The stressed brain - discovering the neural pathways to risk and resilience

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Discovering the neural pathways to risk and resilience

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Chapter 1

General introduction

"The greatest glory in living lies not in never falling, but in rising every time we fall"
- Nelson Mandela - Long Walk to Freedom (1994)

Although often used to refer to a negative state in day-to-day life, responding to stress is an adaptive phenomenon that is essential for the wellbeing of an individual. During our lives we are exposed to a series of challenges that we attempt to overcome. These challenges or stressors may vary from everyday hassles to severe traumatic events. The human organism always strives to reach and maintain a point of equilibrium that enables maximum functionality of the individual, and these balancing scales are a constant interplay between an individual's resources and the situation the individual finds itself in (Selye, 1979). Reaching and maintaining this balance (homeostasis) through biological and behavioral adaptation has been defined by the term allostasis (McEwen, 1998). Under influence of challenges or stressors the body activates various mechanisms, a process that is called the stress response. During a stress response (described in more detail in Box 1) energy is produced and redirected in order to provide resources to deal with the stressor as quickly and efficiently as possible, thus attempting to restore balance. However, there is a high degree of intra-individual variation in how individuals respond to stressors, and in some instances specific types of stressors or frequently repeated stressors can result in maladaptive psychological and neurobiological processes. Furthermore, when these reactions persist they can result in the development of various psychiatric disorders. There is a long history of research into the psychological mechanisms that provide protection (resilience) or confer risk factors (vulnerability) with regard to developing psychiatric symptoms after experiencing chronic or traumatic stress.

So far, research into the biology of vulnerability and resilience to severe stress has largely been driven by animal studies. More recently, the advent of neuroimaging techniques like positron emission tomography (PET), single-photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) has enabled us to start to investigate human brain structure and function in vivo. As this is a relatively recent development, information on brain mechanisms that are related to vulnerability to traumatic stress is less extensive compared to the knowledge on psychological mechanisms. This is even more the case for the neural mechanisms involved in resilience, as traditionally the focus in behavioral neuroscience has always been on vulnerability and psychopathology.

This thesis aims to elucidate on brain mechanisms involved in vulnerability and resilience, and therefore consists of two sections. In the first section (Chapters 2 -5) the neural characteristics associated with a history of stress-related adverse

events of both exogenous nature (childhood maltreatment) and endogenous nature (Cushing's disease) will be addressed. In the second section (Chapters 6 - 8) the neural structural and functional characteristics that are related to resilience to severe stress are investigated using both structural and functional MRI techniques.

Traumatic stress

There is great variety in the types of stressors that elicit a stress response in humans. Situations like losing, winning, physical exercise, social rejection or life threatening situations, all activate the stress system. The most severe acute stressors are called traumatic stressors. In the diagnostic and statistical manual of mental disorders 4th edition (DSM-IV; (American Psychiatric Association, 2000) (exposure to) a traumatic experience is defined as follows:

(A.1.) The person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others. (A.2.) The person's response involved intense fear, helplessness, or horror. Note: In children, this may be expressed instead by disorganized or agitated behavior.

Criterion A.2. has been omitted from the newest version (DSM-V; (American Psychiatric Association, 2013) in order to improve diagnostic accuracy and in order to further objectify the definition (Friedman et al., 2011), but the research in this thesis uses the DSM-IV definition. Traumatic events are quite common as the prevalence of experiencing a traumatic event at least once in our lives is estimated above 80% in Western countries including the Netherlands (de Vries and Olff, 2009), and the majority of people will even experience multiple traumatic events in their respective lives (Ogle et al., 2013).

Animal studies have shown that exposure to severe or prolonged stress in early-life leads to increased reactivity of the HPA axis, which in turn probably enhances the risk to develop psychiatric symptomatology (Liu et al., 1997; Flinn et al., 2011), including negative mood and cognitions, anxiety and symptoms related to memory of the traumatic event (i.e., intrusions and nightmares). In research, a distinction is often been made between exposure to early life stress (like childhood maltreatment) and traumas experienced in later life. In clinical practice most patients with trauma-related symptoms are diagnosed with major depressive disorder, anxiety disorder, posttraumatic stress disorder (PTSD), or a combination of these disorders. As these three classes of disorders are all associated with dysfunction of the HPA-axis, they are also referred to as stress-related disorders (Sachar et al., 1970; Arana et al., 1985; Arborelius et al., 1999; de Kloet et al., 2006). Childhood emotional maltreatment

consists of both emotional abuse (i.e., systematically criticizing or belittling) and emotional neglect (i.e., not providing the emotional support and care needed, ignoring the child's needs). Although childhood emotional maltreatment does not meet the criteria for a traumatic event as defined by the DSM-IV, it is considered one of the major precursors of developing stress-related disorders in later life (Gibb et al., 2007; Spinhoven et al., 2010).

The association between exposure to severe stressful situations and an increased vulnerability to develop psychiatric symptomatology has lead scientists to start investigating changes in neural correlates that are induced by severe stress, particularly when exposed in childhood. Animal studies using adverse environmental paradigms such as maternal separation, loss, or isolation rearing provided evidence of persistent changes in brain structure as a result of exposure to stressful early-life experiences (Fabricius et al., 2008; Oreland et al., 2010). These changes include decreased neurogenesis and reductions in dendritic branching and dendrite length, and were found to be predominantly present in the prefrontal cortex, the amygdala and the hippocampus, which are all constituents of the limbic system (Sanchez et al., 2001; McEwen, 2008). In human studies on the effect of childhood maltreatment on gray matter volumes, affected structures are mostly situated within the medial prefrontal cortex and the hippocampus (Cohen et al., 2006; Tomoda et al., 2009; Treadway et al., 2009; Frodl et al., 2010; van Harmelen et al., 2010). One theory on the pathway through which childhood maltreatment leads to persistent changes in the brain is through exposure to chronic stress and altered HPA-axis functioning (Shonkoff et al., 2009; Doom et al., 2013; Doom et al., 2014).

Although changes in brain structure under influence of exposure to stressful experiences in early life have been clearly demonstrated, changes in brain function are still poorly understood. Because adverse events like childhood maltreatment predispose for the development of psychopathology in later life, it is important to examine these persistent changes in brain function. These changes can tell us something about the pathways that lead from childhood maltreatment to vulnerability for psychiatric disorders. If these pathways are better understood, treatment methods could be adjusted accordingly.

In Chapter 2, the persisting effects of exogenous adverse experiences, in particular repeated exposure to childhood emotional maltreatment, on brain function are investigated. 88 participants (44 participants with a history of childhood emotional maltreatment, and 44 matched healthy controls) were drawn from the large-scale longitudinal Netherlands Study of Depression and Anxiety (NESDA; (Penninx et al., 2008). Using functional magnetic resonance imaging (fMRI), differences in resting-

state functional connectivity (RSFC) were examined for four resting-state networks: the limbic network, the salience network, the default mode network, and a network seeded by a location in the medial prefrontal cortex based on volumetric differences found in previous research (van Harmelen et al., 2010).

Severely stressful experiences, like childhood emotional maltreatment are exogenous factors that typically influence HPA-axis functioning and are associated with an increase in vulnerability towards developing stress-related psychiatric symptomatology. There are also examples of endogenous factors that impose similar effects. The next section will describe such an endogenous cause: Cushing's disease.

Cushing's disease

Cushing's disease represents a unique model for examining the effects of prolonged exposure to high levels of endogenous cortisol on the human brain as well as for examining the relation between these effects and psychiatric symptomatology. In Cushing's disease a pituitary adenoma produces adrenocorticotropic hormone (ACTH), which in turn stimulates the release of glucocorticoids by the adrenal cortex. Normally, the HPA-axis is regulated by a negative feedback mechanism (see box 1), however, as the production of ACTH is stimulated by the pituitary adenoma, the HPA axis is unable to regulate itself, causing an increase in the level of cortisol, which is named hypercortisolism (Nieman and Ilias, 2005; Newell-Price et al., 2006). Cushing's disease is a rare disease with an estimated prevalence of 39.1 per million and an incidence rate of 2.4 cases per million each year (Etxabe and Vazquez, 1994). Physical features that are related to Cushing's disease include, obesity (predominantly abdominal), moon face, high blood pressure, plethora, acne, increased bruisability, striae, weak bones and muscles, hirsutism and glucose intolerance (Newell-Price et al., 1998). In addition to these physical symptoms, psychiatric symptoms have been reported in concert. The psychiatric symptomatology related to Cushing's disease overlaps with the symptomatology present in stress-related disorders, as both symptoms of depression and anxiety are reported (Kelly et al., 1996; Sonino and Fava, 2001).

On a structural brain level hypercortisolism has been associated with decreases in hippocampal volume (Starkman et al., 1992), an area rich in both GR and MR, and therefore very sensitive to hypercortisolism. An important function of the hippocampus is memory functioning, which has also been found to be impaired under influence of hypercortisolism (Whelan et al., 1980; Mauri et al., 1993).

Cushing's disease is treated using transsphenoidal surgery, in some cases followed

by postoperative radiotherapy and/or hydrocortisone substitution, dependent on the outcome of the surgery. Remission occurs with the re-instatement of normalized basal and ACTH-stimulated cortisol values. Following successful treatment of hypercortisolism, both the physical features and the psychiatric symptoms improve substantially (Cohen, 1980; Kelly et al., 1996), which coincides with a reversibility of the reductions in brain volume (Starkman et al., 1999; Bourdeau et al., 2002; Starkman et al., 2003). However, despite improvement, symptoms persist when compared to healthy controls as patients with long-term remission of Cushing's disease display increased prevalence of depression and anxiety (Tiemensma et al., 2010a), cognitive impairments (Tiemensma et al., 2010b), and decreased quality of life (van Aken et al., 2005; Tiemensma et al., 2011). The persistence of these symptoms lead us to hypothesize that in Cushing's disease brain structure and function are permanently changed under influence of long term exposure to hypercortisolism. To examine this, our group conducted an MRI study in which 25 patients in long-term remission of Cushing's disease were compared with 25 healthy controls matched for age, gender and level of education. T1-weighted scans and diffusion tensor imaging (DTI) scans were used to examine gray matter volume and white matter integrity, and resting-state scans were used to examine RSFC, results of which are reported in Chapter 3-5 (see below). In addition, task-related MRI scans were used to examine brain function during cognitive performance, emotional processing, and during encoding and retrieval of emotional stimuli (results are not reported in this thesis).

Reductions in gray matter volume under influence of exposure to hypercortisolism are well documented. However, less is known about the persistence of these reductions and their relation to persistent behavioral impairments. This is addressed in Chapter 3, where differences in brain volume are investigated in patients in long-term remission of Cushing's disease and in healthy controls, using a voxel-based morphometry (VBM) approach. The relation between these brain volumes and psychiatric symptomatology in the patient group are studied in concert.

In Chapter 4 structural brain abnormalities in remitted Cushing's disease are further explored, using diffusion tensor imaging (DTI). DTI is an imaging technique, which enables the mapping of the diffusion of (predominantly water) particles in tissue (for more information see box 2). Using these DTI scans, the degree in which particles are restricted in the direction of their diffusion can be calculated. This is called fractional anisotropy (FA), and FA values in white matter are a measure for the integrity of the white matter. In Chapter 4, white matter integrity in patients in long term remission of Cushing's disease is investigated by extracting FA values from DTI scans, and subsequently using these in a tract-based spatial statistics approach. The

region-of-interest analysis focuses on FA values in the bilateral cingulate cingulum, the bilateral hippocampal cingulum, the bilateral uncinate fasciculus and corpus callosum. An exploratory whole brain analysis is used to detect changes in FA values in white matter outside our a priori defined regions-of-interest. In addition, the association between FA values in the white matter tracts resulting from our region-of-interest analysis and psychiatric symptomatology reported by the patients with long-term remission of Cushing's disease is determined.

In Chapter 5 functional connectivity in remitted Cushing's disease is explored. Using a probabilistic independent component analysis, three a priori hypothesized functional brain networks are identified: The limbic network, the default mode network, and the salience network. Resting-state functional connectivity differences between patients with long-term remission of Cushing's disease and healthy controls are investigated for each of these networks using a dual-regression method. In addition, the strength of resting-state functional connectivity was studied in relation to the psychiatric symptomatology displayed by the patients.

