CORTISOL AND HIPPOCAMPAL INTEGRITY IN A YOUNG HEALTHY POPULATION: PSYCHOENDOCRINOLOGICAL AND NEUROIMAGING FINDINGS

DISSERTATION

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vorgelegt von:

DIPL. PSYCH. MARITA PRÜßNER

betreut von :

PROF. DR. DIRK HELLHAMMNER

PD DR. FRIEDEMANN GERHARDS

PROF. DR. SONIA LUPIEN

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Outline

CHAPTER I:

ADVANCED ORGANIZER

The hypothalamus-pituitary-adrenal (HPA) response to psychological and physiological challenges is regarded as essential to the body's adaptation to stress, maintaining homeostasis by a number of metabolic actions. However, there is considerable evidence for a dysregulation of the HPA axis in aging and various clinical conditions like Alzheimer' s disease, Cushing's disease, depression and chronic stress, mainly characterized by chronically elevated glucocorticoid (GC) levels. Furthermore, animal and human data have demonstrated a particular sensitivity of the hippocampal (HC) formation to elevated GC levels, resulting in cognitive impairment and - when chronically increased as in the above conditions – even in HC atrophy. Little is known about the relationship between HPA activity and functions related to HC integrity like mood, cognition, HC volume and function in young and healthy populations.

In patients and elderly subjects, hippocampal volume is regularly assessed with magnetic resonance imaging (MRI). In young subjects functional MRI has proven effective to determine HC activation in experiments testing explicit and relational memory. Interestingly, structural MRI correlates of HPA function in young healthy subjects are lacking, and functional MRI designs have not been applied in association with HPA activity in either group.

Consequently, the aim of the present work was to investigate the association between cortisol levels and depressive symptomatology, cognition, and hippocampal integrity in young healthy subjects, with a special emphasis on MRI and fMRI derived measures of hippocampal volume and function. Furthermore, it was attempted to identify possible early markers for the frequently observed conditions related to HPA dysfunction in later life.

Chapter 2 outlines current literature describing normal and pathological function of the HPA axis. Special emphasis is put on anatomical and functional features of the hippocampus, as the most prominent brain structure affected by dysregulations of the HPA axis. Furthermore, structural and functional magnetic resonance imaging are introduced as important methods to assess HC volume as well as changes in HC

activation in response to specific stimulation tasks, and their actual applications in the field of Psychoneuroendocrinology are evaluated. Considering this literature, a rational for the research conducted in this thesis is developed.

Chapter 3 describes the results of a first study examining the association between cortisol levels in response to awakening and depressive symptomatology as well as chronic stress in 40 young healthy male university students[1]. In order to examine possible associations between our psychometric test results, cortisol levels, and HC volume and function in this young population, a subgroup of 13 subjects entered the subsequent studies and underwent structural and functional MRI scanning.

Chapter 4 examines the association between hippocampal volume and HPA activity in young healthy subjects. A structural MRI study was performed with the subgroup of 13 subjects. Left and right HC volume were determined manually for each subject employing a newly developed segmentation protocol. Associations between HC volume, explicit memory performance and the cortisol response to awakening as well as to cortisol levels in response to the Trier Social Stress Test (TSST) were investigated.

Chapter 5 investigates stress effects on hippocampal activation during picture encoding. Twelve subjects of the above subgroup underwent functional MRI scanning. Here the "Trier Mental challenge Task" (TMCT) was adapted to the requirements of an fMRI environment. Brain activation during novel picture encoding before the TMCT was compared to activation during encoding after the TMCT. Cortisol was measured before and after the scanning procedure. Thus far, hippocampal activation had not been studied in association with stress.

Chapter 6 provides a general discussion of the three studies and suggestions for future research.

[1] For analyses in subsequent studies, this first study also assessed the cortisol response to the Trier Mental Challenge Test (TSST) in all subjects. Furthermore, in order to assess the effects of stress on memory function, explicit memory was tested before and after the TSST. Results are reported for the subgroup of 13 subjects in association with hippocampal volume (chapter 4).

CHAPTER 2:

THEORETICAL BACKGROUND AND FORMULATION OF HYPOTHESES

2.1 BASICS OF HIPPOCAMPAL MORPHOLOGY AND FUNCTION

The hippocampus (HC) is regarded as a key structure when investigating GC mediated behavioral and brain changes. It is crucially involved in glucocorticoid mediated feedback of the HPA axis (Jacobson & Sapolsky, 1991), and, when looking at effects of chronic exposure to elevated GC levels, it is a frequently targeted structure in magnetic resonance imaging (MRI) studies (O'Brien et al., 1996; Sheline et al., 1996; Lupien et al., 1998; Starkman et al., 1999; Sapolsky, 2000). Furthermore, it has been demonstrated that the HC is associated with specific cognitive processes (Scoville & Milner, 1957; Milner, 1972; Zola-Morgan et al., 1986; Eichenbaum et al., 1996; Maguire et al., 1996; Schacter et al., 1996; Stern et al., 1996; Fernandez et al., 1998; Lepage et al., 1998; Eichenbaum, 1999a, b; Henke et al., 1999), and that both elevated GC levels and hippocampal damage compromise mood (Krishnan et al., 1991; Stokes, 1995; Sheline et al., 1996; Bremner et al., 2000) and hippocampus dependant memory function (Scoville & Milner, 1957; Milner, 1972; Starkman et al., 1992; Lupien et al., 1994; McEwen & Sapolsky, 1995; Lupien et al., 1998; Lupien et al., 1999).

The present chapter provides an introduction in basic HC anatomical and functional features. Aim of the anatomical section is to provide a better understanding of the HC components and localization in the brain when consulting basic research findings and especially MRI and functional MRI results. Furthermore, the integration of the HC within the limbic system and its numerous connections to different brain structures are outlined with the goal to better appreciate the involvement of the HC in various emotional and cognitive states.

2.1.1 ANATOMY OF THE HIPPOCAMPUS

The hippocampus (HC) is a bilaminar formed structure, located symmetrically in the medial temporal lobes of both brain hemispheres. It stretches in a s-shaped scroll along the full length of the floor of the inferior temporal horn of the lateral ventricle. The

hippocampal formation comprises the subiculum, hippocampus proper, the dentate gyrus, the fornix, and the fimbria (Kandel et al., 1991; Fitzgerald, 1996). Anatomically, the HC is divided in four different CA (cornu ammonis) regions. CA1, CA2 and CA3 areas comprise many different afferent and efferent connections to other parts of the hippocampal formation, fornix, entorhinal and association cortex. The HC has three major excitatory afferent pathways. The perforant pathway runs from the subiculum/entorhinal cortex to the granule cells in the dentate gyrus. The mossy fiber pathway, a bundle of axons from the granule cells run to pyramidal cells in the CA3 region. The Schaeffer collaterals, excitatory collaterals from the pyramidal cells in the CA3 region, connect to the pyramidal cells in the CA1 region (Kandel et al., 1991).

For the purpose of volumetric analysis with MRI, the HC can be subdivided in an anterior part, which is referred to as HC head (HH), a medial part, often referred to as HC body (HB), and a posterior part or HC tail (HT). Figure 2.1 illustrates the anatomy of the hippocampus.

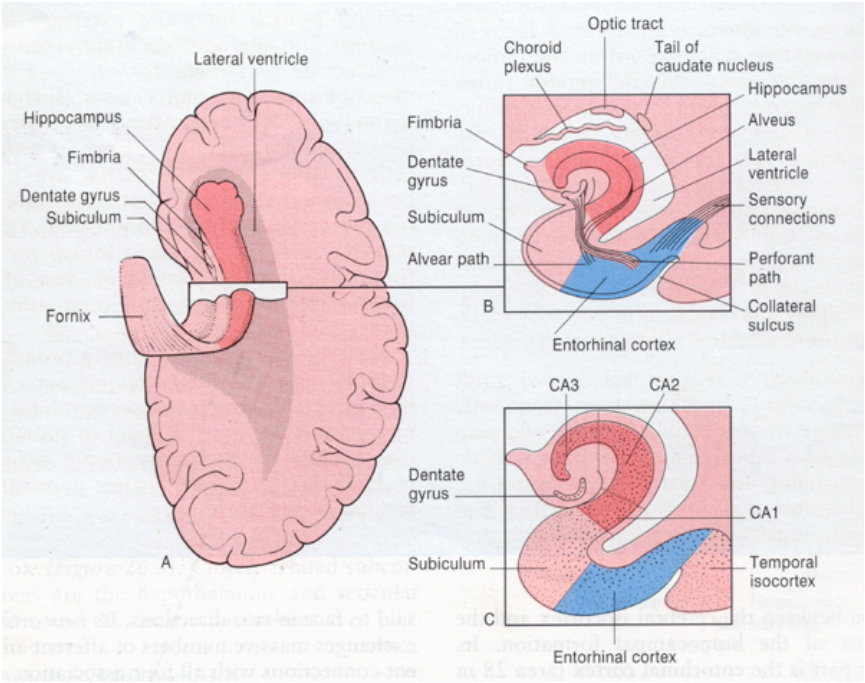

Figure 2.1 Anatomy of the hippocampus [2]

[2] Reprinted from Fitzgerald (1996), Neuroanatomy, 3rd edition, page 260, with permission from Elsevier

The HC is part of the limbic system which plays an important role in learning, memory, and emotion (Kupfermann, 1991a, b; Herman et al., 1996). It comprises the limbic cortex (hippocampal formation, parahippocampal gyrus, cingulate gyrus, and insula) and related subcortical nuclei (amygdala, hypothalamus, reticular formation, nucleus accumbens). Cortical areas closely related to the limbic system are the orbitofrontal cortex and temporal pole (Fitzgerald, 1996).

The parahippocampal gyrus connects the cerebral cortex and the hippocampal formation. The anterior part of the PG is the entorhinal cortex, which exchanges numerous afferent and efferent connections with the association areas of the neocortex as well as with the HC. Information form the neocortex (association area) is channeled through the entorhinal cortex to the hippocampal formation for consolidation. Long-term storage again takes place in association areas. Figure 2.2 shows the different parts of the limbic system.

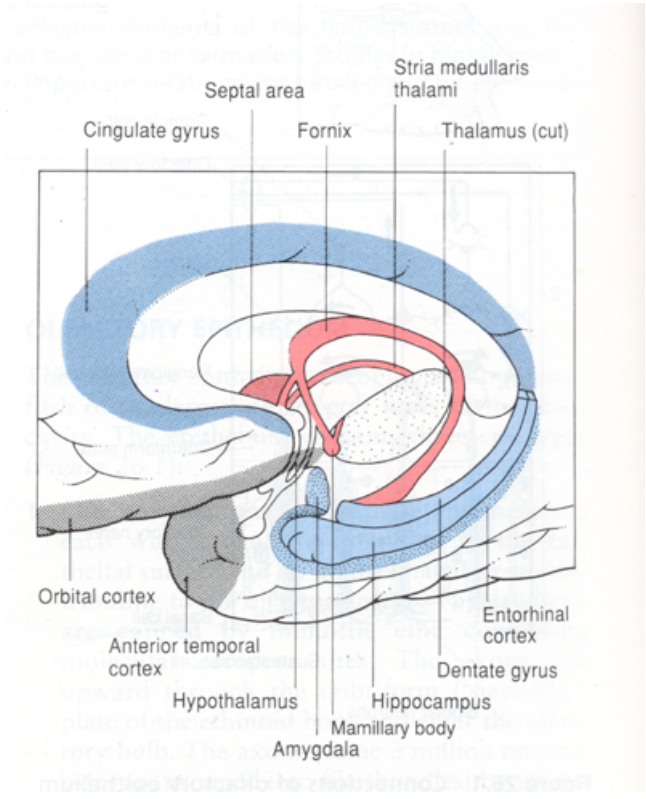

Figure 2.1 Pathways of the limbic system[3]

[3] Reprinted from Fitzgerald (1996), Neuroanatomy, 3rd edition, page 258, with permission from Elsevier

2.1.2 THE HYPOTHALAMUS-PITUITARY-ADRENAL- (HPA-) AXIS

The HPA axis is one of the most well-known hormonal regulation systems. Hypothalamic neurons release corticotrophin releasing factor (CRF), which is generally considered to be the major factor responsible for the secretion of adrenocorticotrophin (ACTH) from the pituitary (Martin, 1992). Adrenocorticotrophin subsequently triggers secretion of glucocorticoids from the adrenal glands. In humans, this final product of the HPA axis is cortisol. Under basal conditions, secretion of cortisol underlies a circadian rhythm with maximal concentrations in the early morning and a continuous decrease over the course of the day (Van Cauter & Refetoff, 1985; Naylor et al., 1988; Van Cauter, 1989).

Physical challenges or psychological threats can result in cortisol levels ten times higher than basal cortisol levels (von Faber & Haid, 1995). Cortisol therefore is considered a "stress hormone". Both physical challenges like a marathon run or stressful psychological situations like a perceived threat can lead to a stimulation of cortisol secretion. Different conditions found to cause elevated cortisol levels are reviewed by Kirschbaum and Hellhammer (Kirschbaum & Hellhammer, 1989, 1994). Already in 1968, Mason defined characteristics of situations that stimulate the HPA axis. The most important variables found were novelty, anticipation, ambiguity and lack of controllability (Mason, 1968). Not only acute but also chronic stress has been shown to be associated with increased basal cortisol levels in both animal studies (Ottenweller et al., 1994; Gomez et al., 1996) and human studies (Ockenfels et al., 1995; Wust et al., 2000; Pruessner et al., 2003).

Physiological changes observed under stress include a direction of energy to muscles including mobilization of stored energy, inhibition of further energy storage and gluconeogenesis (Sapolsky et al., 2000). A higher vascular tone ensures better energy transport to muscles. Immune function is stimulated initially, maybe for enhanced resistance to infections. After the first wave of the stress response, higher GC levels

contribute to a suppression of immune function and prevent it from overshooting (Munck & Guyre, 1986; Sapolsky et al., 2000). Because of its immunosuppressive and anti-inflammatory characteristics, the synthetic derivates of cortisol are used for treatment of autoimmune diseases (Andersson & Goodkin, 1998; Neeck, 2002).

Glucocorticoid actions are exerted through two types of receptors, which show the highest density in the HC and differ in both their affinity for GCs and their cellular effects (Reul & de Kloet, 1985). Mineralocorticoid- or Type I receptors (MRs) have a limited distribution, and the highest amounts are found in the hippocampus. They have a tenfold higher affinity for GCs than glucocorticoid- or Type II receptors (GRs) and are already occupied to a large extent at baseline conditions and at the trough of the circadian rhythm, which is the time of day with the lowest GC levels. Glucocorticoid receptors are widely distributed in the CNS and are also present in amygdala, hypothalamus, cerebral cortex and most brainstem monoaminergic nuclei. Additional occupation of the lower affinity GRs is observed at the peak of the circadian rhythm and after exposure to a stressor (Reul & de Kloet, 1985; de Kloet et al., 1999).

Both under basal and stress conditions, the HPA axis is sensitive to feedback inhibition by circulating GCs. Negative feedback is exerted on all areas of the HPA, resulting in inhibition of CRF and ACTH secretion from the hypothalamus and pituitary. Because of its high content of both MR and GR receptors, the HC is a major target site for glucocorticoids and plays another important role in feedback regulation (Jacobson & Sapolsky, 1991). Apparently, there is a MR specificity for the inhibition of basal HPA activity, whereas stress induced or circadian peak HPA responses are strongly related with occupation of the lower affinity GR receptors. Hippocampal inhibitory effects on basal and stress-induced HPA activity can be observed at all levels of the HPA axis. Reduced ACTH secretion from the hypothalamus appears to be mediated by hippocampal effects on CRF, vasopressin, and oxytocin (Jacobson & Sapolsky, 1991). Also, cortical areas, especially the prefrontal cortex seem to participate in the inhibition of HPA axis activity (Feldman & Conforti, 1985).

2.1.3 MEASUREMENT OF HPA REACTIVITY

Several tests have been developed that aim at investigating the reactivity of the HPA axis to external or internal stimulation, or measuring basal regulation of the axis. Pharmacological tests are performed to investigate the reactivity of the HPA axis and its feedback loops. CRF administration allows investigation of the ACTH response from the pituitary, and the GC response from the adrenal glands and has become an important challenge test in clinical endocrinology and psychiatry (Muller et al., 1982; Schreiber et al., 1993; von Werder & Muller, 1993; Dickstein & Shechner, 1997). The ACTH test is administered to test the pituitary reserve and GC release from the adrenal glands (Hasinski, 1998; Thaler & Blevins, 1998; Dorin et al., 2003).

The dexamethasone suppression test (DST) tests normal HPA function by occupation of pituitary GR receptors causing inhibition of ACTH and cortisol release (Carroll et al., 1981; Holsboer, 1983; Arana et al., 1985). If cortisol is secreted above a certain threshold despite the strong negative feedback, this suggests abnormal functioning of the HPA axis. In posttraumatic stress disorder (PTSD), a low dose DST is used to test increased sensitivity of the HPA axis; if cortisol release is suppressed as a consequence of a very low dose of dexamethasone, this suggests increased sensitivity of the axis to stimulation (Yehuda, 2000, 2001). Metyrapone administration pharmacologically decreases cortisol release form the pituitary and thus prevents negative feedback. As a consequence, ACTH secretion from the pituitary is increased, and the HPA function can be observed in the case of missing GCs and negative feedback. Elevated ACTH levels indicate hypofunction of the adrenal cortex (Thomas, 1992; Norman & Litwack, 1997).

In 1993, Kirschbaum and collaborators developed the Trier Social Stress Test (TSST), a tool for the induction of moderate psychosocial stress in a laboratory setting. The TSST consists of a ten minutes anticipation period followed by a ten minutes test period, during which the subject has to deliver a public speech and perform mental arithmetic in front of an audience. This test of acute HPA responsiveness has been shown to lead to 2 to 4-fold elevations above baseline in salivary cortisol concentrations

(Kirschbaum et al., 1993). Furthermore, significant increases in heart rate as well as in ACTH, serum cortisol, GH, and prolactin levels could be demonstrated.

Another approach to measure HPA regulation is to determine the cortisol response to awakening. It could be demonstrated that cortisol increases by 50-75% within 30 minutes after awakening, and that this response shows high intraindividual stability (Pruessner et al., 1997b). Awakening appears to endogenously stimulate the HPA axis at the level of the pituitary (Schmidt-Reinwald et al., 1999). Several studies have shown a relationship between the cortisol response to awakening and measures of chronic stress (Schulz et al., 1998; Wust et al., 2000), burnout (Pruessner et al., 1999), and chronic pain (Geiss et al., 1997).

Over the last decade, cortisol sampling in saliva has been established as a non-invasive and inexpensive alternative to blood sampling for the assessment of adrenocortical activity (Kirschbaum & Hellhammer, 1989; Ehlert et al., 1990; Kirschbaum, 1991; Ockenfels et al., 1995; Pruessner et al., 1997a; Heim et al., 1998; Hucklebridge et al., 1998; Kudielka et al., 1998). Salivary cortisol measures the unbound "free' fraction of cortisol (5-10% of the total cortisol). The remaining 90-95% are bound mainly to corticosteroid binding globulin (CBG; 70-85%) and albumin (10-15%) (Kirschbaum & Hellhammer, 1989; Ekins, 1990). Only the free fraction has the capacity to cross the blood-brain barrier and diffuse through cell membranes, and thus only this part is biologically active. The correlation coefficients between cortisol in saliva and cortisol in plasma range from $r = .71$ to $r = .96$ (Kirschbaum & Hellhammer, 1994). Cortisol is rapidly transferred from serum to saliva, peak values can be observed 1-2 minutes after maximal concentration in serum (Kirschbaum & Hellhammer, 1989). Peak cortisol concentrations in saliva can be observed ten minutes after cessation of a psychobiological stress task (Kirschbaum et al., 1993) and within 30 minutes after awakening (Pruessner et al., 1997b).

Different saliva sampling techniques have been described like pipette, paper or cotton swab (for overview see (Kirschbaum & Hellhammer, 1994). A convenient

sampling device is the so-called "Salivette" (Sarstedt Inc., Rommelsdorf, Germany), which consists of a small cotton roll in a plastic centrifugation tube. At sampling times, the subject is instructed to leave the cotton swab in his/her mouth for 30-60 seconds until it is saturated with saliva. It is then stored in the plastic tube until analysis. It is recommended to freeze the samples until they can be analyzed, although storage at room temperature does not seem to affect the cortisol measures (Kirschbaum & Hellhammer, 1994).

Analysis of salivary cortisol levels is mostly done with a radioimmunoassay (RIA), which uses protein molecules with specific antibodies. Several adaptations from existing radioimmunoassays for the assessment of serum cortisol have been described that allow cortisol assessment in saliva (for overview see Kirschbaum & Hellhammer, 1989). A nonisotopic time-resolved fluorescence immunoassay for cortisol measurement in saliva has been developed by Dressendörfer and collaborators (Dressendorfer et al., 1992). This analysis method yields comparable results to the RIA (Kirschbaum & Hellhammer, 1994) and has been used in numerous studies in psychoneuroendocrinological research (Ockenfels et al., 1995; Kudielka et al., 1998; Pruessner et al., 1999; Wust et al., 2000; Wolf et al., 2001).

2.1.4 THE HIPPOCAMPUS AND COGNITION

Early evidence for a specific role of the medial temporal lobe, and especially the HC in memory came from studies with epileptic patients who underwent surgery for bilateral removal of the HC and neighboring temporal lobe structures. Scoville and Milner described profound impairments of new memory storage in these patients (Scoville & Milner, 1957; Milner, 1972). Subsequent lesion studies confirmed the special role of the HC for memory processes (Zola-Morgan et al., 1986; Zola-Morgan et al., 1992; Giovagnoli, 2001).

