t=0, +30 and +60 minutes after awakening. All subjects were scanned in summer. A time sheet was given to participants to log the exact time of sampling in order to control for compliance-related errors. To ensure that the samples would not be contaminated, the subjects were asked to refrain from eating, drinking, caffeinated or sweet beverages, and brushing their teeth prior to taking the samples.

Saliva samples were analyzed for cortisol using a time-resolved fluorescence immunoassay. Intra- and inter-assay variability was less than 10% and 12%, respectively (Dressendorfer et al., 1992).

Preliminary analysis consisted of a mixed model ANOVA to test the interactions of awakening cortisol levels (measured at t=0, +30 and +60), days (1 and 2) and sex. Three-way ANOVA was performed using DATASIM (Bradley, 1998).

To reduce data dimension, variables AUC_G (Area under the curve with respect to ground) and AUC_I (increased area under the curve with respect to baseline) were calculated for each day (Pruessner et al., 2003a). Pearson correlation analysis was performed to determine the CAR variable that was correlated

[6] Also known by acronyms ACR (awakening cortisol response) and CRA (cortisol response to awakening)

across measurement days.

MRI acquisition, preprocessing, and volumetry

Subjects were scanned on a 1.5-T Siemens Sonata Scanner (Siemens AG, Erlangen, Germany). Anatomical MRIs were acquired using a T1-weighted ICBM (International Consortium for Brain Mapping) protocol (3D SPGRE, TR/TE = 18/10, flip angle = 30°. 176 1-mm contiguous sagittal slices, FOV = 256 x 256 mm2). Images were processed using CIVET[7] a processing pipeline that includes several steps such as correction for intensity nonuniformity, transformation to standardized MNI152 atlas, partial volume correction, tissue classification and skull striping, as described by (Zijdenbos et al., 2002). Within the pipeline, the volumes of the cerebrospinal fluid, gray- and white-matter volumes, as well as the scale factor to transform the volumes between native and standard space were computed.

The hippocampal segmentation was performed manually, using the interactive software package DISPLAY developed at the Brain Imaging Center of the Montreal Neurological Institute. Anatomical boundaries used for the hippocampus and a step-by-step segmentation protocol are described in detail elsewhere (Pruessner et al., 2000).

Because the head size (estimated from scale factors that transformed the native data onto MNI152 average) in men was significantly larger than women ($t42=5.88$, $p<.0001$), to avoid adding a gender bias, all morphometric measures were transformed to subject's native space.

[7] http://wiki.bic.mni.mcgill.ca/index.php/CIVET

Generalized linear modeling (GLM) was used to investigate the effect of age, sex and CAR variables on hippocampal volume using SPSS11 for Mac OS X (SPSS, Chicago, IL).

Cortical thickness analysis

Automated cortical thickness estimation was performed using the CLASP algorithm (Kim et al., 2005) that generates accurate surfaces of deep sulci by using skeletonized CSF maps. The cortical surface for each hemisphere was extracted by deforming a spherical polygon model to match the white matter boundary and then expanding it along a Laplacian field to reach the boundary between GM and CSF (Kim et al., 2005). Cortical thickness is defined simply as the distance between these linked vertices on the inner and outer cortical surfaces (t-link). This method has been validated using both manual measurements (Kabani et al., 2001) and simulation (Lerch and Evans, 2005).

Diffusion blurring (Chung et al., 2003) based on the surface curvatures was performed (FWHM = 30 mm) to normalize the data while preserving the anatomical boundaries. This method increases statistical sensitivity, by minimizing false positives (Lerch and Evans, 2005).

For each hemisphere, vertex-based GLM was performed to detect areas of the brain where the variations in cortical thickness correlated with the CAR. Effect of age and sex were controlled for in the model.

To correct for multiple comparisons, the resulting statistical maps were thresholded using a false discovery rate (FDR) of 10% of false positives (Genovese et al., 2002). To increase statistical sensitivity, the q-value was calculated after pooling the p-values from all regressions within the target

region of interest that encompassed the medial prefrontal and medial temporal areas.

Results

Awakening cortisol response

Because the raw cortisol values were not normally distributed, the cortisol molar weights were transformed on a logarithmic scale. Table 1 presents the awakening cortisol values for each day.

Table 1: Awakening cortisol variables (Mean ± SD)

	Male			Female		
	Day1	Day2	Average	Day1	Day2	Average
Awaken. hr.	6:40±52'	6.26±60'	6:33±56'	6:32±1:14'	6:49±50'	6:41±1:04'
Cortisol t=0	0.88±0.34	0.83±0.29	0.85±0.31	0.83±0.36	0.93±0.23	0.88±0.30
Cortisol t=30	1.01±0.33	1.06±0.33	1.03±0.33	1.01±0.45	1.04±0.31	1.03±0.38
Cortisol t=60	0.91±0.42	0.94±0.30	0.92±0.36	0.93±0.43	0.93±0.40	0.93±0.41
AUC_G	56.06±17.78	58.42±16.48	57.27±16.97	55.97±22.48	58.96±16.44	57.50±19.47
AUC_I	4.21±14.22	8.57±9.47	6.44±12.09	6.43±15.88	3.11±11.86	4.74±13.92

Cortisol values are on logarithmic scale

A three way mixed design ANOVA (sex × 2 days × 3 times of cortisol measurement) did not reveal any interaction ($F_{1,84}=1.84$, $p > .20$). There was also no interaction of sex by awakening measurement, sex by day, or day by time of measurement (p's > .60). Main effect of days ($F_{1,42}=.24$, $p > .60$) and sex ($F_{1,42}=.49$, $p > .40$) was non-significant. The main effect of time was highly significant ($F_{2,84}=18$, $p < .0001$) Post-hoc Tukey's test revealed significant difference between cortisol measured at time of awakening, +30 and +60 minutes.

The AUC_G of days one and two were significantly correlated ($r=.48$, $p < .001$); therefore, we averaged the AUC_G over two days. By contrast, the AUC_I measurements for day one and day two were not correlated ($r=.104$, $p > .40$), perhaps reflecting increased load of state factors on the AUC_I, as suggested by a recent study (Hellhammer et al., 2007). Because our hypothesis tests the relation between a stable trait measure of the HPA axis activity and brain morphology, we excluded AUC_I measures from further analysis.

Independent sample t-test revealed no sex difference in AUC_G ($t42 = -.2$, $p > .80$). Age and sex entered together in a generalized linear model did not predict any variations in the AUC_G ($F2,41= 1.3$, $p > .20$).

Covariation of the volumetric data with CAR

Figure 1 summarizes the volumetric data. The percentage of GM over total intracranial volume in men (45 ± 1.9) was significantly less than in women (47 ± 1.5; $t42=-3.9$; $p<.001$); however differences in WM (men: 40 ± 2.9 ; women: 39 ± 2.7, $t42=.87$) left HC volume (men: 3328 ± 479; women: 3224 ± 397; $t42=.79$) and right HC volume men: 3421 ± 400; women:3294 ± 419; $t42=1.03$) were non-significant.

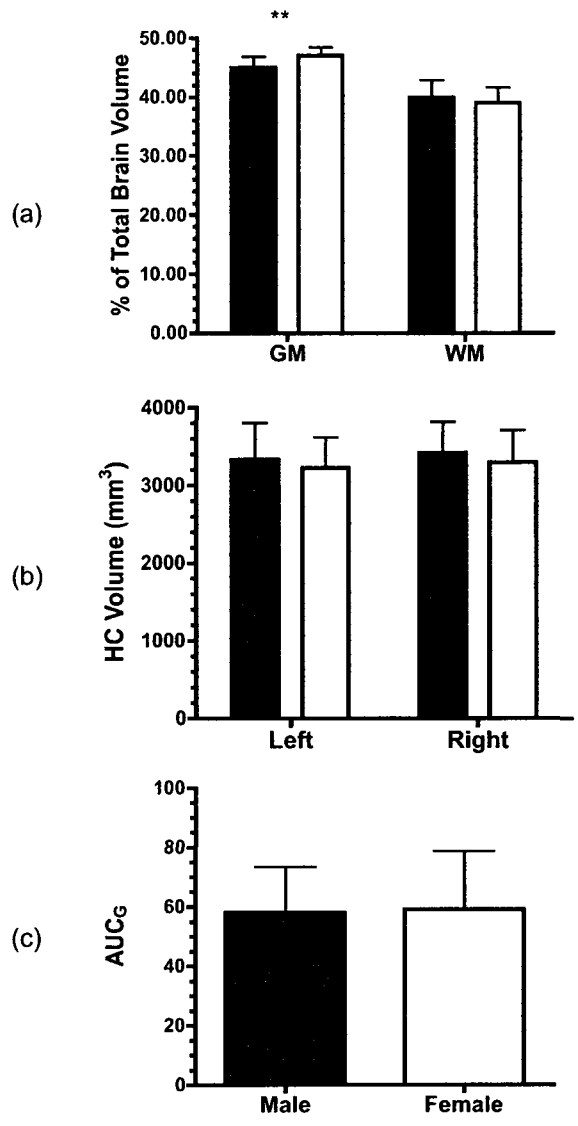

Figure 1: Volumetric data. (a) After correcting for head size, ratio of GM volume over entire intracranial cerebral volume (WM+GM+CSF) was significantly less in men than in women. (b) No sex differences in HC volume. (c) No sex differences in AUC_G. (Men black, and women white.)

For the total sample, the white matter (WM) ratio (percentage of WM volume over total brain volume) decreased with age ($r = -.312$, $p < .05$), however gray matter (GM) was not significantly correlated with age ($r = -.178$, $p > .20$), indicating an age-related increase of ventricular volume. WM and GM ratios

were not correlated with AUC_G.