The brain characteristics of resilience to trauma

As described in a previous section experiencing a traumatic event is quite common, with an estimated prevalence of around 80% in Western countries (de Vries and Olff, 2009). Prevalence estimations for the development of PTSD after trauma exposure vary greatly based on type of trauma and age of exposure (Stein et al., 1997; Creamer et al., 2001; Alisic et al., 2014). However, it is clear that not everyone who experiences a traumatic event will develop trauma-related psychiatric symptoms. This notion is supported by figures from The United States National Comorbidity Survey showing an estimated 7.8% of the population in the US develops a PTSD at least once in their lives, which is a lower incidence rate compared to trauma exposure (Kessler et al., 1995). Clearly, there is a degree of inter-individual variation in the way individuals respond and adapt to severe stress, with a spectrum ranging from vulnerable individuals on the one side to resilient individuals on the other. In simple terms of outcome, resilience can be defined as the absence of psychopathology after experiencing a traumatic event. Usually, however, more complex and dynamic definitions of resilience are used in the literature. These describe resilience as a dynamic, multidimensional process encompassing positive adaptation within the context of significant adversity, and also, from a more psychobiological standpoint, as short-term and long-term responses that reduce allostatic load (Curtis and Cicchetti, 2003; Charney, 2004; Cicchetti and Rogosch, 2009).

In the search for protective and risk factors, the first evidence came from large-scale epidemiological studies that attempt to map characteristics and incidence

rates of trauma exposure and PTSD. Evidently, the nature of the traumatic event is an important risk factor, with sexual assault being the strongest predictor of PTSD for women and combat exposure for men (Kessler et al., 1995; Stein et al., 1997; Creamer et al., 2001). Although men are more prone to experience a traumatic event, women are more likely to develop PTSD (Kessler et al., 1995; Breslau et al., 1999). The experience of prior trauma and especially trauma in childhood was found to be a risk factor, but also the lack of social support posttrauma and having a family history of psychopathology were found to be important determinants in the development of PTSD (Ozer et al., 2003). These risks or vulnerability factors however do not give an answer to the question why some individuals thrive, adapt or 'bounce back' in the face of adverse events, while others break down and develop psychiatric symptoms. Over the years, research has therefore focused on investigating psychological mechanisms that protect an individual from the negative effects of trauma. These include, but are not limited to, emotion regulation, self-esteem, executive functioning (i.e., problem-solving skills and planning), active coping strategies, optimism and internal locus of control (Masten, 2001; Southwick et al., 2005; Masten, 2007; Cicchetti, 2010). In addition, personality traits are also associated with an individual's level of resilience. High neuroticism is directly related to an increased chance for developing PTSD (Breslau et al., 1991; Nakaya et al., 2006), whereas low neuroticism, and high traits of extraversion, conscientiousness, openness and agreeableness are related to a resilient personality profile (Friborg et al., 2005; Campbell-Sills et al., 2006).

In contrast to the knowledge on psychological factors that are involved in resilience, not much is known about the neural mechanisms that underlie resilience to traumatic stress. To review the state of the art knowledge regarding these mechanisms at the start of this part of the thesis we conducted a thorough literature review, resulting in Chapter 6, which provides an oversight of studies that investigate characteristics of brain structure and function related to resilience to trauma. One of the key conclusions of this review concerns the designs that are being used to examine resilience. Most studies use a design comparing trauma-exposed non-PTSD (resilient) individuals with PTSD individuals. With this comparison it remains unclear whether differences found between these two groups should be attributed to trauma-related symptomatology in the patient group, or to the resilience in the control group. Therefore, some suggestions on how to operationalize resilience in behavioral neuroscience are provided. One of these suggestions is to add another control group to the design, consisting of individuals who have not experienced trauma and do not have a history of psychopathology. The advantage of including this control group to the design is that it enables the disentangling of effects related to resilience from effects related to psychopathology. In both chapter 7 and 8 this

design is applied to investigate neural mechanisms that are specific to resilience to traumatic stress.

In Chapter 7, resting-state functional connectivity related to resilience to childhood maltreatment is investigated. Individuals with a history of childhood maltreatment without psychopathology (n = 11; resilient group) are compared to both individuals with a history of maltreatment and psychopathology (n = 11; vulnerable group) and a healthy control group (n = 11), consisting of individuals without a history of childhood maltreatment and without psychopathology. The same networks as in Chapter 3 are examined: limbic network, default mode network, salience network, and a network seeded by a location in the medial prefrontal cortex.

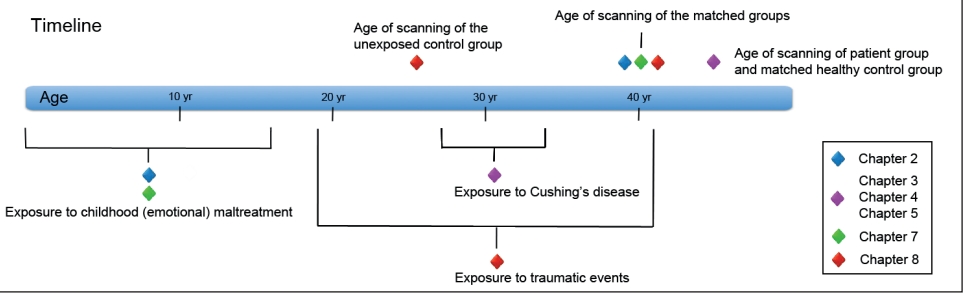
To be able to further investigate the neural mechanisms of resilience to trauma an MRI study was conducted using a highly relevant group within the context of trauma exposure and resilience: Dutch police officers. By virtue of their profession, Dutch police officers have a very high chance of encountering multiple traumatic events. It is therefore very important that these individuals are as resilient as possible, and to ensure this police officers are subjected to strict selection criteria and training methods. There are indications that these methods are effective as there is no evidence that police officers suffer from more trauma-related psychopathology compared to non trauma-related occupations (van der Velden et al., 2013). However, these training and selection methods are based on psychological and personality constructs that are known be indicative of resilience, might potentially be enhanced by additional information on the neural mechanisms that underlie resilience to trauma. In our study, police officers that were exposed to multiple profession related traumatic events but with no history of psychopathology (n = 29; resilient group) are compared to police officers with trauma exposure and a history of psychopathology (n = 33; vulnerable group) and to recruits from the police academy who were not exposed to traumatic events and had no history of psychopathology (n = 19; control group). T1-weighted scans and diffusion tensor imaging scans are used to examine gray matter volume and white matter integrity, and resting-state scans are used to examine resting-state functional connectivity. In addition, task-related fMRI scans are used to examine brain function during implicit emotion regulation, emotional working memory performance, and during social stress (results of the task-related fMRI scans are not reported in this thesis).

Using the T1-weighted scans and diffusion tensor imaging scans characteristics of gray matter volume and white matter integrity related to resilience to stress are reported in Chapter 8. Moreover, the relationship between these characteristics and resilience related behavior are examined, as well as the association between

white matter integrity characteristics, which is a measure for structural connectivity, and functional connectivity measured with resting-state MRI scans.

Finally, in Chapter 9, a summary is provided of the empirical studies reported in this thesis and the findings are integrated and discussed. A timeline is provided (Figure 1) to give an oversight for each of the Chapters when severe stress / hypercortisolism exposure occurred and when MRI measurements where assessed.

Figure 1. Timeline of severe stress / hypercortisolism exposure and time of scanning for each of the Chapters.



The stress response (BOX 1)

The primary system in the brain that initiates and regulates the stress response is the limbic system. It consists of various brain structures including the medial prefrontal cortex, insula, amygdala, hippocampus, hypothalamus and nucleus accumbens (Morgane et al., 2005; Shin and Liberzon, 2010). The amygdala is a key structure in the initiation of the stress response, as it is activated when faced with a stressor (Cahill et al., 1996; Irwin et al., 1996; Whalen, 1998; Shin and Liberzon, 2010), signaling the hypothalamus to activate the autonomic nervous system. The autonomic nervous system controls involuntary functions like heartbeat, blood pressure, constriction, and dilation of blood vessels, and regulates these through the sympathetic nervous system and the parasympathic nervous system. The sympathetic nervous system promotes action, whereas the parasympathic nervous system promotes generation and preservation of energy, mainly by putting all systems to a phase of rest, aside from digestion, which is activated. During a stressor the autonomic nervous system switches on the sympathic nervous system through the release of the hormone epinephrine by the adrenal glands as well as norepinephrine by the locus coeruleus (Berridge and Waterhouse, 2003). The release of these hormones result in a number of physical reactions, including increases in heart rate, increases in respiratory rate, stimulation of glycogenolysis in the liver and muscles (Arnall et al., 1986). These reactions all result in increases

of energy availability throughout the body, but most importantly in the muscles. This rise in energy provides an individual with the resources needed to adapt to the situation as best as possible. On a behavioral level, this results in the fight-or-flight response (Cannon, 1929), although responses of freeze, fright and faint have also been observed when exposed to severe stressors (Bracha, 2004). The sympathetic nervous system reaction on a stressor is a rather fast process, with the release of epinephrine and norepinephrine being initiated within milliseconds after the stressor presents itself (Morilak et al., 2005).

A simultaneously activated, but much slower mechanism is the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is activated under influence of the same signaling of the amygdala to the hypothalamus. The hypothalamus secretes corticotropin-releasing hormone (CRH), which in turn stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH signals the adrenal cortex to produce and release glucocorticoid hormones (in humans also known as cortisol) to the bloodstream (Riedemann et al., 2010). The release of glucocorticoids by the adrenal cortex happens between three and seven minutes after the initial activation of the HPA axis (Bassett and Cairncross, 1975). Glucocorticoids can pass the blood-brain barrier, reaching the brain where they will bind on either the mineralocorticoid receptors (MR) or the glucocorticoid receptors (GR). MR have a seven to ten fold greater affinity for binding glucocorticoids when compared to GR (Reul et al., 2000), which means they are occupied the majority of the time, whereas GR only get occupied when substantial amount of glucocorticoids are available (i.e., during awakening or during a stress response). The GR are expressed throughout most of the brain, with the strongest expression in the hippocampus. MR expression is more localized in specific areas of the brain, with the strongest expression in the hippocampus as well, and moderate expression in the amygdala and hypothalamus (Reul and de Kloet, 1986). By binding to these receptors, glucocorticoids regulate the HPA axis as negative feedback occurs on the secretion of both CRH in the hypothalamus and ACTH in the pituitary gland (Wintermantel et al., 2005).

The activation of the HPA-axis is a much slower process compared to the epinephrine/norepinephrine release, and the role of the HPA-axis is to fine-tune and terminate the initial stress response (Radley et al., 2008). Glucocorticoids facilitate the fight-or-flight response by increasing the availability of energy. This happens through increasing the mobilization of glucose (Rizza et al., 1982; Dinneen et al., 1993), by increasing metabolism of proteins and fatty acids (Horber and Haymond, 1990; Djurhuus et al., 2002), and by inducing insulin resistance (Rizza et al., 1982). In addition, glucocorticoids protect an individual

from overactivation of the immune system in the case of tissue damage by suppressing the immune response (Palacios and Sugawara, 1982; Elenkov, 2004). This is the reason that glucorticoids are also used as an immunosuppressive drug proscribed for the treatment of various disease related to a dysfunction of the immune system.