With the development of neuroimaging methods, it became possible to investigate the basis of human memory in the living brain. Structural magnetic resonance imaging (MRI) identified alterations in HC shape and HC volume reductions in association with memory deficits (Press et al., 1989; Deweer et al., 1995; Horn et al., 1996; O'Brien et al., 1997; Lupien et al., 1998; see also chapter 2.3.6). Functional neuroimaging studies reported medial temporal and HC activation in association with characteristics like novelty (Stern et al., 1996), memory encoding processes (Stern et al., 1996; Fernandez et al., 1998; Rombouts et al., 1999; Schacter & Wagner, 1999; Killgore et al., 2000), retrieval success (Kapur et al., 1995; Nyberg et al., 1996), and spatial mapping (Maguire et al., 1997).

With the use of more advanced research methods it became obvious that the hippocampal memory system is rather heterogeneous (Stern & Hasselmo, 1999). Most prominently, the HC is associated with declarative (also termed explicit, episodic, conscious, and relational) memory (Squire, 1992; Eichenbaum, 1999a) and spatial memory (Handelmann & Olton, 1981; Bohbot et al., 1998). Regarding implicit (or unconscious) memory, results are inconclusive (Squire, 1992; Schacter et al., 1996; Beauregard et al., 1998; Ouchi et al., 1998; Chun & Phelps, 1999). A recent review proposes the need to integrate results from lesion, cellular and imaging studies (Stern & Hasselmo, 1999). Another review already identified common characteristics observed in different studies involving the hippocampus. Here, the relational nature of memory processes is emphasized, and it is proposed that the HC system plays a critical role in binding together multiple inputs to permit representations of the relations among the constituting elements of scenes or events (Cohen et al., 1999). Also, it appears to be episodic encoding rather than retrieval that activates the HC system (Schacter & Wagner, 1999; see also Chapter 2.3.7).

Moreover, it should be considered that various memory functions appear to be associated with a different localization in the HC. Thus, lesions in the CA1 region seem to impair conditional learning and anterograde memory (Ridley et al., 1995; Rempel-Clower et al., 1996; Govindaiah et al., 1997; Virley et al., 1999), whereas the CA3 region appears to be especially important for spatial memory (Handelmann & Olton, 1981;

Roozendaal et al., 2001; Steffenach et al., 2002). Episodic memory *encoding* appears to be associated with rostral portions of the HC, whereas the basis for episodic memory *retrieval* seems to be primarily located in caudal areas (Lepage et al., 1998). Hemispheric differences in the contribution to memory performance have also been reported. Whereas there are conflicting reports regarding the hemisphere that is more strongly involved in explicit memory processes (Lehtovirta et al., 1995; Iidaka et al., 2000; Johnson et al., 2001), a predominant role of the right HC for spatial memory processes seems to be secured (Maguire et al., 1997; Bohbot et al., 1998; Barrash et al., 2000).

2.2 GLUCOCORTICOID EFFECTS ON HIPPOCAMPAL MORPHOLOGY AND FUNCTION

2.2.1 GLUCOCORTICOIDS AND HIPPOCAMPAL ATROPHY

Its high number of GC receptors makes the HC a primary target for glucocorticoids. Adverse effects of prolonged GC exposure on the HC have been extensively demonstrated in animals (Uno et al., 1994; Magarinos et al., 1997; McEwen & Magarinos, 1997; Raber, 1998; McEwen, 1999; Fuchs et al., 2001). In humans, chronic exposure to elevated GC levels in several neuropsychiatric disorders seems to have a similar effect. In patients with major depression, Cushing syndrome, or Alzheimer disease the extent of glucocorticoid hypersecretion is found to be correlated to the extent of HC atrophy (Starkman et al., 1992; O'Brien et al., 1996; Sheline et al., 1996; Sapolsky, 2000). Neuron loss occurs over the course of months and seems to be reversible, suggesting the atrophic process to be a physiologically adaptive mechanism to stress (McEwen & Magarinos, 1997; Sapolsky, 2000). Additional evidence for an association between GCs and HC atrophy comes from patients with Posttraumatic stress disorder (PTSD) following combat trauma or childhood abuse (Bremner et al., 1995; Bremner et al., 1997; Sapolsky, 2000; Yehuda, 2001). However, GC levels in PTSD have been reported to be lower than normal, making it more difficult to attribute HC atrophy to excess GC levels (Sapolsky, 2000; Yehuda, 2001).

Several mechanisms have been identified that underlie the decrease in HC volume. First, GC overexposure over several weeks leads to HC atrophy by decreasing the number and length of apical dendrites in HC CA3 neurons (Sapolsky, 2000). This process seems to be mediated by a GC induced increase of glutamate, an excitatory amino acid neurotransmitter, in HC synapses. Second, GCs can disturb dendritic branching by impairing the efficacy of neurotrophins. Third, elevated GC levels inhibit neurogenesis in the HC dentate gyrus (Reagan & McEwen, 1997; Gould & Tanapat, 1999). This effect is in part due to glutamate effects on N-methyl-D-aspartic acid- (NMDA) receptor activation (Cameron et al., 1995), a process that seems to be reversible (McEwen & Magarinos, 1997).

Finally, GCs have a well-described effect on glucose metabolism. Peripherally, an inhibition of glucose transport and metabolism and an increase in serum glucose levels are observed (Munck, 1971; Horner et al., 1987). Effects on central nervous system tissues include an inhibition of glucose transport metabolism in several brain regions including the HC (Horner et al., 1990; Virgin et al., 1991; de Leon et al., 1997). This loss of energy in neurons and astrocytes probably contributes to hippocampal sensitivity to stress.

2.2.2 GLUCOCORTICOIDS AND COGNITION

High levels of glucocorticoids have been repeatedly found to be associated with cognitive impairments. Acute administration of hydrocortisone or exposure to an acute stressor leads to significant decreases in declarative memory performance (Wolkowitz et al., 1990; Kirschbaum et al., 1996). Remarkably, only memory functions dependent on hippocampal activity are impaired (Wolkowitz et al., 1990). Memory impairments and attention deficits are also described as a side effect of corticosteroid treatment (Ling et al., 1981; Varney et al., 1984).

Since the hippocampus is both critically involved in the feedback regulation of HPA activity and essential for declarative and spatial memory processes, the association between elevated cortisol levels and memory impairments seems to be mediated via the hippocampus. However the direction of the causal relationship is not yet clear.

In animals, chronic stress and elevated GC levels have been shown to impair HC-mediated memory (Luine et al., 1994; Conrad et al., 1996; Magarinos et al., 1997; Ohl et al., 2000). In humans, long-term exposure to elevated GC levels in certain conditions like aging (Lupien et al., 1994; Lupien et al., 1997; Lupien et al., 1998), Cushing's syndrome (Starkman & Schteingart, 1981; Starkman et al., 1992; Forget et al., 2000), and depression (Sheline et al., 1999; Belanoff et al., 2001; Neylan et al., 2001) has been reported in association with memory deficits. At the same time, the above described conditions are frequently characterized by HC atrophy (Starkman et al., 1992; O'Brien et al., 1996; Bremner & Narayan, 1998; Lupien et al., 1998), making it more difficult to identify the actual cause for cognitive impairment in these cases. Recently, elevated GC levels have been reported in association with memory deficits and HC atrophy in a group of flight attendants suffering from chronic disruption of their circadian rhythms (Cho, 2001).

Memory formation is believed to be at least in part based on a phenomenon called long-term potentiation (LTP) (Diamond et al., 1988; Shors & Matzel, 1997). Long-term potentiation is defined as a long-lasting enhancement in the excitatory synaptic potential of postsynaptic HC neurons induced by high-frequency electrical stimulation of afferent fibers (Kandel et al., 1991; Diamond et al., 1992). Mediated by MRs and GRs, stress and glucocorticoids appear to modulate the excitability of hippocampal neurons and thus LTP in the CA1 field and dentate gyrus (Bliss & Lomo, 1973; Foy et al., 1987; Diamond et al., 1992). Activation of the MR receptor decreases the slow afterhyperpolarization (AHP) (Joels & de Kloet, 1990), thereby increasing the firing rate of a neuron to a stimulus and enhancing neuronal excitability. On the other hand, GR activation enhances the slow AHP (Joels & de Kloet, 1989), resulting in neuronal inhibition. The relationship between the GC levels and LTP seems to follow an inverted u-shape (Diamond et al., 1992), with

activation of MR enhancing LTP, and activation of GR leading to a suppression of LTP (Pavlides et al., 1996; Kim & Yoon, 1998; Pavlides & McEwen, 1999). In fact, *in vitro* studies have shown that LTP seems to be induced optimally when corticosteroids are mildly elevated and all MRs and some of the GRs are activated (de Kloet et al., 1999). Through additional GR activation homeostasis seems to be disturbed and damaging glucocorticoid effects can be exerted (de Kloet et al., 1999). The inverted u-shape relationship observed between glucocorticoid levels and LTP is regarded as relevant to the dose-response relationship between GC levels and memory performance (Lupien & McEwen, 1997).

2.2.3 GLUCOCORTICOIDS AND DEPRESSION

Major depression is frequently characterized by hypercortisolemia which becomes evident in increased 24h cortisol plasma concentrations (Halbreich et al., 1985; Linkowski et al., 1985; Trestman et al., 1995; Weber et al., 2000). Furthermore, negative feedback control in depressed patients appears to be impaired. After administration of the synthetic corticosteroid dexamethasone, 40-60% of patients fail to inhibit cortisol secretion, a phenomenon called 'dexamethasone resistance' (Carroll et al., 1976; Asnis et al., 1981; Kocsis et al., 1984). Other dysregulations of the HPA axis in major depression include hypersecretion of corticotropin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus (Nemeroff, 1988), and hypertrophy of the adrenal glands (Rubin et al., 1995).

Apart from HPA dysregulations in association with clinical depression, depressive symptomatology and negative affect have been reported as secondary symptoms in disorders characterized by hypercortisolism like Cushing' s syndrome (Starkman et al., 1981; Kelly et al., 1983; Loosen et al., 1992) or multiple sclerosis (Fassbender et al., 1998). Other studies report associations between post-partum blues and elevated cortisol levels (Taylor et al., 1994) or abnormal responses to the Dexamethasone Suppression Test (DST; Singh et al., 1986). In a study by van Eck and collaborators (van Eck et al.,

1996), negative affectivity (state) and depressive symptomatology (trait) in male white-collar workers both showed positive associations with cortisol. Hypercortisolism in clinical depression has been linked to HC atrophy (O'Brien et al., 1996; Sheline et al., 1996), and patients with Cushing's syndrome display both, depressive symptoms and HC atrophy (Starkman et al., 1992; Kelly, 1996).

2.3 MAGNETIC RESONANCE IMAGING (MRI) IN PSYCHO-ENDOCRINOLOGICAL RESEARCH

Magnetic Resonance Imaging (MRI) was first developed in the early 1950s to measure the atomic components of chemical samples. Since then, it has evolved into a powerful tool that depicts soft tissues of the human body and brain with high spatial resolution. In some cases, these in vivo structural images even surpass photographs of pathologic specimen. The technique distinguishes itself from other procedures using X-rays by being non-invasive (Kandel et al., 1991; Jackson, 1994; Pavlides & McEwen, 1999). Thus, it can be used repeatedly to study tissue features in healthy volunteers and patients.

The development of functional MRI (fMRI) revolutionized the field of neuroimaging only a decade ago. The technique enables researchers to depict local blood flow and blood oxygenation changes that accompany neural activity during sensory, motor, and cognitive stimulation and performance. Both MRI and fMRI have an established role in the neurosciences. Brain anatomy and function can be studied in vivo and related to a variety of conditions like clinical status, psychological tests performance rates, and differences in cellular and endocrinological parameters.

In the following sections, an overview of the basic principles and physical features of both MRI and fMRI is provided. The content of these chapters is based on several reviews and books (Martin et al., 1991; Cohen et al., 1993; Cohen & Bookheimer, 1994; David et al., 1994; Köchli & Marinek, 1994; Le Bihan et al., 1995; Barinaga, 1997). Subsequently, applications and findings of structural MRI in psycho-endocrinological

settings are described. Emphasis is put on a recently developed protocol for HC segmentation. Finally, applications of functional MRI in psychological and clinical settings are outlined, demonstrating the potential of this method for psychoneuroendocrinological research.

2.3.1 PHYSICAL PRINCIPLES OF MRI

In brief, MRI can be described as an externally applied magnetic field with systematically varying strength, causing protons in atomic nuclei to be temporally perturbed and to create a signal as they return to their resting state. This signal is different depending on tissue density and properties, and its recording allows the generation of structural images. In the following, a more detailed description is provided.

Magnetic field properties of protons

The nucleus consists of a proton and an electron spinning around it. Protons and their positive electrical charge are constantly spinning (they have a 'spin'), and thus they produce a little magnetic field like electrical currents.

Alignment of protons to an external magnetic field

When a patient is exposed to a strong magnetic field, the protons in his body tissues line up longitudinally to the magnetic field. In a strong magnetic field, protons make a 'precession' movement. That means, like a spinning top, the axis the protons are rotating around is itself circling, forming a cone shape. The precession frequency describes how many times the protons precess per second. The stronger the magnetic field, the higher is the precession rate. The Larmor equation states that the precession frequency is exactly proportional to the strength of the external magnetic field. Slowly, the spins align parallel to the magnetic field and the magnetic force of single spins adds up to a magnetic field.

Radio frequency pulse, T1 and T2, TR and TE

The alignment with the external magnetic field can be disturbed by a radio frequency (RF) pulse. Only when the RF pulse corresponds to the Larmor-frequency of the protons, can they pick up energy, a phenomenon called 'resonance'. As a consequence, the longitudinal magnetization is decreased, and transversal magnetization emerges, which causes the protons to precess in phase. As soon as the RF signal ends, the transverse magnetization starts to disappear. The time that it takes for the longitudinal magnetization to recover is called longitudinal relaxation time (T1). Also, after the RF pulse is switched off, protons no longer precess in phase. The time it takes for transversal magnetization to fade is called transversal relaxation time (T2). T1 is about 300 to 2000msec long, T2 only 30 to 150 msec. Tissues with high water content have longer T1 and T2 relaxation times than fatty tissues.

A RF pulse that tilts the magnetization 90° is called a 90° pulse. Other RF pulses are also possible and are named accordingly, e.g. 180° pulse. The sum vector of longitudinal and transversal magnetization is called 'net magnetization'. The transversal component of this net magnetization induces a signal, which can be measured by an antenna. A second RF pulse is sent in shortly after the first. When this second pulse comes in before longitudinal relaxation has recovered completely, the signal will be different. The time distance between two RF pulses is called TR (time to repeat). A TR of less than 500msec is considered to be short, a TR greater than 1500msec to be long. Since different tissues have different relaxation times, the TR determines the signal intensity of tissues and thus the contrast of an MR image. With long TRs, signal intensity is mainly caused by proton density. We talk about proton density-weighted images.

A T2 weighted image is obtained by sending a 180° pulse in a certain time distance ('Time to echo' : TE/2) after the RF pulse (spin echo sequence). As a consequence the dephasing protons precess in the opposite direction and rephase again. This results in a stronger transversal magnetization and signal again. This signal is called 'spin echo'. The 180° pulse neutralizes constant external inhomogeneities.

The shorter TE, the stronger is the signal, but the weaker is the tissue contrast. On the other hand, a weaker signal after a long TE makes it more difficult to differentiate the signal from the background noise. This problem is referred to as 'signal-to noise-ratio'. A TE of less than 30msec is considered to be short, a TE greater than 80msec to be long. A short TR results in a T1 weighted image whereas a long TE gives a T2 weighted image. In T1 weighted images fluids appear darker than other tissues, whereas in T2 fluid appears white.

MRI images have high spatial resolution and contrast. A standard clinical scanner uses a magnetic field of 1.5 Tesla and can routinely exceed a three-dimensional resolution of 1mm x1mm x1mm. Images can be created in coronal, horizontal and sagittal sections.

2.3.2 PHYSICAL PRINCIPLES OF FUNCTIONAL MRI

Functional MRI identifies areas of brain activity by comparing signals of MRI images recorded in activated and non-activated states (Belliveau et al., 1992). The technique localizes brain function with an accuracy of millimeters and a temporal resolution of seconds (for reviews see Cohen & Bookheimer, 1994; Barinaga, 1997).

Early fMRI studies were still invasive and used paramagnetic contrast agents (e.g. Gadolinium DTPA) to introduce local inhomogeneities in the magnetic field. As a result of dephasing protons, brain regions receiving greater blood flow produce weaker MR signals than other regions. Shortly later, it was detected that the use of externally injected contrast agents is not necessary, since hemoglobin becomes highly paramagnetic in its deoxygenated state and thus can be regarded as a naturally occurring contrast agent (Ogawa et al., 1990; Kwong et al., 1992). FMRI takes advantage of the fact that local blood flow and oxygenation increase substantially during neuronal activity. As a consequence, the local deoxyhemoglobin concentration decreases and produces larger MR signals in T2*-weighted images. The image resulting from a comparison of images

acquired in activated and control conditions is called BOLD (blood oxygenation-level dependant) contrast.

FMRI has several advantages compared to Positron Emission Tomography (PET), which was the technique of choice for a long time. PET is more invasive than fMRI since it requires the injection of radioactive isotopes for the mapping of active brain areas. The effective resolution for PET in between-subjects designs is about 5mm and the temporal resolution depends on the radioactive agent used, but cannot exceed 1 minute.

2.3.3 EXPERIMENTAL PROCEDURE OF FMRI

The vast majority of fMRI studies are conducted at 1.5 Tesla scanners, although some centers now use machines with higher field strength (up to 4 Tesla) to achieve higher spatial resolution. In a typical fMRI study, first, a series of anatomical scans are collected. These are of higher spatial resolution (typically 1mm x 1mm x 1mm) than the subsequently recorded functional images (e.g.3mm x 3mm x 5mm). Functional images are later superimposed on the high-resolution anatomical images, which allows exact anatomical location of activated brain areas. Auditory stimuli can be presented via headphones, visual stimuli are displayed on a rear projection screen, that the subject sees via a mirror.

In a classical block design the experimental task ("A") is repeatedly alternated with a control task ("B") that is identical except for one small difference. Frequently a kind of low-level control task ("C) such as simple fixation or rest is chosen to contrast the main condition of interest. Data is collected for a block of time (e.g. 60 seconds) for each condition ("AB"- or "ABC"- design). Different orders of block designs are possible (Buckner, 1998; Savoy, 1998).

With the development of event-related (ER-) fMRI designs, more flexibility was brought to the field. It enables researchers to test the effect of sudden changes of

conditions in the experiment (Buckner et al., 1996). Thus, experimental and baseline tasks can be intermixed randomly and BOLD images can be sorted a posteriori, for example according to the subject's performance in a certain task. ER-fMRI is based on the observation that hemodynamic changes start to occur within milliseconds after a neuronal event (Rosen et al., 1998). It could be demonstrated that fMRI is sensitive to even subtle signal changes produced by isolated trials. The separate contributions of rapidly occurring events to signal intensity can be detected with procedures that take into account overlapping hemodynamic responses (Buckner et al., 1998b; Rosen et al., 1998).

2.3.4 FMRI DATA ACQUISITION AND ANALYSIS

In a typical fMRI study, a single image is between 4 to 8mm thick, and a set of 10 to 20 images covers an entire plane across the brain. The in-plane resolution of a slice is about 3mm or more. The entire brain is repeatedly sampled in about every two seconds. In a typical fMRI experiment, 128 sequential images separated by 2 seconds might be acquired during one run. An experimental manipulation might occur every 30 seconds. As a result, successive series of images are acquired during each condition that are equal with regard to anatomy but with different signal intensity. For a brief sensory event, the hemodynamic response evolves over a 10-12 sec period. The onset of the hemodynamic response is about 2 seconds after neuronal activity, and it is prolonged in duration. These small signal changes are usually not obvious at first glance and have to be extracted by statistical procedures, which produce an activation map. Each voxel is given a value corresponding to its intensity. A voxel is a three-dimensional area defined by slice thickness and in-plane resolution (eg. 3x3x5mm). The simplest way to produce an activation map is to subtract signal intensities occurring during a rest or baseline condition from signal intensity during the experimental condition. Besides pairwise comparisons, other statistics like ANOVA can be used to create an activation map.

Another commonly constructed map is a correlation map. High correlations are seen when changes in signal intensity accompany task changes. Statistics used for hypothesis

testing are mostly based on the standard general linear model of statistics and are applied on a voxel-by-voxel basis, thus resulting in t-, z-, or f- activation maps. Of course, these thousands of statistical tests require correction for multiple comparisons.

On the activation map, voxels that contain task-related BOLD-contrast are highlighted, and areas of activation can be identified by superimposing them on the high-resolution volumetric images. Increases in intensity represent increases in statistical significance. The grey scale activation maps images are mostly transformed into spectral color images, which facilitates the identification of peak activation.

Event-related fMRI data can be analyzed with the same method, applying the general linear model (Josephs et al., 1997; Friston et al., 1998). Another approach for ER-fMRI analysis is to determine response contributions of separate events to the hemodynamic signal. For the generation of a statistical map, ER-fMRI data can be selectively averaged in relation to trial onsets, as it is done with EEG and MEG data (Buckner et al., 1998b). A crucial issue for this analysis procedure is the variance of the hemodynamic response. It appears that the hemodynamic response during a set of successive events is a linear summation of the isolated events (Cohen, 1997), and that it is rather stable across subjects (Buckner et al., 1998c) when observed in similar cortex regions. However, marked variations in timing and shape of the responses have been observed across different brain regions (Schacter et al., 1997; Buckner et al., 1998c). Thus, in order to obtain information about delays of neuronal activity in certain areas and related activity between regions within milliseconds, new developments in analysis procedures are required that are sensitive to different timing and shape of the hemodynamic response curve.

2.3.5 LIMITATIONS OF FUNCTIONAL MRI

Functional MRI is very sensitive to motion artifacts. Not only overt body movement, speaking or coughing, but also breathing and heartbeat can cause motion artifacts in the

resulting images. Because of the smaller voxel size in fMRI images, the same movement will have a higher impact on fMRI than on PET images. Motion reduction is attempted a priori by instructions to the subjects and certain head immobilization techniques. Motion can be measured after the experiment. Before the actual analysis, preprocessing for fMRI images includes motion correction techniques, like realignment of each image to one image (David et al., 1994; Buckner, 1998). Since speaking would result in significant head movement, fMRI studies of higher functions like language and memory require covert performance, which might not lead to the expected activation (Cohen & Bookheimer, 1994).