Multivariate GLM (Y~ Intercept + AUC_G) with left and right HC volumes as dependent variables did not reveal a significant effect ($F_{2,41}$=.061, p>.90). Instead, the GLM (Y~ Intercept + age×sex +AUC_G×sex) predicted significant variations in the left (corrected model: $F_{4,39}$ = 3.06, p<.03) and the right (corrected model $F_{4,39}$ = 2.96, p<.04). Most of the variation in the model was explained by age×sex interaction (for both sides $F_{2,39}$ >4.5, p<.02). Effect of the AUC_G×sex interaction was only marginally significant (for both sides $F_{2,39}$ >2.57, p<.09). Age alone predicted over 23% of variations in the left and right HC volumes in women, but not in men. The AUC_G alone did not reveal significant variations in the HC volume of men or women. However, multiple regression analysis including age and the AUC_G as independent variables revealed a positive correlation between the AUC_G and the HC volume in men, which was significant on the right side (b=.49, t=2.2, p<.04) but not on the left side (b=.43, t=1.9, p<.08). This relation was not observed in women (left: b=-.13, t=-.7, p>.40; and right b=-.11, t=-.6, p>.50; see Figure 2-b for partial regression plots). In this model, significant effect of age on HC volume reduction in women remained significant (left: b=-.50, t=-2.63, p<.02; right: b=-.46, t=-2.3, p<.03) and effect of age on hippocampal volume in men was not significant (left: b=-.33, t=-1.5, p > .15; right: b=-.30, t=-1.4, p > .18; see Figure 2-c for partial regression plots). We note again that correlations between age and the AUC_G were not significant in men: r=.32, p > .15; or women: r=.19, p > .40 (Figure 2-a). These results indicate that variations in HC volume in men are dependent on both the AUC_G and age. By contrast, variations in the HC volumes of women were independent of the AUC_G but predicted by age.

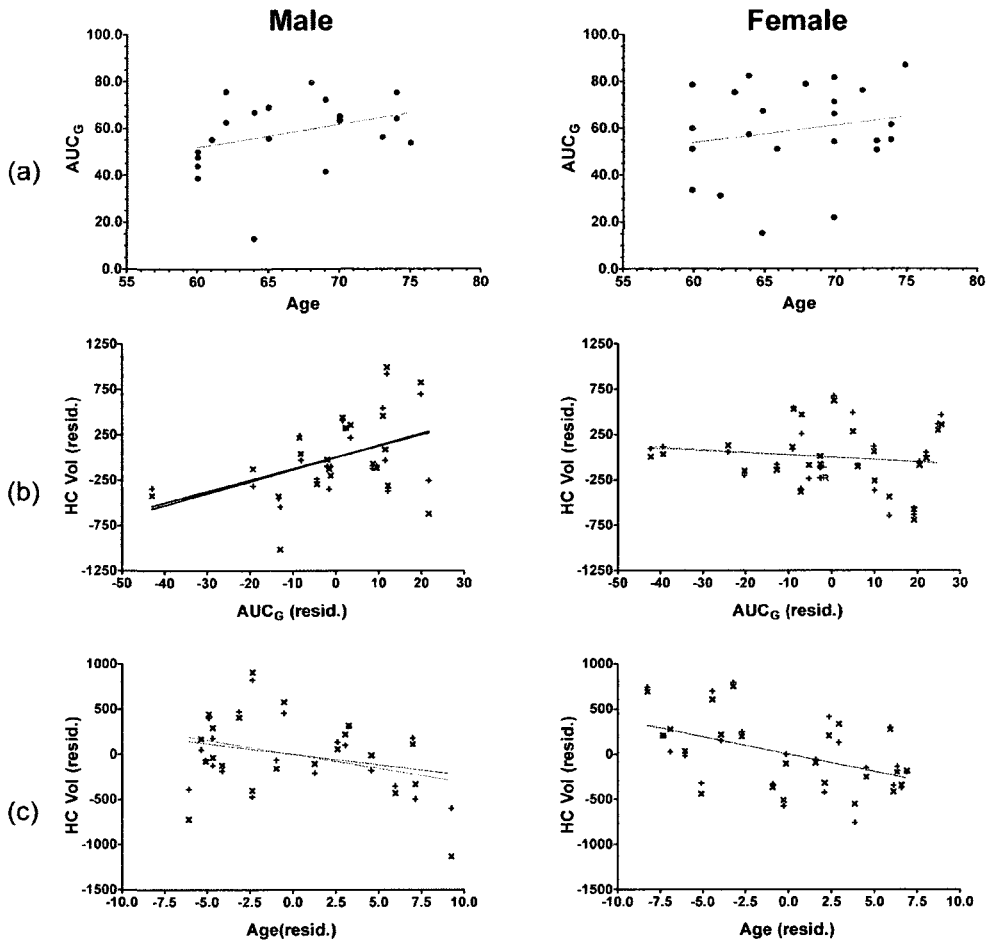

Figure 2 : Regression plots. (a) Linear relation between age and AUC_G is not significant. (b) Partial regression plot of residuals of HC volume (y-axis) and AUC_G (x-axis), removing age effect. (c) Partial regression plot of residuals of HC volume (y-axis) and Age (x-axis), removing AUC_G effect (× left HC; + right HC).

Cortical thickness correlations

Vertex-based GLM ($Y = I + AUC_G$) showed a focal effect of the AUC_G on variations of cortical thickness in the left pregenual anterior cingulate cortex (ACC). The highest peak of correlation was observed at stereotaxic [-6, 36, 17]: $t_{42} = -3.42$; $p < .001$, uncorrected; Figure 3). Including age and sex in this model did not change the main effect of the AUC_G on the ACC thickness. (Note that age-related cortical thickness variations did not overlap with the ACC region

predicted by the AUC_G, see Supplemental Figure.) The cluster of connected vertices that satisfied false discovery rate of 10% was exclusive to the anterior cingulate region. We chose the peak of this cluster to investigate correlations between the cortical thickness of this region and other variables of interest. This peak represents the average thickness of vertices smoothed with a 30 mm diffusion blurring kernel. Univariate GLM with the ACC cortical thickness at [-6, 36, 17] as dependent and age, sex and the AUC_G as predicting factors confirmed that only the main effect of the AUC_G on this cortical region was significant ($F_{1,40}$ = 9.96, p < .004). Effects of sex ($F_{1,40}$ = 1.42 p > .20) and age (($F_{1,40}$ = .63, p > .40) were non-significant. Variations in the ACC thickness were not correlated with the left HC (men: r = .13, p > .50; women: r = .28, p > .20) or the right HC (men: r = .02, p > .90; women: r = .32, p > .10) volumes.

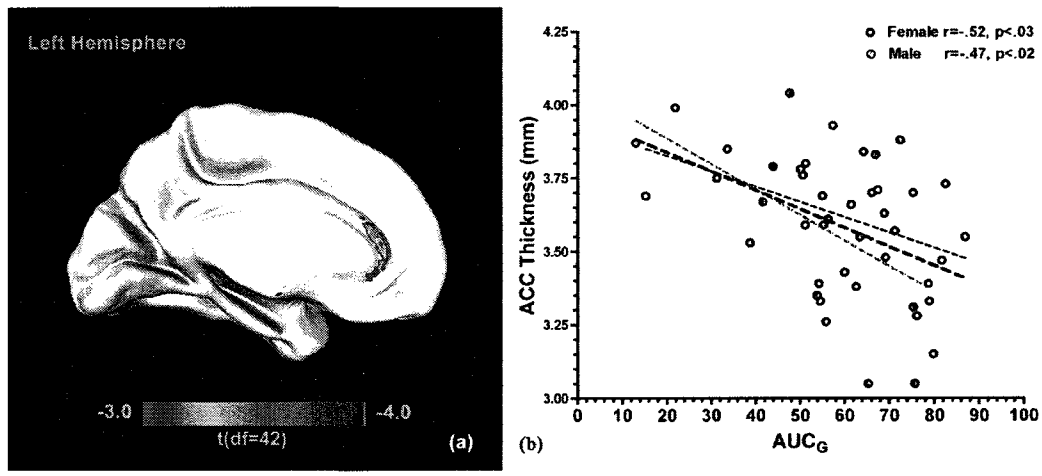

Figure 3: Variation of cortical thickness with awakening cortisol. (a) t-statistics maps overlaid on the average surface of the sample (N=44, linearly registered to the MNI152 model). (b) Cortical thickness measured at stereotaxic [-6, 36,17] (with 30mm diffusion blurring) versus the AUC_G of CAR. No sex interaction is present (Slopes:$F_{1,40}$ =.79, p>.38; Intercepts: $F_{1,41}$=1.29, p>.26). Correlation coefficient for total sample: r=-.46, p<.002.

Discussion

This study aimed to investigate (in a healthy older population) the relation between brain morphology and basal HPA axis activity measured from the awakening cortisol levels. We chose the AUC_G of the CAR because it showed significant within-subject stability across days one and two. Interestingly, variations in the AUC_G were not predicted by age or by sex. The AUC_G revealed a distinct reduction of the left pregenual ACC thickness in the entire sample, and a male-specific increase in the hippocampal volume.