Diffusion Tensor Imaging (Box 2)

Diffusion tensor imaging (DTI) is a special imaging technique, which allows the mapping of diffusional properties of predominantly water molecules in brain tissue. In neuroscience, DTI is typically used to measure integrity of white matter tracts throughout the brain. White matter tracts are myelinated nerve cell projections, which connect various gray matter areas enabling signal transmission between these areas. The myelin sheath acts as an insulator, thus increasing the speed of transmission of signals through the nerve. Diffusion inside the white matter tracts is restricted by the myelin sheath and the cell membrane. Hence, it is faster in the direction aligned with the tract and slower in the perpendicular direction. A DTI scan uses information obtained by scanning in multiple gradient directions (in the case of the studies described in this thesis 32 directions were used) to compute for each voxel the diffusion tensor, which has the shape of an ellipsoid (See Figure 2). The shape of the ellipsoid can be defined by three eigenvectors: one describing the principal long axis, one describing the width, and one describing the depth of the ellipsoid. Each of the eigenvectors has a length, which is called the eigenvalue (λ). The eigenvalue along the principal axis (λ1) is also called the axial diffusivity (AD). The average of the other two eigenvalues (λ2 and λ3) is called the radial diffusivity (RD). Furthermore the mean of the three eigenvalues is called the mean diffusivity (MD). Using the three eigenvalues the fractional anisotropy (FA) value can be calculated (See formula 1 (Mori and Zhang, 2006). FA is a sensitive marker for measuring white matter integrity and its values are scaled between 0 (isotropic) and 1 (anisotropic). However, it is also non-specific, meaning it gives no information about the structural properties underlying abnormalities in white matter tissue. In this thesis, finding differences in FA values is therefore always followed by post-hoc analyses of the AD, RD, and MD in those regions. Decreased diffusion along the principal direction of the fiber (AD) indicates axonal loss (Budde et al., 2009), while increased diffusion perpendicular to the principal direction of the fiber (RD) indicates demyelination (Song et al., 2005; Alexander et al., 2007). In addition, an increase in overall water diffusion in all directions (MD) is also an indication for demyelination (Horsfield and Jones, 2002), but could be caused by the presence of edema as well (Alexander et al., 2007).

Figure 2. Eigenvectors of the diffusion tensor model

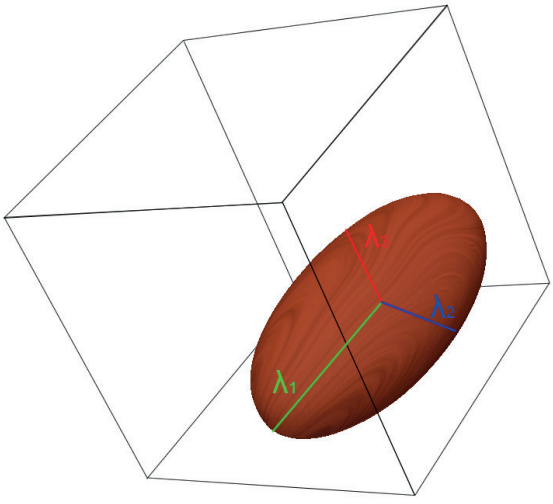


Figure 2 depicts the three eigenvectors describing the ellipsoid of the diffusion tensor model. λ1 (green) describes the length of the first eigenvector, describing the length of the ellipsoid. λ2 and λ3 describe the length of the second and third eigenvectors, which describe the width and the depth of the ellipsoid.

Formula 1. Computing FA from the eigenvalues

$$\mathrm{FA} = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}},$$

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Chapter 2

Resting-state functional connectivity in adults with childhood emotional maltreatment

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Abstract

Background

Childhood emotional maltreatment (CEM) has been associated with disturbances in emotional and behavioral functioning, and with changes in regional brain morphology. However, whether CEM has any effect on the intrinsic organization of the brain is not known. In this study, we investigated the effects of CEM on resting-state functional connectivity (RSFC) using seeds in the limbic network, the default-mode network (DMN) and the salience network, and the left dorsomedial prefrontal cortex (dmPFC).

Methods

Using 3-T magnetic resonance imaging (MRI), resting-state functional MRI (RS-fMRI) scans were obtained. We defined seeds in the bilateral amygdala, the dorsal anterior cingulate cortex (dACC), the posterior cingulate cortex (PCC) and the left dmPFC, and used these to examine whether individuals reporting CEM (n=44) differed from individuals reporting no CEM (n=44) in RSFC with other brain regions. The two groups were matched for age, gender, handedness and the presence of psychopathology.

Results

CEM was associated with decreased RSFC between the right amygdala and the bilateral precuneus and a cluster extending from the left insula to the hippocampus and putamen. In addition, CEM was associated with decreased RSFC between the dACC and the precuneus and also frontal regions of the brain.

Conclusions

We found that CEM has a profound effect on RSFC in the limbic network and the salience network. Regions that show aberrant connectivity are related to episodic memory encoding, retrieval and self-processing operations.

Introduction

In 2009, an estimated 9.3% of all children living in the USA experienced maltreatment (U.S. Department of Health and Human Services, 2009). Emotional maltreatment involves any act or series of acts of commission (i.e. verbal abuse) or omission (i.e. emotional neglect) by a parent or other caregiver that results in harm, potential for harm, or threat of harm to a child's emotional development (Leeb et al., 2008; Egeland, 2009). The experience of emotional neglect and emotional abuse has a substantial impact on an individual's life. This impact is enhanced when the maltreatment is experienced in childhood, partly due to the dependence of children on the perpetrator for various necessities of life, such as food, shelter and protection from harm. Consequences of childhood emotional maltreatment (CEM) include effects on mental well being (Gibb, 2002; Teicher et al., 2006; Leeb et al., 2008; Egeland, 2009; Wright et al., 2009), internalizing attribution styles (Taussig and Culhane, 2010), emotion regulation (Rellini et al., 2012) and behavior (Gilbert et al., 2009). In addition, the experience of CEM increases the chance of developing various psychopathologies (Egeland, 2009), including anxiety and depression (Gibb et al., 2007; Spinhoven et al., 2010). These consequences have been found to continue or become evident long after the maltreatment ended, even after the child reaches adulthood. Although CEM has not received as much attention as physical abuse and sexual abuse, it has become increasingly clear that CEM occurs more frequently and has its own specific disruptive effects on the development, functioning and attachment styles of an individual (Finzi et al., 2000; McLewin and Muller, 2006; Egeland, 2009; van Harmelen et al., 2010a).

From animal studies it is known that paradigms resembling emotional maltreatment in humans, such as maternal separation, have a profound effect on brain morphology and behavior of animals (McEwen, 2001; Fabricius et al., 2008). Regions of the brain predominantly being affected by maternal separation include the hippocampus (McEwen, 2001; Fabricius et al., 2008; Joels et al., 2008), the amygdala (Joels et al., 2008) and the medial prefrontal cortex (mPFC; (Muhammad et al., 2012). In line with these animal studies, previous work by our group found CEM to be associated with abnormalities of regional brain morphology in humans (van Harmelen et al., 2010b). We demonstrated reduced gray matter volumes in the left dorsal mPFC (dmPFC) in subjects who reported having experienced CEM.

The amygdala, hippocampus and mPFC are important constituents of a limbic network known to be involved in stress responses and emotion regulation

(Vermetten and Bremner, 2002; Bremner, 2007a; Shin and Liberzon, 2010). The hippocampus is involved in declarative memory and is connected reciprocally to the amygdala, which plays a crucial role in the acquisition of fear responses and in memory consolidation of emotional experiences and stimuli (Bremner, 2007b). The mPFC has a more controlling function in the neural circuitry of stress and emotion, as it inhibits fear responses and emotional responsiveness mediated by the amygdala, and is important for self-referential processes (Bremner, 2007b; Roy et al., 2009). Amygdala activation has been found to increase during and after stressful situations(van Marle et al., 2009; Oei et al., 2012; van Wingen et al., 2012). Moreover, we found increased amygdala activation in individuals reporting CEM during the processing of faces (van Harmelen et al., 2013). An increase in functional connectivity between the amygdala and cortical midline structures was found during a recovery period after the induction of social stress (Veer et al., 2011), highlighting the importance of functional connectivity for understanding responsiveness to (chronic) stress.

Functional magnetic resonance imaging (fMRI) is widely used to study functional connectivity within the context of task paradigms, but it is also being used increasingly to study activation and connectivity during the resting state, that is in the absence of an externally controlled task or stimulus (Biswal et al., 1995; Raichle et al., 2001). During the resting state, several networks of functionally connected brain areas have been identified consistently (Damoiseaux et al., 2006). Given the influence of a history of CEM on brain structure and on emotional processing and regulation, episodic memory and self-referential processing, it can be hypothesized that resting-state networks of brain areas involved in these processes show abnormalities in individuals reporting CEM (Danese and McEwen, 2012). This is especially the case for the default-mode network (DMN), the salience network and limbic network. The DMN is a network containing the precuneus cortex, posterior cingulate cortex (PCC), mPFC, lateral and inferior parietal cortex and ventral anterior cingulate cortex (vACC; (Raichle et al., 2001; Greicius et al., 2003). The DMN is thought to be involved in the retrieval and manipulation of episodic memories and semantic knowledge, self-referential processing and prospective memory (Raichle et al., 2001; Buckner et al., 2008; Kim, 2012). The function of the salience network is the identification of the most important internal and extrapersonal stimuli with respect to reaching or protecting a state of homeostatic equilibrium (Seeley et al., 2007). The salience network contains the dorsal anterior cingulate cortex (dACC) and orbital frontoinsular cortex, along with several subcortical and limbic structures (Seeley et al., 2007). The limbic network is involved in emotional processing and

regulation and contains structures such as the amygdala and hippocampus and medial prefrontal structures such as the ACC.

Abnormalities in resting-state functional connectivity (RSFC) have been found in a variety of (neuro)psychiatric disorders known to involve aberrant stress system reactivity and disturbed emotion regulation and self-processing, such as depression and anxiety (Greicius, 2008; Broyd et al., 2009; Liao et al., 2010; Veer et al., 2010). Moreover, CEM has been identified as an important risk factor for these disorders (Egeland, 2009; Spinhoven et al., 2010). However, at present it is unknown whether exposure to CEM is associated with altered RSFC in adulthood.

Therefore, in the current study we aimed to evaluate whether there are differences in RSFC between individuals who reported having experienced CEM compared to individuals who reported not having experienced CEM. Taking into consideration the role of the limbic network in the neural circuitry of stress and emotion, we hypothesized that individuals with a history of CEM would show aberrant connectivity in the limbic network during the resting state. In addition, we hypothesized that individuals with a history of CEM would typically also display altered RSFC within the salience network and the DMN, given the roles of these networks in emotional processing, episodic memory and self-processing. Finally, given our previous finding of morphological abnormalities in the left dmPFC in individuals reporting CEM (van Harmelen et al., 2010b), we also expected to find differences in RSFC of this area.

Methods

Assessment of CEM

Childhood maltreatment was assessed through the use of The Netherlands Mental Health Survey and Incidence Study (NEMESIS) trauma interview(Robins et al., 1988; de Graaf et al., 2002). In this interview, respondents were asked whether they had experienced emotional neglect, emotional abuse, physical abuse and/or sexual abuse before the age of 16 years, how often the childhood maltreatment had occurred (responses were recorded as: 'never', 'once', 'sometimes', 'regularly', 'often' or 'very often') and what their relationship to the perpetrator was. Emotional neglect was described as: 'people at home didn't listen to you, your problems were ignored, you felt unable to find any attention or support from the people in your house'. Emotional abuse was described as: 'you were cursed at, unjustly punished, your brothers and sisters were favored – but no bodily harm was done'. Our definition of CEM (i.e. emotional neglect and/or emotional abuse before the age of 16 years)

is based on the definition from the American Professional Society on the Abuse of Children (APSAC; (Binggeli et al., 2006; Egeland, 2009). This definition states that emotional child maltreatment consists of acts of commission (emotional abuse such as degrading, terrorizing, belittling, blaming, exploiting) and/or omission (emotional neglect, for example isolation, rejection, denying emotional responsiveness) that convey to the child that they are worthless, unloved and unwanted, and are harmful to the child's emotional developmental needs.
Sample

Participants were drawn from the large-scale longitudinal Netherlands Study of Depression and Anxiety (NESDA; (Penninx et al., 2008). From the 301 subjects who underwent the MRI scanning protocol, 97 reported having experienced CEM (emotional neglect and/or emotional abuse) more than once before the age of 16 years. We discarded data from 15 subjects due to excessive head motion (>3 mm in any direction) during resting-state data acquisition. Next, 38 subjects who reported having experienced either sexual or physical abuse or both once or more before the age of 16 were removed from the data to obtain a CEM group without sexual and physical abuse. This resulted in a CEM group of 44 subjects. In the CEM group, 97.7% (n=43) reported having been emotionally neglected and 29.5% (n=13) reported having experienced emotional abuse. The control group, NoCEM (n=44), consisted of subjects who reported having experienced no childhood maltreatment of any kind before the age of 16 and was group-wised matched to the CEM group for age, gender, handedness and presence of psychopathology. The demographics of each group together with the distribution of psychiatric diagnoses are reported in Table 1.