Ferromagnetic material cannot be brought into close proximity of the scanner's strong magnet. Patients with pacemakers and magnetic implants must be excluded, metal parts in the mouth can cause areas of signal loss in the image (Le Bihan et al., 1995). Another common problem is the partial voluming effect, which occurs when the scanner's spatial resolution is lower that the size of the structure of interest (Savoy, 1998). Finally, the temporal resolution of fMRI is still limited. Whereas EEG and MEG measure electrical activity of neurons directly, changes in the hemodynamic response usually occur only after a delay of about 2 seconds (Rosen & Savoy, 1998).

2.3.6 APPLICATION OF STRUCTURAL MRI

Volume quantification of the HC and other medial temporal lobe structures is an established research tool in the neurosciences to investigate the association of these structures with different disease states (Convit et al., 1993; Laakso et al., 1996; Sheline et al., 1996; De Leon et al., 1997; Jack et al., 1997; Baulac et al., 1998; Jack et al., 1999; Laakso et al., 2000). Because of its important role in conditions characterized by HPA dysfunction, the HC is one of the most frequently targeted structures in structural imaging. Thus, HC volume assessment plays an important role in association with conditions like major depression (O'Brien et al., 1996; Sheline et al., 1996; Sheline et al., 1999; Sapolsky, 2001), Cushing syndrome (Starkman et al., 1992; Starkman et al., 1999),

Alzheimer's disease (Convit et al., 1993; Horn et al., 1996; De Leon et al., 1997; Jack et al., 2000; Laakso et al., 2000), aging (De Leon et al., 1997; Jack et al., 1997; Lupien et al., 1998), and posttraumatic stress disorder (PTSD) (Bremner et al., 1995; Bremner et al., 1997; Stein et al., 1997; Bremner, 1999; Pavlides & McEwen, 1999; Yehuda, 1999; see also Chapter 2.2.1).

Depending on different data acquisition techniques and application of different analysis software with different precision, results of HC volume quantification from MR images vary between laboratories. Moreover, there are controversies regarding the exact anatomical boundaries that should be used for hippocampal segmentation (Jack et al., 1995; Pruessner et al., 2000). In order to overcome these discrepancies, a protocol for segmentation and quantification of HC volume was established with 40 healthy normal control subjects (Pruessner et al., 2000), taking advantage of high resolution 3D visualization of structural MRI. In addition, the high resolution T1 weighted MR images were prepared for analysis by a correction for image non-uniformities (Sled et al., 1998), linear stereotaxic transformation (Collins et al., 1994) into coordinates based on the Talairach Atlas (Talairach & Tournoux, 1988), and resampling onto a 1mm voxel grid using a linear interpolation kernel. These preprocessing steps allowed for higher precision of the anatomical boundary definition. Using the protocol, the HC structure is manually outlined in coronal sections by simultaneous access to sagittal and horizontal sections. Figure 2.3 provides an example for the hippocampal boundaries in coronal and sagittal sections. The exact anatomical boundaries, and the segmentation procedure are described in detail by Pruessner and collaborators (Pruessner et al., 2000).

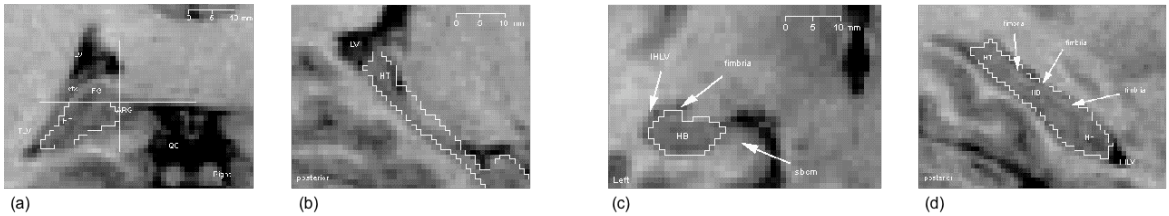

Figure 2.3 Examples for segmentation of the hippocampal tail (a,b) and body (c,d) in coronal and sagittal sections

2.3.7 APPLICATION OF FUNCTIONAL MRI

Early fMRI studies have concentrated on the visual system (Belliveau, 1991; Kwong, 1992; Ogawa. 1992) and the sensory motor cortex (Bandetti, 1992; Kim, 1993). Areas of increased BOLD signal were calcarine/occipital and sensory/motor cortices, respectively, as predicted from lesion and PET studies. Of more interest to the field of neuroscience are subsequent applications, which examine higher order cognitive function such as language (Bellemann et al., 1995; Binder et al., 1997; Phelps et al., 1997), attention (Pugh et al., 1996; Buchel & Friston, 1997), and learning and memory (Spitzer et al., 1996; Braver et al., 1997; Schacter et al., 1997; Buckner et al., 1998a; Buckner et al., 1998b; Fernandez et al., 1998; Schacter & Buckner, 1998; Cabeza & Nyberg, 2000). The results replicate and often extent previous PET findings (Cohen & Bookheimer, 1994). FMRI studies are increasingly employed in clinical and psychiatric fields (Bookheimer, 1996; Kalin et al., 1997; Bellgowan et al., 1998; Detre et al., 1998; Johnson et al., 2000; Dupont et al., 2002).

Of great importance to the field of Psychoneuroendocrinology, the development of PET and fMRI provides new methods to investigate the role of the HC in human subjects. Interestingly, functional imaging studies have mainly investigated HC and medial temporal lobe activation with regard to its role in memory (see Chapter 2.1.4). The development of functional neuroimaging methods such as Positron Emission Tomography (PET) and functional MRI (fMRI) brought about high expectations. However, early neuroimaging studies often failed to show HC activation and to substantiate neuropsychological findings (Shallice et al., 1994; Buckner et al., 1995; Kapur et al., 1995). This lack of HC activation was often explained by methodological difficulties in imaging the HC structure. However, subsequent studies reported medial temporal and HC activation in association with tasks that involve declarative relational memory processes (Cohen et al., 1999; see Chapter 2.1.4). The authors of these studies claim that studies cited in support of characteristics like novelty (Stern et al., 1996), explicit memory (Schacter, 1997), retrieval success (Kapur et al., 1995; Nyberg et al.,

1996), or spatial mapping (Maguire et al., 1997) to activate the MTL, provide equally strong support for the relational account.

In a typical relational memory task described in a PET study by Henke and collaborators (Henke et al., 1997), subjects had to make relations between pictures of a person and of a house by deciding, if the person was an inhabitant or a visitor of the house. Another example for an experimental task that meets the characteristics of relational memory and that has produced HC activation in fMRI is the 'encoding of visual complex scenes' (Stern et al., 1996; Detre et al., 1998).

Some studies have investigated blood flow changes in the brain in response to physiological changes. Brain activation changes were observed after sleep deprivation (Drummond et al., 1999; Drummond & Brown, 2001) and cocaine abuse (Breiter et al., 1997; London et al., 1999). Respiratory and blood pressure challenges have been shown to affect HC activation (Harper et al., 1998). A PET study investigated regional blood flow changes in subjects with snake phobia. Presenting phobogenic visual stimuli resulted in increased activity of the sympathetic nervous system and in reduced activation in several brain areas including the HC and frontal lobes (Wik et al., 1993). A PET study reports reduced glucose utilization in elderly subjects after glucocorticoid administration (de Leon et al., 1997). Another recent PET study demonstrates effects of cortisone administration on cued recall of word pairs learned 24 h earlier and a large decrease in regional cerebral blood flow in the right posterior medial temporal lobe, especially in the parahippocampal gyrus, a region associated with successful verbal memory retrieval (de Quervain et al., 2003). However, up to the present, the effects of stress and cortisol levels on HC activation have neither been tested in young and healthy subjects nor with the promising new method of functional magnetic resonance imaging.

2.4 SUMMARY AND IMPLICATIONS FOR STUDY DESIGN

The previous chapters provided an overview of basic HC and HPA characteristics and outlined the literature describing associations of elevated cortisol levels with cognition, depression and mood. Special emphasis was put on the HC as a key structure regulating GC actions and its particular vulnerability to chronic GC exposure, potentially resulting in HC atrophy.

HC volume changes in conditions of HPA dysregulations in humans are regularly assessed with MRI. The basics principles of structural and functional MRI were described, and an overview of applications in the field of Neurosciences and Psychoneuroendocrinology was provided.

The literature overview presented evidence that elevated GC levels are described mostly in association with pathological conditions and advanced age. Studies on the effects of elevated cortisol levels in young healthy subjects suggest some effects on cognition and mood, but do not investigate associations with HC volume. Also, several functional neuroimaging studies report hippocampal activation in explicit memory paradigms. However, until now, stress and cortisol effects on this activation have not been assessed.

Aim of the present work was to investigate associations between GC levels and HC integrity in young, healthy subjects. Different studies were performed referring to various aspects of HPA related dysfunction. The hypotheses developed in the present thesis are each based on indications for HPA related changes observed in clinical and aged populations. Each study covers a different aspect of function related to the HPA axis. None of the associations investigated in the present thesis have been explored in such a way in a young healthy human population. Associations between GC levels and measures of stress and depression were investigated, and a special focus was put on MRI derived measures of HC volume and function.

2.4.1 RATIONALE AND HYPOTHESES FOR PARTICULAR STUDIES

Chapter 2.2.3 reviews evidence for hypercortisolemia in subjects with depression and negative affectivity. In these studies, plasma cortisol levels have been regularly assessed as diurnal profile or dexamethasone resistance. A higher cortisol response to awakening could be demonstrated in subjects with increased levels of chronic stress in recent studies. Depressive symptoms and their association to cortisol have not been investigated in young healthy subjects. Furthermore, the potential of the awakening cortisol response as an alternative to diurnal cortisol assessment in plasma has not been explored. Thus, the aim of the first study was to assess the association between depressive symptomatology and the cortisol response to awakening in a young healthy population. According to the reported findings, the specific hypothesis formulated for the first study was:

Hypothesis 1: Higher depressive symptomatology in healthy young subjects is positively associated to awakening cortisol assessed over four weeks

Chapter 2.2.1 describes findings of HC volume reduction in association with chronically elevated cortisol levels. Furthermore, findings described in chapter 2.2.2 point to impairment of HC dependant cognitive function in association with elevated GC levels and HC atrophy. HC volume assessment with MRI has recently been improved by the establishment of a new segmentation protocol (chapter 2.3.6). The association between cortisol levels and HC volume has not been investigated in healthy young subjects. Goal was to relate basal and acute cortisol levels to HC volume quantified with this new protocol. The hypothesis tested in the second study was:

Hypothesis 2: Higher cortisol levels are associated with lower hippocampal volume

As outlined in chapters 2.1.3 and 2.3.7, functional MRI is a new but recognized method to determine HC activation during memory tasks. Furthermore, chapters 2.2.1 and 2.2.2 point to GC effects on HC metabolism and long-term potentiation in animal and in vitro studies. It can be expected that changes in HC function precede HC volume loss.

Functional MRI might have a high potential to investigate HC activation changes associated with a modulation in GC levels in humans. Goal of the third study was to establish a stress task for the fMRI environment and to compare HC activation during a memory task before and after stress. The specific hypothesis for the third study was:

Hypothesis 3: Acute stress and elevated cortisol levels are associated with reduced hippocampal activation.

CHAPTER 3:

SELF-REPORTED DEPRESSIVE SYMPTOMS AND STRESS LEVELS IN HEALTHY YOUNG MEN: ASSOCIATIONS WITH THE CORTISOL RESPONSE TO AWAKENING

3.1 ABSTRACT

There is evidence that clinical depression as well as negative mood is associated with elevated basal cortisol levels. Recently, measuring the cortisol response during the first hour in the morning with strict reference to the time of awakening has been established as a reliable marker of individual adrenocortical activity. In studies using this marker, a relationship with self-reported stress levels and psychosomatic symptoms has been found. Goal of the present study was to investigate the association of self-reported depressive symptomatology with early morning free cortisol levels and their relationship to measures of stress.

The severity of depressive symptoms using the Hamilton Depression Inventory (HDI) as well as chronic and acute stress perception was assessed in 40 healthy young men. Once a week, for four consecutive weeks, subjects provided saliva samples at 0, 30, and 60 minutes after awakening. Higher levels of depressive symptomatology were associated with a greater cortisol response after awakening. This association appeared to be stronger when only subjects in the non-clinical range of depression were included. Furthermore, cortisol levels and depressive symptomatology were significantly positively correlated with measures of chronic and acute stress perception. The present study extends earlier findings of HPA hyperactivity in clinical depression to healthy young men with mild levels of depressive symptomatology. Measuring the cortisol response to awakening is proposed as an economical alternative to traditional approaches determining basal HPA activity. Associations between depressive symptomatology and chronic stress, as well as implications for future studies are discussed.

3.2 INTRODUCTION

Hyperactivity of the Hypothalamus-Pituitary-Adrenal (HPA) axis in major depression has been frequently reported in recent decades (for reviews see (O'Brien et al., 1993; Plotsky et al., 1998). Alterations at different levels of the HPA system are discussed to contribute to the observed elevation in cortisol levels. First, there seems to be an increased central

drive with CRF hypersecretion from the paraventricular nucleus (PVN) of the hypothalamus (Checkley, 1996), probably potentiated by the action of arginin vasopressin (AVP; (Holsboer & Barden, 1996). Second, negative feedback control of the HPA is impaired in depression, probably due to altered capacity or function of glucocorticoid receptors (Modell et al., 1997). Employing the Dexamethasone Suppression Test (DST) as a tool to examine glucocorticoid mediated feedback of the HPA axis, 50-60% of patients with depression (primarily severe endogenous depression) fail to display a subsequent suppression of cortisol (Carroll et al., 1976a; Carroll, 1984). Third, patients with major depression show an enlargement of the adrenal glands (Nemeroff et al., 1992), which seems to be reversible upon remission from the acute depressive episode after treatment (Rubin et al., 1995). Apart from HPA dysregulations in association with clinical depression, numerous studies report negative affect in conditions characterized by hypercortisolism (Starkman et al., 1981; Kelly et al., 1983; Singh et al., 1986; Loosen et al., 1992; Taylor et al., 1994; Fassbender et al., 1998).

Traditionally, cortisol secretion in depression or negative mood is determined as diurnal profile in plasma (Asnis et al., 1981; Halbreich et al., 1985; Maes et al., 1986; Dahl et al., 1991; Trestman et al., 1995; Yehuda et al., 1996) or urine (Carroll et al., 1976b; Rubin et al., 1987; von Zerssen et al., 1987). Only few studies employed saliva samples of cortisol (Ockenfels et al., 1995; Goodyer et al., 1996; van Eck et al., 1996). Some studies report an association between depressive symptomatology and elevated cortisol levels especially in the morning hours (Maes et al., 1986; von Zerssen et al., 1987; Ockenfels et al., 1995; Yehuda et al., 1996), while others conclude that the evening cortisol levels show the highest association with depression (Gold, 1988).

In recent years, the observation of a pronounced cortisol response to awakening (Spath Schwalbe et al., 1991; Linkowski et al., 1993; Van Cauter et al., 1994) has triggered a series of studies testing the usefulness of that marker to determine basal HPA regulation. Cortisol levels in saliva with strict reference to the time of awakening increased by 50-70% during the first 30 minutes and showed test-retest correlations of r=.45 to r=.70 (Pruessner et al., 1997). These results clearly demonstrate higher

intraindividual stability than single morning cortisol assessment or measurement at predefined times (Coste et al., 1994; Pruessner et al., 1997). Several studies investigated the association of the cortisol response to awakening with psychological variables and found elevated cortisol levels 30 to 60 minutes after awakening in subjects with high levels of chronic stress during the past year (Schulz et al., 1998; Wust et al., 2000). In a similar way, perceived stress during the last month was correlated with increases of cortisol levels during the first hour after awakening after dexamethasone pretreatment (Pruessner et al., 1999). In contrast, a diagnosis of burnout (Pruessner et al., 1999) or chronic pain (Geiss et al., 1997) was characterized by attenuated cortisol levels after awakening.

The above findings clearly encourage the measurement of salivary free cortisol after awakening to identify HPA hyperactivity in association with depression and negative mood. In a group of 40 healthy young men we assessed the relationship between self-reported depressive symptoms and the cortisol response to awakening as well as the association between depressive symptomatology and cortisol levels with measures of acute and chronic stress.

3.3 METHODS

3.3.1 SUBJECTS

Forty healthy male university students aged 18 to 35 years (mean age 24.3 ± 4.33) were recruited via postings on university bulletin boards. Women were excluded from this study due to possible confounding effects of menstrual cycle and use of oral contraceptives on HPA responsivity (Kirschbaum et al., 1999). Exclusion criteria were any history of psychiatric disorder, cardiovascular problems, and alcohol abuse. Subjects had to be medication free at the time of testing and were asked to indicate any history of chronic health problems, which were assessed in order to detect any factors that could affect cortisol reactivity. Out of 40 subjects, six were light to moderate smokers. They

reported smoking between 1 and 15 cigarettes per day. However, statistical analysis revealed that smoking was not associated with any of our dependent variables. The study was approved by the local hospital's ethics board, and written consent was obtained from each subject before participation.

3.3.2 CORTISOL ASSESSMENT

Cortisol was assessed from saliva with the "Salivette" sampling device (Sarstedt, Rommelsdorf, Germany). This noninvasive technique can be used at home and interferes only minimally with normal daily routines. Cortisol in saliva reliably reflects the free (unbound) fraction of cortisol in plasma (Kirschbaum & Hellhammer, 1994).

Subjects collected saliva once a week (Wednesday or Thursday) for four consecutive weeks at 0, 30 and 60 minutes after awakening in the morning. Awakening was either spontaneous or by alarm clock. Previous studies have shown that the cortisol response is not affected by this variable (Pruessner et al., 1997). Subjects were asked to refrain from caffeinated beverages and smoking prior to saliva sampling. Furthermore, they were instructed not to brush their teeth before the end of the sampling time in the morning, nor to eat or drink in the 10 minutes before sampling and to rinse their mouth with water before sampling. All psychological testing and an acute stress rating were performed within the month after cortisol assessment. The individual time delay between cortisol and psychological assessment did not create any effects.

3.3.3 ASSESSMENT OF DEPRESSIVE SYMPTOMATOLOGY

Depressive symptomatology was assessed with the Hamilton Depression Inventory (Reynolds, 1995), a revised and extended self-report version of the Hamilton Depression Rating Scale (HDRS; (Hamilton, 1967). The HDI consists of 23 items and serves as a severity measure of depressive symptoms. Subjects are asked to refer to their feelings and

behavior during the past two weeks, which has become a standard on self-report measures of depression (Reynolds, 1995). According to the authors of the HDI, a cutoff score of 19 best distinguishes between non-depressed and clinically depressed persons rated with DSM-III-R, while levels between 14 and 18.5 are regarded as sub-clinical and suggest a general level of psychological distress. The internal consistency reliability coefficient for the HDI is .93 (Cronbach's α), the test-retest reliability coefficient is .95 (Pearson product moment correlation). Criterion related validity of the HDI was proven by high correlations ($r = .94$; $p < .001$) with the HDRS clinical interview, construct validity was demonstrated by strong correlations ($r = .93$; $p < .001$) with the Beck Depression Inventory (BDI).

3.3.4 ASSESSMENT OF CHRONIC AND ACUTE STRESS

Subjective stress levels were assessed with an English version of the "Trier Inventory for the Assessment of Chronic Stress" (TICS; (Schulz & Schlotz, 1999). The TICS is a 39 item self-report scale for the assessment of chronic stress. It consists of the six scales "work overload", "worries", "social stress", "lack of recognition", "work discontent" and "intrusive memories". Internal consistency coefficients for the six scales vary between .76 and .88. Subjects are asked to assess on a five point rating scale how frequently they experienced specific stressful situations over the past year. As a measure of acute stress, subjects were asked to indicate their actual stress level on a ten-point rating scale with 1 being very low and 10 being very high.

3.3.5 PSYCHOLOGICAL, ENDOCRINOLOGICAL AND STATISTICAL ANALYSIS

The saliva samples were analyzed using a time-resolved immunoassay with fluorescence detection (Dressendorfer et al., 1992). The intra- and interassay variability was below 10% and 12%, respectively. One subject had to be excluded because of cortisol levels that were more than three standard deviations above the group mean. One subject

obtained a HDI score of 36,5 (cutoff score 19) and thus is referred to as potentially clinically depressed. Two subjects obtained a score of 19 and three subjects scored between 14 and 18.5, which according to the authors of this questionnaire corresponds to the sub-clinical range of depression. These subjects were excluded from analysis of the non-clinical range of depression.

In order to analyze the cortisol increase after awakening for the four sampling days, two-way within-subject ANOVAs were computed with repeated measures on factors time (3 levels) and day (4 levels). To analyze the linear relationship between depressive symptoms or chronic stress with cortisol regulation, Pearson correlations were computed between the scores on the HDI as well as the TICS and the cortisol response after awakening. Pearson correlations were also calculated between HDI and TICS scores. Spearman rank correlations were employed to determine associations between the 10-point rating scale for acute stress and HDI scores as well as cortisol levels. For all correlational analysis, cortisol levels were transformed into a single value by calculating the area under the curve (AUC) for each day, and then computing the median from the four individual cortisol (day) values.

Since previous studies had shown, that cortisol levels 30 and 60 minutes after awakening best differentiate between groups (Schulz et al., 1998; Wust et al., 2000), the impact of cortisol levels at different times after awakening was assessed. Therefore, groups of subjects with high and low depressive symptomatology as well as high and low stress levels were determined by median split and entered in two-way ANOVAs (group by time) as independent variables. Here, for the individual cortisol levels over four weeks, the median was calculated for each sampling time (0, 30 and 60 minutes after awakening). These aggregated levels served as dependent variable in the ANOVA. Significant interaction effects were specified by Newman-Keuls post hoc tests.