On of the most important finding of the current study was the detection of a focal inverse correlation between the thickness of the left pregenual anterior cingulate cortex (ACC) and the total levels of cortisol awakening response (Figure 1). It is known that the bidirectional neural pathways between the ACC and the hypothalamus are important for regulation of the HPA axis stress responses (Feldman et al., 1995; Herman et al., 2003). In humans stress-related psychopathologies such as posttraumatic stress disorder (Drevets et al., 2008) and depression (Drevets et al., 2008) are linked to ACC atrophy. Recent functional neuroimaging studies confirm a correlation between the neural activity of the ACC and cortisol response to psychological stressors (Kern et al., 2008; Liberzon et al., 2007; Taylor et al., 2008). Anatomical neuroimaging studies also indicate that the ACC's morphology relates to stress-modulating factors such harm avoidance (Pujol et al., 2002), perception of self's low social standing (Gianaros et al., 2007a), even early life stress (Cohen et al., 2006) in healthy subjects. Altogether, both function and morphology of the ACC seems to relate to individual variations in vulnerability to stress. Higher CAR values are also reported in association with risk factors for chronic stress; e.g. attachment anxiety (Quirin et al., 2008), trait anxiety (Greaves-Lord et al.,

2007), lower socioeconomic status (Steptoe et al., 2003; Wright and Steptoe, 2005), early life experience (Buske-Kirschbaum et al., 2007), as well as depression (Bhagwagar et al., 2005; Pruessner et al., 2003b), and posttraumatic stress disorder (Wessa et al., 2006). To our knowledge, our data is the first to report a link between the ACC and the CAR. In the absence of psychometric data, we cannot conclude that inverse correlation between the CAR and the ACC is maladaptive and linked to chronic stress, but it is likely that factors leading to hypersecretion of the awakening cortisol also impact on the ACC plasticity.

The hypothesis that higher CAR is causally related to neuronal reconfiguration of the ACC is plausible. An inverse correlation between the left ACC volume and ability to attenuate HPA axis response to low dose of dexamethasone has been reported in older adults (MacLullich et al., 2006). Notwithstanding the unique cytoarchitecture of the ACC in humans—characterized by spindle neurons particular to humans and great apes (Nimchinsky et al., 1999)—precedent rodent evidence has shown a left lateralized ACC reconfiguration caused by hypercortisolism (Cerqueira et al., 2005; Cerqueira et al., 2007a) and chronic stress (Cook and Wellman, 2004; Perez-Cruz et al., 2007; Radley et al., 2004). In fact, Cerqueira and colleagues used histology and MRI together to show that hypercortisolism induced by high-dose dexamethasone and adrenalectomy treatment indeed predicted an MRI-detectable reduction of the left ACC volume (Cerqueira et al., 2005). The reason for left lateralization of the glucocorticoid effects on the cingulate cortex morphology is not yet clear, but it has been suggested that behavioral topology of the medial prefrontal cortex contributes to lateralized neuronal remodeling (Cerqueira et al., 2008). Currently, we cannot speculate on behavioral correlates of this lateralization.

However, the similarity between our finding and animal models makes it plausible that the ACC thickness reduction might be an acquired effect due to increased daily cortisol exposure (e.g. due to chronic stress).

The second important observation is that variations in the gray matter density (T1-weighted) predicted by the AUC_G were not similar in the ACC and the HC. Unlike the ACC, variations in the HC volume were affected by age×sex interactions. In men, the AUC_G predicted an increase in the HC volume (more prominent on the right side), but only after controlling for age. This observation indirectly supports those studies that show a blunted CAR associated with hippocampal damage (Buchanan et al., 2004) and with hippocampal atrophy in type II diabetic subjects (Bruehl et al., 2009). Interestingly, a positive correlation between the CAR and the HC volume is reported in young healthy men as well (Pruessner et al., 2007). It is possible that positive correlation between the HC volume and the AUC_G might reflect an adaptive mechanism. By contrast, in women, the AUC_G did not predict HC volume variations—although slopes of regression in men and women were significantly different. Instead, the HC volume reduction in women was significantly predicted by age—an effect that approached a trend in men, but only after controlling for the AUC_G. This might suggest that higher AUC_G protected against age-related effects in men, but not in women.

Sex interaction with HC volume variation (with age and with the CAR) draws attention to significance of estrogen effects on hippocampal function and plasticity (Spencer et al., 2008). In rats, for instance, dendritic spine density of CA1 neurons is enhanced by acute stress in males but reduced in females (Shors et al., 2001). In our previous studies, we have shown that the HC volume is

sensitive to factors such as the history of estrogen exposure (Lord et al., 2008). We have also shown that sex interacts with correlation between the HC volume and maternal care (Buss et al., 2007) and self esteem (Pruessner et al., 2005)—factors that are likely to mediate the effects of chronic stress on hippocampal plasticity. Correlation between the HC volume and the AUC_G in women might have been occluded by interindividual heterogeneity in estrogen exposure (e.g. due to difference in onset of menses, menopause, or different courses of hormonal replacement therapy). Sex differences deserve further investigation, but proper treatment of behavioral or hormonal predictors of sex interaction with HC plasticity is beyond the scope of this current article.

Nevertheless, it is interesting that the correlation between the ACC and the AUC_G is robust to these possible sex factors. Moreover, it surprising that, in men, the AUC_G predicts increased HC volume but reduced ACC thickness. Both the ACC and the HC have high concentration of corticosteroid receptors (Diorio et al., 1993; McEwen et al., 1968); are interconnected (Cenquizca and Swanson, 2007a); contribute to the negative feed back inhibition of the HPA axis responses via connections to GABAergic neurons of the paraventricular nucleus of the hypothalamus (Herman et al., 2003); and show similar dendritic reconfiguration in response to glucocorticoid manipulation (Cerqueira et al., 2007b; Woolley et al., 1990). However, we did not observe a correlation between the HC volume and the ACC thickness, not even in men. Thus the effect of awakening cortisol on the morphology of these structures is likely to be mediated by different factors.

We propose a hypothesis for this observed dissimilarity by noting that central actions of cortisol on neuronal configuration are mediated by mineralocorticoid

and glucocorticoid receptors (MRs and GRs, respectively). MRs and GRs are ligand activated transcription factors important for different aspects of genomic control(Revsin et al., 2005). Whereas MRs are essential for maintaining basal HPA axis sensitivity, the GRs control (and down regulate) the initial stress responses (De Kloet et al., 1998). Cortisol has a much higher affinity for nuclear MR (Reul and de Kloet, 1985), and although MRs and GRs are co-localized in the limbic system (De Kloet et al., 1998), the ratio of MR/GR binding capacity is greater in the hippocampus compared to the cingulate (Chao et al., 1989). Sousa and colleagues have recently reviewed effects of MR and GR activation on neuronal configuration and have concluded that MRs are necessary for neurogenesis and proliferation; and that exclusive GR activation leads to neuronal loss and apical dendritic atrophy (Sousa et al., 2008). The hippocampal MR activation also plays an important role in facilitating long term potentiation (LTP), increasing calcium conductance in CA1 and CA3, and controlling cell proliferation in the dentate gyrus (Joels, 2008). Interestingly, a behavior-independent right-preferred concentration of MRs is reported in the mice hippocampus (Neveu et al., 1998). Of course, we do not know the link between the CAR and MR occupancy. Plus, T1-W MRI does not inform whether morphological variations we observe reflect apoptosis, neurogenesis or dendritic reconfigurations. However, in as far as the CAR is linked to basal HPA axis activity, it is plausible to speculate that increased MR binding capacity in the hippocampus, or different ratio of MR/GR co-localization in the hippocampus compared to the ACC would affect the neural plasticity of this region differently. Future studies are needed to examine the factors that dissociate neuronal plasticity of different regions of the limbic system.

Certain limitations of this report must be noted. First, our study lacks

behavioral measures; therefore, we cannot assess the adaptive or maladaptive nature of correlations we report here. Second, we have not discussed amygdala, although patterns of amygdalar and hippocampal plasticity might give clues to behavioral substrates of neuronal reconfiguration (Vyas et al., 2006; Vyas et al., 2002). Amygdala will be examined in future studies. Third, we considered only one aspect of the CAR, the AUC_G, because we were interested in a variable that was stable across days. It is worth noting that the hippocampus seems to be particularly important for cortisol increase 30 minutes after awakening, but not the rest of the diurnal levels (Buchanan et al., 2004). By increasing number of sampling days we can minimize the within subject variance in the AUC_I (Hellhammer et al., 2007). Thus, further studies must also examine whether morphological correlates of the AUC_I and the AUC_G are overlapping or dissociated. Last but not least, the age range of our participants is limited (between 60-75 years), and the participants are recruited from clinically healthy and socially active population. Therefore our findings might not fully capture the effect of age on variations of the AUC_G with brain morphology. Despite these limitations, this is the first study to examine neural correlates of the CAR across the entire cortical mantle of healthy individuals. Cortical thickness analysis reveals a very focal but robust link between the CAR and the pregenual anterior cingulate cortex, which is different from correlations observed in the hippocampus. Considering that the ACC is critical for visceral and behavioral aspects of self-regulation, its morphological correlation with different aspects of HPA axis control might help elucidate determinants of interindividual differences in aging and behavioral adaptation.

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Supplemental Material

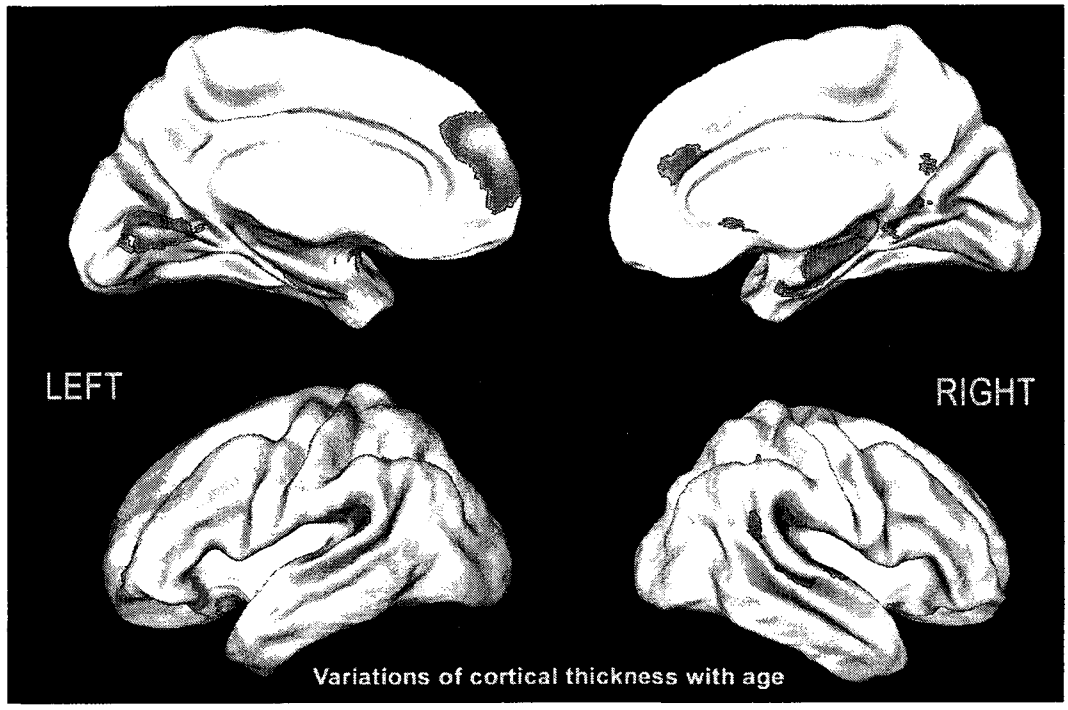

Figure S1: Age-related reduction in cortical thickness ($p<.05$, corrected). This figure illustrates age-related reduction of medial prefrontal, parahippocampal, superiror temporal and medial occipital areas. No overlap between CAR-related reduction in cortical thickness in the ACC and age-related cortical thickness reductions is present.