Data acquisition
Imaging data were acquired at one of the three participating scanning locations, situated in the University Medical Centers in Leiden, Amsterdam and Groningen, using Philips 3-T MR systems (Philips Healthcare, The Netherlands). These systems were equipped with a SENSE-8 (Leiden and Groningen) and a SENSE-6 (Amsterdam) channel head coil respectively. A recent study demonstrated that multi-center datasets can be aggregated and shared, even when different scan sequences were used (Biswal et al., 2010). As part of a fixed imaging protocol, resting-state fMRI (RS-fMRI) data were acquired for each subject. Subjects were instructed to lie as still as possible and not to fall asleep. After completion of the scan, all subjects confirmed not having fallen asleep. To obtain RS-fMRI data, T2*-weighted gradient-echo echo-planar imaging (EPI) was used with the following scan parameters in Amsterdam and

Table 1. Demographic and Clinical Characteristics of Individuals Reporting CEM versus Individuals Reporting No CEM.

	Individuals reporting CEM (N=44)	Individuals reporting No CEM (N=44)	p
Gender, % M/F	50/50	45.5/54.5	.669[a]
Handedness, % L/R	0/100	0/100	1.000[a]
Age, Mean (SD)	39.0 (10.3)	37.6 (9.7)	.506[b]
Current Diagnosis,			
MDD, *n*	13	19	.184[a]
ANX, *n*	9	7	.580[a]
CDA, *n*	14	10	.338[a]
HC, *n*	8	8	1.000[a]
# Lifetime Disorders			
# MDD episodes, Mean (SD)	4.8 (8.39)	2.86 (6.90)	.223[c]
# ANX diagnoses, Mean (SD)	1,4 (0.94)	0.9 (1.02)	.018[c]
Scan Location Amsterdam, *n*	11	13	.632[a]
Scan Location Leiden, *n*	23	22	.831[a]
Scan Location Groningen, *n*	10	9	.796[a]
NEO-FFI neuroticism, Mean (SD)	39.9 (8.8)	37.1 (9.4)	.088[c]
NEO-FFI extraversion, Mean (SD)	33.3 (7.6)	35.9 (7.4)	.103[b]
NEO-FFI openness, Mean (SD)	33.1 (5.6)	31.1 (5.2)	.069[c]
NEO-FFI agreeableness, Mean (SD)	42.7 (6.4)	44.5 (5.3)	.156[b]
NEO-FFI conscientiousness, Mean (SD)	35.1 (5.4)	36.7 (6.1)	.204[b]
BAI at baseline, Mean (SD)	12.9 (8.9)	10.6 (9.0)	.183[c]
BAI at scanning, Mean (SD)	11.3 (8.5)	9.9 (9.9)	.224[c]
MADRS at scanning, Mean (SD)	13.8 (9.6)	11.1 (11.2)	.114[c]
IDS at scanning, Mean (SD)	20.5 (11.2)	18.4 (13.9)	.271[c]

ANX = anxiety disorder; BAI = Beck Anxiety Inventory; CDA = comorbid major depressive disorder and anxiety disorder; CEM = Childhood emotional maltreatment; HC = Healthy control subjects; IDS = Inventory of depressive symptomatology; MADRS = Montgomery-Åsberg Depression Rating Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire.

[a] = Chi-Square Test

[b] = Independent Sample t-test

[c] = Mann-Whitney U Test

Leiden: 200 whole-brain volumes, repetition time (TR) 2300 ms, echo time (TE) 30 ms, flip angle 80°, 35 transverse slices, no slice gap, matrix 220 × 220 mm, voxel size 2.3 × 2.3 mm, slice thickness 3 mm. The scan parameters in Groningen were similar except for: TE 28 ms, 39 axial slices, voxel size 3.45 × 3.45 mm. For registration purposes and for gray matter density analysis, anatomical images were acquired using a sagittal three-dimensional (3D) gradient-echo T1-weighted sequence with the following scan parameters: TR 9 ms, TE 3.5 ms, flip angle 80°, 170 sagittal slices, no slice gap, matrix 256 × 256 mm, voxel size 1 mm isotropic. All anatomical images were examined by a neuroradiologist. No abnormalities were found.

Data preprocessing

The structural and RS-fMRI images were preprocessed using FEAT (FMRIB's Expert Analysis Tool) version 5.90, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). Non-brain tissue removal was applied to the structural images. Motion correction was applied to the RS-fMRI data along with non-brain

tissue removal, spatial smoothing using a 6-mm full-width at half-maximum (FWHM) Gaussian kernel, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, 0.01 Hz cut-off). RS-fMRI data were registered to the high-resolution structural image (T1) and subsequently the T1 image was registered to the 2-mm isotropic MNI-152 (T1 standard brain average over 152 subjects; Montreal Neurological Institute, Canada) images. The resulting transformation matrices derived from these registration steps were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

Statistical analysis

After preprocessing, the data were analyzed using seed-based correlations assessing three networks of interest: the limbic network, the DMN and the salience network. The following seed regions of interest (ROIs) were selected: the bilateral amygdala (limbic network), the bilateral dACC (salience network; (Margulies et al., 2007) and the PCC (DMN; (Fox et al., 2005). The bilateral seeds for the amygdala were created in standard space using the Harvard–Oxford Subcortical Structural Probability Atlas. In addition, a mask was created for the area showing decreased gray matter density earlier identified in individuals reporting CEM, in the left dmPFC (van Harmelen et al., 2010b), along with a white matter mask and a cerebrospinal fluid (CSF) mask. MNI coordinates for each of the masks are reported in Table 2.

Table 2. MNI coordinates of the seed regions.

Mask	Seed region	MNI Coordinates		
		x	y	z
Limbic network	Left Amygdala	-20	-6	-16
	Right Amygdala	26	-2	-18
Salience Network	Left dACC	-6	18	28
	Right dACC	6	18	28
Default Mode Network	PCC	-2	-36	36
Left dmPFC	Left dmPFC	-11	23	40
Confound Regressors	Left White Matter	-24	26	18
	Right White Matter	24	26	18
	Left CSF	-4	4	14
	Right CSF	4	4	14

MNI = Montreal Neurological institute; dACC = dorsal anterior cingulate cortex; PCC = posterior cingulated cortex; dmPFC = dorsomedial prefrontal cortex; CSF = cerebrospinal fluid.

A sphere with 4-mm radius was created around the single voxel seed. These spheres were then transformed to the native space using the inverse transformation matrices obtained during registration in the preprocessing phase. Spatially averaged time series were extracted for each seed and each subject. A time series was also extracted for the global mean signal. For each subject and for each network separately, a multiple regression analysis was performed using the general linear model implemented in FSL (Smith et al., 2004). The time courses that were extracted from the voxels in all of our seed regions were entered as a regressor in a general linear model for each network. Nine nuisance regressors were included in the model: the signal from the white matter, the CSF signal and the global signal, and six motion parameters (three translations and three rotations). The global signal was included to reduce artifacts associated with physiological signal sources (i.e. cardiac and respiratory)(Birn et al., 2006; Fox and Raichle, 2007). After reslicing the resulting parameter estimate maps and their corresponding within-subject variance maps into 2-mm isotropic MNI space, they were entered into a higher-level within- and between-groups mixed effects analysis (one- and two-sample t tests). For each subject, gray matter density maps were derived from the anatomical scans using FSL. Subjects in this study were drawn from the same sample (the NESDA) as the subjects used to investigate the structural abnormalities of CEM (van Harmelen et al., 2010b). Therefore, to control for structural differences possibly confounding differences in functional connectivity and to correct for the effects of possible misregistration (Oakes et al., 2007), information about gray matter density of each subject was included as a voxelwise confound regressor. Groups were compared using the general linear model including age and scan location as additional confound regressors in each comparison. Cluster correction was applied in all group analyses with an initial cluster-forming threshold of $z > 2.3$ and a corrected $p < 0.05$.

Results

Psychometric data

There was no significant difference between the CEM group and the control group in anxiety rates based on Beck Anxiety Inventory (BAI) scores (Beck et al., 1988) both at baseline and immediately before scanning or in depressive symptoms measured by the Montgomery–Åsberg Depression Rating Scale (MADRS; (Montgomery and Asberg, 1979) and the Inventory of Depressive Symptomatology (IDS; (Rush et al., 1996). No differences between the groups were found in neuroticism, extraversion, agreeableness, openness or conscientiousness as measured by the subscales of the Neuroticism–Extroversion–Openness Five-Factor Inventory (NEO-FFI; (Costa Jr and

McCrea, 1992). In terms of past psychopathology, no between-group differences were found in the number of episodes of major depressive disorder (MDD). However, our CEM group did report experiencing significantly more episodes of anxiety disorders in the past (Table 1).

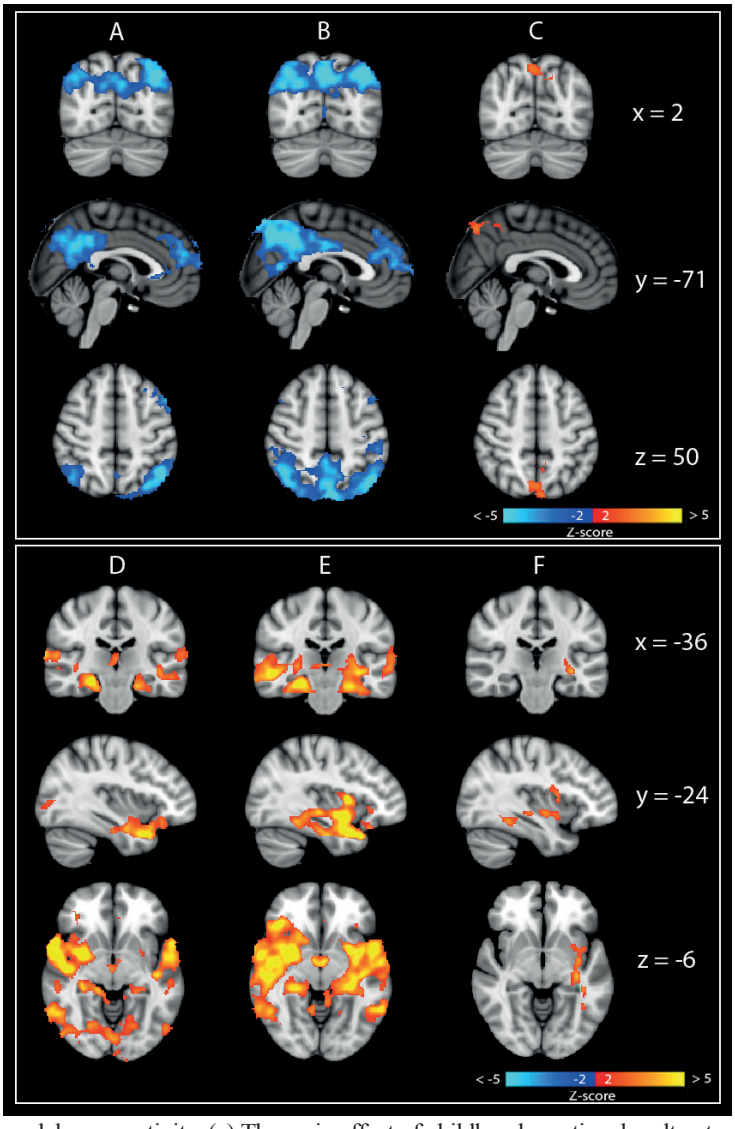
Resting-state functional connectivity

Analysis of the main effects of both the CEM group and the NoCEM group showed connectivity between the seed chosen for the specific networks and other structures known to be implicated in these networks in both groups, indicating correct positioning of our seeds. Analysis of the amygdala seeds showed a decrease in negative connectivity between the right amygdala and the superior division of the bilateral occipital cortex and the bilateral precuneus cortex in the CEM group (Fig. 1). Furthermore, a decrease in positive connectivity was found in the CEM group between the right amygdala and a large cluster stretching from the orbitofrontal cortex and the insular to subcortical structures including the hippocampus and the putamen of the left hemisphere of the brain (Fig. 1). The left amygdala seed yielded no differences between the two groups but, when taken together, the bilateral amygdala seeds showed a decrease in negative connectivity with the cuneus, the superior division of the lateral occipital cortex and the precuneus in the left hemisphere of the brain in the CEM group.