3.4 RESULTS

Cortisol levels after awakening showed a highly significant increase during the first 30 minutes on all four sampling days [$F(2,76) = 22.9$; $p < .001$]. At the time of awakening a mean cortisol level of 17.64 nmol/l was observed which increased by approximately 40% to 24.55 nmol/l 30 minutes after awakening. The response was not significantly different between the four sampling days (all $p > .10$). The effect size for the factor time was $f^2 = .26$, explaining 21% of the variability of the cortisol response to awakening ($w^2=.21$). Figure 3.1 illustrates the cortisol response after awakening for the three sampling times and four sampling days.

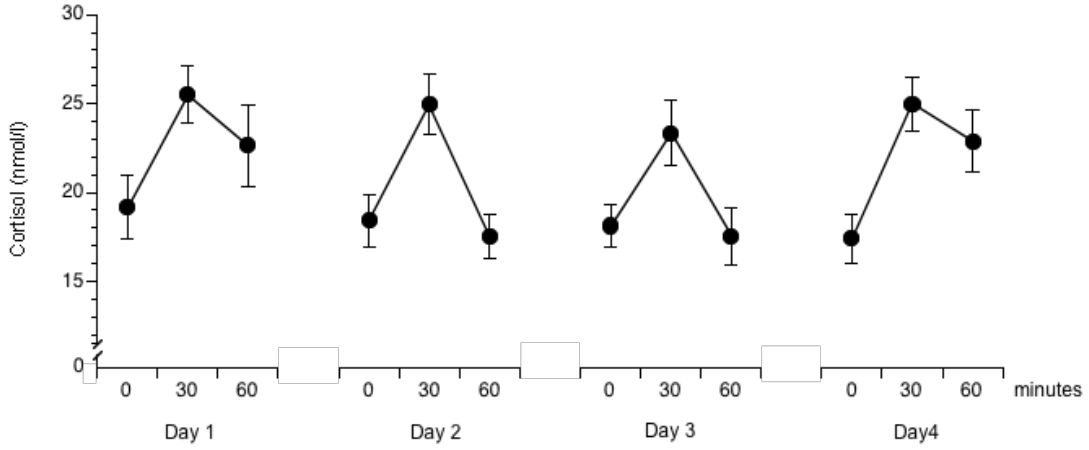

Figure 3.1: Mean cortisol levels 0, 30, and 60 minutes after awakening on the four sampling days (one day each for four consecutive weeks, n = 39)

In order to describe possible associations between depressive symptomatology and cortisol levels after awakening, first the scores for the Hamilton Depression Inventory were determined. Analysis revealed a HDI mean score of 8.88, which is slightly lower than the mean score for male college students of 9.44 reported by Reynolds & Koback (Reynolds, 1995).

Pearson correlations between the total HDI score and the aggregated cortisol response after awakening showed a trend for significance (r = .30; p = 0.06) for the total group (n=39). There appeared to be a stronger association (r = .34, p = 0.05) when subjects in the sub-clinical and clinical range of depression were excluded. Figure 3.2 shows the scatterplot between HDI score and the cortisol response after awakening for the non-clinical group.

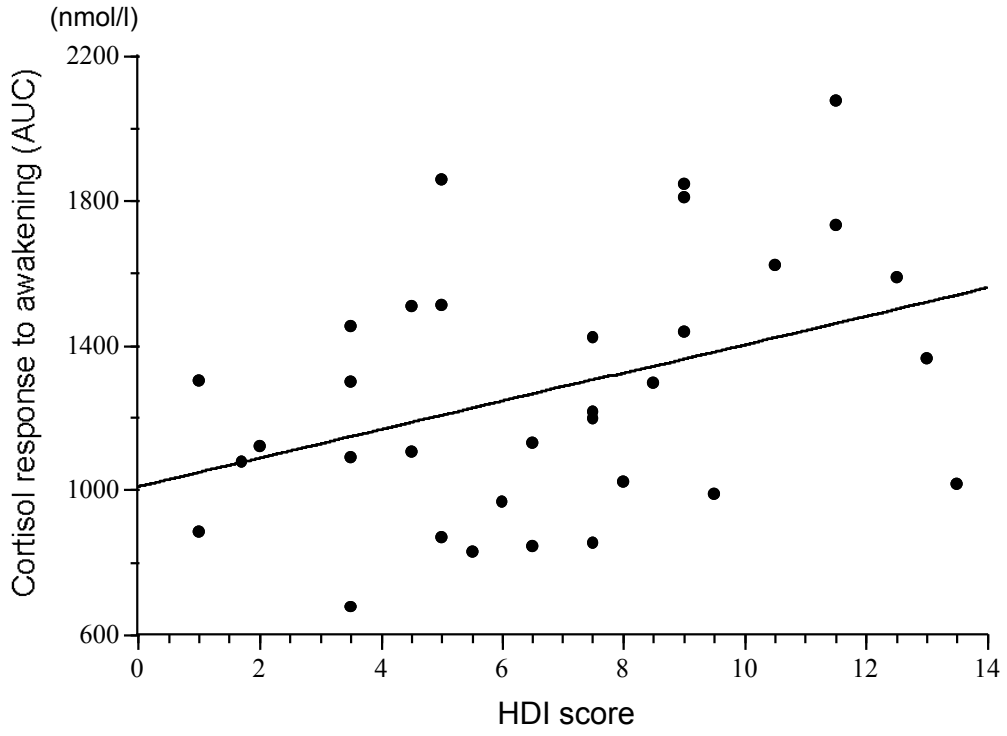

Figure 3.2: Scattergram of cortisol response to awakening (area under the curve, median over four sampling days) plotted against the total score of the Hamilton Depression Inventory; Pearson correlation: r = .34, p = 0.05, n = 33

Subjects were then assigned to groups with low and high depression scores by employing a median split for the HDI scores (low: HDI ≤ 7.5; high: HDI > 7.5). For the total group, an ANOVA (group by time) revealed a significantly higher cortisol response after awakening in subjects with high HDI scores [$F(1,37) = 7.56$; $p < 0.01$]. The group by time interaction effect showed a trend to be significant [$F(2,74) = 2.91$; $p = 0.06$].

Excluding all subjects in the sub-clinical and clinical range of depression still resulted in a significant group effect [$F(1,31 = 7.26; p = 0.01$]. Moreover, a significant group by time interaction was observed [$F(2,62) = 5.4; p = 0.007$]. Post-hoc analysis revealed that the cortisol levels 30 and 60 minutes after awakening reflected the group differences best. Figure 3.3a and b shows the cortisol levels during the first hour after awakening for the total and non-clinical group in subjects with high and low scores on the HDI.

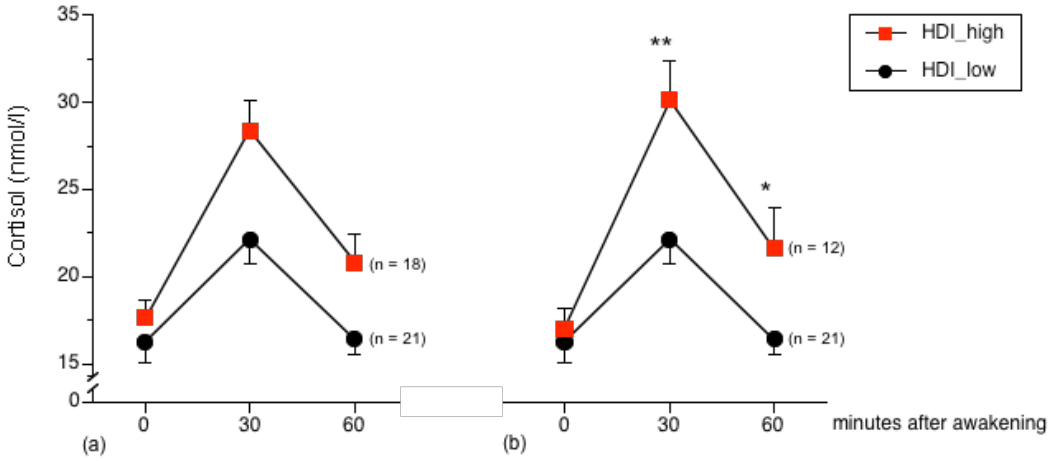

Figure 3.3: Cortisol response during the first hour after awakening (median over four weeks) in subjects with high (>7.5) and low (≤7.5) scores on the Hamilton Depression Inventory. (a) Total group, main effect $p < 0.01$, interaction effect $p = 0.06$; (b) non-clinical group, all subjects in the clinical and sub-clinical range of depression excluded, main effect $p = 0.01$, interaction effect $p < 0.007$. Difference according to Newman Keuls post-hoc analysis: ** $p < 0.001$, * $p < 0.01$.

The total HDI score was significantly correlated with the total score of the TICS ($r = .67$; $p < 0.001$) as well as to scores on its subscales 'work overload' ($r = .44$; $p < 0.01$), 'worries' ($r = .44$; $p < 0.01$), 'social stress' ($r = .65$; $p < 0.001$), 'lack of recognition' ($r = .59$; $p < 0.001$), and intrusive memories ($r = .67$; $p < 0.001$), suggesting similarities between the concepts.

Next, associations between chronic stress and the cortisol response after awakening were investigated. Correlational analyses revealed a trend for a significant association

between the total score of the TICS and cortisol after awakening (area under the curve, median over four weeks; $r = .31$; $p < 0.06$). The only subscale which demonstrated a significant association with cortisol was 'work overload' ($r = .37$; $p = 0.02$). However, after correcting for the number of comparisons, the result failed to remain significant. Separating the group by median split according to the subjects' total score on the stress scale, a two-way ANOVA showed no main effect for the factor 'group' but revealed a significant group by time interaction effect [$F(2,74) = 3.78$; $p = 0.027$]. Newman-Keuls post-hoc analysis revealed significant differences in the cortisol levels 30 minutes after awakening.

Assigning subjects to groups with high and low levels on each of the six subscales again revealed no main effect on cortisol for the factor group. However, for the scale "work overload" a significant interaction effect was shown [$F(2,74) = 6.78$; $p = 0.002$] which was still significant after adjustment of the significance level according to the number of comparisons. Post-hoc analysis revealed significant differences in the cortisol levels at the time of awakening and 30 minutes thereafter. Figure 3.4 a and b show the cortisol levels after awakening in the groups with high and low total scores on the TICS, as well as in the group with high and low scores for work overload.

Significant associations between the ten point rating scale for momentary feelings of stress and the HDI were demonstrated by Spearman rank correlations ($r = .42$; $p = 0.008$). This result further demonstrates a partial overlap of self-perceived stress and feelings of depression. Also, this measure showed the highest correlation of all stress measures with cortisol levels after awakening ($r = .46$; $p = 0.004$; see figure 3.5).

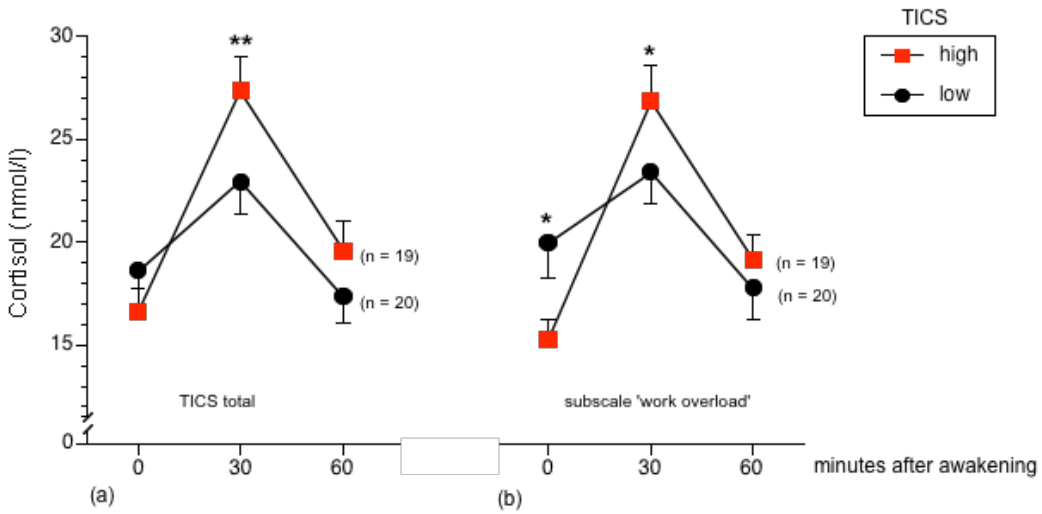

Figure 3.4: Cortisol response during the first hour after awakening (median over four weeks). (a) Subjects with high and low total scores on the Trier Inventory for the assessment of Chronic Stress, interaction effect group by time $p < 0.03$. (b) Subjects with high and low scores on the subscale 'work overload', interaction effect $p = 0.002$. Difference according to Newman Keuls post-hoc analysis: ** $p < 0.01$, * $p < 0.05$.

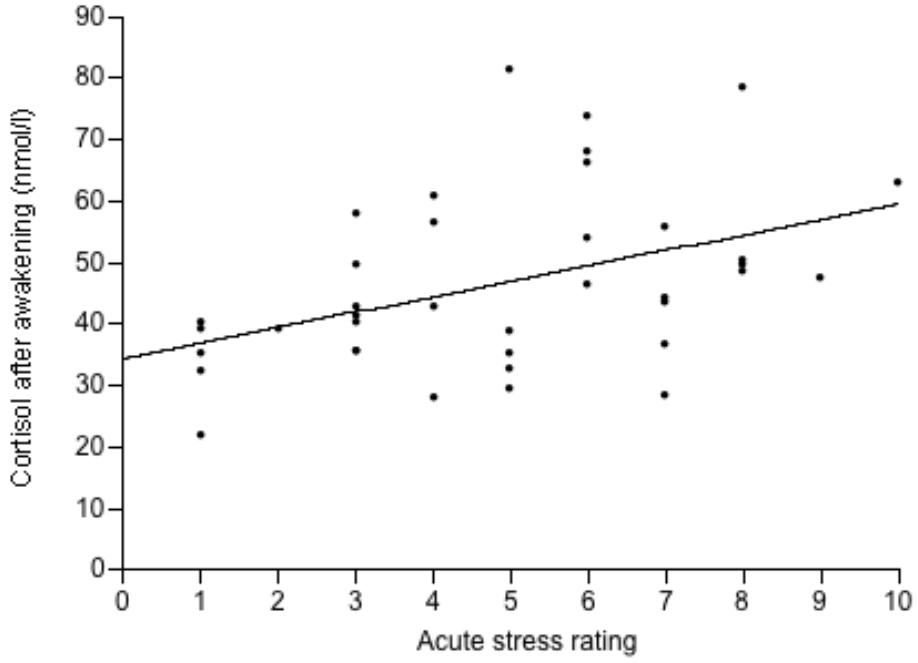

Figure 3.5: Scattergram of the cortisol response after awakening (area under the curve, median over four sampling days) plotted against the acute stress rating (Spearman correlation: R= .46; p<.004)

3.5 DISCUSSION

The present study examined the relationship between depressive symptomatology, measures of stress, and the cortisol response to awakening in healthy male college students. Most importantly, a positive association between elevated cortisol levels after awakening and the self-reported severity of depressive symptoms could be demonstrated. The present finding differs from earlier studies in several aspects. First, whereas numerous studies in the past reported hypercortisolism in association with clinical depression (Murphy, 1991; O'Brien et al., 1993; Plotsky et al., 1998), the present study investigated changes in HPA activation due to differences in the severity of depressive symptoms in a normal population. In contrast to the diagnosis of clinical depression, which regularly is confirmed with a structured clinical interview, the present study assessed the severity of depressive symptomatology with a self-report measure. However, since a clinical interview was not performed, we cannot exclude the possibility that clinically depressed subjects were part of our population.

Second, compared to previous studies measuring changes in HPA activation and mood due to predefined situational factors (O'Connor et al., 1989; Taylor et al., 1994; Ockenfels et al., 1995; McCleery et al., 2000), the HDI is believed to measure the severity of depressive symptomatology on a more general level. However, it could be argued that the two-week period the HDI is referring to is too short to measure depressive symptomatology beyond a state. On the other hand, it has to be noted that this reference time is consistent with other self-report measures of depression and the time period of symptom duration in the DSM-IV (Reynolds, 1995).

Third, cortisol regulation was assessed during one hour in the morning with strict reference to the time of awakening. We propose that this method has several advantages compared to traditional approaches determining cortisol regulation. Diurnal assessments of cortisol secretion in depressed patients and controls (Asnis et al., 1981; Halbreich et al., 1985; Maes et al., 1986; Dahl et al., 1991; Trestman et al., 1995; Yehuda et al., 1996) led to inconsistent results regarding the time of day that best differentiates between

groups. Whereas measurement of cortisol levels at predefined times underlies large inter- and intra-individual variation (Coste et al., 1994), cortisol sampling during the first hour in the morning with strict reference to the time of awakening appears to produce more reliable results (Pruessner et al., 1997). Moreover, determining early morning free cortisol in saliva is noninvasive, less time consuming than diurnal sampling and can be done at home, without disturbing individual morning habits.

It was intriguing to find group differences in the cortisol response to awakening already in the non-clinical range of depression. When we included subjects with more severe depressive symptoms according to the HDI, the association with cortisol was less clear than for the non-clinical group alone. The common finding that only a small proportion of clinically depressed subjects reacts with cortisol non-suppression to the DST (Carroll et al., 1976a; Carroll, 1984) suggests that clinical depression is characterized by an increased variability of cortisol levels and that additional mechanisms might have an impact on HPA regulation.

Previous reports describing a significant relation between measures of stress and early morning free cortisol (Schulz et al., 1998; Pruessner et al., 1999; Wust et al., 2000) could be confirmed and extended in the current study. Consistent with the results by Schulz and collaborators (Schulz et al., 1998), the present study reports significantly higher cortisol levels after awakening in subjects with high 'work overload'. Moreover, we report a significant cortisol difference between subjects with high and low scores on the total TICS score 30 minutes after awakening, a finding which has not been reported before. Differences to a previous study which reports associations between other TICS sub-scales and cortisol (Wust et al., 2000) might be explained by the smaller sample size and the restriction to male subjects in the present study. Furthermore, the median cortisol level over a period of four weeks assessed here is expected to better represent baseline HPA activity than measurement at a single day.

Interestingly, the highest correlations of the cortisol response after awakening were found with a ten point rating scale assessing momentary feelings of stress. Although a

simple statement about the level of acute stress is rather unspecific and should not be over-interpreted, this finding points to the relevance of actual mood variations in determining cortisol differences. The observed correlation between measures of chronic stress and depressive symptomatology confirmed studies which have provided similar evidence (Breslau & Davis, 1986; Krause, 1986; Phelan et al., 1991; Bifulco et al., 2000). The association between the acute stress measure and the HDI score was even stronger. It can be argued that the time frame both scales are referring to is overlapping. Thus, the acute stress measure might have better reflected the subject's actual situation than the TICS, which is referring to the past year.

It can only be speculated about the causal relationship between stress, depression and elevated cortisol levels, but a mediating role of life stressors for the development of depressive illness has been suggested (Monroe, 1991; Brugha et al., 1997). The underlying dysregulations of the HPA axis in depression and chronic stress states appear to follow a similar pattern. Both conditions are characterized by increases in cortisol secretion, for both an increased central drive, impaired glucocorticoid feedback and hypertrophy of the adrenal gland have been observed (Checkley, 1996; Holsboer & Barden, 1996). The consistent co-appearance of hypercortisolism and depression led several authors to suggest that elevated cortisol levels cause depressive symptoms (Checkley, 1992; O'Brien et al., 1993; Stokes, 1995). Additional evidence for such a relationship comes from studies reporting that depressive symptomatology seems to be reversible by antiglucocorticoid therapy and antidepressant actions on HPA regulation (Murphy et al., 1991; O'Brien et al., 1993; Barden et al., 1995; Holsboer & Barden, 1996; Wolkowitz et al., 1999).

Future studies still need to compare the usefulness of measuring the cortisol response to awakening with traditional approaches to assess HPA dysregulations. It will be particularly interesting to investigate if the proposed method is applicable for patients with clinical depression. Furthermore, future studies should include more detailed questionnaires assessing the association between acute stress perception and the cortisol response to awakening. Knowing about the higher prevalence of depressive

symptomatology in woman (Piccinelli & Wilkinson, 2000), it would also be interesting to include both genders in similar investigations in the future.

CHAPTER 4:

THE ASSOCIATION OF HIPPOCAMPAL VOLUME WITH CORTISOL MEASURES AND COGNITION IN HEALTHY YOUNG MEN:

A STRUCTURAL MRI STUDY

4.1 ABSTRACT

In aged and pathological populations, chronically elevated basal glucocorticoid levels have been repeatedly described in association with reduced hippocampal volume and impairment of hippocampus dependent cognitive processes. Investigation of this relationship in young healthy populations, however, is lacking. Thus, in a group of 13 healthy young men, we investigated the association between hippocampal volume and cortisol levels (basal and stimulated) as well as explicit memory performance. High-resolution 3D Magnetic Resonance Imaging (MRI) was performed, and hippocampal volumes were determined manually according to a recently developed segmentation protocol. Cortisol levels were determined in response to a psychosocial stress test, and in response to awakening, over a period of four weeks. Explicit memory was tested before and after stress with a 'paired associates' paradigm.

Larger hippocampal volume was significantly associated with higher cortisol responses to stress. Subjects with larger hippocampal volumes also showed a significantly higher cortisol increase during the first hour after awakening. Moreover, higher hippocampal volume was associated with lower performance rates in the explicit memory task before stress but not thereafter. The results in this young population stand in contrast to findings in clinical and aged populations showing a positive correlation between the variables in question. However, our findings correspond to and extend a recent study reporting a negative correlation between explicit memory and hippocampal volume in young healthy volunteers (Chantome et al., 1999), and suggest that in young and healthy populations the relationship between hippocampal volume and glucocorticoid regulation might be reversed.