CHAPTER 6

GENERAL DISCUSSION

Summary

The global objective of this work was two-fold. First, it aimed to investigate whether there was an association between the neural activity of the medial prefrontal cortex and the hippocampus and the neuroendocrine HPA response to an experimental task recently developed in our lab. Second, it aimed to identify an objective index of brain- and HPA axis interactions that could best characterize interindividual variances in stress sensitivity. To address these questions, we used pre-existing data sets that were collected based on two main hypotheses:

1) The HPA axis stress-related activity is predicted by an individual's subjective perception of social evaluative threat (Dickerson and Kemeny, 2004). This theory is the basis of the methodology of the Montreal Imaging Stress Task (Dedovic et al., 2005), which constituted most of the fMRI data examined here.
2) The hippocampus and the medial prefrontal cortex play an important role in negative feedback inhibition of HPA axis stress activity (Herman

et al., 2005); we used this theory to interpret the increased limbic deactivation in response to the MIST (Pruessner et al., 2008a).

In total, 112 neuroimaging and cortisol data sets (68 young and 44 old) were available, with the MIST being the common factor between three sets of independent studies.

In one study (Chapters 2 and 3), the MIST was administered together with a novel-picture encoding (pre-MIST) and delayed paired picture recognition (post-MIST) fMRI tasks performed by 19 healthy young male college students. In two other studies, the MIST was performed on 49 young and 41 older men and women (Chapter 4). Although the MIST sessions for both cohorts were identical, these groups were were tested using different experimental protocols.

We made the following observations that tie the functional studies together:

a. The cortisol variations due to the MIST were highly variant between subjects, while significant stress cortisol response was observed in about half of subjects in each study. Overall, the stress cortisol response was more pronounced in the young compared to the older group.

b. The experimental condition of the MIST leads to a significant reduction of the BOLD signal compared to the control condition (deactivation). Despite experimental heterogeneity, patterns of brain activity during the MIST were similar in the young subjects: The experimental condition of the MIST was associated with a significant reduction of the BOLD signal (deactivation) in the medial prefrontal and medial temporal areas. By contrast, in the older subjects, the deactivation of the posterior parietal

areas dominated the topography of the BOLD signal change during the experimental condition of the MIST.

c. We observed a significant modulatory effect of cortisol on medial prefrontal, medial temporal, insular and precuneal areas; however, the direction of this correlation was not uniform. For instance, whereas in young subjects in both studies we observed a negative association between cortisol response and the BOLD signal in the rostral ACC or hippocampal areas, we observed a positive correlation in the dorsal part of the ACC and the hippocampus in the older subjects (Chapter 4).

d. For all three studies, posthoc analysis revealed a modulatory effect of self-esteem and locus of control on neural activity of the rostral ACC and precuneus. Self-esteem accounted for ACC variations in the young (Chapter 2, Supplementary analysis 2) and perception of other's control accounting for variations in the precuneus in the older subjects (Supplementary analysis 2).

e. The consistent stress-group differences in the deactivation of the ACC and the precuneus allude to the possibility of baseline differences in the "default mode network" activity. (As will be discussed, this important observation helps conceptualize a novel approach to studying the neural correlates of HPA axis activity in humans)

Moreover, we uncovered associations between the HPA axis and the hippocampus and ACC that were unrelated to the MIST. We showed that:

f) The state of hippocampal activity during a memory function might explain later HPA axis stress responses (Chapter 2).

g) The prefrontal activity related to HPA-axis response to stress was significantly correlated during different tasks (Chapter 3).

h) The baseline HPA axis activity (estimated from the cortisol awakening response) predicted morphological variations in the pregenual ACC and the hippocampus, although the directions of correlations and the interactions of sex and age with CAR-related morphological variations were dissimilar (Chapter 5).

Altogether, observations made in these analyses corroborate the animal evidence that the hippocampus and medial prefrontal cortex are closely related to HPA axis regulation. In addition, they highlight several limitations of the current methodology. In the following sections, these limitations are discussed and a new model and a methodology are proposed that allow for an integral examination of the neural correlates of the HPA-axis activity.

The Hippocampus and the Medial Prefrontal Cortex

The hippocampus and the medial prefrontal cortex are the most studied CNS controllers of stress-related HPA axis activity. The focus on these structures stems from the early discovery that the adrenal steroids crossed the blood-brain barrier to target corticosteroid receptors in the hippocampus (McEwen et al., 1968). Corticosteroids mediate a number of rapid non-genomic and slower genomic actions in the hippocampus and MPFC (in particular, the cingulate cortex). For instance, they can rapidly increase cellular excitability (via MR activation) in subfields of the hippocampus (e.g. CA1 area), thus facilitating

long-term potentiation during the encoding of a stressful event or learning experience within that context (Joels, 2008)—presumably an evolutionary mechanism to prepare organism for coping with future stress (Sousa et al., 2008). By contrast, the slow and genomic actions mediated via GR-activated pathways—operating in a more inhibitory and suppressive mode (Sapolsky et al., 2000)—can hamper learning after a stressful event (Joels, 2008). Across the lifespan, chronic exposure to corticosteroids, alongside the species' environmental context, can also impact the neuroplasticity of these brain areas (Sousa et al., 2008). The closed loop of HPA axis regulation and gene transcription in the hippocampal and prefrontal structures during early development (Meaney et al., 1993) and aging (De Kloet et al., 1991) has made these structures regions of interest for examining biobehavioral mediators of the CNS and HPA axis correlations in humans. For instance, several researchers have shown a structural association between the hippocampus, ACC and HPA axis activity ((Buchanan et al., 2004; Lupien et al., 1998; MacLullich et al., 2005; Pruessner et al., 2005; Wolf et al., 2002). The modulatory effect of cortisol on neural activity of the hippocampus and MPFC is also observed in a handful of functional neuroimaging studies(de Leon et al., 1997; de Quervain et al., 2003; Ganguli et al., 2002; Kern et al., 2008; Liberzon et al., 2007; Pruessner et al., 2008a; Wang et al., 2007; Wang et al., 2005).

Our findings in this study corroborate several existing theories regarding the role of the hippocampus and the MPFC in HPA axis regulation, but also challenge a few. In the first study, we illustrated a link between hippocampal activity and cortisol stress response: subjects who showed a cortisol stress response differed in the extent of hippocampal activation prior to stress and hippocampal deactivation after stress. As expected (Kirschbaum et al., 1996;

Kuhlmann et al., 2005; Wolf et al., 2001b), the stress responders performed less well in the recognition task, but this performance decline was unrelated to cortisol levels. In fact, higher cortisol levels during recognition predicted better performance, but only in the responder group. Therefore, our findings suggested a complex role for the link between the HPA axis and hippocampal function that goes beyond metabolic modulations caused by cortisol (de Quervain et al., 2003; Sapolsky et al., 2000). In our interpretation of the findings of this study, we focused on the function of the hippocampus as an integrator of information, as recently conceptualized by Gray and McNaughton (Gray and McNaughton, 2003). Their "septo-hippocampal theory" posits that the hippocampal formation contains a set of topographically represented comparators involved in resolving stimulus- or response-based conflicts (McNaughton, 2006). The subfields of hippocampal formation detect particular environmental situations that will result in the activation of goal representations in other executive brain areas. The activation of the hippocampus thus depends on detecting competing or conflicting options or environmental situations in terms of risk and/or benefit. This interpretation of the hippocampal function falls in line with Herman's theory that the subicular outputs from the hippocampus play a prominent role in inhibition of psychologically induced hypothalamo-pituitary-adrenocortical (HPA) axis activity (Herman and Mueller, 2006). Similar to the hippocampus, the medial prefrontal cortex plays a complex role in behavioral regulation. For instance, the ACC (which is also important for stress-specific HPA axis regulation (Diorio et al., 1993) as it is structurally susceptible to chronic exposure to stress and HPA axis dysregulation) (Cerqueira et al., 2005), integrates visceral, executive, attentional, emotional and cognitive signals (Botvinick et al., 2004; Paus, 2001; Critchley, 2005). It helps optimize goal-directed action planning (Rushworth et

al., 2004; Walton et al., 2004; Yarkoni et al., 2005a) and counter-balances the emotional and cognitive components of perception (Bush et al., 2000; Critchley, 2005; Simpson et al., 2001a; Simpson et al., 2001b). In fact, interactions of the hippocampus and the MPFC with the HPA axis are mediated via intricate synaptic projections between these structures and the rest of the forebrain (Herman et al., 2005,Feldman et al., 1995).