Analysis of the RSFC of the bilateral dACC seeds probing the salience network showed decreased negative connectivity between the left dACC seed and the angular cortex and the precuneus of the right hemisphere in CEM (Fig. 2). Furthermore, decreased positive connectivity was found in the CEM group between the left dACC seed and a bilateral frontal cluster containing the mPFC, the paracingulate gyrus and the frontal pole (Fig. 2). Contrasts for the right dACC seed and the left and the right dACC seeds together yielded no differences between the CEM group and the NoCEM group.

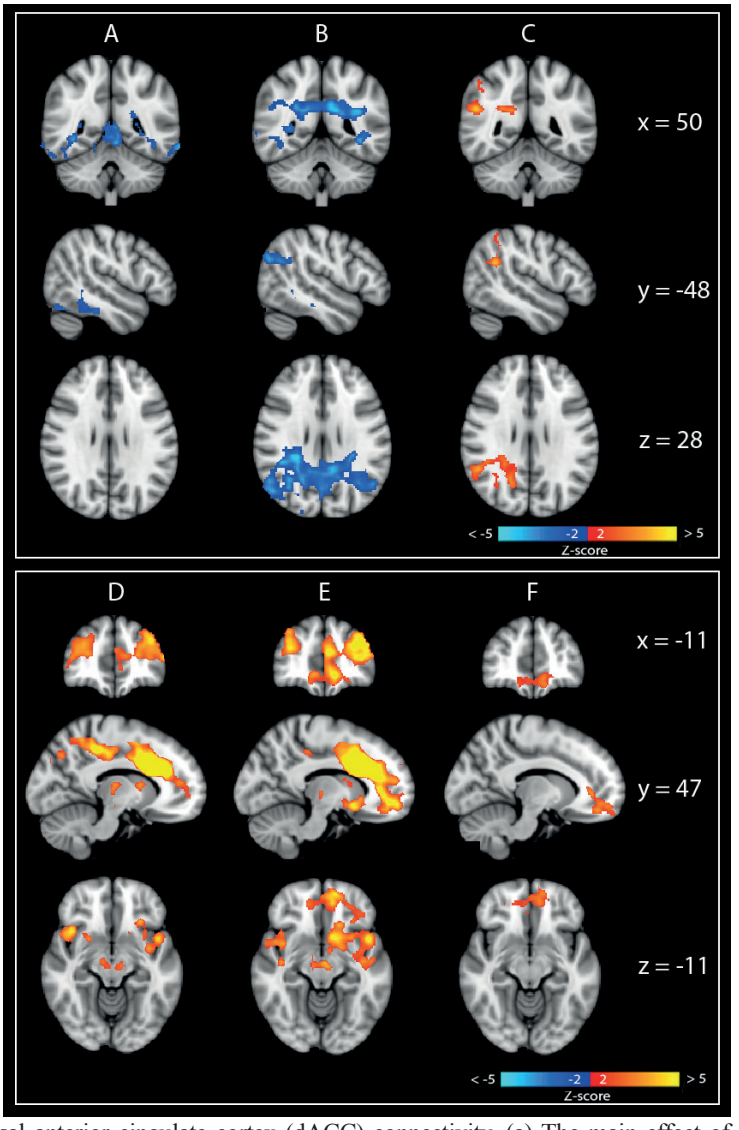
Analysis of the seed in the left dmPFC, the area implicated in the structural effects of CEM, and also the PCC seed probing the DMN yielded no differences between the CEM and the control group. Information about all significant between-group effects is listed in the online Supplementary Tables S1 and S2.

Figure 1. Right Amygdala connectivity



Right amygdala connectivity. (a) The main effect of childhood emotional maltreatment (CEM) for negative connectivity with the right amygdala, (b) the main effect of NoCEM for negative connectivity with the right amygdala, (c) the between-group effect of negative connectivity with the right amygdala, (d) the main effect of CEM for positive connectivity with the right amygdala, (e) the main effect of NoCEM for positive connectivity with the right amygdala and (f) the between-group effect of positive connectivity with the right amygdala. Images are z statistics, overlaid on the MNI-152 1 mm standard brain. The left hemisphere of the brain corresponds to the right side of the image.

Figure 2. Left dorsal anterior cingulate cortex (dACC) connectivity



Left dorsal anterior cingulate cortex (dACC) connectivity. (a) The main effect of childhood emotional maltreatment (CEM) for negative connectivity with the left dACC, (b) the main effect of NoCEM for negative connectivity with the left dACC, (c) the between-group effect of negative connectivity with the left dACC, (d) the main effect of CEM for positive connectivity with the left dACC, (e) the main effect of NoCEM for positive connectivity with left dACC and (f) the between-group effect of positive connectivity with the left dACC. Images are z statistics, overlaid on the MNI-152 1 mm standard brain. The left hemisphere of the brain corresponds to the right side of the image.

Discussion

The aim of this study was to investigate differences in RSFC between adult individuals reporting CEM and a control group without maltreatment and matched for the presence of psychopathology, using a seed-based correlation approach. We hypothesized aberrant connectivity of seed regions in the limbic network (amygdala), salience network and DMN seeds and of a dmPFC region previously found to exhibit significant gray matter loss in this group of individuals (van Harmelen et al., 2010b). We found aberrant connectivity of the amygdala and salience network seeds but, contrary to our hypotheses, no aberrant connectivity was found for the seed in the DMN and the previously identified brain region within the dmPFC that showed structural abnormalities in the CEM group.

Of note, we found decreased negative RSFC in individuals reporting CEM between the right amygdala and a brain region containing the precuneus and parts of the superior division of the lateral occipital cortex. Task-related neuroimaging studies have shown the precuneus to be involved in visuospatial imagery (Frings et al., 2006), episodic memory encoding and retrieval (Fletcher et al., 1995; Cavanna and Trimble, 2006) and self-referential processing (Kircher et al., 2000; Kjaer et al., 2002; Lou et al., 2004). Studies have shown that a history of CEM is associated with specific disturbances in emotional and cognitive processing, including negative explicit and automatic self-associations and increased amygdala reactivity (van Harmelen et al., 2013). Taking into account the role of the amygdala in the acquisition of fear responses and in the memory consolidation of emotional experiences, the decrease in connectivity between the right amygdala and the precuneus in individuals reporting CEM could reflect or underlie specific disturbances in emotional and cognitive (self) processing in individuals with a history of CEM.

Another finding in our study was decreased positive connectivity in the CEM group between the right amygdala and a large area in the left hemisphere stretching from the orbitofrontal cortex and the insula to subcortical structures including the hippocampus and the putamen. The hippocampus and the insula are regions known to be involved in emotion processing and affect regulation (Pessoa, 2008; Veer et al., 2011). Of note, reduced connectivity in a resting-state network containing the insular cortex and the amygdala has also been found in patients with MDD (Veer et al., 2010). Because of the matching for presence of psychopathology, our results cannot be attributed to a higher prevalence of depression in our CEM group, suggesting a possible shared RSFC abnormality between CEM and MDD that could

be associated with, or underlie, the elevated risk for developing recurrent and persistent depressive episodes (Nanni et al., 2012).

With regard to the altered RSFC of the right amygdala with the putamen and the orbitofrontal cortex, it should be noted that both are part of an intricate functional network also containing the dorsolateral PFC, the ventral medial pallidum and thalamic regions (Bennett, 2011). This prefrontal-limbic network is thought to be involved in goal-directed activity and also insight into an individual's well-being (Bennett, 2011). The latter function includes the ability to suppress negative feelings, an ability that is usually found to be reduced in individuals who have experienced CEM (Taussig and Culhane, 2010).

Analysis of the left and right amygdala seeds together demonstrated a decrease in negative connectivity with a brain region including the cuneus, the superior division of the lateral occipital cortex and the precuneus cortex in the left hemisphere of the brain in the CEM group. As this region was also found in the analysis for the right amygdala seed, we conclude that this result is mostly driven by the differences in connectivity with the right amygdala.

Functional connectivity analysis of the bilateral dACC seeds, probing the salience network, showed altered RSFC in individuals reporting CEM. Decreased negative RSFC was found between the left dACC and the right angular cortex and the right precuneus. As self-referential processing is an important function ascribed to the precuneus, a decrease in connectivity with the precuneus within the salience network might be related to the disturbances in relating internal and external stimuli to oneself in individuals reporting CEM (Gibb, 2002; Wright et al., 2009; van Harmelen et al., 2010a). We also found a decrease in positive connectivity between the left dACC seed and a bilateral frontal region containing both the mPFC and the frontal pole in individuals reporting CEM. Previous studies implicate the ACC, the mPFC and the frontal pole in reward-guided learning, decision making and adjusting problem-solving strategies (Kahnt et al., 2011; Koechlin, 2011; Tsujimoto et al., 2011). The altered connectivity of the left dACC with these regions might be interpreted as underlying certain disturbances of reward-guided learning and decision-making strategies such as those reported by Guyer et al. (2006), who showed that maltreated children made more risky decisions and responded less quickly as the chance of winning increased (Guyer et al., 2006).

As the precuneus cortex is an important part of the DMN, it could be argued that

differences in RSFC with the precuneus cortex are caused by group differences in DMN activity, rather than in connectivity with the precuneus cortex. However, both groups showed similar patterns of DMN connectivity and no between-group differences were found. Similarly, the seed derived from our previous study showing structural effects of CEM did not yield group differences.

To the best of our knowledge, this is the first study examining RSFC in individuals reporting CEM. Our sample size (n=88) was fairly large with respect to MRI studies in the field of psychiatry. We matched the groups for presence of psychopathology, improving homogeneity of our two groups, which did not differ in neurotic personality characteristics, anxiety symptoms, depressive symptoms and history of experienced depressive episodes. Finally, this study facilitates replication as a seed-based ROI approach was used to analyze the data. There are also some limitations to consider. The cross-sectional design of this study precludes any claim of causality or developmental trajectory, as we cannot establish whether the differences found were already present before the experience of CEM or were a consequence of the experience of CEM or its developmental and social sequelae. The presence of CEM was assessed retrospectively based on self-report and not corroborated with other sources. A bias in recall, either over- or under-reporting the experiences, cannot therefore be excluded. Clearly, the interpretation of abnormalities in RSFC in our cross-sectional observational design is more speculative, as the relationship between abnormalities in RSFC and abnormalities in task-related functional connectivity in CEM has not yet been studied directly. Our seed-based analysis is also a possible limitation as it focuses on certain networks, ignoring possibly valuable information about other networks in the brain. Another possible limitation is the influence of between-group differences in heart rate variability and respiratory rate on the results. As this physiological activity was not monitored in the current study, it remains unclear whether any differences between the two groups have influenced the results. However, regressing out global signal changes has been shown to at least partly filter out the effects of cardiac and respiratory fluctuations (Birn et al., 2006; Fox and Raichle, 2007). Finally, our RS-fMRI data were acquired at the end of a fixed imaging protocol: after completion of three task-related fMRI runs and the acquisition of an anatomical scan (scan sequence: Tower of London, word encoding, T1-weighted scan, word recognition, perception of facial expression, resting-state scan; (van Tol et al., 2011). It is therefore possible that the facial expression task influenced the RSFC (i.e. carryover effect), with subjects from our CEM group showing aberrant connectivity in areas involved in the processing of emotional faces while the facial stimulus was no longer present.

In summary, this study is the first study to demonstrate patterns of aberrant RSFC in adult individuals reporting CEM, between areas in the brain known to be involved in (emotional) stimulus processing, emotion regulation, decision making and self-referential processing. The aberrant connectivity of the precuneus with both the limbic network and the salience network in CEM is a novel finding and its possible relationship with disturbances of self-referential processing, typically found in CEM, should be investigated in future studies.