4.2 INTRODUCTION

Elevated glucocorticoid levels are believed to contribute to hippocampal atrophy and impairment in hippocampus dependent memory function. While cognitive deficits can already be observed in healthy young subjects in response to acute elevations of GCs

(Lupien et al., 2002), significant hippocampal volume reduction is mostly reported in aged and pathological populations characterized by chronic exposure to high GC levels (O'Brien et al., 1996; Sheline et al., 1996; Lupien et al., 1998). In these conditions, lower HC volume is commonly accompanied by cognitive deficits (Starkman et al., 1992; Tsolaki et al., 1994; Deweer et al., 1995; De Leon et al., 1997; Kohler et al., 1998; Pavlides & McEwen, 1999; Sapolsky, 2000a). A recent study reported temporal lobe atrophy together with spatial memory deficits and elevated cortisol levels in a group of young female flight attendants characterized by chronic disruption of circadian rhythms (Cho, 2001). A study including young and old subjects reported an inverse relation between HC volumes and basal cortisol levels, controlling for age. Since the association was not investigated separately for young subjects, conclusions about the relationship between hippocampal volume and HPA activity in this age group can not be drawn from this study (Wolf et al., 2002). In another recent study, the association between HC volume and explicit memory performance was investigated in 70 healthy young subjects. The authors were surprised to find a negative association between these variables (Chantome et al., 1999).

Studies on the relationship between GC levels, memory performance, and HC volume in young healthy subjects are lacking or do not include all three variables. Accordingly, the aim of the present study was to investigate the relationship between hippocampal volume, cortisol levels, and explicit memory performance in healthy young subjects. Hippocampal volume was determined manually, separately for the left and right hemisphere using a recently developed protocol (Pruessner et al., 2000). Cortisol was assessed in response to an acute psychosocial stressor and after awakening, which has been shown to be a biological marker for HPA activity (Pruessner et al., 1997). Explicit memory performance was assessed at low and high cortisol levels (before and after the psychosocial stress test).

4.3 METHODS

4.3.1 SUBJECTS

Thirteen healthy young male volunteers (age range 19-32 years, mean age 23.85) participated in the study. Any history of neurological or psychiatric disorder, and alcohol or drug abuse served as exclusion criteria. Furthermore, subjects had to be medication free at the time of testing. Handedness was assessed with the Edinburgh Handedness Inventory. One subject was left-handed, all other subjects were right-handed. The study was approved by the Montreal Neurological Institute' s ethics board, and subjects gave written informed consent prior to the experiment.

4.3.2 EXPERIMENTAL PROTOCOL

Subjects collected saliva samples during the first hour after awakening once a week for four weeks. Subjects reported to the laboratory two times. During the first session, the cortisol response to a psycho-social stress task was determined and explicit memory was tested before and after stress. The second time, magnetic resonance imaging scans were taken of each subject for hippocampal volumetry.

4.3.3 HIPPOCAMPAL VOLUME ASSESSMENT

High-resolution T1 weighted anatomical MRI volumes (1x1x1mm) were acquired on a 1.5 T Siemens Magnetom Vision Scanner. The raw images were transferred to a Silicon Graphics workstation (Silicon Graphics, Mountain View, CA). Pre-processing included correction for image intensity non-uniformities (Sled et al., 1998) and linear stereotaxic transformation (Collins et al., 1994) into coordinates based on the Talairach atlas (Talairach & Tournoux, 1988).

Hippocampal volume analysis was performed using the interactive software package DISPLAY developed at the Brain Imaging Center of the Montreal Neurological Institute. This program allows simultaneous segmentation of brain structures in coronal, sagittal and axial orientations and calculates their volumes. Anatomical boundaries used for the hippocampus and the segmentation process are described in detail in a recently developed protocol (Pruessner et al., 2000).

4.3.4 THE TRIER SOCIAL STRESS TEST (TSST)

The "Trier Social Stress Test" (TSST) is a well-established and highly effective instrument to provoke activation of the HPA axis. In several studies, the TSST has induced considerable changes of ACTH, cortisol (serum and saliva), growth hormone and prolactine as well as significant increases in heart rate (Kirschbaum et al., 1993). Salivary cortisol levels after the TSST reliably show 2-4 fold elevations above baseline within 30 minutes. In the present study, subjects were asked to deliver a free speech and to perform mental arithmetic in front of a false mirror. Immediately before study onset, a person in a white lab-coat was introduced to the subject as an expert judging his non-verbal behavior and linguistic skills. Both the expert and the experimenter were sitting behind the false mirror during the performance. Instructions were given to the subject via a microphone. The TSST lasted 10 minutes. A total of eight saliva samples for cortisol assessment were taken 55, 30 and 15 minutes and immediately before the TSST and immediately, 10, 30, and 50 minutes thereafter.

4.3.5 CORTISOL RESPONSE TO AWAKENING

The pronounced cortisol increase during the first hour after awakening in the morning has been found to be associated with higher measures of self-reported stress (Schulz et al., 1998; Pruessner et al., 1999; Wust et al., 2000; Pruessner et al., 2003b). We were interested in the association of this marker for basal HPA activity with hippocampal

volume. Subjects collected saliva once a week (Wednesday or Thursday) for 4 consecutive weeks at 0, 30, and 60 minutes after awakening in the morning. They were asked to refrain from drinking caffeinated beverages and smoking before saliva sampling. Furthermore, subjects were instructed not to brush their teeth before the end of the sampling time in the morning, not to eat or drink in the 10 minutes before sampling, and to rinse their mouth with water before sampling.

4.3.6 EXPLICIT MEMORY ASSESSMENT

Explicit memory was assessed by a cued recall test described in detail elsewhere (Lupien et al., 1994). The experimental list was comprised of six moderately related word pairs (related-pairs) and six unrelated word pairs (unrelated-pairs). The related pairs correspond to third associates in the free association norms, to minimize guessing in the cued-recall task. The unrelated-pairs were matched to the related pairs in terms of word frequency, word length and grammatical category. The task was presented on a laptop computer. Subjects had to read aloud the list of word pairs. The list was presented twice in different order. Following the presentation, subjects were asked to recall a member of a pair when confronted with the other. Parallel lists with different word-pairs were presented in counterbalanced order and were tested for recall within 30 minutes before and after the TSST.

4.3.7 BIOCHEMICAL ANALYSES

Salivary free cortisol was measured using the Salivette sampling device (Sarstedt, Rommelsdorf, Germany). Biochemical analysis of the saliva samples was performed using a time-resolved immunoassay with fluorescence detection (DELFIA, Dressendörfer et al., 1992]. Inter-assay and intra-assay coefficients of variance were below 12% for all analyses.

4.3.8 STATISTICAL ANALYSES

The average HC volume (right+left/2) was calculated for each individual, and subjects were separated by median split into groups with large and small HC volumes. Furthermore, cortisol measures at different times were transformed into a single value by calculating the area under the curve (AUC; Pruessner et al., 2003a). In order to create a single value for the cortisol response to awakening assessed over four weeks, the AUC was calculated for each day, and the median of these four values was determined. T-tests were calculated to investigate differences in the cortisol response to the TSST and to awakening. Pearson correlations were performed to observe associations between right and left hippocampal volume, the cortisol responses to the TSST and to awakening, and performance on the explicit memory task before and after stress.

4.4 RESULTS

Hippocampal volume and cortisol

Mean right hippocampal volume was higher than mean left hippocampal volume (4200mm^3 and 4090mm^3, respectively). Mean cortisol levels increased from 12.32 nmol/l before the acute psychosocial stress task to 21.09 nmol/l 20 minutes thereafter ($t = -2.65$; $p < .02$). Mean cortisol levels after awakening increased from 17.9 nmol/l to 26.4 nmol/l during the first 30 minutes ($t = -3.37$; $p < .01$).

Separating the group by median split into subjects with large and small mean hippocampal volume ($N = 6$ and $N = 7$, respectively) revealed a stronger cortisol AUC response to the TSST ($t = -3$; $p < .01$) and to awakening ($t = - 4.4$; $p < .001$) for subjects with larger HC volume. Figure 4.1 shows the cortisol response to the TSST for subjects with high and low hippocampal volume. Figure 4.2 shows differences in the cortisol response to awakening in both groups.

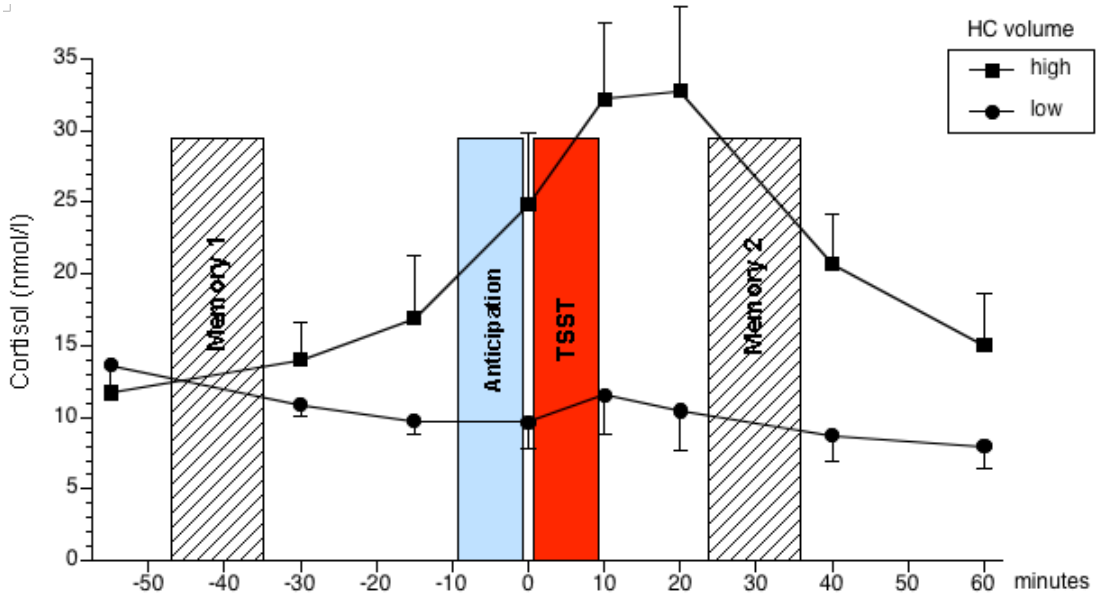

Figure 4.1: Cortisol response to the Trier Social Stress Test (TSST) (+/- SE) in subjects with high and low hippocampal volume

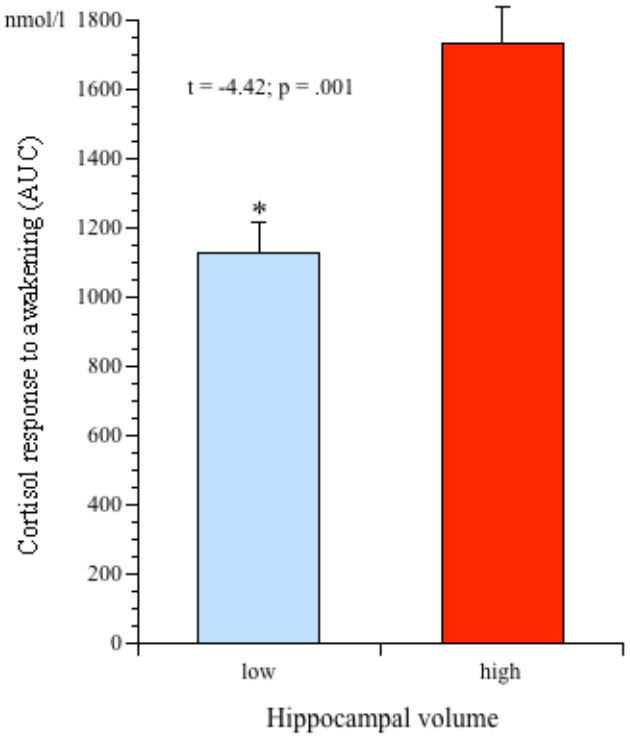

Figure 4.2: Average cortisol response to awakening (+SE) in subjects with low (n = 7) and high (n = 6) mean hippocampal volume (R+L/2).

Higher right and left hippocampal volumes were found to be correlated with a higher cortisol response (AUC) to the acute psycho-social stress test ($r = .74$; $p < .004$ and $r = .67$; $p = .01$, respectively; see Figure 4.3). Correlations for hippocampal volume and the cortisol response to awakening revealed a trend for the right hemisphere ($r = .52$; $p < .07$) and failed to be significant for the left hemisphere ($r = .42$; $p < .16$).

Hippocampal volume and cognitive performance

Performance in the explicit memory task was not significantly different before and after the TSST for both related and unrelated word pairs (each $p > .50$).

Higher right and left hippocampal volumes were correlated with impaired performance in the 'related-pairs' memory task before the TSST ($r = -.57$ and $r = -.58$, both $p < .04$; see Figure 4.4). For related word pairs encoded and recalled after stress, the association with HC was not significant anymore (each $r < .20$, each $p > .50$). No effect was observed for recall of the unrelated word pairs (each $p > .50$).

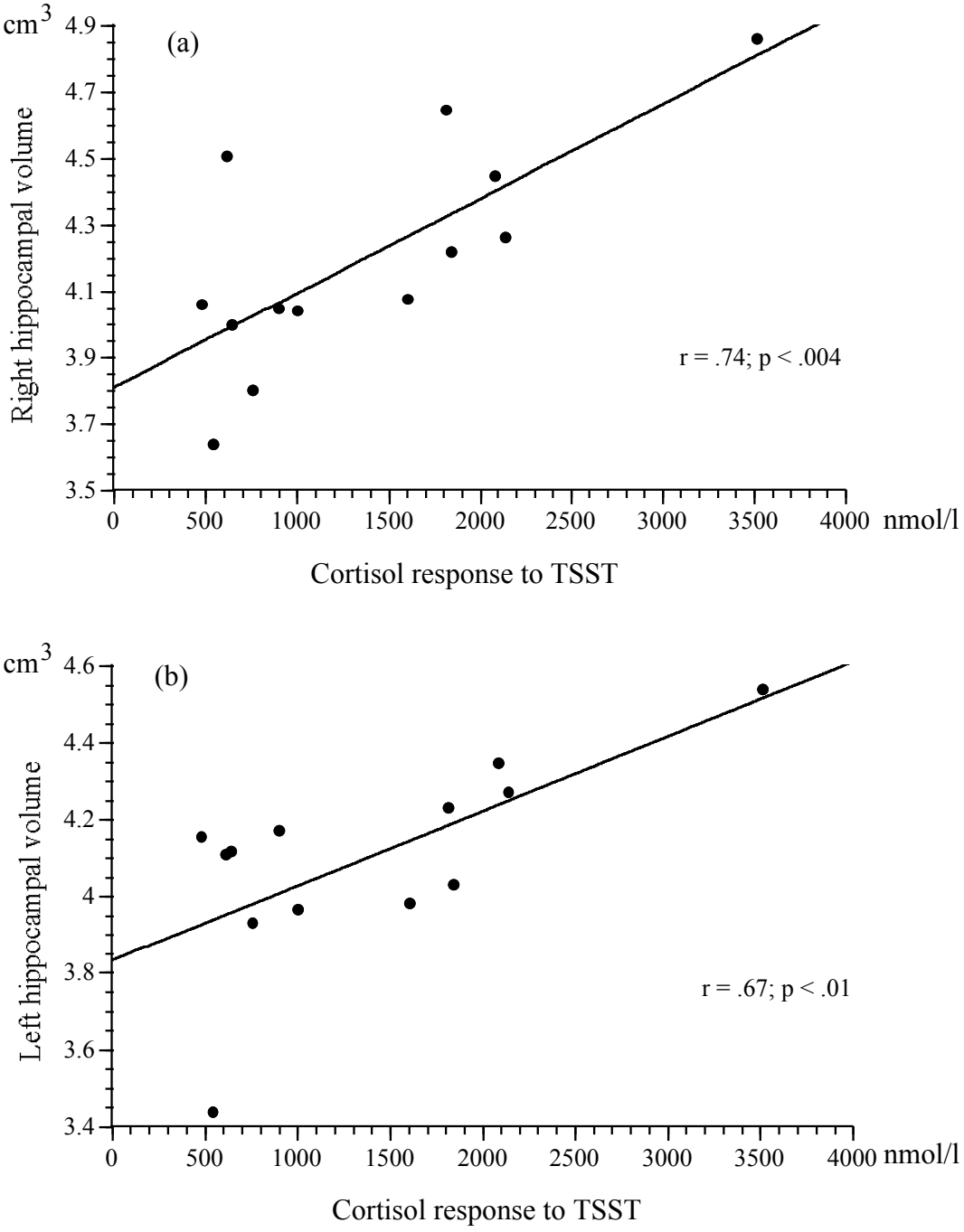

Figure 4.3: Pearson correlation between right (a) and left (b) hippocampal volume and the cortisol response to the Trier Social Stress Test (TSST)

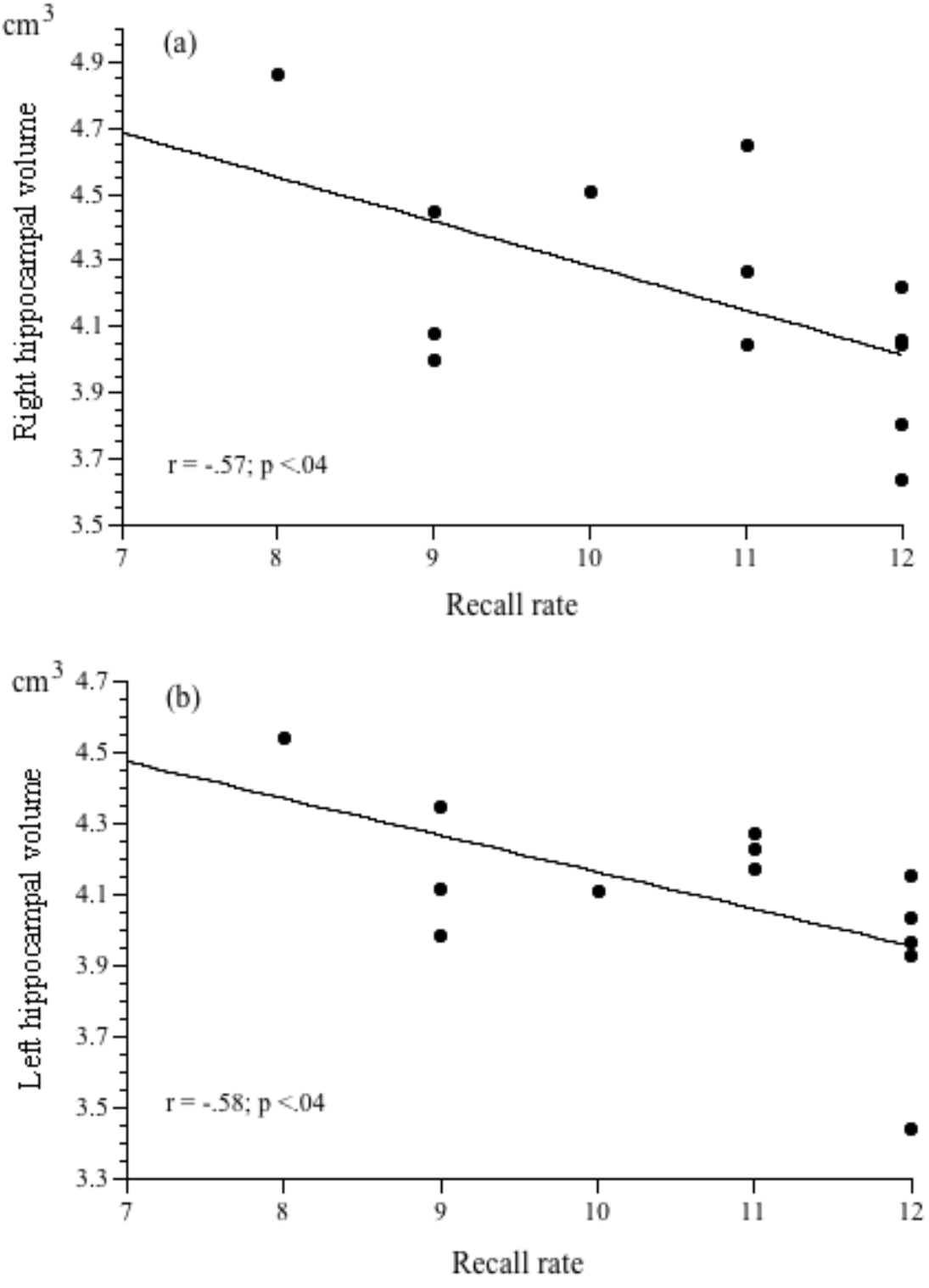

Figure 4.4 Pearson correlation between right (a) and left (b) hippocampal volume and recall rate in a 'paired associates' memory task before the TSST

Cortisol response and cognitive performance

Subjects with lower recall of related words in the 'paired associates' task before stress showed a trend for a higher cortisol response to the psychosocial stress task ($r = -.54$; $p < .06$). This association became insignificant after an 'outlier' was excluded with a cortisol response more than two standard deviations above the group mean. Cognitive performance was not related to the cortisol response to awakening ($p > .50$). Cortisol responses to awakening and to the TSST were not significantly related ($p > .10$)

4.5 DISCUSSION

The present study investigated the association between cortisol levels, HC volume and explicit memory performance in young healthy individuals. Larger hippocampal volume was related to a higher cortisol response to both a psycho-social stress test and awakening in the morning. Whereas the cortisol response to the first test represents the responsiveness of the HPA axis to a single acute challenge, repeated assessment of the cortisol increase during the first hour after awakening in the morning is believed to represent general HPA activity in an individual (Pruessner et al., 1997). Furthermore, a larger hippocampal volume was negatively correlated to performance in an explicit memory test carried out before the TSST, but not to a parallel test performed after this psychosocial stress task.

In previous studies, the association between HC volume and cortisol levels has mostly been investigated in pathological and aged populations, characterized by HPA dysfunction. Chronically elevated GC levels in these studies were repeatedly found to be associated with HC atrophy (Starkman et al., 1992; O'Brien et al., 1996; Sheline et al., 1996; Lupien et al., 1998). The observed association is usually explained by neurotoxic effects of GCs on the HC, with disturbances in dendrite branching, neurogenesis and glucose metabolism, eventually resulting in atrophy of the structure (Reagan & McEwen, 1997; Gould & Tanapat, 1999; Sapolsky, 2000a). Furthermore, earlier studies frequently report an association between reduced HC volume and impaired explicit memory

performance (O'Brien et al., 1997; Kohler et al., 1998; Lupien et al., 1998; Pavlides & McEwen, 1999; Cho, 2001). These populations are also characterized by elevated basal cortisol levels due to different reasons, and a simple determination of the causal relationships between the factors becomes difficult.