Therefore, while examining the link between the hippocampus and the HPA axis activity, at least two factors need to be considered. One is the electrophysiological modulation of hippocampal activity caused by the activation of different types of corticosteroid receptors. The other is the signaling pathways activated depending on context- and individual-specific processing of the given stimuli. Our data underline the importance of such considerations. In all three functional data sets, we observed a modulatory relationship between cortisol and BOLD signal in the medial temporal and medial prefrontal areas. However, the loci where such correlations were significant were not identical, and the direction of these linear relationships were not similar. In our morphometric analysis, we found a significant correlation between basal cortisol levels (measured from the CAR) and the morphology of the hippocampus and the ACC. However, the morphological variations predicted by the CAR were not similar between these structures, and were likely related to endocrinologic or behavioral gender differences. Without quantitative neuroimaging (e.g. measuring glucose metabolism, or oxygen extraction factor in the functional studies and spectroscopy in the morphometric study), physiological interpretation of these observed correlations is not possible. Without controlled behavioral assessments (e.g. accurate recording of performance metrics, error detection, response time, or subjective rating of task

difficulty, anxiety and reward), we are also limited in our ability to interpret the causal relations between the neural subdivisions engaged by the task (or behavioral traits) and their inhibitory and excitatory modulation of the HPA axis.

Another factor to consider while studying hippocampal or prefrontal control of the HPA axis is functional organization of these structures. The hippocampus is an anatomically heterogeneous structure that is topographically connected to the hypothalamus (Swanson and Cowan, 1975) and the cerebral cortex (Cenquizca and Swanson, 2007b). This topography delineates its functionally specialized subregions (Eichenbaum et al., 2007; Kohler et al., 2002; Rombouts et al., 2001; Small et al., 2001). The same holds true for the medial prefrontal cortex (Barbas and Pandya, 1989; Petrides and Pandya, 1999); for instance, the ACC is a heterogeneous region in terms of its cytoarchitecture, connectivity and function (Bush et al., 2000; Paus et al., 1998; Vogt and Pandya, 1987). Although the varying effects of corticosteroids on different subregions of the hippocampus have to some extent been uncovered (Joels, 2008), the topography of corticosteroid actions on different subdivisions of the medial prefrontal cortex have remained largely unstudied.

Interestingly, our results highlight the importance of considering such subdivisions. For instance, the MIST-related deactivation of the head of the hippocampus in the first study was correlated with increased cortisol response; but there was no correlation between cortisol levels and hippocampal-tail activation during recognition—although the levels of cortisol were still high. Outside the hippocampus, when comparing brain activity in stress-responders and nonresponders performing encoding, MIST and recognition tasks, we

noticed a dichotomous pattern of activity in the dorsocaudal (the 'cognitive') and rostroventral (the 'emotional') subdivisions of the ACC (Chapter 3). When performing regression analysis to predict regional variations of the BOLD signal with cortisol variables (Chapter 4), we noticed a positive correlation between total cortisol levels and a large part of the MPFC. This encompassed Brodmann areas 6,8,9,10,24 and 32 in the old subjects. By contrast, in the young subjects, total cortisol levels predicted a lower BOLD signal in small regions in Brodmann areas 10, 11, 32 and the hippocampus. Although our data do not allow for an interpretation of the behavioral significance of such subdivisions, they do provide evidence for functional connectivity amongst subregions that were co-linearly modulated by cortisol. This information can potentially be useful in designing experiments for mapping cortical influences on HPA axis activity or investigating functional connectivity in relation to HPA axis control.

To demonstrate the feasibility of predicting HPA axis activity from the patterns of MPFC activation, we performed a posthoc conjunction analysis on the correlation maps (i.e. the voxel-wise t-values obtained from regression of BOLD against cortisol variables) of the young and old subjects who underwent identical MIST sessions. Despite overall differences in patterns of correlation, we found co-linear variations of the BOLD with cortisol in B32 and B11 in both age groups. Exploring the significant interindividual heterogeneity in the amplitude of the BOLD in these two ROIs (i.e. splitting groups based on those showing a positive BOLD in both regions, a negative BOLD in both regions, and a positive BOLD in one and a negative BOLD in the other region), we uncovered significant differences in the patterns of HPA axis regulation. What makes this observation noteworthy is that in the heterogeneity of the BOLD signal in response to stress was also encoded the heterogeneity of the HPA axis

activity prior to stress. This observation is in line with emerging evidence that differences in context or motivation can alter the ways in which the subregions of the medial prefrontal cortex interact to process and respond to cognitive or emotional stimuli (Hajcak and Foti, 2008; Rushworth et al., 2007). Ongoing cognitive processing has been shown to lead to instantaneous behavioral adaptation, manifested as functional dissociations in different parts of the medial prefrontal cortex (Rushworth et al., 2007). Such considerations add a new level of complexity to all the factors that should be accounted for while studying the neural correlates of the HPA axis activity. However, to link heterogeneity of the MPFC neural response to variations in the HPA axis activity even prior to stress (as we showed in Chapters 3 and 4) might provide a methodological tool to objectively control the interactions between pre-task factors and task-related cognitive outcomes.

The Neural Correlates of the Self

In our examination of the overall differences in the patterns of neural response to the MIST, we observed significant group differences in patterns of deactivation of the ACC and the precuneus in all functional studies—although it has to be noted that the topography of deactivations was task- and group-related. In Chapter 3, we observed a dichotomous pattern of precuneal and ACC activation in the stress responders and non-responders. In Chapter 4, we observed a linear variation of the ACC and the precuneus with cortisol variables. Moreover, in the young subjects of both functional studies (who extensively deactivated the ventromedial ACC in response to the MIST), we noticed a positive relationship between self-esteem and ACC activity (especially B32). By contrast, in the older subjects who showed significant deactivation of the posterior medial parietal areas, we observed a positive correlation between

perception of other's control and precuneal and posterior cingulate activity. In other words, the higher the self esteem, the higher the BOLD signal in the ACC;, while, the lower the perception of control over the outcomes of one's life, the higher the precuneal activity. Previous studies have shown that self-esteem and the locus of control are important predictors of the HPA axis response to psychological stress (Gruenewald et al., 2004; Pruessner et al., 2005; Pruessner et al., 2004b). A substantial amount of evidence indicates that the ACC and precuneus are important for self-awareness (Gusnard, 2005), self-regulation (Posner et al., 2007) and self-referential thoughts (Cavanna and Trimble, 2006; Ochsner et al., 2005). Hence, differential patterns of ACC and precuneal deactivation in stress responders and non-responders, combined with personality-related modulation of these regions' response to an experimental stress task is noteworthy. Our observations add to the growing body of evidence that personality plays an important role in the neural processing of cognitive and emotional stimuli (Engels et al., 2007; Gray et al., 2005; Kumari et al., 2004; Vrticka et al., 2008). By showing an association between personality factors and stress-related activity in brain regions considered important for processing self-related information, we provide support for the theory that "stress" is an experience tied closely to the individual's perception of threat to self (Dickerson and Kemeny, 2004). Our findings emphasize the need for including personality factors in models that test neural correlates of HPA axis activity.

The HPA Axis and the Energetic Basis of Neural Activity

Because we observed significant group (responder vs non-responder and old versus young) differences in the topography of cerebral deactivations, we focused much of our discussion on the "default mode network" theory. The default mode network theory originated based on evidence indicating consistent

deactivation (i.e. reduced hemodynamic and metabolic activity during a task compared to resting condition) in the medial prefrontal, precuneal and posterior parietal areas in response to goal-directed functions (Raichle et al., 2001). It has to be noted that none of our functional analyses examined resting-state neural activity, which relates to the energetic basis of neural activity in conscious states (Raichle and Gusnard, 2002; Shulman et al., 2004), or the synchronized low-frequency fluctuations in resting state activity of functionally connected networks (Biswal et al., 1997; Fox et al., 2005; Fransson, 2005). Importantly, our reported "deactivations" both in this thesis and the original article (Purssner et a., 2008) are based on subtracting the control condition (mental arithmetic) from experimental condition (mental arithmetic + uncontrollability + social evaluative threat). Therefore, the deactivations do not reflect reduced neuronal activity compared to the unstimulated brain. Rather, they show average BOLD signal differences caused by the social evaluative component of the MIST. As far as the goal of determining neural correlates of stress, this approach is more beneficial than comparing the experimental condition to a true baseline, which would conflate neural response to cognitive, attentional, motor and emotional responses to stress. We have thus interpreted group differences observed in the topology of stress-induced neural activations and deactivations in relation to "baseline" behavioral traits that determine perception and coping. To some extent, the link between activity in these areas and personality variables supports this assumption. However, more precise data recording is needed to dissociate specific components of perception or coping. Nevertheless, that these differences are manifested in the frontoparietal default mode network is remarkable because, increasingly, the activation topology of these areas has been linked to intrinsic states of consciousness (Fox et al., 2006) or behavioral adaptations (Fox et al., 2007), both which are subject to

interindividual variation (Goncalves et al., 2006; Kelly et al., 2008). It is becoming clear that intrinsic fluctuations in the resting brain represent functional organization (Vincent et al., 2007). Recent research by Vincent et al (Vincent et al., 2008) has shown intrinsic correlation in three functionally distinct brain networks: 1) dorsal attention network, including intraparietal sulcus and the junction of the precentral and superior frontal sulcus, involved in attention to the external world; 2) hippocampal-cortical memory network, including hippocampus, retrosplenial cortex, posterior cingulate, inferior parietal lobule and precuneus, related to internally directed mental activity; and, 3) frontoparietal network, which is spatially interposed between the attention and memory system and includes the lateral prefrontal cortex, anterior cingulate cortex, and inferior parietal lobule; it is involved in cognitive control and decision-making. Plausibly, these researchers argue that interactions between the anti-correlated attention- and memory-systems (Fox et al., 2005) are mediated by the frontoparietal system, as it is placed to integrate information from theses systems.