Supplementary Table S1

Right amygdala seed negative connectivity NoCEM[1] > CEM[2]

MNI[3] Coordinates			Region	Side	Z-value	p-value
x	y	z				
-22	-84	36	Lateral Occipital Cortex	L	4.12729	<0.0001
6	-70	52	Precuneus Cortex	R	3.57515	0.0004
-10	-86	42	Lateral Occipital Cortex	L	3.51271	0.0004
-34	-94	22	Occipital Pole	L	3.47727	0.0005
0	-72	50	Precuneus Cortex	L	3.41088	0.0006
-4	-80	50	Precuneus Cortex	L	3.39125	0.0007

Right amygdala seed positive connectivity NoCEM[1] > CEM[2]

MNI[3] Coordinates			Region	Side	Z-value	p-value
x	y	z				
-8	4	-22	Cerebral cortex	L	4.26867	<0.0001
-34	-24	-6	Hippocampus	L	4.23788	<0.0001
-40	-44	-12	Temporal Occipital Fusiform Cortex	L	4.08975	<0.0001
-36	-8	-6	Insular Cortex	L	3.74475	0.0002
-34	-12	-4	Putamen	L	3.693	0.0002
-40	4	4	Insular Cortex	L	3.66699	0.0002

Bilateral amygdala seed positive connectivity CEM[1] > NoCEM[2]

MNI[3] Coordinates			Region	Side	Z-value	p-value
x	y	z				
-12	-88	22	Cunual Cortex	L	4.04	<0.0001
-20	-84	32	Lateral Occipital Cortex	L	3.65	0.0003
-18	-76	42	Lateral Occipital Cortex	L	3.43	0.0006
-16	-82	40	Lateral Occipital Cortex	L	3.33	0.0009
-32	-74	-2	Occipital Fusiform Gyrus	L	3.29	0.0010
-30	-88	18	Lateral Occipital Cortex	L	3.17	0.0015

[1] NoCEM = individuals reporting having experienced no childhood emotional maltreatment

[2] CEM = individuals reporting having experienced childhood emotional maltreatment

[3] MNI = Montreal Neurologic Institute

Voxel size is 2x2x2 mm. Reported voxels are the locations of the local maxima, spread throughout the cluster.

Supplementary Table S2

Left anterior cingulate seed negative connectivity NoCEM[1] > CEM[2]

MNI[3] Coordinates			Region	Side	Z-value	p-value
x	y	z				
52	-50	26	Angular Cortex	R	4.81742	<0.0001
20	-60	24	Precuneus Cortex	R	4.14162	<0.0001
16	-50	24	Precuneus Cortex	R	4.10182	<0.0001
26	-44	26	White Matter	R	3.92834	<0.0001
42	-42	26	Supramarginal Gyrus, posterior division	R	3.61115	0.0003
32	-38	30	Supramarginal Gyrus, posterior division	R	3.08196	0.0021

Left anterior cingulate seed positive connectivity NoCEM[1] > CEM[2]

MNI[3] Coordinates			Region	Side	Z-value	p-value
x	y	z				
-8	38	0	Paracingulate Gyrus	L	4.02746	<0.0001
-12	44	-16	Frontal Medial Cortex	L	3.92824	<0.0001
10	38	-6	Paracingulate Gyrus	R	3.84837	0.0001
-8	50	-10	Frontal Medial Cortex	L	3.69775	0.0002
-12	56	-10	Frontal Pole	L	3.64786	0.0003
16	38	-20	Frontal Pole	R	3.44047	0.0006

[1] NoCEM = individuals reporting having experienced no childhood emotional maltreatment

[2] CEM = individuals reporting having experienced childhood emotional maltreatment

[3] MNI = Montreal Neurologic Institute

Voxel size is 2x2x2 mm. Reported voxels are the locations of the local maxima, spread throughout the cluster.

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Chapter 3

Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing's disease: a case–control study

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Abstract

Background
Patients with long-term remission of Cushing's disease (CD) have persistent psychological and cognitive impairments. It is unknown whether, and to what extent, these impairments are accompanied by structural abnormalities in the brain. We aim to investigate structural changes in the brain in patients with predominantly long-term remission of CD and to examine whether these changes are associated with psychological and cognitive dysfunction and clinical severity.

Design
A cross-sectional, case–control study.

Methods
In 25 patients with predominantly long-term remission of CD and 25 matched healthy controls, grey matter volumes in the regions of interest (hippocampus, amygdala, and anterior cingulate cortex (ACC)) and in the whole brain were examined, using 3T magnetic resonance imaging and a voxel-based morphometry approach. Psychological and cognitive functioning were assessed using validated questionnaires and clinical severity was assessed using the Cushing's syndrome severity index.

Results
Compared with controls, patients had smaller grey matter volumes of areas in the ACC (on average 14%, $P<0.05$) and greater volume of the left posterior lobe of the cerebellum (on average 34%, $P<0.05$). As expected, patients with remitted CD reported more depressive symptoms ($P=0.005$), more anxiety ($P=0.003$), more social phobia ($P=0.034$), more apathy ($P=0.002$), and more cognitive failure ($P=0.023$) compared with controls, but the differences in grey matter volumes were not associated with psychological or cognitive measures, nor with clinical severity.

Conclusion
Patients with predominantly long-term remission of CD showed specific structural brain abnormalities, in the presence of psychological dysfunction. Our data form a basis for future work aimed at elucidating the relation of the structural brain abnormalities and the sustained psychological deficits after long-term exposure to high cortisol levels.

Introduction

Cushing's disease (CD) is caused by excessive endogenous cortisol exposure (Newell-Price et al., 2006). After successful surgical correction of hypercortisolism, the physical, psychological, and cognitive symptoms improve substantially (Starkman et al., 1986; Dorn et al., 1997). However, despite curative treatment of the adenomas per se, multiple physical, psychological, and cognitive complaints may persist and morbidity and mortality remain increased even in the case of long-term remission (Pereira et al., 2010; Feelders et al., 2012).

Cortisol is the main hormonal mediator of the stress response and acts via stimulation of both the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the CNS. Hypothalamic-pituitary-adrenal (HPA)-axis activity is regulated by limbic structures such as the hippocampus and amygdala and the anterior cingulate cortex (ACC; (de Kloet et al., 2005). These areas are also important target areas for glucocorticoid hormones via activation of MR and GR. In accordance, long-term exposure to elevated cortisol levels has been linked to functional and structural changes of these limbic structures both in humans and preclinical studies (Cerqueira et al., 2005; McEwen et al., 2012). For example, prolonged cortisol elevations predict memory dysfunction and reduced volume of the hippocampus and ACC during aging (Lupien et al., 1998; MacLullich et al., 2006). Moreover, in patients with Cushing's syndrome, hypercortisolism was associated with smaller hippocampal volumes and overall brain atrophy (Starkman et al., 1992; Simmons et al., 2000; Bourdeau et al., 2002), with increasing hippocampal volumes and improving emotional and cognitive functioning after correction of hypercortisolism (Starkman et al., 1999; Bourdeau et al., 2002; Starkman et al., 2003; Hook et al., 2007; Starkman et al., 2007; Toffanin et al., 2011).

To date, the long-term effects of chronic overexposure to cortisol, such as in CD, on the brain has been evaluated in only one study (Resmini et al., 2012). In that study, focusing on memory function and hippocampal volume in 33 patients with Cushing's syndrome and 34 matched healthy controls, no overall differences in hippocampal volume between patients and controls were found. However, there was a considerable heterogeneity within the patient group in terms of disease status and treatment. Both patients with active CD and patients with CD in remission, with either pituitary or adrenal disease, were included and analyzed as one group, precluding definite conclusions (Resmini et al., 2012). Furthermore, volumetric

analyses were limited to the hippocampus and did not include other brain regions known to be important in emotional and cognitive functioning.

Recently, we performed a large cross-sectional study in a well-characterized cohort of patients with long-term biochemical remission, i.e. successful treatment for CD. We found a decreased quality of life (van Aken et al., 2005; Tiemensma et al., 2011), a higher prevalence of psychopathology (e.g. depression, anxiety, and apathy) (Tiemensma et al., 2010a), maladaptive personality traits (Tiemensma et al., 2010a), and subtle cognitive impairments (Tiemensma et al., 2010b), despite long-term cure. The results of these studies suggest irreversible effects of longer periods with glucocorticoid excess on brain function and possibly brain structure. These findings were associated with clinical characteristics (e.g. hydrocortisone dependency).

The primary aim of the present cross-sectional study was to investigate whether this cohort of patients with predominantly long-term biochemical remission of pituitary-dependent CD shows structural brain abnormalities, using a voxel-based morphometry approach. In particular, given the results of our previous study, we aimed to evaluate structural changes in important cerebral regions of the limbic system, i.e. the hippocampus, the amygdala, and also in a cerebral key region for both cognitive and emotional functioning: the ACC. Furthermore, we performed an explorative whole brain analysis to detect possible structural changes in areas outside these a priori defined regions of interest (ROI). In addition, we aimed to explore associations between structural changes and measures of psychological and cognitive dysfunction and to take clinical characteristics, such as hydrocortisone dependency, into account.

Methods

Subjects

All patients in long-term remission of CD of pituitary origin, monitored at our institute (n=49) and between 18 and 60 years of age, were invited by letter and those who did not respond were contacted by phone. The response rate was 96% and 31 patients were screened for eligibility. Exclusion criteria were (history of) drug or alcohol abuse, neurological problems, contraindications for undergoing a magnetic resonance imaging (MRI) scan, and left-handedness. A total of 25 CD patients and 25 matched healthy controls were included in this study. All CD patients had been treated by transsphenoidal surgery, two patients (8%) additionally underwent

bilateral adrenalectomy, whereas six patients (24%) had received additional radiotherapy. One patient (4%) used antidepressants. Healthy controls were pair-wise matched for gender, age, and education and recruited by advertisements in grocery stores and via Internet. Inclusion criteria for healthy controls were aged between 18 and 60 years, right-handedness, no current or prior drug or alcohol abuse, no present and past history of psychiatric or neurological disorders, no use of psychotropic medication, and no contraindications for MRI scanning.

The diagnosis of CD had been confirmed in all patients. Adrenocorticotrophic hormone (ACTH)-dependent Cushing's syndrome had been diagnosed based on internationally agreed guidelines, with clinical manifestations and positive biochemical tests, including increased urinary excretion rates of free cortisol, decreased overnight suppression by dexamethasone (1 mg), and elevated midnight salivary cortisol values. Cure of CD had been achieved by transsphenoidal surgery and, if necessary, followed by repeated surgery and/or postoperative radiotherapy. Cure of CD was defined by normal overnight suppression of plasma cortisol levels (<50 nmol/l) after administration of dexamethasone (1 mg) and normal 24-h urinary excretion rates of cortisol (<220 nmol/24 h). Hydrocortisone independency was defined as a normal cortisol response to corticotrophin-releasing hormone (CRH) or insulin tolerance test (>500 nmol/l). Patients were followed at our department with yearly intervals, and pituitary hormone substitution was prescribed in accordance with the results of the yearly evaluation. In patients who were glucocorticoid dependent after treatment, recovery of the pituitary–adrenal axis was tested twice a year. The dose of hydrocortisone was on average 20 mg/day divided into two to three dosages. After withdrawal of hydrocortisone replacement for 24 h, a fasting morning blood sample was taken for the measurement of serum cortisol concentrations. Patients with serum cortisol concentration <120 nmol/l were considered to have ongoing glucocorticoid dependency, and hydrocortisone treatment was restarted. Patients with serum cortisol levels of 120–500 nmol/l were tested by ACTH stimulation tests (250 µg). A normal response to ACTH stimulation was defined as a stimulated cortisol >550 nmol/l. When the cortisol response to ACTH was normal, patients were tested by insulin tolerance test (ITT) or CRH stimulation test. When cortisol responses to these tests were <550 nmol/l, hydrocortisone treatment was restarted. Evaluation of growth hormone (GH) deficiency was done by insulin-tolerance test or arginine–GHRH test only in patients under the age of 70 years and only after at least 2 years of remission. Patients with an inadequate stimulation of GH by one of these tests were treated with recombinant human GH, aiming at insulin-like growth factor 1 levels between 0 and +2 S.D. values. In addition, the twice-

yearly evaluation consisted of measurement of free thyroxine and testosterone levels (in male patients). If results were below the lower limit of the respective reference ranges, L-thyroxine and/or testosterone substitution was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. Persistent cure of CD was documented by normal values of a dexamethasone (1 mg) suppression test, urinary cortisol excretion rates, and midnight salivary cortisol levels before participation in the current study.

The estimated duration of disease was determined through patients' history by looking for the earliest physical/somatic signs. Duration of remission was calculated from the date of curative transsphenoidal surgery, or in case of persistent disease, from the date of normalization of biochemical tests after postoperative radiotherapy. Patient and treatment characteristics were collected from the patient records.
Written informed consent was obtained from all participants before the clinical assessment and the MRI-scan session. Our institutional review board approved the study protocol. This study was in accordance with the principles of the declaration of Helsinki.