Current literature focuses on detrimental effects of stress and glucocorticoid increase on HPA function and hippocampal integrity. The association between hippocampal volume, cortisol levels and memory performance in healthy young populations has not been investigated. The results of the present study contradict the commonly reported findings, suggesting that this relationship might be reversed in the absence of stress or chronically elevated cortisol levels. It has to be kept in mind, that a cortisol response is regarded as beneficial and essential in helping the body to cope with a challenging situation (Selye, 1936; Mason, 1968; Sapolsky, 2000b). In our group all cortisol levels are in a non-pathological range (Thomas, 1992). Also, mean hippocampal volumes correspond to earlier findings for young adulthood (Pruessner et al., 2001). In a young healthy population, both a pronounced cortisol response to acute stress and awakening and a large hippocampus could be regarded as parts of a healthy and functional HPA system, allowing successful adaptation to short-term stress. Thus, a positive correlation between both measures seems possible. Only in the case of cumulative overexposure to high GC levels, permanent atrophy would be expected (Sapolsky, 1996).

A recent study is in support of our findings. In a group of seventy healthy young adults, Chantome and coworkers (Chantome et al., 1999) report a negative correlation between explicit memory performance and hippocampal volume. However, no cortisol measures were performed in this study.

Considering the inverted u-shape relationship that has been proposed between GC levels and hippocampal function (Lupien & McEwen, 1997), it could be speculated that a larger HC needs a higher cortisol response to ensure optimal hippocampal functioning, maybe due to a larger number of GC receptors. GC levels appear to be optimal for HC dependent processes when all mineralocorticoid receptors (MRs) and some of the

glucocorticoid receptors (GRs) in the HC are occupied (de Kloet et al., 1999). Initial cortisol levels might not have been optimal in subjects with larger hippocampi, explaining the negative correlation between HC volume and memory performance before the psychosocial stress task but not afterwards. There is increasing evidence for beneficial effects of moderately elevated GC levels on hippocampus dependent memory (Lupien & McEwen, 1997; Lupien et al., 2002).

It cannot be ruled out that the observed associations of cortisol levels and explicit memory performance with HC volume are explained by another, non-identified variable. Also, the observed correlation between cortisol and HC volume could be an accidental finding, thus being not representative of a young healthy population.

The results of the present study have to be regarded as preliminary. Future studies should include female participants and larger groups. In order to gain insight in the changing relationship between cortisol levels, HC volume and explicit memory performance over the life span, subjects of different age phases should be included or even a longitudinal study design should be attempted. The identification of characteristics of 'healthy' HPA function is expected to contribute to a better understanding of pathological processes.

CHAPTER 5:

EFFECTS OF PSYCHOSOCIAL STRESS ON HIPPOCAMPAL ACTIVATION DURING PICTURE ENCODING:

A FUNCTIONAL MRI STUDY

5.1 ABSTRACT

Stress and elevated glucocorticoid (GC) levels are considered harmful to the hippocampus (HC), with acute increase resulting in impaired memory function, and chronic increase resulting in HC atrophy. Animal- and *in vitro* studies revealed GC dependent metabolic changes within the HC. The present functional magnetic resonance imaging (fMRI) study was performed to determine brain activation during a stressful mental challenge task (TMCT) and to assess the effect of this stressor on HC activation and memory performance during picture encoding.

12 healthy male students participated in the study. The stressful task led to activation of several brain structures including amygdala and HC. The encoding task resulted in bilateral HC, parahippocampal, fusiform and lingual, and right amygdala activation. When comparing brain activation during encoding before and after the TMCT, a distinct reduction in right HC head activation after stress became apparent. Right and left HC activation was highly correlated with encoding success for pictures presented before the TMCT. Following stress, this association became weaker for the left and disappeared for the right HC. Recognition in the total group was significantly improved when pictures were presented after the TMCT, probably due to a delay effect. However, high HC activation during the TMCT resulted in impairment of subsequent right HC activation during encoding and a loss of the memory delay effect. Mean cortisol levels as well as HC activation during the mental challenge task were significantly and negatively correlated with performance levels during the TMCT. The present findings suggest that stress and HC activation during stress impair subsequent HC activation and performance during a memory task.

5.2 INTRODUCTION

The hippocampus plays an important role for explicit memory processes (Scoville & Milner, 1957). Functional imaging studies have identified HC activation in association with novelty assessment (Tulving et al., 1994), spatial memory tasks (Maguire et al., 1997) and the establishment of relations between components of memory (Henke et al., 1997; Henke et al., 1999). It seems that forming associations between different components during encoding tasks is the critical quality of stimuli to evoke HC activation in neuroimaging studies (Cohen et al., 1999). A memory task that meets the characteristics of novelty and relational memory and that has consistently produced HC activation in the past is encoding of visual complex scenes (Stern et al., 1996; Detre et al., 1998).

A large number of findings from animal and human studies suggest that the hippocampus is vulnerable to stress and glucocorticoid (GC) actions (for reviews see Sapolsky et al., 1987; Lupien & McEwen, 1997; McEwen & Magarinos, 1997; Bremner, 1999; Sapolsky, 2000). Prolonged stress or chronic GC exposure can cause HC volume loss via dendritic atrophy in HC pyramidal cells (McEwen & Sapolsky, 1995) or inhibition of neurogenesis in the dentate gyrus (Sapolsky, 2000). Acute GC effects include a reduction in neuronal excitability (Joels & de Kloet, 1989) and glucose metabolism (de Leon et al., 1997).

Moreover, stress and high GC levels appear to impair long-term potentiation (LTP) in the HC, which has been discussed as a neurobiological mechanism critically involved in memory formation (Diamond et al., 1988; Shors & Matzel, 1997). In line with these findings, it has been shown that elevated GC levels impair memory function (Starkman et al., 1992; Lupien et al., 1994; Newcomer et al., 1994; Kirschbaum et al., 1996; O'Brien et al., 1996; Lupien et al., 1997; Lupien et al., 1998). Although most studies report a negative association between GC levels and memory performance, recent research suggests that the association between GCs and HC processes might follow an inverted u-

shape function, with moderate GC levels being necessary for LTP and optimal memory performance (Lupien & McEwen, 1997; de Kloet et al., 1999).

A hypothesis that can be derived from the above findings is that stress modulates HC activation during memory processing. The present functional magnetic resonance imaging study was designed to investigate this relationship in humans. Subjects performed novel picture encoding before and after a fMRI version of a stressful mental challenge task. Similar tasks have shown their potential to increase cortisol levels (Kirschbaum, 1991; Pruessner et al., 1999) and to modulate HC activation (Soufer et al., 1998; Critchley et al., 2000) in the past. During both encoding and mental challenge conditions, blood-oxygen-dependent (BOLD) signal changes in the brain were measured. Effects of stress on brain activation during encoding and associations between HC activation, memory performance and cortisol levels were investigated.

5.3 MATERIALS AND METHODS

5.3.1 SUBJECTS

Twelve healthy young male volunteers (age range 19-32 years, mean age 24) participated in the study. Any history of neurological or psychiatric disorder, and alcohol or drug abuse served as exclusion criteria. Furthermore, subjects had to be medication free at the time of testing. Handedness was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). One subject was left-handed, all other subjects were right-handed. The study was approved by the Montreal Neurological Institute's ethics board, and subjects gave written informed consent prior to the experiment.

5.3.2 PROCEDURE

Figure 5.1 illustrates the order and time frame of the different experimental materials and methods used. Immediately before and after the fMRI session, outside the scanner, saliva samples for cortisol assessment were taken. After an anatomical scan, subjects were asked to perform three runs of picture encoding. Then, subjects performed four runs of a mental arithmetic task, expected to modulate cortisol levels, followed by three more runs of picture encoding. Finally, picture recognition was tested outside the scanner. In order to control for diurnal variations in cortisol secretion, all subjects were tested in the afternoon between 2 and 4pm.

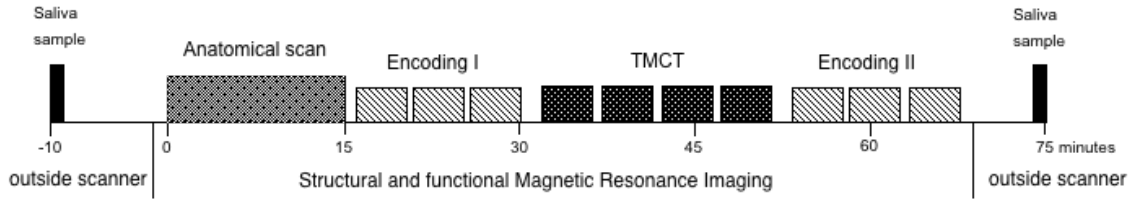

Figure 5.1 Time frame of experimental parts

5.3.3 FMRI EXPERIMENTAL TASK

Scene Encoding and Baseline Tasks

252 novel complex scenes were selected from a database (Art Explosion, Nova Development Corp. USA, 1996) and separated into two sets with 126 pictures each for counterbalancing purposes. The stimulus criteria were based on the study by Stern and collaborators (Stern et al., 1996). All scenes consisted of a set of different elements (objects, nature, buildings, people, and animals), difficult to describe with a simple name. Pictures for the first and second encoding conditions were counterbalanced in a way that half of the subjects saw the first set of scenes before the mental challenge test and the second set thereafter, for the other subjects the order was reversed. Subjects were

instructed to attempt memorization of the novel pictures during scanning. Pictures were projected onto a screen behind the subject 's head, and were visible for the subject through a mirror attached to the head-coil. A new item was presented every three seconds. For the purpose of comparison, subjects also performed a baseline task during scanning. In this task, two scenes alternated every three seconds. Here, subjects were asked to simply look at the pictures. 126 functional volumes per scanning run were acquired at a rate of one volume every 2 seconds. 14 novel pictures were followed by 14 baseline presentations. This sequence was repeated three times per run. Each run contained 42 novel complex scenes and 42 presentations of the two baseline scenes. Each subject completed three encoding runs before and three encoding runs after the mental challenge condition.

The Trier Mental Challenge Test (TMCT)

The TMCT is a psychosocial stress task employing mental arithmetic. Time limitation and continuous negative feedback create a stressful environment. This test has been shown to successfully increase cortisol levels in the past (Kirschbaum, 1991; Pruessner et al., 1999). In order to fit the design of a functional MRI study, the TMCT was modified from its original version. In the version used in this experiment, computer-generated equations containing addition, subtraction, division and multiplication (e.g. 3 x 18 - 49 = 5) were presented on the screen. Subjects were asked to decide if the result shown was correct or incorrect by pressing the right or left mouse button. For each task they were given 5 seconds to decide. A progress bar above the equation indicated the time left to answer. When the time limit was exceeded, the message 'TIME OUT' flashed on the screen for two seconds and a new equation was presented. If the subject pressed a mouse key within the time limit, immediate feedback was given by the message 'CORRECT' or 'INCORRECT'. In the lower left corner of the screen the subject' s score was presented (e.g. 5/10, meaning 5 out of 10 tasks were answered correctly). In order to meet the requirements for fMRI stimulus presentation, a baseline task was added to the TMCT, where subjects had to decide via mouse click if a given number was odd or even. Feedback was given like in the other task, but there was no time limit. Experimental and

baseline task alternated in one-minute intervals. Each run consisted of two experimental and two baseline conditions and lasted four minutes. In total, subjects had to perform four runs of the mental challenge task with 120 frames each acquired every two seconds.

Picture recognition

Five to ten minutes after the entire scanning procedure, picture recognition was tested with a forced choice paradigm. Subjects were presented with all 252 scenes in a randomized order on a laptop computer. Each picture of the scanning period was paired with a new one side-by-side, and subjects were asked to decide which of the two they had seen before.

5.3.4 CORTISOL ASSESSMENT

Salivary cortisol was obtained 10 minutes before and immediately after the end of the scanning session using the Salivette sampling device (Sarstedt, Rommelsdorf, Germany). In order to control for diurnal variations in cortisol secretion, all subjects were tested in the afternoon between 2 and 4pm. Biochemical analysis of the saliva samples was performed using a time-resolved immunoassay with fluorescence detection (DELFIA, Dressendörfer et al., 1992).

5.3.5 MRI IMAGE ACQUISITION

Subjects were scanned on a 1.5 T Siemens Magnetom Vision Scanner. Head immobilization was achieved by foam padded ear cups and a nose bar mounted to the head coil. During the first fifteen minutes, high-resolution T1 weighted anatomical images (1x1x1mm) were acquired. During the following 60 minutes, T2* weighted volumes with blood oxygenation level-dependent (BOLD) contrast were obtained for functional MRI scanning using the Mosaic 64 sequence. 17 axial 7mm thick slices were

acquired continuously in an angle along the long axis of the HC (In-plane resolution: 3,59 X 3,59 mm; FOV 230mm, TR: 0.8sec; flip angle: 90 degrees; TE: 50ms). Scanning time and stimulus presentation were synchronized by a trigger signal from the scanner at the beginning of every volume.

5.3.6 Data analysis

Functional MRI analyses

Images were preprocessed by applying a 6mm Gaussian smoothing kernel and motion correction with alignment to the third frame of each run. Statistical analysis of fMRI images was performed with the in-house software packages fmristat and multistat (Worsley et al., 2002). In order to verify that our task led to similar results than previous studies (Stern et al., 1996), in a first step only brain activation during encoding before the TMCT was determined. Likewise, brain activation during encoding after stress and during the TMCT was determined. Therefore, the dynamic data sets were transformed into standard stereotaxic space, and blood flow differences between experimental and baseline conditions were computed as t-maps. In order to identify changes in brain activation between the encoding condition before and after stress, the t-maps resulting from picture encoding after stress were subtracted from activation during encoding before the TMCT.

Statistical analysis

For further statistical analyses, a region of interest analysis was performed for the right and left hippocampus with identification of the individual peaks of activation (t-values). For the determination of boundaries, a recently developed protocol for HC segmentation was employed (Pruessner et al., 2000). The obtained HC activation values entered further statistical analyses as dependent variables. Pearson correlations were calculated between right and left HC peak activation and picture memory separately for the data acquired before and after stress.

The cortisol response to the TMCT was determined by testing the difference between both samples with a t-test for correlated samples. Memory performance was quantified after the scanning procedure as the number of correctly recognized pictures separately for both encoding conditions (before and after stress). In order to create an index for math performance, the relation of correct answers to all answers given by the individual subject was multiplied by the quotient of the individual number of answers and the highest number of answers given by any of the subjects. (MATH_COR – [MATH_ANS-MATH_COR])*(MATH_ANS/35.38). Due to technical problems during the stress condition, data on math performance rates was only available for n=10 subjects. Pearson correlations were calculated between cortisol levels, cognitive data and HC activation. In order to determine the effects of HC activation during stress on subsequent HC activation and performance during picture encoding, t-Tests for independent samples were calculated with groups showing high and low HC activation during stress.

5.4 RESULTS

Effects of novel picture encoding on brain activation

Subtracting the baseline condition from the encoding of novel complex scenes across all subjects before the mental arithmetic task resulted in bilateral (right>left) activation in HC areas. In line with earlier findings (Stern et al., 1996), this comparison also revealed activation in parahippocampal, fusiform, and lingual areas bilaterally and in the right amygdala (Table 5.1). Figure 5.2 shows the activation for the total group before the TMCT in coronal sections.

Changes in brain activation and cognitive performance during encoding after the TMCT

The activation pattern during encoding after the TMCT appeared to be similar to the first encoding condition. In order to determine possible effects of our stress task, the t-maps for the second encoding condition were subtracted from those before the TMCT. Results revealed a decrease in blood flow in our region of interest, which was specific to the right

HC head (t =2.6; p < .05, uncorrected). The individual peak t-values of left and right HC activation served as dependent variables for subsequent analyses. A two-factor ANOVA with time (before and after TMCT) and hemisphere (right and left) as independent variables revealed a significant interaction effect of time by hemisphere on HC activation. Figures 5.3a and b illustrate that especially right HC activation was reduced after the TMCT. Right and left HC activation at each time point were significantly correlated with each other (r = .71; p < 0.01 and r = .84; p <0.001, respectively).

		Coordinates			t-value
Brain area		x	y	z	
R	fusiform gyrus	32	-46	-18.3	10.51
R	Lingual gyrus	18.9	-52.7	-4.0	6.16
R	Parahippocampal gyrus	24.4	-40	-12	9.57
R	Pulvinar (posterior thalamus)	11.8	-28	-1.6	7.04
R	Hippocampus (posterior)	28	-33.8	-8.6	7.47
R	Hippocampus (anterior, head)	28	-19.7	-18.1	6.24
R	Amygdala	21.3	-8	-13.7	6.9
R	Occipital gyrus (medial)	34.7	-80	4.5	11.67
R	Cingulate gyrus	16.4	-50.8	6.2	5.66
L	Fusiform gyrus	-31.9	-36	-14.1	10.08
L	Parahippocampal gyrus	-30.1	-30	-22.3	7.61
L	Pulvinar	-17.2	-30	-0.7	7.54
L	Hippocampus (posterior)	-22	-31.8	-8.2	6.39
L	Hippocampus (anterior, head)	-24	-17.8	-13.8	5.44
L	Occipital gyrus (medial)	-40.9	-78	4.5	8.19
L	Cingulate gyrus	-12	-55.9	6.2	5.59
L	Superior parietal	-26.6	-63.7	58	6.67

Table 5.1: Brain areas showing significant BOLD signal increases during encoding of visual complex scenes (peak t-values in each area with Talairach coordinates; critical threshold: t = 4.66; p<0.0001)

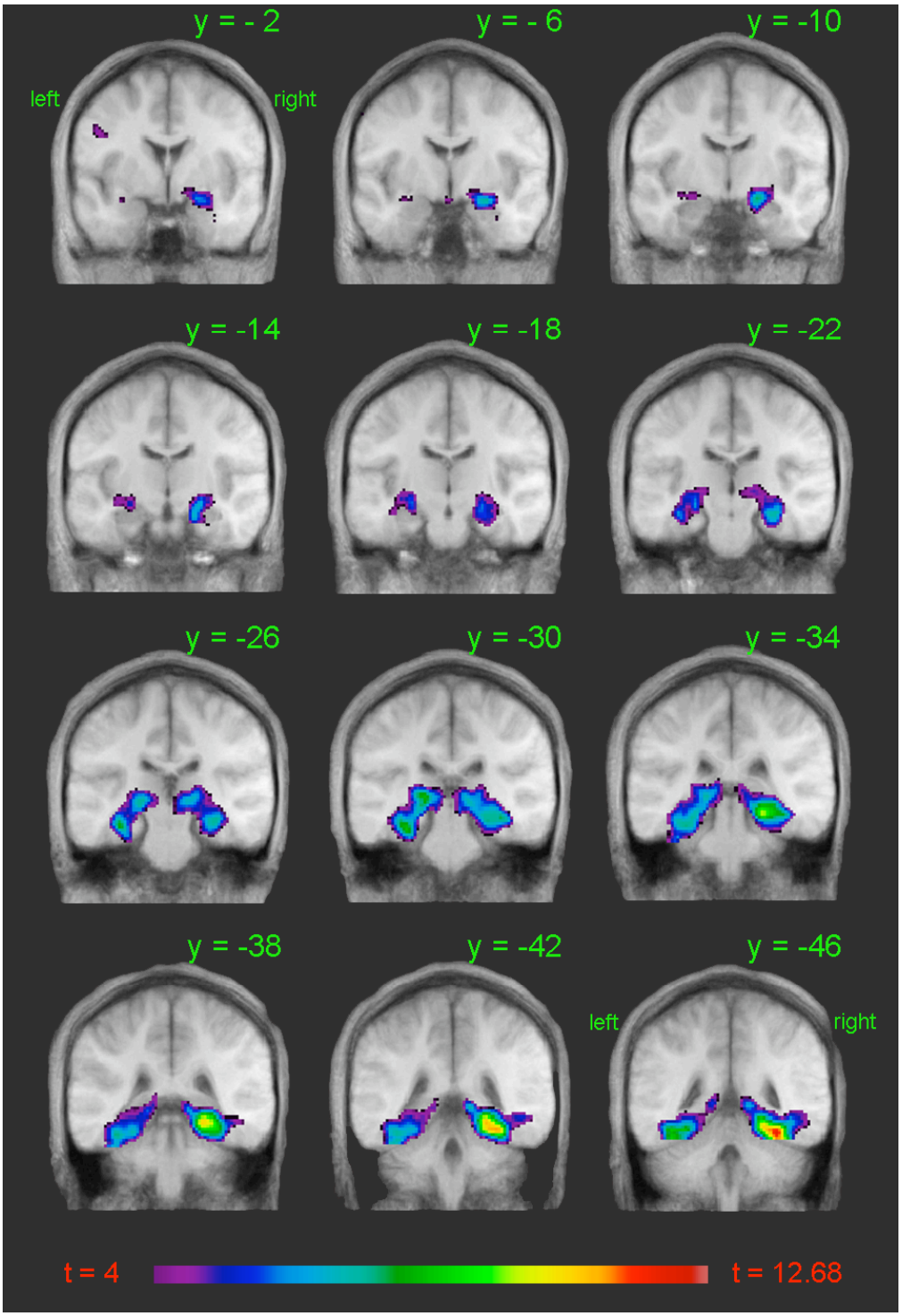

Figure 5.2: T-maps of functional activation during encoding of visual complex scenes. The average of all 12 subjects before the mental challenge task is shown in coronal slices superimposed on the average Talairach transformed brain of all subjects; picture threshold at t = 4, statistically significant at $p < 0.0001$ when $t > 4.66$.