Indeed, our data illustrate the heterogeneity of such default mode network deactivations across different populations doing a similar task (Chapter 4, Supplemental analysis 1). Whereas the control condition of the MIST produces identical patterns of activation in the midline postcentral and cerebellar areas in all three fMRI tests, the extent of deactivation patterns is dissimilar between responders and non-responders in each group. Comparing the average BOLD signal of experimental vs. control conditions further highlights these group differences. For instance, although the old and the young non-responders showed similar patterns of deactivation in the precuneal and ventromedial prefrontal regions when doing simple math (i.e. control condition compared to

baseline), they had different patterns of deactivation when presented with stressful task. In response to stress, the young non-responders further deactivated the ventromedial prefrontal area—albeit less strongly than responders, while the old nonresponders deactivated the postcentral and cerebellar regions (which were activated when doing simple math.) Deactivation of postcentral and cerebellar regions, which was more pronounced in the older non-responder than responder subjects, might indicate that, when presented with the added challenge of stress, or the verbal instructions, the older subjects simply stopped trying! This interpretation is consistent with the notion that motivation to achieve a certain goal and level of task engagement predicts the HPA axis stress response (Dickerson and Kemeny, 2004; Lazarus, 1993; Tops et al., 2006). Deactivation differences in the responders of each young group are also noteworthy. Young responders in both studies had significantly more deactivated prefrontal brains than nonresponders. However, the young responders in the first study also demonstrated deactivated dorsomedial and precuneal regions. One explanation for this might be differences in experimental paradigms tested on these groups: in study 1, the young subjects were given a picture-encoding task, and anticipated an upcoming recognition test. This was not the case in study 2 or in the older group.

While the behavioral substrates of these observed differences are complex, to detect them objectively based on variations in the HPA axis profile offers a methodological approach to investigating the neurophysiologic bases of interindividual variations in behavioral adaptation, of which 'stress' is only one manifestation. In fact, the parametric statistical analysis performed in Chapter 4 appears to better capture the covariations of neural activity of a set of cortical and subcortical regions with different HPA axis activity indices: the total

amount of cortisol present during measurement of the BOLD signal, and changes in cortisol levels with respect to individual's baseline. Although these variables were correlated, only 22% of variance in these variables is shared. Thus, the AUC_{total} and $AUC_{increase}$ might explain dissociable variances in the neuronal activity. One advantage of using such compound variables is that they do not rely on a predicted model of stress response (e.g. an expected increase in cortisol circulation lagging with respect to the onset of stress). In that sense, irrespective of "stress," per se, these variables can serve as objective biomarkers explaining, at least partially, some of the interindividual heterogeneities in neural activity. For example, we showed that in older subjects (who did not show a large neuroendocrine stress response) the AUC_{total} modulated neural activity in the prefrontal regions, even though those regions were not activated or deactivated by the task. Cortisol variables also modulated the neural signals in parietal areas such as precuneus (important for self-referential thought) (Cavanna, 2007), inferior parietal lobule (important for perception of the other) (Raffi and Siegel, 2007; Rizzolatti et al., 2006; Uddin et al., 2006; Vickery and Jiang, 2009), as well as insular and opercular areas (associated with visceral awareness) (Critchley et al., 2004). These brain regions form a network that represents intrinsic conscious activity (aimed at maintaining the individual's physiological and psychological stasis) that is not related directly to sensory or motor events (Raichle and Snyder, 2007). Because the HPA axis is also charged with maintaining homeostatic stability through changing environments, these correlations are significant as they demonstrate the coupling between the HPA axis and the default-mode network activity.

Although variations in states of mind are important when considering adaptive behavioral responses to stress, it is equally important to consider the metabolic

effects of unbound cortisol on energetic bases of default neuronal function (Rothman et al., 2003; Shulman et al., 2002). To date, research on the neurophysiology of neuronal activity has shown that a large proportion (> 80%) of energy consumed by the brain (20% of body's total energy consumption) is provided by glycolysis that leads to oxidative phosphorylation (Raichle and Mintun, 2006). Although oxidative phosphorylation is the main source of adenosine triphosphate (ATP) energy units, glycolysis (following glucose production from glycogen, and pyruvate production) provides energy much more rapidly than do than oxidative processes—especially in the astrocytes that are important for glutamate recycling (Pellerin and Magistretti, 2004). Because the brain has limited storage, it has been suggested that the limbic-HPA axis pathway functions to send a 'glucose signal' when the metabolic demands of cerebral activity exceed available resources (e.g. in the presence of challenging tasks) (Peters et al., 2004). The HPA axis activity is followed by increased cortisol secretion that crosses the blood-brain barrier and increases glucose allocation. At the same time, this glucose allocation is controlled via interactions of MRs and GRs that stabilize the deployment of energy resources to the brain as well as the rest of the body (Peters et al., 2004). The role of MRs and GRs in mediating glutamate signaling is most extensively studied in the hippocampus (Joels, 2008), where default intrinsic activity is linked to states of vigilance and monitoring (Gray and McNaughton, 2003; McNaughton, 1997). However, to the best of our knowledge, the relationship between corticosteroid receptor activity and the behavioral and metabolic substrates of resting state activity have not yet been examined. Our observations, combined with other reports of cortisol-related variations in glucose metabolism (de Leon et al., 1997; Kern et al., 2008) and cerebral blood flow (de Quervain et al., 2003; Liberzon et al., 2007; Wang et al., 2005) in areas known for their default

intrinsic activity, supports the theory that the HPA axis (especially cortisol) is linked to metabolic control of the CNS. Although research has established that glucose metabolism accounts for most of energetic requirements of cerebral activity, the relationship between glycolysis, oxidative phosphorylation and hemodynamics of neuronal activity remains elusive. Determining a physiological baseline is critical in interpretation of the BOLD signal (Shulman et al., 2007), especially since evidence is emerging that physiological baselines determine the amplitude of the BOLD response (Pasley et al., 2007). In that sense, our findings offer incentive to consider the profiles of HPA axis activity (which can be easily and non-invasively measured using saliva) as a proximal index of baseline physiological state in models that examine the physiological basis of neuronal activity.

The Shortcomings of the MIST

So far, we have discussed the perspective offered by the analysis of data collected using the MIST. From this vantage point, several limitations of the MIST become visible, helping us to address them in future.

Let us dissect the elements designed in the MIST:

a) The version of the MIST offered a block-design paradigm consisting of
b) The baseline block: look at the task interface without taking any action;
c) The control block: perform mental arithmetic; use a button to select the correct answer on the visual dial; press a second button to select the answer; observe feedback on screen (correct, incorrect, timeout)
d) The experimental (stress) block: perform mental arithmetic, which becomes incrementally harder (more operands and more division/multiplication operators) if subject performs well; try to

memorize words appearing in a box (although the subjects do not know that this is a distraction designed to increase pressure); be able to see a time bar showing how fast the subject is running out of time, use a button to select the correct answer on the visual dial; press a second button to select the answer; see feedback on screen (correct, incorrect, timeout), keep an eye on performance bar (which would lower the visual score if an error is made) and try to keep performance high enough to be "acceptable."

e) In addition, the subjects are given verbal instructions to keep improving their performance in order for their participation to be "useful" to the study.

To some extent, the experimental condition of the MIST conflates several behavioral components to accomplish the objective of inducing a cortisol stress response by challenging the participant's ego when he or she performs an uncontrollable, mentally challenging task under social evaluative threat. One limitation of the MIST is that it is blind to individual's motivations, which would affect their appraisal of a task in relation to personal goals, and which account for variations in biological responsiveness to stress (Dickerson et al., 2004; Dickerson and Kemeny, 2004; Gaab et al., 2005; Lazarus, 1993; Riolli and Savicki, 2003). This also makes the interpretation of the functional brain maps ambiguous. For instance, the ACC—which seems to be predominantly associated with different aspects of HPA axis stress regulation—produces distinct neural responses to error detection (Carter et al., 1999; Hajcak et al., 2004), which depends on reward (Amiez et al., 2006; Kennerley et al., 2006), punishment (Taylor et al., 2006) or motivation (Tops et al., 2006; Walton et al., 2003). However, we are currently unable to dissociate the behavioral significance of correlations we observe in the ACC. Of course, it is virtually

impossible to design a task that accounts for individual differences in motivation. However, controlling for selective attention of the participants to elements such as reward, punishment, fear, or shame would help in interpretation of the prefrontal activity that, as we observed, interacts with the HPA axis function.

Furthermore, our results suggest that pre-MIST states of cognitive activity alter baseline endocrine and neural activity, which would predict later neuroendocrine response to stress. Hence, inter-subject comparability of results produced by the MIST might depend on highly controlled protocols that ensure minimal variation in experimental design. For example, in the studies presented in Chapter 4, we illustrated significant age-group differences in neural and HPA axis activity in response to an identical block-design MIST protocol. However, several factors such as the pre-stress experience (e.g. performing a memory task before MIST, or participating in TSST, thus being aware to the "deceptive" design of the MIST); novelty of scanning session, MIST interface (e.g. the word distractor that may have attracted attention of the older subjects who were recruited for a "memory and aging" study, thus cared more about the well-being of their memory than arithmetic skills) or even interaction with different examiners (e.g. young examiners being less intimidating with the older subjects) might have contributed to behavioral or perceptual differences manifested in heterogeneous neural and endocrine response patterns. Therefore, the MIST must be considered a highly subjective paradigm that depends on myriad factors, from the subject's motivations or mental states, to his or her interactions with examiners. A subjective rating of pre-, and peri-stress experience, plus assessment of personality factors are essential to untangle the heterogeneities observed in our experiments (especially between the old and the young groups).