Study design
We scheduled a single study visit of 2 h for MRI scanning (60 min) and an interview for the evaluation of the clinical data and the assessment of psychological and cognitive functioning. Scan sessions took place between 0900 and 1200 h. After the examination, participants were asked to complete several self-rating questionnaires at home for the assessment of psychopathology and cognitive functioning and to return them within a week.

Assessment of psychopathology and cognitive functioning
Presence and severity of depressive symptoms were evaluated using the Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery and Asberg, 1979; Snaith et al., 1986), which was the only scale that was assessed by the interviewer, and the Inventory of Depression Symptomatology (IDS; (Rush et al., 1996). Anxiety was evaluated using the Beck Anxiety Inventory (BAI; (Beck et al., 1988) and the Fear Questionnaire (FQ; (Marks and Mathews, 1979). Apathy and irritability were assessed using the Apathy Scale (AS) and the Irritability Scale (IS) respectively (Starkstein et al., 2001; Chatterjee et al., 2005). The Cognitive Failures Questionnaire (CFQ) was used to assess failures in perception, memory, and motor function (Broadbent et al., 1982).

Cushing's syndrome severity index

The Cushing's syndrome severity index (CSI; (Sonino et al., 2000) was used to assess current severity of symptoms and to retrospectively estimate (clinical) severity at the time of active disease. The CSI contains eight clinical features and can be scored on a 3-point scale, ranging from 0 to 2. A higher total score on the CSI indicates greater severity, with a range of 0–16. The information necessary for completing this index was derived from clinical history and medical files. Two raters, who reached consensus on each feature in case of discrepancy, scored the CSI. For the active phase, the CSI was scored retrospectively. The current score was evaluated based on the last yearly evaluation. The total score of the active phase and the total score of the remission phase were used in the analyses.

MRI data acquisition

Images were acquired on a Philips 3T MRI system (Philips Healthcare, Best, The Netherlands; software version 3.2.1). A SENSE-32 channel head coil was used for radio frequency transmission and reception. For each subject, anatomical images were obtained using a sagittal three-dimensional gradient-echo T1-weighted sequence (repetition time=9.8 ms, echo time=4.6 ms, matrix size 256×256, voxel size 1.17×1.17×1.2 mm, 140 slices, scan duration 4:56 min) as part of a larger imaging protocol. A neuroradiologist, blinded for the clinical details of the subjects, examined all anatomical images. Apart from incidental age-related white matter hyperintensities and effects of the post-transsphenoidal surgery in the perisellar area, no other macroscopic abnormalities were observed in the patients and controls.

Statistical analyses and data preprocessing

The first analysis comprised the voxel-based comparison of grey matter volumes in the ROI (i.e. hippocampus, amygdala, and ACC) and across the whole brain between patients with predominantly long-term remission of CD and their matched healthy controls. Structural data were analyzed with FSL-VBM, a voxel-based morphometry style analysis (FMRIB's Software Library; (Smith et al., 2004). First, structural images were brain-extracted and grey matter-segmented (Zhang et al., 2001). The resulting grey matter partial volume images were then aligned to MNI-152 (T1 standard brain average over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) standard space, using affine registration (Jenkinson et al., 2002), followed by nonlinear registration. The resulting images of all participants were averaged to create a study-specific template, to which the native grey matter images were then nonlinearly reregistered.

The Jacobian of the warp field obtained in this registration reflects the voxel-wise relative volume change between the original and the study-specific template. In order to correct for local expansion or contraction, the registered partial volume images were then modulated by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. The Gaussian outputs a weighted average of each voxel's neighborhood, with the average weighted more toward the value of the centrally located voxels. The application of this type of smoothing reduces the noise in the data substantially.

The Harvard–Oxford Cortical and Subcortical Structural Atlases implemented in FSL were used to create masks for our ROI: the bilateral hippocampus, the bilateral amygdala, and the ACC. Probability range was set to 50–100% for all three structures. The study-specific template was then applied to this mask to create a study-specific template of the grey matter values in the ROI only. Finally, groups were compared using a general linear model (GLM) including age, gender, and level of education as confound regressors. A voxel-wise GLM was applied using permutation-based (5000 permutations) non-parametric testing, correcting for multiple comparisons across space. First, groups were compared in our ROI, using the created mask. Second, an exploratory whole brain VBM analysis was done using the study-specific grey matter image as a mask to investigate whether any unpredicted differences existed between CD patients and controls. To explore possible differences between patients with hydrocortisone substitution (n=13) and patients without substitution (n=12), these two steps were repeated contrasting these two groups. Threshold-free Cluster Enhancement was used for finding clusters in the data (Smith and Nichols, 2009), with thresholds for both the ROI comparison as well as the whole brain analysis set on $P<0.05$ corrected. In addition to the VBM analysis, we used FMRIB's integrated registration and segmentation tool (FIRST) to perform an automated segmentation of the amygdala and the hippocampus, allowing both shape and volume analyses.

The second analysis compared patients with predominantly long-term remission of CD and their matched healthy controls on measures of psychological and cognitive functioning. Data from questionnaires were analyzed using SPSS for Windows version 20.0 (SPSS, Inc.). All data are presented as numbers and percentages, means and S.D.s, or median and interquartile range (IQR). The assumption of normal distribution was tested using the Kolmogorov–Smirnov test and the assumption of equal variances with a Levene's test. With respect to psychological and cognitive functioning, normally distributed continuous variables between patients and

matched controls were compared using t-tests, and non-normally distributed continuous variables (MADRS, IDS, BAI, FQ, AS, and CFQ) using Mann–Whitney U tests. Considering the overlap in phenomenology assessed by the questionnaires, a strict correction for multiple testing might be too conservative, therefore all tests were two-sided with $P<0.05$ uncorrected.

A third analysis was conducted in the patient group. In this analysis, we examined voxel-wise correlations of behavioural and clinical characteristics with grey matter volume in the areas resulting from the ROI analysis and the whole brain analysis. The possible influence of radiotherapy could not be properly examined, considering the small number of patients that had received radiotherapy. The level of significance was set at $P<0.05$.

Results

Patient characteristics

As expected, patients and matched healthy controls did not differ in age, gender, or education. The mean estimated duration of active disease was 7.9±7.9 years (range 0.8–37.0). The mean duration of remission was 11.2±8.2 years (range 0.8–29.4). Hydrocortisone replacement therapy was given to 13 patients (52%). The mean CSI score during active disease was 8.1±2.0 and 2.5±1.5 at the time of evaluation (i.e. long-term remission; Table 1).

Table 1. Clinical characteristics of patients with predominantly long-term remission of Cushing's disease (n=25). Data are presented as mean±S.D. or number (%) or by median IQR.

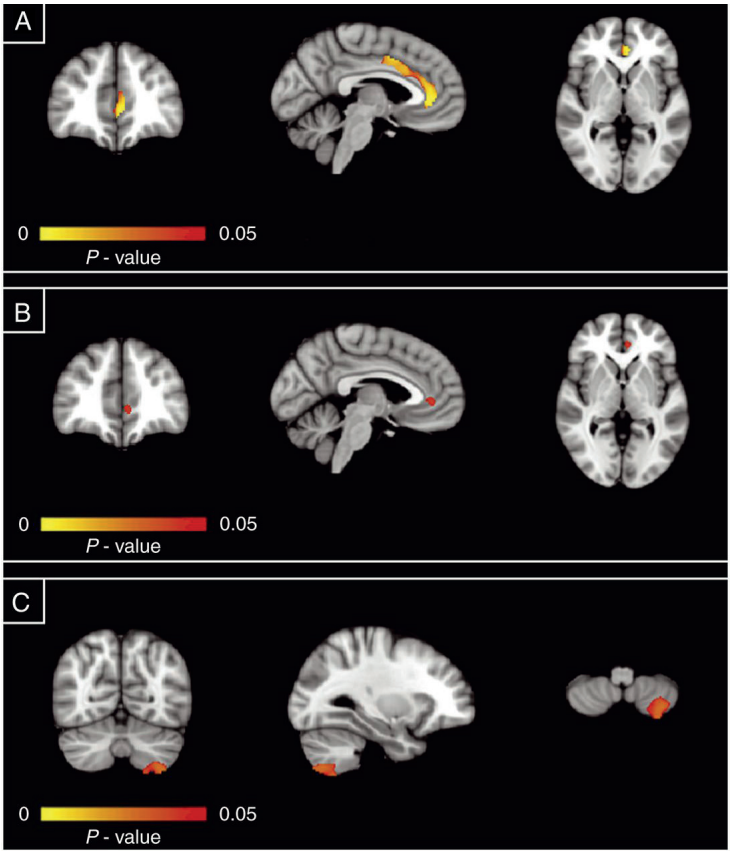
	CD patients (n=25)	**Matched controls** (n=25)	***P* value**
Gender (male/female)	4/21	4/21	1.000[a]
Age (years)	45 ± 8	47±7	0.471[b]
Education			0.946[a]
Low	6 (24%)	6 (24%)	
Medium	12 (48%)	11 (44%)	
High	7 (28%)	8 (32%)	
Surgery			
Transsphenoidal adenomectomy	25 (100%)		
Bilateral adrenalectomy	2 (8%)		
Radiotherapy	6 (24%)		
Disease duration (years)	7.9 ± 7.9		
Duration of remission (years)	11.2 ± 8.2		
Hypopituitarism			
Any axis	14 (56%)		
GH	10 (40%)		
LH/FSH	9 (36%)		
TSH	10 (40%)		
ADH	3 (12%)		
Hydrocortisone substitution	13 (52%)		
Hydrocortisone dose (mg/d)	20.0 (0.0 – 20.0)		
Clinical severity index			
Active phase, total	8.1 ± 2.0		
Remission phase, total	2.5 ± 1.5		

P values were tested with: [a] Chi-square test and [b] independent-sample t-test.

ROI analyses

The VBM analysis, in patients in with predominantly long-term remission of CD, showed smaller grey matter volumes in a large part of the bilateral ACC in comparison with controls. Closer examination of the data revealed that the patients had an average of 14% smaller grey matter volumes in the ACC compared with matched healthy controls. There were no grey matter volume differences in the bilateral hippocampus and amygdala (Fig. 1A). We observed no greater grey matter volumes in any of the ROIs in CD patients compared with controls. Furthermore, within the patient group no differences were found in grey matter volumes between patients with hydrocortisone substitution and patients without substitution. The FIRST analysis showed similar results, with no differences in both shape and volume of the bilateral amygdala and bilateral hippocampus, between patients and controls.

Figure 1. VBM analysis results



(A) Results of regions of interest analysis, with lesser grey matter volumes in patients than that in controls (P<0.05; 617 voxels, 2mm isotropic). (B) Results of whole brain analysis with lesser grey matter volumes in patients than that in controls (P<0.05; 37 voxels, 2mm isotropic). (C) Results of whole brain analysis with lesser grey matter volumes in patients than that in controls (P<0.05; 323 voxels, 2mm isotropic). Effects are presented on the MNI-152 1mm standard brain at a threshold of P<0.05. Coordinates are x=−4, y=42, and z=0 for (A and B) and x=−29, y=−66, and z=−56 for (C). The left hemisphere corresponds with the right side of the image.

Whole brain analysis

Patients with predominantly long-term remission of CD showed smaller grey matter volumes in the left perigenual region (Brodman's area between BA 32 and BA 12) of the ACC, compared with controls (Fig. 1B). Greater grey matter volumes were found in the posterior lobe of the left cerebellum in CD patients compared with controls (Fig. 1C). On average patients showed 34% larger grey matter volumes in

the left posterior lobe of the cerebellum compared with controls (P<0.05). When the threshold was lowered to P<0.10, an additional similar effect was observed in grey matter volumes of the right posterior lobe of the cerebellum. Within the patient group, no differences were found in grey matter volumes between patients with hydrocortisone substitution and patients without substitution.