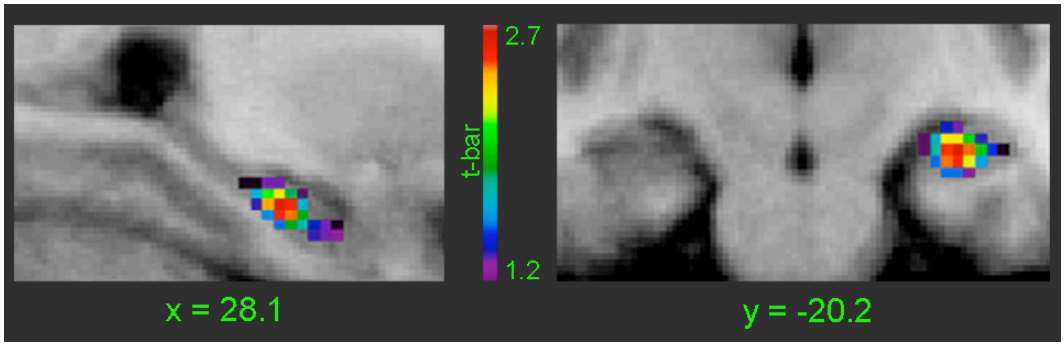

Figure 5.3a: T-map resulting from subtraction of brain activation during picture encoding after the mental challenge task (TMCT) from activation during a parallel task before the TMCT. Activation is superimposed on the Talairach transformed average anatomical scan of all subjects and shown for a sagittal and coronal slice of the right hippocampus; Picture threshold: t = 1.2; peak activation: t =2.6; $p < .05$, uncorrected.

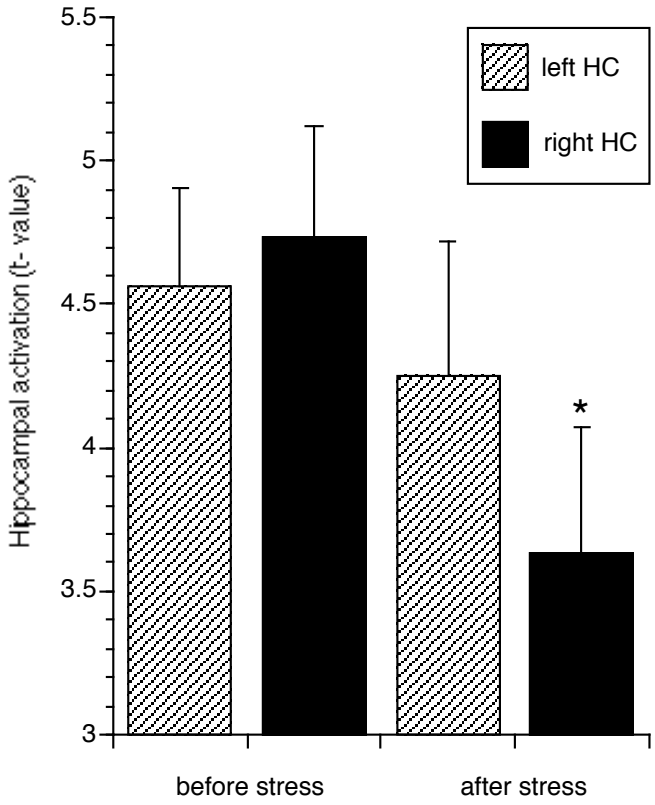

Figure 5.3b: Peak hippocampal activation before and after the mental challenge task ($F = 5.31$; $p < .04$)

Cortisol response to the TMCT and associations with HC activation and cognitive performance

For the total group, cortisol levels did not change between the two times of measurement before and after the fMRI scanning procedure [t (11) = .24; p = .81]. However, cortisol levels increased in seven subjects and decreased in five subjects over the course of the experiment, suggesting the presence of responders and non-responders to psychosocial stress in this group (Lupien et al., 1997). Average cortisol levels in the responder group were increased by 47% at the end of the experiment.

Higher mean cortisol levels were correlated with a lower percentage of correct answers out of all answered math tasks ($r = -.70$; $p < .02$). Cortisol levels were not associated to recall success of pictures. Also, there was no correlation between cortisol levels and HC activation during the TMCT, but a trend for lower right HC activation after stress when cortisol levels were initially high ($r = .53$; $p < .08$).

During the recall condition, subjects remembered 83.3% of the scenes presented before the TMCT and 86.4% of the pictures presented after ($t= 2.4$; $p < 0.03$). A higher encoding rate before the TMCT was significantly correlated with higher right and left activation of the HC head at that time ($r = .78$; $p < 0.003$ and $r = .77$; $p < 0.003$, respectively). After the TMCT this effect faded, and only activation of the left HC head remained correlated with the amount of memorized scenes presented at that time ($r = .52$; $p < 0.08$). Activation of the right HC head after the TMCT was no longer significantly correlated with the encoding rate ($r = -.31$; $p = 0.33$). Figures 5.4a to d illustrate these associations.

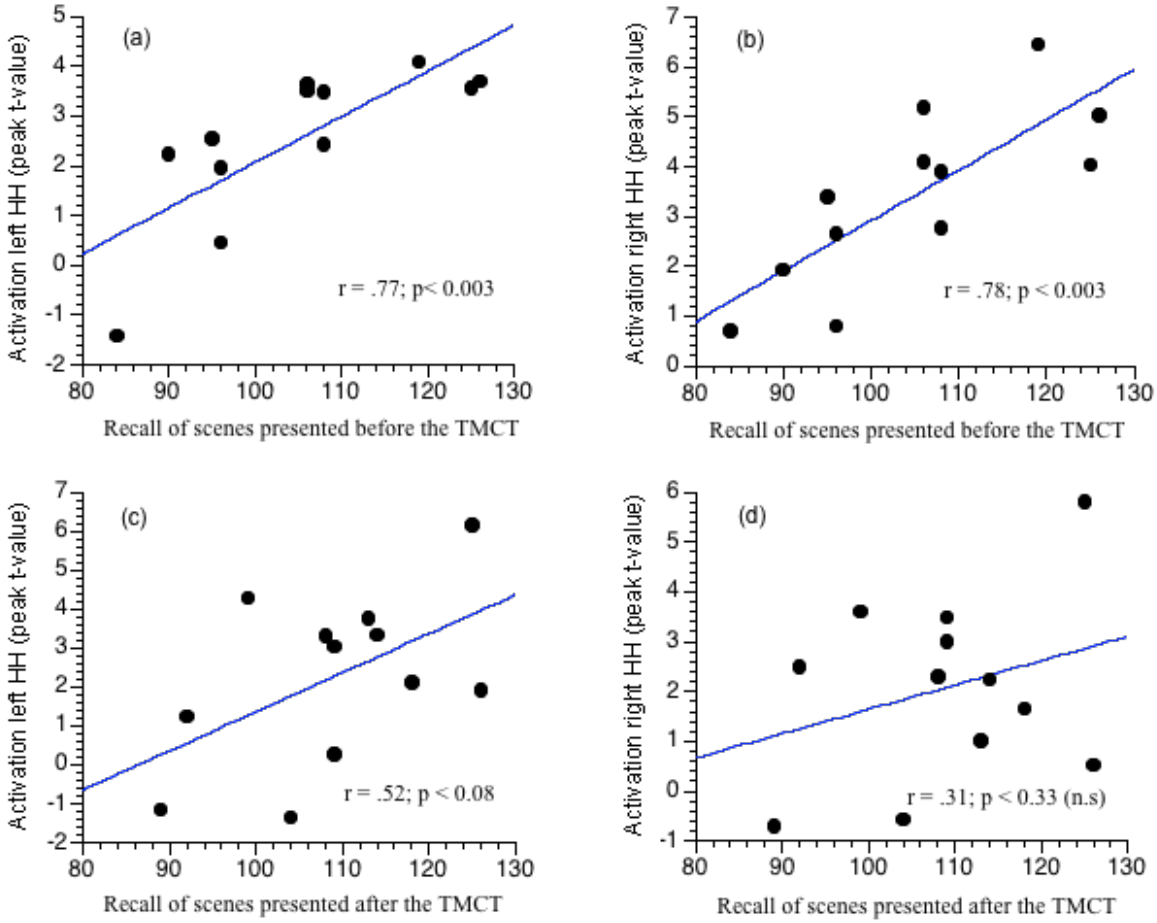

Figure 5.4: Pearson correlations between hippocampal head activation and number of recognized visual scenes. (a) left and (b) right HC before the TMCT; (c) left and (d) right HC after the TMCT

Brain activation during the TMCT and effects on cognitive performance and hippocampal activation during subsequent encoding

The mental challenge task compared to baseline revealed activation in Cingulate gyrus (R: 5.7/-10/35.4; t =6.0 and L: x=-10.1/6.0/31.9; t = 5.38), Medial frontal gyrus (R:14.0/66/1.5; t=6.2), Medial parietal lobe (R:-4.0/-47.6/35.1; t=3.9 and L: 4.0/47.1/36.8; t=3.9), Amygdala (R: 20.1/-4.0/-15.9; t=3.8 and L:-24/-10.1/-13.7; t=2.9) and Hippocampus (R:32.1/-14.0/-15.9; t=3.16 and L: –25.6/-12/-17.9; t=2.4). Figure 5.5

shows the HC activation during the mental challenge task in a coronal section across the HC head.

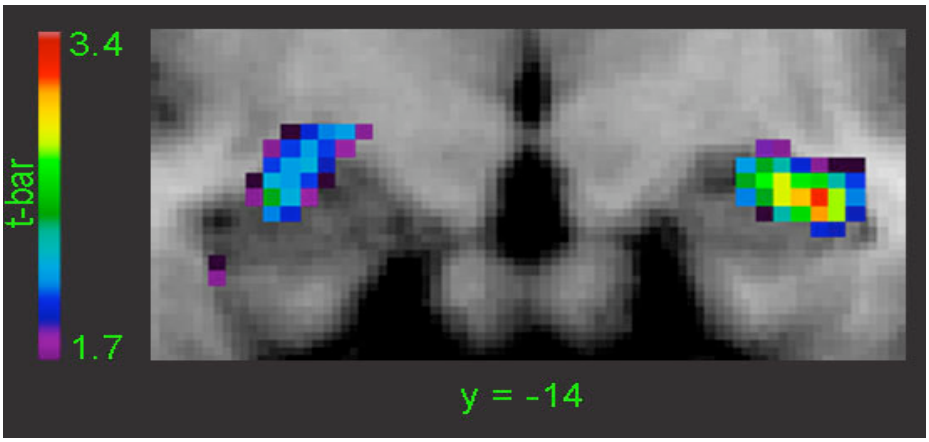

Figure 5.5: Hippocampal activation during performance of the mental challenge task

Using the index for math performance, HC activation during the TMCT was negatively correlated with performance in the arithmetic task constituting the stressful condition ($r = -.74$; $p < .01$, see figure 5.6).

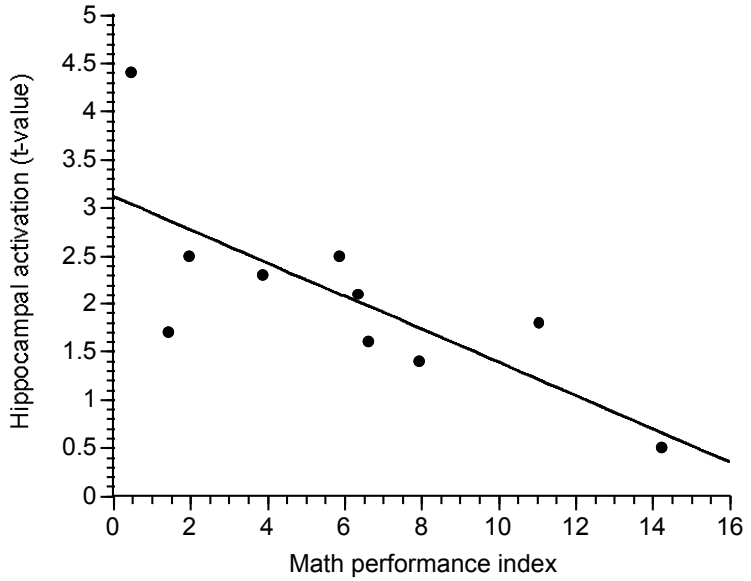

Figure 5.6: Pearson correlation between hippocampal activation during the TMCT (peak t-value right or left) and math performance; $r = -.74$, $p < .01$, $n = 10$

High hippocampal activation during the TMCT resulted in a reduction of right hippocampal activation during encoding ($t = 2.76$; $p < .02$; see figure 5.7a). Likewise, only subjects with low HC activation during the TMCT presented the delay effect with improvement of memory under the second encoding condition resulting in 7.34% better recall, whereas subjects with high HC activation during the mental challenge task only showed a 0.6% improvement in picture recall ($t = 2.26$; $p < .05$, see figure 5.7b).

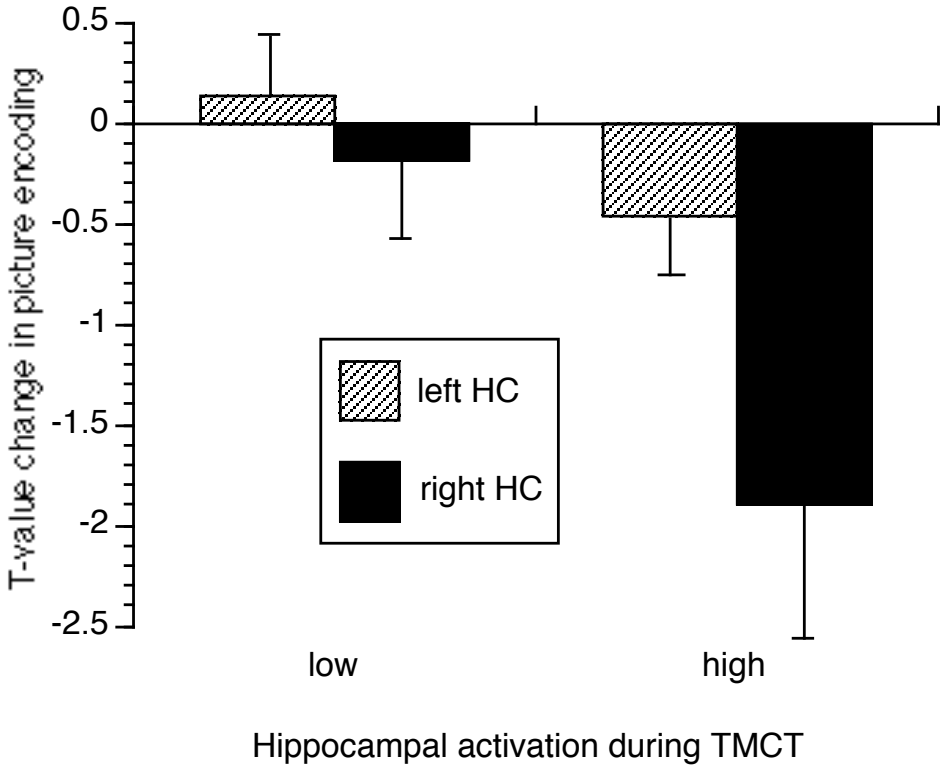

Figure 5.7a: Effect of low or high hippocampal activation during the TMCT on HC activation during picture encoding; group effect: $F = 5.43$, $p < .05$; hemisphere effect: $F = 4.9$, $p < .06$; interaction effect group x hemisphere: $F = 6.48$, $p < .03$

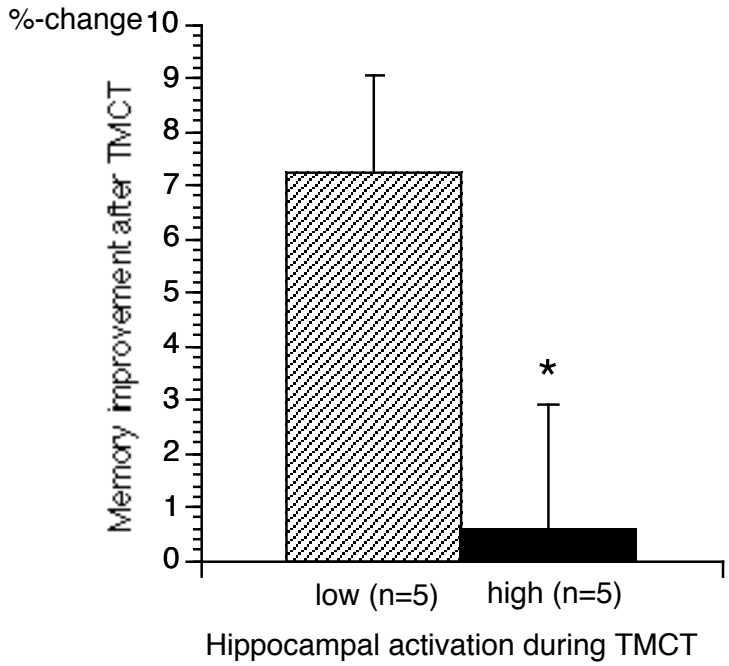

Figure 5.7b: Memory improvement (% -change between first and second encoding condition) is impaired by hippocampal activation during the mental challenge task; $t = 2.26$; $p < .05$.

5.5 DISCUSSION

A requirement for the investigation of hippocampal blood flow changes in response to a stressor is to employ a memory paradigm that effectively activates the HC. As expected, the encoding of novel complex visual scenes used here produced activation in the HC bilaterally, which is comparable to previous imaging studies using this kind of paradigm (Stern et al., 1996; Detre et al., 1998). In addition we found significant activation in bilateral parahippocampal, fusiform, and lingual areas as well as in the right amygdala. The observed pattern is in line with recent studies, showing bilateral and right-lateralized medial temporal lobe activation for episodic memory encoding of non-verbal material (Cabeza & Nyberg, 2000). Finding activation of the right amygdala supports the view

that this structure plays a role in memory encoding and moreover indicates a potential emotional component of the stimulus material (Hamann et al., 1999; Canli et al., 2000).

Demonstrating a decrease in HC activation after the mental challenge task, the present study for the first time provided evidence for stress effects on cerebral blood flow in humans. Results from animal and in vitro studies are consistent with the assumption that this effect was caused by stress and the associated increase in cortisol levels. Reduced HC activity due to elevated glucocorticoid levels has been reported for different levels of HC functioning like LTP (Diamond et al., 1988; Shors & Matzel, 1997) and glucose metabolism (de Leon et al., 1997). Some indirect evidence for GC effects on HC activation has come from electrophysiological studies (Joels & Vreugdenhil, 1998; Joels, 2001).

Interestingly, a clear asymmetry with especially right hemispheric reduction of HC activation was observed. This finding is in line with a recent study reporting a stress induced de-activation in the right HC (Critchley et al., 2000). It is speculated that the right HC is more sensitive towards glucocorticoid actions than the left. Evidence in this direction comes from authors postulating a dominant role of the right hemisphere in the control of cortisol secretion (Wittling & Pfluger, 1990; Sullivan & Gratton, 1999).

The observation that both left and right HC head activation were strongly correlated with encoding success prior to the mental challenge task corresponds with findings showing that the magnitude of focal activation is associated with cognitive performance rates (Brewer et al., 1998; Wagner et al., 1998; Fernandez et al., 1999). We extended this finding by demonstrating that this relationship appears to be modulated by stress. The correlation with encoding success became considerably weaker for the left and vanished for the right HC after the TSST. This observation suggests a disrupting effect of the stressful task on the association between HC activation and related memory processes, possibly mediated by elevated glucocorticoid levels. Again, the right hemisphere seemed to be affected more strongly, supporting the assumption of a higher sensitivity of the right HC to stress effects.

Pictures presented after the stress task were remembered better than those presented before. An explanation for this finding is that pictures presented more closely to the time of recall are remembered better. This 'delay effect' could have just masked a possible association between recall rate and HC activation in the second memory condition. Furthermore, it can be speculated whether the mental challenge task increased the subjects' arousal and attention. As a consequence, less effort to perform the encoding task could have resulted in the observed decrease in HC activation. The possibility that more efficient cognitive strategies require less metabolic activation has been discussed before (Parks et al., 1988). Also, reduced HC activation could have caused other brain structures to compensate for lost HC activation, a phenomenon that has been observed in atrophic brains (Garrido et al., 2002), and thus still maintain cognitive function.

Interestingly, the observed stress effects on activation and the association between activation and cognitive performance seem to be specific to the HC head. This finding agrees with results from recent imaging studies suggesting that this area plays a specific role in cognitive processing (Ouchi et al., 1998; Strange et al., 1999) and is stronger affected by individual differences (Pruessner et al., 2001).

The stressful character of mental arithmetic has been established in previous studies (Kirschbaum, 1991; Soufer et al., 1998; Pruessner et al., 1999; Critchley et al., 2000). In addition, we determined endocrine and cognitive measurements as well as brain activation during the TMCT in the present study. Due to access restrictions inside the scanner, only two cortisol samples could be taken just before and after the scanning procedure. Since the samples could not be synchronized with the experimental tasks they only provide a rough estimate of GC changes in response to the stressful situation. With these two measurements, we observed cortisol levels that remained at the same height across time. Without external stimulation, cortisol secretion follows a diurnal rhythm with high levels after awakening and a continuous decrease over the course of the day (Weitzman et al., 1971; Desir et al., 1980). The increase in cortisol levels in a subgroup of subjects suggest a moderate stress effect for at least part of the population. It is not surprising that some subjects did not show a cortisol increase since earlier studies have

already identified inter-individual differences in this response (Malarkey et al., 1995; Lupien et al., 1997; Biondi & Picardi, 1999).

We identified a negative correlation between cortisol response and performance rate in the mental challenge task, which further supports the view of the TMCT as a stressful task. The finding is in line with several studies reporting cognitive impairment with increasing cortisol levels (Wolkowitz et al., 1990; Kirschbaum et al., 1996; Lupien et al., 1997; Vedhara et al., 2000). The cortisol response was not related to brain activation in any condition. This does not rule out an association between those variables and might be a consequence of the addressed time distance in the present study. In fact, reduced medial temporal lobe activation after GC administration has been reported in recent PET studies (de Leon et al., 1997; de Quervain et al., 2003). However, another recent study reports that cortisol increases were only weakly correlated with changes in the hemodynamic response (Gregg et al., 1999).