Finally, as discussed earlier, the HPA axis activity supports adaptive adjustment to metabolic demands of stressors. Therefore, in examining neural correlates of stress, it is important to test both the behavioral and the physiological basis of brain activity. In its current form, the MIST does not allow for proper measurement of baseline states and intrinsic fluctuations in neural activity. It would be very interesting, for example, to compare the pre- and post-MIST resting state BOLD signals; or to compare the functional connectivity of different default mode networks prior to and after MIST application. Such comparisons might be informative in delineating the neural correlates of HPA axis activity in relation to psychological stress.

A New Region of Interest

To date, research on the neural correlates of HPA axis activity has been dominated by an interest in the hippocampus—and justifiably so. The role of the hippocampus in HPA axis regulation is well established. The hippocampus has a high concentration of corticosteroid receptors. Over four decades of research has shown that activation of these receptors affects function and morphology of the hippocampal neurons in different subfields. More practically, the hippocampus is a structurally distinct region that can be easily delineated even from standard MRIs. However, the current analyses conducted using fMRI data indicate that the neurophysiology of stress needs to be examined in distributed networks. Nevertheless, variations observed in model-free networks might be hard to interpret, therefore it is still important to define a seed or a target region based on existing theories to test specific hypothesis against. We propose the ACC as such a region of interest. As Figure 1 illustrates, almost all of HPA axis-related variations are significantly mapped onto the ACC. Of

course, as Figure 1 shows, significant variations in spatial distribution of the observed effects exist. Nevertheless, we might discern an important trend: The dorsal regions of the ACC show positive correlation with HPA axis activity (higher BOLD associated with higher cortisol amounts in the older subjects, depicted in orange; higher BOLD in cortisol responder subjects during encoding, depicted in green). By contrast, the more rostroventral parts are correlated with HPA axis stress response (lower BOLD associated with higher rostroventral ACC in the young, depicted in red and magenta; lower BOLD response to recognition in cortisol responders in blue; lower BOLD associated with lower self-esteem in cyan). The picture that emerges from this figure suggests a possible anti-correlation between the dorsal and ventral parts of the ACC in relation to HPA axis control. This anti-correlation, or dichotomous functionality of the dorsocaudal and rostroventral parts of the ACC, has been previously reported (Bush et al., 2000) and is linked with emotional and cognitive interactions (Simpson et al., 2001a; Simpson et al., 2001b). Here, we can infer that increased HPA axis activity was associated with greater activation of the dorsocaudal or the "cognitive" part of the ACC during encoding in stressed subjects, or during mental arithmetic in older subjects. In line with theory proposed by Fehm and Peters (Peters et al., 2004), we might postulate that higher cortisol mobilization is aimed at meeting the metabolic demands of the cognitive tasks that are likely to be harder for the older or stress-sensitive subjects. On the other hand, the rostroventral or the "emotional" part of the ACC seems to be involved in negative feedback inhibition of the HPA axis stress response; this is consistent with Herman's theory that the ACC projections innervate the GABAergic PVN neurons that put a break on HPA axis activity (Herman et al., 2003). This summary picture thus calls for a careful examination of the interactions between the dorsocaudal and ventromedial

parts of the ACC on balancing the HPA axis function.

Moreover, we observed a significant negative correlation between the CAR and the left pregenual ACC (depicted in white), with striking resemblance to several animal models of chronic stress or HPA axis dysregulation. What made this observation interesting was that the CAR predicted robust age- and sex-independent variations in the ACC; however, the same was not true for the hippocampus. This finding also highlighted the importance of considering the role of the HPA axis in neuronal reconfiguration, rather than simply seeking its neurotoxic effects in the GR/MR-rich hippocampus.

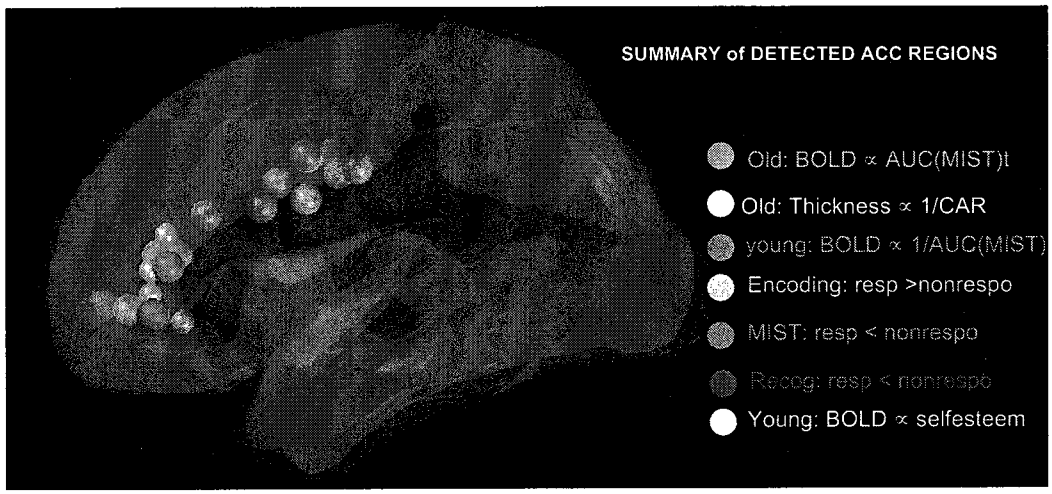

Figure 1: The ACC peaks that were significantly related to stress BOLD response variations predicted by HPA axis activity. The balls correspond to the highest peak of identified clusters (at least 200 contiguous voxels), statistically significant after correction for multiple comparisons.

Although lack of appropriate behavioral data impedes our interpretation of the functional anatomy depicted in Figure 1, the significance of the ACC in the context of behavioral adaptation deserves attention.

First, it is plausible that the link between the ACC and the HPA axis is

mediated by epigenetic factors. The ACC is a multisensory area that processes input from the visual, auditory, olfactory, tactile, nociceptory and sensorimotor areas (Banati et al., 2000; Coghill et al., 1994; de Araujo et al., 2005; Kawashima et al., 1999; Koski and Paus, 2000; Kuo and Yen, 2005; Paus et al., 1991; Paus et al., 1993). Also, the ACC plays an essential role in autonomic, affective and cognitive adaptation (for review see Botvinick, 2007; Bush et al., 2000; Critchley et al., 2003; Paus, 2001). Focusing on the critical role of the ACC in self-regulation, Posner and colleagues have proposed that the ACC plays an important role in neural development (Posner et al., 2007). The unique cytoarchitecture of the ACC, characterized by large spindle neurons in layer Vb of the more anterior region of the Brodmann area 24 (Nimchinsky et al., 1995), corroborates this proposed developmental theory. Spindle neurons are unique to humans and great apes, and their average size and number grows relative to brain size and functional evolution (Nimchinsky et al., 1999). The larger size of the neurons might reflect the increased arborization and axonal connectivity associated with higher intelligence (Allman et al., 2001). In humans, the spindle neurons can be discerned four months after birth; therefore, they have been linked to the functional maturation of the ACC (Allman et al., 2002). Moreover, neuroimaging evidence that the metabolic activity of the ACC increases from childhood to adulthood (Van Bogaert et al., 1998) confirms the role of this structure in brain maturation. Additional evidence exists for the morphological association of the ACC with IQ scores in developing children (Lerch et al., 2006) based on early life stress events (Cohen et al., 2006), perception of self's social standing (Gianaros et al., 2007a), and with personality traits such as harm avoidance (Pujol et al., 2002). Emerging theories suggest that epigenetic factors such as early life experience, environment, and personality traits are linked to HPA-axis programming that occurs early in development, but sets the stage for

a lifetime of behavioral adaptation to stress (Meaney et al., 2007; Szyf et al., 2007). Therefore, the epigenetic link between the ACC and the HPA axis activity deserves investigation.

Secondly, several functional features of the ACC make it a good candidate for testing the neural basis of psychological stress. One is the phenomenon of error-related negativity (ERN; a transient event-related negative potential observed in the ACC activity when subject makes an error or detects one; (Falkenstein et al., 2000; Gehring et al., 1995). Thus, similar to the hippocampus (McNaughton, 2006), the ACC detects response conflict (Botvinick et al., 2004; Braver et al., 2001; Carter et al., 1998), be it a conflict generated by competing responses (Yeung et al., 2004), by feedback on an error (Luu et al., 2003), or a conflict between expected and actual outcomes (Gehring and Fencsik, 2001). In this way, the ACC triggers a strategic adaptation of the cognitive control system based on cost-benefit analysis and reward-action evaluation in goal-directed decision-making (Kennerley et al., 2006; Rushworth et al., 2004; Taylor et al., 2006; Walton et al., 2003; Walton et al., 2004; Yarkoni et al., 2005a). This well-established function of the ACC assists in designing experiments for assessing the impact of goal engagement in relation to personality and HPA axis reactivity to stress—as recently shown by (Tops et al., 2006).

Moreover, ACC lesion studies have shown that the ACC modulates the HPA axis activity at several levels of connection to the amygdala, hippocampus and visceral areas such as the hypothalamus and brainstem (Feldman and Conforti, 1985; Feldman and Conforti, 1987; Feldman et al., 1995). Lesion studies (Ballantine et al., 1987; Cohen et al., 1999; Cohen et al., 2001; Jenike, 1998; Richter et al., 2004), as well as neuroimaging research (Chang et al., 2004;

Kitayama et al., 2006; Maltby et al., 2005; Pizzagalli et al., 2006; Rauch et al., 2003; Stadler et al., 2006), have provided substantial evidence for the influence of the ACC on behavior and affective disorders. In humans, neuronal dysfunction in the anterior cingulate and the medial prefrontal cortex is associated with mood and affective disorders such as depression, anxiety and post-traumatic stress that also involve HPA dysregulation (Carey et al., 2004; Drevets, 1999; Kumari et al., 2003; Liotti et al., 2002; Vermetten and Bremner, 2002). Of course, the ACC is not a single, homogeneous structure. Using neuroimaging, functional subdivisions of the ACC are described in terms of differences in effective connectivity to the rest of the brain (Koski and Paus, 2000). As discussed above, our results hint that the affective (rostroventral) and cognitive (dorsocaudal) subdivisions of the ACC (Bush et al., 2000) might exert differential control on the HPA axis activity, perhaps depending on the context in which a stressor is presented or perceived. Our current findings encourage an inquiry into effective connectivity between these ACC subregions and the rest of the brain to better understand the neural basis of interindividual variations in HPA axis activity.