Brain areas activated during mental arithmetic under time pressure included prefrontal and parietal areas. This is in line with studies investigating blood flow changes due to mental arithmetics (Chochon et al., 1999; Rickard et al., 2000; Gruber et al., 2001; Menon et al., 2002). The observed amygdala activation could be a sign for emotional arousal induced by the mental challenge task. A recent review has identified amygdala activation specifically in association with fear (Phan et al., 2002). Posterior cingulate activation is probably associated with the response to sensory stimuli and attention (Vogt et al., 1992; Yamasaki et al., 2002), but has been also discussed in association with presentation of threatening stimuli (Maddock & Buonocore, 1997).

Like for the encoding condition, we determined the individual peak activation in our region of interest. Higher HC activation during stress was associated with lower performance rates in the arithmetic task at that time, a finding that again strengthens the possibility that reduced activation is indeed beneficial for cognitive performance. Like increased cortisol levels, HC activation could represent increased arousal and stress. As pointed out before, stress seems to disrupt a positive association between HC activation

and cognitive performance. It might even invert this association, with higher activation leading to lower cognitive performance, suggesting an inverted u-shape relationship between activation and cognitive performance rates as it has been discussed for cortisol levels and cognitive performance (Lupien & McEwen, 1997; de Kloet et al., 1999).

Subjects with high HC activation during the TMCT showed low activation in this area during the subsequent memory task and did not show the expected improvement in encoding success. Only subjects with low HC activation during stress did profit from the delay effect. Again, these findings indicate an impairing effect of stress on HC function. From the present findings it seems reasonable to assume that changes in HC blood flow are a consequence of processes occurring under stress (e.g. GR receptor induced mechanisms). It can be assumed that these processes have consequences also for the subsequent encoding task and compromise further activation. This does not exclude the possibility that the mechanisms responsible for HC activation during stress and encoding are different. Furthermore, HC activation during stress seems to impair HC dependent memory processes and can be responsible for lacking associations between HC activation and cognitive performance. Thus, the present findings suggest that HC activation is affected by stress. Considering HC activation in addition to cortisol changes might be a fruitful way to explain and understand stress effects on the human body and brain in the future.

Taken together, it can not be finally decided from this study, to what extent cortisol levels are responsible for changes in brain activation and if a reduction in HC activation can be considered as beneficial or non-beneficial for HC dependant processes. Continued investigation of these physiological alterations under stress is clearly indicated. In particular, it will be necessary to find a way to monitor cortisol changes over the course of a fMRI study without inducing additional subject motion, or drastically increasing the invasiveness of the study. This way, cortisol levels could be determined in closer proximity to the different experimental phases and possible associations between GC levels and blood flow changes in the brain are more likely to be detected. What can be concluded from this study, however, is that HC activation during stress seems to have an

effect on memory dependent processes, and that both memory and stress paradigms are reflected by HC activation in fMRI. It will be interesting to continue investigations in this area, since the identification of the association between HC activation, memory processes and stress is one of the essential questions related to Psychoneuroendocrinology.

CHAPTER 6:

GENERAL DISCUSSION

After a short recapitulation of the scientific background, this final chapter brings to mind the rationale and hypotheses for the three studies, conducted as part of the doctoral thesis, and summarizes the results. Subsequently, integration of the results is attempted considering relevant literature, and a model is suggested illustrating associations between the relevant variables. Finally, limitations of the study design are presented and recommendations for future research continuing the path taken in this thesis are made.

6.1 SYNOPSIS AND INTEGRATION WITH RELEVANT LITERATURE

In *Chapter 2* the reader was provided with the theoretical framework for the present thesis. Literature about normal and pathological function of the HPA and HC was presented with the aim to draw attention to the particular vulnerability of the HC in clinical and aged populations. Furthermore, structural and functional imaging methods were introduced and their potential for psychoneuroendocrinological research was outlined. The final section of this chapter describes the development of the questions addressed in this thesis and concludes with the formulation of the specific goal to investigate conditions associated with HPA regulation in young healthy subjects with special emphasis on MRI derived measures of HC volume and function.

In order to have a pool of subjects for subsequent MRI testing available, cortisol levels were assessed after awakening and in response to the Trier Social Stress Test. Chronic stress and depression were assessed with questionnaires, and explicit memory performance was tested before and after the stress test in a group of 40 healthy young men.

Based on the well-known findings of elevated basal glucocorticoid levels in patients with major depression (see chapter 2.2.3), a first study *(Chapter 3)* investigated the relationship between depressive symptomatology and cortisol measures in this group. In previous studies, the assessment of HPA activity was both time consuming (mostly diurnal assessment) and invasive (mostly blood sampling, administration of

dexamethasone). Recently, the cortisol response to awakening has been established as a reliable biological marker for individual HPA activity, and associations of this marker with measures of chronic stress have been repeatedly reported in healthy subjects. The strong associations between chronic stress and depression reported in the literature were further encouragement to investigate the association between symptoms of depression and the cortisol response to awakening in young healthy subjects. *Hypothesis 1,* predicting a positive association between the cortisol response to awakening and depressive symptomatology in this population was confirmed. Indeed, in the group of 40 male university students, higher cortisol levels to awakening were associated with increased depressive symptomatology as assessed with the Hamilton Depression Inventory.

Together with earlier studies providing evidence in the same direction (*Chapter 2.2.3*), it can be concluded that associations between cortisol levels and mood and stress levels are naturally occurring in young healthy subjects. However, the causal direction can not be determined. Taken together, evidence clearly indicates an association between cortisol levels and psychological and cognitive variables, that is already evident in young healthy populations. This association is well accepted and constitutes one of the basic interests of Psychoneuroendocrinology.

In a second study (*Chapter 4*) another aspect of HPA function was investigated. HC volume reductions have been repeatedly described in association with chronically elevated basal glucocorticoid levels and impairment of HC dependent cognitive processes (see chapters 2.2.1 and 2.1.4). In order to obtain information about such a relationship in young healthy subjects, a subgroup of 13 students from the first study participated in a study assessing HC volume and relating it to cortisol and cognitive measures. HC volume was determined manually employing a newly developed segmentation protocol. According to *hypothesis 2*, it was expected to find higher cortisol levels in association with lower hippocampal volume. Interestingly, right as well as left HC volume were found to be positively related to cortisol levels in response to the Trier Social Stress Test (TSST) and the cortisol response to awakening. Furthermore, higher HC volume was

related to impaired cognitive performance in an explicit memory task before the TSST but not afterwards. In contrast to the results of the first study, the findings of this second study do not agree with observations in clinical and aged populations. Thus, *hypothesis 2* had to be rejected. However, the results correspond to, and extend a recent study reporting a negative correlation between explicit memory and HC volume in a young healthy population (Chantome et al., 1999). It is assumed that a large HC and a strong cortisol response are both qualities of a healthy and fully functional HPA system. Furthermore, an inverted u-shape relationship between cortisol levels and cognitive performance is proposed here. In this case, a large HC with a possibly higher number of GC receptors indicates flexibility of the HPA system. The question raised from the opposite findings in this healthy/young and pathological/aged populations is: What are the mechanisms that turn an adaptive into a maladaptive HPA system?

One approach to address this question is to look at changes in HC function in response to endogenous glucocorticoid increase. Thus, a third experiment (*Chapter 5*) was designed to investigate the effects of stress on HC activation. On a molecular and electrophysiological level, elevated GC levels have been shown to modulate HC activation (see chapter 2.2.1 and 2.2.2). The effects of stress and elevated GC levels on HC activation in neuroimaging studies have not been investigated in young healthy subjects. Also, recent functional MRI findings of HC activation in association with the performance of explicit memory tasks render this method a promising tool to investigate changes in HC activation related to stress (*Chapter 2.3.7*). Thus, in a functional MRI study with a subgroup of 12 subjects from the initial study, brain activation was assessed during novel picture encoding before and after a mental challenge task. *Hypothesis 3* postulated reduced HC activation after stress and could be confirmed. Right HC activation was reduced after the stress task. Similarly, two recent PET studies report that glucocorticoid administration reduces hippocampal/medial temporal lobe activation (de Leon et al., 1997; de Quervain et al., 2003). Furthermore, the highly positive correlation between HC activation and memory performance that had been observed in the present study before the stressful task, was not apparent anymore afterwards, suggesting an interfering effect of the stress task on this association. However, memory performance for

picture encoding after stress was improved, and higher HC activation during the TMCT was associated with reduced performance in the math task as well as in subsequent encoding success and HC activation. Contrary to the results of the depression study, it cannot be finally decided from these preliminary results if reduced HC blood flow is generally beneficial or detrimental for HC functioning. Studies reporting positive associations between the magnitude of focal activation and cognitive performance rates (Brewer et al., 1998; Wagner et al., 1998; Fernandez et al., 1999) support the view of beneficial effects of HC activation, whereas the present finding of improved memory performance with decreased activation suggests the contrary. However, permanently reduced HC blood flow due to chronically elevated cortisol levels might in the long run be responsible for atrophic processes.

6.2 A MODEL OF TRANSITION FROM ADAPTIVE TO NON-ADAPTIVE GLUCOCORTICOID MEDIATED OUTCOMES (TANGO)

An important question that can be raised from the results of this thesis is: Which changes related to cortisol increases can be regarded as healthy and adaptive, and at what point can the HPA axis and changes related to elevated cortisol levels be regarded as dysfunctional and maladaptive?

From the available literature and the results of the studies presented in this thesis, a model is postulated that places the observed HPA related changes on a continuum according to the persons cumulative/chronic exposure to cortisol levels. Cumulative GC exposure is investigated as a consequence of age, social support, environmental and personality factors and serves as independent variable for HC related changes at different levels. Changes related to elevated GC levels can occur on a behavioral, functional, and structural level. On the behavioral level, GC effects on HC dependant memory function are distinguished from associations with chronic stress and depression. The functional level includes changes in HC glucose levels and long-term potentiation as observed in animal and in vitro studies and changes in HC blood flow as assessed in humans with

functional imaging methods such as PET and fMRI. The structural level includes changes in dendritic branching and actual HC atrophy.

It is speculated that functional and behavioral changes precede structural changes in the HC. Several cellular processes have been identified in animals and in vitro studies that result in HC atrophy. Furthermore, mostly from animal and in vitro studies, there is some indication that cortisol affects HC function in the form of electrophysiological cell activity or glucose utilization. Changes in long-term potentiation and the extent of HC glucocorticoid receptor occupation have been related to cognitive performance in animals (see chapter 2.2.1). It can be assumed that similar processes are present in human populations.

The studies executed as part of this thesis all aimed at looking at associations between HPA activity and measures related to hippocampal integrity in young healthy subjects. It was expected to find rather transient and moderate changes in behavioral and HC variables, indicating normal variations in HPA regulation and associated variables. In this population with supposedly low cumulative exposure to cortisol, HPA related changes can be observed on behavioral and functional levels. The association is believed to be dynamic and adaptive to ensure homeostasis. At this level, HC volume is believed not to be affected by cortisol changes.

In the present model, changes in memory, depressive symptomatology and feelings of chronic stress vary with acute cortisol levels and HC activation. Finding increased depressive symptomatology (retrospective assessment over the past two weeks) in association with elevated cortisol levels after awakening over a period of four weeks in the first study suggests an already moderate dysregulation in this age group on a behavioral level. Thus, behavioral alteration could be an early indication for mal-adaptive changes related to cortisol elevations.

Changes on the functional and behavioral level are believed to influence each other. Research on LTP suggests that changes in HC long-term potentiation are associated with cognitive changes (*Chapter 2.2.2*). Results of the present fMRI study point in a similar

direction, suggesting that changes in HC blood flow are associated with performance in a math and an explicit memory task.

The observation of reduced HC activation after an acute stress task and the disappearance of correlations between HC activation and cognitive performance after stress can be regarded as a transient stress effect. In light of the improved memory performance after the stress task and the negative effects of high HC activation during the TMCT on math and subsequent memory performance, reduced HC activation and the fading of positive correlations between HC activation and memory do not seem to have short-term negative effects on a behavioral level. It could be speculated that other brain areas effectively take over memory encoding functions during stress.

With higher cumulative glucocorticoid exposure, changes in behavioral and functional levels are expected to become more permanent. It is believed that permanent functional changes precede structural changes. Whereas normal cortisol exposure helps to maintain homeostasis in the body, chronically elevated cortisol levels can develop a neurotoxic quality. The finding of higher cortisol levels in association with higher HC volume in the young population is interpreted as indication for a healthy and functional HPA system. The observation that a larger HC is associated with lower memory performance before stress but not thereafter is explained by the possibility that a larger HC needs higher cortisol levels to ensure optimal HC functioning. This relationship has been described in the inverted u-shape model (see *Chapter 2.2.2*). The HC structure will be the last level affected by cortisol levels. Whereas behavioral level and HC function are already affected by acute modulation of cortisol levels, structural changes are only expected with chronic GC. Thus, it is not surprising to not find a negative correlation in this young population.

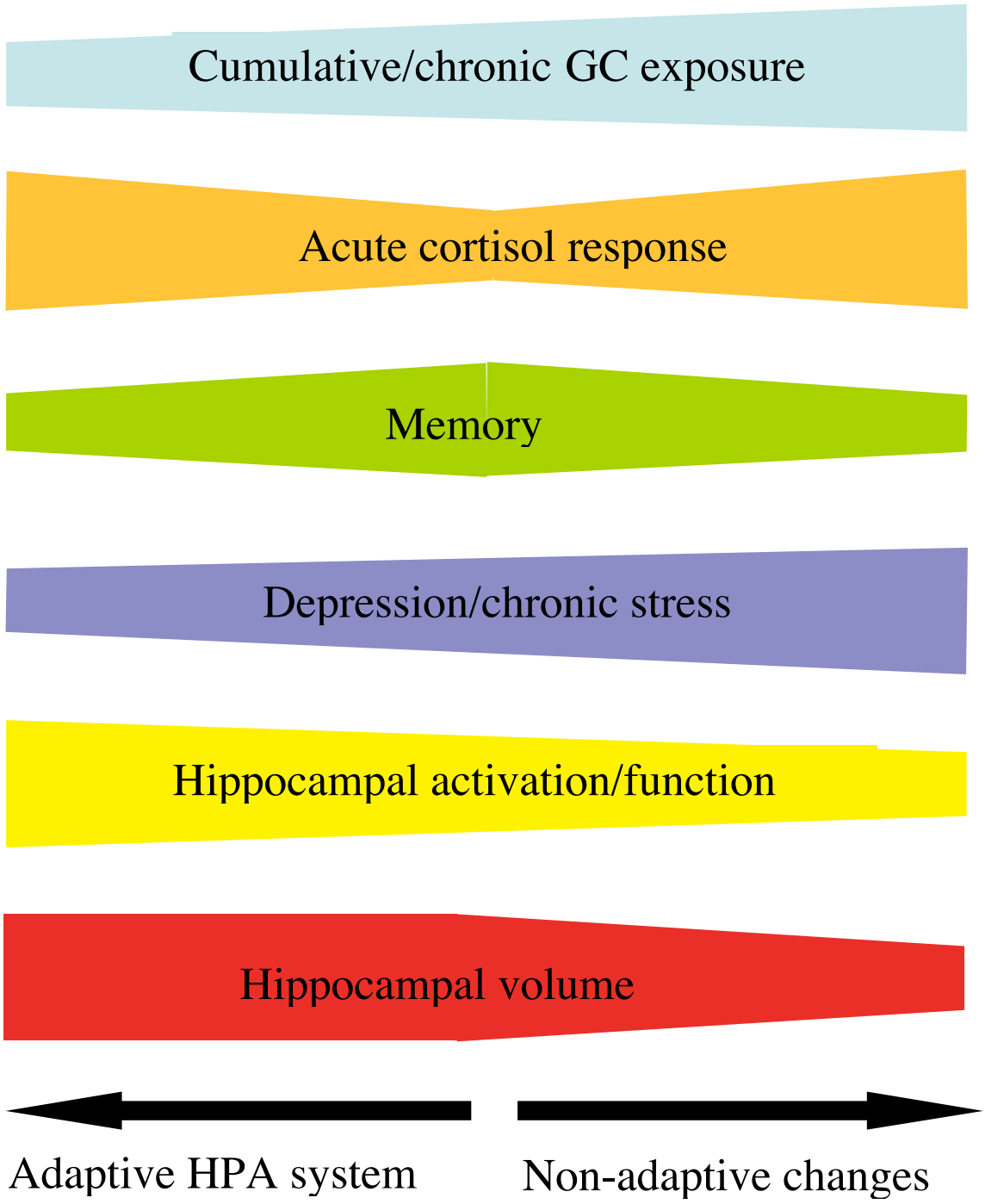

Figure 6.1 A model of transition from adaptive to non-adaptive glucocorticoid mediated outcomes

6.3 NEW APPROACHES TO HPA RESEARCH

All tests and methods used in the studies presented here are established research tools in the neurosciences, but have not been applied in this particular combination and population before. Furthermore, specific improvements have been made to increase the reliability of some of the measures used in this thesis.

In the first study we used the Hamilton Depression Inventory (HDI) to assess the extent of depressive symptomatology in a non-clinical population. The HDI is a well-known instrument to assess clinical depression. Normative data have been provided for young healthy subjects, allowing for use of this measure to obtain measures of depressive symptomatology in this population. It can be speculated that other measures of mood and depression would have yielded similar results.

The assessment of the cortisol response to awakening has been proven effective in chronic stress research and is regarded as a reliable biological marker for individual HPA activity. Although cortisol is regularly assessed in association with major depression, our data demonstrates for the first time the effectiveness of this tool in determining elevated cortisol levels in association with depression. In order to improve reliability of the measure, the cortisol response to awakening was assessed once a week over a period of four weeks. The area under the curve was calculated for each sampling day and the mean of the two median AUC measures entered further statistical analyses. Measuring the cortisol response to awakening repeatedly and then using the described aggregated measure for statistical analyses was expected to reduce the potential influence of acute events and mood swings on this variable and thus, to better reflect the individual HPA function than measurements on a single day.

HC volume is frequently assessed in conditions characterized by HPA dysfunction. However, normative data on HC volume provided by different laboratories show great variability, due to different boundaries used for HC segmentation, differences in MRI acquisition procedures and segmentation software. In order to improve the reliability and

validity of our HC measure, a new segmentation protocol was developed and applied to the current data set.

Changes in HC blood flow have been shown in studies employing explicit memory paradigms in functional neuroimaging studies. The Trier Mental Challenge Task reliably provokes elevations in cortisol levels. However, the effects of stress on HC activation have not been investigated before. As part of the fMRI study, the TMCT was modified to comply with the requirements of an fMRI environment, which is mostly a restriction of movement including overt speech.

6.4 LIMITATIONS

A general limitation of this thesis is the fact that only selected associations between HPA regulation and indices of HC integrity were analyzed in this young population. This is especially true for the first study, which only reports associations between cortisol levels after awakening and depressive symptomatology. However, data for explicit memory performance and cortisol response to the TSST were assessed as part of the first study with 40 subjects. It was decided not to report these results for the whole group, first because this subject has already been covered in other studies (Kirschbaum et al., 1996), and second, because it was felt that extensive coverage of all possible associations would have directed the attention away from the actual interest of this thesis, the association of HPA function with measures of structural and functional MRI. Nevertheless, the cognitive and acute cortisol measures were important variables to enter in the neuroimaging design.

Similarly, the reported association between depressive symptomatology and cortisol levels after awakening appears to be an isolated result that has not been further pursued. In fact, in the groups of 13 and 12 subjects, HC volume and activation were not related to depressive symptomatology or chronic stress (all $p > .20$). Thus, depression and chronic stress do not appear as variables in the subsequent studies investigating HC volume and

activation. However, since such associations have been shown in major depression and animal studies, it would be interesting to determine the factors that influence the association. It can be assumed that a higher age and more severe symptoms play a role, or/and that subjects have not yet reached a maladaptive state as proposed in the TANGO model.

Some limitations have to be considered in association with the fMRI study. A technical issue concerns the requirement of motion restriction during scanning, which allowed cortisol sampling in the fMRI experiment only outside the scanner. The large delay in time between cortisol samples could thus be responsible for the fact that interpretable associations between cortisol levels and HC activation were not found.

For the statistical analyses, HC activation was defined as peak activation. Another way to look at HC activation would have been to determine the extent of activation, which is the number of activated voxels in our region of interest over a certain threshold.

Also, HC de-activation was observed during the TMCT in a comparable magnitude to HC activation.The exact implication of de-activation in the brain is not known but is currently being discussed in the research community (Gusnard & Raichle, 2001). However, looking at associations of these alternative measures of brain activation with memory performance and cortisol levels might have created different results. Since the present fMRI study was a pioneer work, it is assumed that these particularly concerns can be overcome in subsequent studies.

Female subjects were not included in the population since there is some evidence of a greater heterogeneity of HPA activity in women related to the menstrual cycle phase (Kudielka et al., 1998; Kudielka & Kirschbaum, 2003). Thus, caution must be taken to generalize the results. The relatively small sample size is another reason to regard the findings as preliminary.

6.5 OUTLOOK

Structural and functional magnetic resonance imaging appear to have a high potential for the further development in the field of Psychoneuroendocrinology. Whereas structural MRI has already been proven effective in clinical and aged populations, the availability of functional MRI adds a promising new dimension to the field. Intensifying the use of both methods to investigate brain changes associated with HPA activity in young and middle aged populations could aid in determining the transition from functional to less functional states.

It will be a challenge for subsequent fMRI studies investigating stress effects to find a way to monitor cortisol levels over the course of the experiment, without increasing subject motion or disturbing the magnetic field by having the experimenter repeatedly walk into the scanning room during the scanning procedure. Considering blood instead of saliva sampling might be less disturbing for the experimental course and image quality. However, actions have to be taken to assure catheter functioning over the up to two hours scanning period. Also, there appear to be some issues related to the required length of the catheter in such settings. In order to being able to make a statement about more permanent changes in fMRI activation, longitudinal studies would have to be pursued, comparing subjects with high and low chronic stress and/or basal cortisol levels.

Future studies need to further examine the effects of elevated GC levels on HC function and structure in young healthy populations and in different phases of life. This might help to better understand the difference between healthy and damaging GC effects, as well as the transition from large HC volumes to HC atrophy with the respective associated behavioral and functional consequences.

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