Overall, the link between the HPA axis function and the ACC task-related function, intrinsic activity, morphology, or connectivity might constitute a methodological approach to objective quantification of interindividual variability in stress reactivity. As new trends in the network properties of behavior emerge, we can examine possible aberrations in networks that are functionally or structurally linked to this 'stress ROI', in relation to disease, genes, or other epiphenomenal factors.

A Proposal for Future Research

In the conclusion of *Stress of Life*, Selye writes, "For our scientific research in the laboratory we need an operational definition of stress, that is, one which shows us what to do in order to see stress." (Selye 1956, p273) To some extent, this research has been successful in showing *in vivo* an operational state of a brain under stress. The picture of stress seen through the lens of the neuroimaging methods applied here is plausible, as it points out significant correlations between the MPFC, the hippocampus and the HPA axis activity. As important as these correlations are, we cannot interpret the relationship between the hippocampus or MPF and the endocrine stress response to be a causal one. Clearly, not only these structures, but several other brain regions (such as precuneal and posterior cingulate regions) can constitute the differences observed in brain activity of stress responders and non-responders. When splitting data in our three different studies based on cortisol increase during the period of MIST administration, we found clear differences in extent of task-related deactivations. However, patterns of deactivation for stress-responders and nonresponders of different groups were not overlapping. These observations clearly indicate that binary categorization of subjects to groups does not fully capture the nuances of neurological substrates of stress. Then, what have we learned from the "phenomenological" outlook in this research?

Overall, the results of both correlational and group analyses in this study indicate that cortisol stress response is linked to brain regions known for states of awareness. In our interpretations we have drawn links between baseline HPA axis function and baseline CNS activity that are important for maintenance of organism's stability within changing physiological and psychological environments. In providing evidence for involvement of the ACC and the

hippocampus in HPA axis regulation, we have discussed the literature that links these structures to general states of arousal, environmental monitoring and adaptability that perhaps determine an organism's sensitivity in terms of 'perceiving' and 'reacting' to a stressor. Figure 2 depicts a schematic representation of our interpretations.

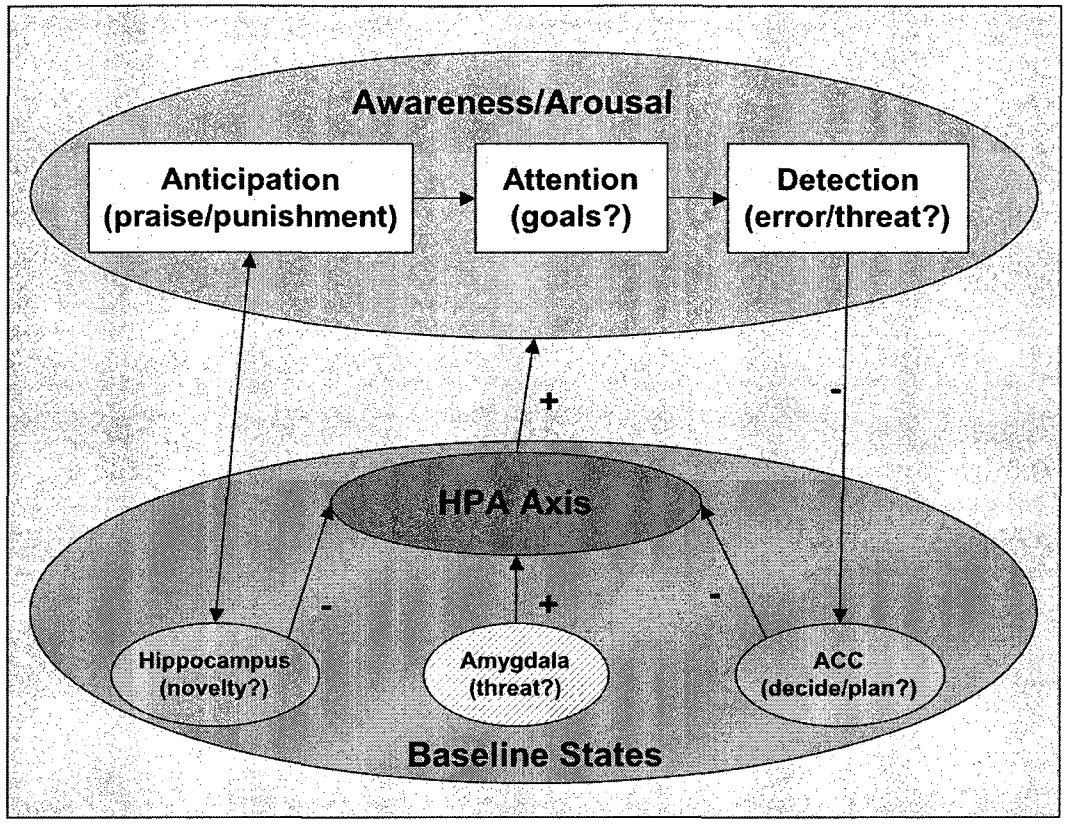

Figure 2: A schematic representation of possible interactions between neural and perceptual factors that affect the HPA axis function. Interindividual variations in sensitivity to stress are introduced by differences in anticipation, goals and differences in attentional biases (e.g. emotional or cognitive attention) and perception of sources of threat to those goals. In the case of anticipated stress, the hippocampus and the ACC play a role in integrating perceptual signals and mediating individual-specific behavioral adaptations. The ACC and the hippocampus are considered important for negative feedback inhibition of the HPA axis stress response; while the amygdala is more important for excitation of the HPA axis. Altogether, the baseline states of arousal are likely to influence the thresholds of activation or deactivation in these brain structures, which will lead to variations in stimulation or inhibition of the PVN neurons that initiate the HPA axis activity.

A model that aims to predict stress-sensitivity in different groups needs to account for several factors. One of the main predictors of a psychoneuroendocrinological stress response is "anticipation." Therefore, the first cause of interindividual variance in stress reactivity is whether they "anticipate" a negative outcome that threatens the goal of self-preservation. Here, the second source of variation appears: "goals". For instance, "memory" might be a more important goal for an older population who anticipates the negative aspects of age-related cognitive impairment; while competency in arithmetic skills might be more relevant to young college students under peer pressure. The goals also determine how individuals "pay attention", "make decisions" and plan and execute "actions". Whereas it is plausible to assume similar goals will direct attention and decision similarly, it is important to consider that individuals vary in the resources that are available to them. Variations in availability of resources can lead to variations in behavioral adaptation and coping. If we consider the physiological function of the HPA axis: providing "adaptive" metabolic support to an organism under stress, then all these factors (anticipation, goal, attention, decision, coping) might stimulate the HPA axis at different stages of "stress processing". In this regard, it is perhaps more relevant to ask "how" the brain initiates a stress response—rather than "which" anatomical structures activate to stress.

Recently, neuroimaging research has been moving beyond "where" (i.e. localization of a function of interest in the brain), and has been taking an interest in "how" the brain generates a hemodynamic signal. This approach depends on biophysical models of neurovascular coupling, plus models of functional integration that consider context-dependent interactions among functionally (and anatomically) related brain regions (Friston et al., 2006;

Friston, 2005). The strength of such models depends on an objective, easily measured biometric index of energy coordination for the brain (i.e. the HPA axis activity in baseline and in stimulated conditions). Hence, even beyond the scope of stress research, considering the HPA axis profiles might be valuable in constructing models that examine the "how" of neural responses. At the same time, examining the topology of neural networks that interact with the HPA axis might help understand the causes of interindividual variations in stress sensitivity—or cognitive and emotional regulation in general. Such objective methodology would complement psychometric assessments that are subject to demographic, cultural, or even linguistic variations, and which are not easily reducible. We propose that variations in the HPA axis encode variations in activity states of intrinsically connected attentional, emotional and memory networks. To understand which network enforces "eustress," and which is bogged down by "distress," would advance our understanding of stress. This could help us devise preventative or therapeutic strategies that alleviate the high costs associated with the inevitable stress of daily life today.

Conclusion

The research presented in this thesis aimed at two main objectives. First, we wanted to test whether the MIST could reveal the neural correlates of psychosocial stress. We noticed that the heterogeneity of activation and deactivation patterns in the MIST were linked to variations in HPA axis stress response. From these observations we learned the importance of careful measurement and control of the baseline states (behavioral and physiological) which appear to influence the amplitude of the neural and the HPA axis responses.

Our second aim was to identify an objective index that could best characterize the variances in relation between the brain and the HPA axis activity. We showed a robust coupling between cortisol and the ACC (both functional and structural), which can be further explored to identify interindividual variations in vulnerability to adverse stress effects.

Beyond the scope of stress research, our observations offer neuroimagers a methodology to measure an index of interindividual variations in physiological adaptation marked by HPA axis fluctuations (easily measured from cortisol); while offering neuroendocrinologists a methodology to investigate the interindividual variation in the topology of neural networks that interact with the neuroendocrine system. Such methodology complements psychometric assessments that are subject to factors such as emotional states or even cultural and linguistic variations, which are not easily reducible.

The most important conclusion of this study is that the neural correlates of stress need to be examined in an integrated fashion, where interactions of psychology, physiology and the world outside of one's self or control are well accounted for. Stress does not occur in one brain structure; rather it reflects the sum total of brain networks adaptations. These networks remain to be tested with more advanced neuroimaging methodologies.

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ETHICS APPROVALS