Psychopathology and cognitive functioning among patients and controls

Table 2 shows that patients with predominantly long-term remission of CD had more depressive symptoms (P<0.005) compared with controls, as assessed with the MADRS and the IDS. The mean total score on the MADRS was 6.3, indicating mild depressive symptoms. Furthermore, CD patients experienced more anxiety (P=0.003), more social phobia (P=0.034), and a greater degree of apathy (P=0.002), with 44% of patients having a score of 14 or higher, which is indicative of clinically relevant apathy. On the IS, 36% of the patients had a score of 14 or higher, which is indicative of clinically relevant irritability. In addition, CD patients reported more cognitive failure (P=0.023) compared with controls. No other significant between-group differences were found. Within the patient group, no significant differences were found in psychopathology and cognitive functioning between patients with hydrocortisone substitution and patients without substitution.

Table 2. Psychopathology and cognitive failure questionnaires: patients with predominantly long-term remission of Cushing's disease vs. matched controls. Data are presented as mean ± SD or number (%).

	Cushing's disease (n = 25)	**Matched controls** (n = 25)	***P* value**
MADRS	6.3 ± 5.5	1.4 ± 1.8	**0.000**[b]
Inventory depression scale (IDS)	46.8 ± 13.0	36.3 ± 5.8	**0.005**[b]
Beck Anxiety inventory (BAI)	28.4 ± 5.7	24.0 ± 3.1	**0.003**[b]
Fear Questionnaire (FQ)	24.5 ± 17.4	14.2 ± 10.0	0.051[b]
agoraphobia subscale	6.1 ± 7.9	3.4 ± 4.7	0.477[b]
blood injury phobia subscale	6.2 ± 8.3	3.2 ± 4.1	0.118[a]
social phobia subscale	12.2 ± 8.0	7.6 ± 4.9	**0.034**[b]
Irritability scale (IS)	12.1 ± 8.7	8.0 ± 6.1	0.066[a]
Total score > 14	9 (36%)	6 (24%)	
Apathy scale (AS)	13.6 ± 6.6	7.8± 3.8	**0.002**[b]
Total score > 14	11 (44%)	2 (8%)	
Cognitive failure questionnaire (CFQ)	38.0 ± 16.5	27.6 ± 9.7	**0.023**[b]

P values were tested with: [a] independent-sample t-test and [b] Mann-Whitney U test.
Level of significance was set at *P*<0.05.

Furthermore, in the patient group no significant associations between grey matter volumes in the ACC and cerebellum, and scores on the distinguishing psychometric

instruments (MADRS, IDS, BAI, AS, and CFQ), were found using a voxel-wise correlation approach. Also, no significant associations between grey matter volumes of the areas of effect and clinical characteristics (i.e. estimated disease duration, duration of remission, clinical severity indexes, active and remission subscale, data not shown) were found.

Discussion

This study demonstrates that structural abnormalities in the brain are present in patients cured from CD, despite long-term remission. The data indicate that in comparison with matched healthy controls, volumes of areas in the ACC were smaller, whereas grey matter volumes of the left posterior lobe of the cerebellum were larger in patients. There were no significant differences in grey matter volumes in the hippocampus or amygdala between the two groups. These findings may support the hypothesis that the increased prevalence of depressive symptoms, anxiety, apathy, and cognitive impairments observed in patients with long-term cured CD (Tiemensma et al., 2010a; Tiemensma et al., 2010b) is associated with structural brain changes. However, in these patients no significant correlations were found between psychological dysfunction and clinical characteristics on the one hand and the grey matter volumes of the ACC and left posterior lobe of the cerebellum on the other hand.

We confirmed our hypothesis that the ACC would be affected in cured CD. The amygdala and hippocampus are connected to the anterior regions of the ACC and constitute a neural circuitry for stress reactivity and modulation (Shin and Liberzon, 2010). Dysfunction of this circuitry is implicated in mood and anxiety disorders (Bremner, 2007). In addition, patients with stress-related psychopathology show a reduced volume of the ACC (Woodward et al., 2006; van Tol et al., 2010). In accordance, reduction of ACC volume is also found in animals exposed to hypercortisolism (Cerqueira et al., 2005) and in elderly humans with dysregulation of the HPA-axis (MacLullich et al., 2006). Importantly, the ACC is involved in cognitive-affective processes, such as assessing the projection of emotional and motivational stimuli and the regulation of emotional responses (Bush et al., 2000), and mediates ongoing behavioral adaptation (Sheth et al., 2012). Therefore, the identified abnormalities of the ACC may be involved in disturbances of cognitive and emotional functioning identified in CD (Starkman and Schteingart, 1981) and in patients after long-term remission of CD (Pereira et al., 2010; Tiemensma et al., 2010a; Tiemensma et al., 2010b). However, in the current study we were not able

to demonstrate a correlation between the observed brain changes and quantitative estimates of psychopathology. This may be due to power problems or limitations of the clinical rating scales for psychopathology. An alternative hypothesis could be that the identified structural abnormalities may also underlie or reflect abnormalities in functional or structural connectivity.

In the exploratory whole brain analysis, we found an enlarged volume of the left cerebellum in patients with predominantly long-term remission of CD. When we lowered the threshold, grey matter volumes of the right cerebellum were also found to be enlarged in patients with predominantly long-term remission of CD, indicating that this effect might be bilateral. Interestingly, the cerebellum is susceptible to increased cortisol levels (Teicher et al., 2003) and it is involved in motor functioning, as well as cognitive and emotional functioning (Baumann and Mattingley, 2012). Intriguingly, a study by Spinelli et al. reported that individuals exposed to an extremely stressful environment developed a larger cerebellum (Spinelli et al., 2009). Another research group investigated the effect of chronic stress on cortical and striatal circuits (required for goal-directed behavior and habits) in rats. They found global hypertrophy of the dorsolateral striatum and atrophy of the dorsomedial striatum and suggested that the reorganization of the corticostriatal circuits after chronic stress is bidirectional, based on hypertrophy and atrophy of neuronal dendritic trees (Dias-Ferreira et al., 2009). This mechanism of bidirectional reorganization could also provide an explanation for the larger volume of the cerebellum in our patients treated for CD.

Contrary to our hypotheses, we did not find alterations in the hippocampus and amygdala. However, it might be that these brain structures were affected during active disease (Starkman et al., 1992; Simmons et al., 2000; Bourdeau et al., 2002), but that grey matter volumes increased after biochemical cure. This would be in accordance with the previously found increase in hippocampal volume in CD patients after correction of hypercortisolism (Starkman et al., 1999; Bourdeau et al., 2002; Hook et al., 2007; Toffanin et al., 2011) and the well-documented plasticity of hippocampal neurons in animal models (Schubert et al., 2008). Nevertheless, children experienced cognitive decline despite reversal of brain atrophy 1 year after surgical remission (Merke et al., 2005) and adult patients with long-term remission of CD still demonstrated impaired memory function (Tiemensma et al., 2010b). Recently, a potential mechanism was provided for this persisted memory impairment, by demonstrating that in comparison with healthy matched controls, patients in remission of CD show biochemical abnormalities in the hippocampus,

without reduction in hippocampal volume (Resmini et al., 2013). Studies on animals have documented that other brain areas also show structural changes in response to increased cortisol levels (Dias-Ferreira et al., 2009). However, the plasticity (in this case the extent of reversibility) of these non-hippocampal structures in CD is still unknown. As there are no studies that have focused on other brain structures in patients with active CD, like the ACC or amygdala, it is not clear when these structural changes occur or how they develop over time.

For direct effects of glucocorticoids on a brain area, either the MR or GR has to be present in this area. Using data on human brain tissue arrays available from The Allen Institute for Brain Science, a high expression was demonstrated of both MR and/or GR, not only in the hippocampus, ACC, and amygdala but also in the cerebellum (Hawrylycz et al., 2012). Taking into account the effects found in our study, which were limited to the ACC and cerebellum, one can conclude that expression of MR and/or GR in a brain area is necessary, but not predictive of structural changes following chronic overexposure to glucocorticoids. A possible alternative explanation is that structural changes may also occur via transsynaptic mechanisms. Such mechanisms have been suggested for (transient) morphological changes in the hippocampal CA3 area, which itself expresses very low numbers of GRs, but receives input from the cortisol-sensitive dentate gyrus (Fuchs et al., 2006).

To our knowledge, our study is the first to show that structural abnormalities in the brain are present in patients cured from CD, despite long-term remission. Strengths of our study are the homogeneity of our patient cohort with regard to treatment (i.e. all patients had been treated with transsphenoidal surgery) and the careful selection of controls. Nevertheless, heterogeneity still existed in the patient group with regard to disease duration and duration of remission, which may have decreased the power of this study. Although a sample size of 25 in both groups is appropriate for the evaluation of structural changes with MRI (Pell et al., 2008), our study might have been underpowered to detect possible correlations between clinical data, psychological and cognitive measures, and grey matter volumes within the patient group, and to detect grey matter differences between patients with or without hydrocortisone substitution. In addition, cognitive functions were assessed using a questionnaire (i.e. CFQ), and although this questionnaire has been validated repeatedly, it is no substitute for extensive neuropsychological testing, which gives a more accurate representation of cognitive functioning. Furthermore, because of our cross-sectional design it cannot be excluded that structural abnormalities were already present in patients before onset of CD. The use of a longitudinal

design in future research could provide more insight into the course of the found abnormalities.

In general, alterations in grey matter volume in adults with pathology have been found to be associated with dysfunctions of specific areas or related circuitry. However, the absence of volumetric differences does not exclude functional alterations in brain areas and circuits. It should also be acknowledged that a volumetric VBM approach does not reveal the underlying changes or pathology in grey matter microstructure, i.e. at the level of neurons or glia cells. Subsequently, at present there are no data available on abnormalities at the level of neurons or glia cells after chronic overexposure to glucocorticoids that may shed more light on the nature of the observed structural abnormalities. Therefore, conclusions about functional alterations in the specific brain areas cannot be drawn based solely on our findings. Exploring functional brain characteristics in our sample would be an important next step to further elucidate the neurobiological basis of psychological dysfunction in patients with remitted CD.

The data presented in this study provide a further perspective toward detailed phenotyping of patients after treatment of CD, who have always been considered cured after long-term remission of hypercortisolism. In agreement with others, CD and possibly Cushing's syndrome as well, could be a unique model to study the apparently prolonged, or even irreversible, effects of increased cortisol exposure on the brain. It is tempting to speculate that these findings, to a certain extent, could also apply to patients with chronic or recurrent forms of highly prevalent stress-related disorders and, in addition, to patients chronically treated with exogenous corticosteroids that are commonly prescribed to suppress the immune system (54).

In summary, this study demonstrates that patients with long-term cure after treatment for CD have profound structural alterations in the brain, with smaller volumes of an area in the ACC and greater volumes of the left posterior lobe of the cerebellum, and report more depressive symptoms, anxiety, social phobia, apathy, and cognitive failure, compared with healthy controls. The findings suggest possible structural substrates for long-term psychological effects of hypercortisolism in CD. Clearly, more research is needed to increase our insight into the underlying mechanisms and the trajectory of changes, which may also lead to the identification of 'critical time windows' or potential targets for prevention.

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Chapter 4

Widespread reductions of white matter integrity in patients with long-term remission of Cushing's disease

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Abstract

Background
Hypercortisolism leads to various physical, psychological and cognitive symptoms, which may partly persist after the treatment of Cushing's disease. The aim of the present study was to investigate abnormalities in white matter integrity in patients with long-term remission of Cushing's disease, and their relation with psychological symptoms, cognitive impairment and clinical characteristics.

Methods
In patients with long-term remission of Cushing's disease (n=22) and matched healthy controls (n=22) we examined fractional anisotropy (FA) values of white matter in a region-of-interest (ROI; bilateral cingulate cingulum, bilateral hippocampal cingulum, bilateral uncinate fasciculus and corpus callosum) and the whole brain, using 3 T diffusion tensor imaging (DTI) and a tract-based spatial statistics (TBSS) approach. Psychological and cognitive functioning were assessed with validated questionnaires and clinical severity was assessed using the Cushing's syndrome Severity Index.

Results
The ROI analysis showed FA reductions in all of the hypothesized regions, with the exception of the bilateral hippocampal cingulum, in patients when compared to controls. The exploratory whole brain analysis showed multiple regions with lower FA values throughout the brain. Patients reported more apathy (p =.003) and more depressive symptoms (p b.001), whereas depression symptom severity in the patient group was negatively associated with FA in the left uncinate fasciculus (p b 0.05). Post-hoc analyses showed increased radial and mean diffusivity in the patient group.

Conclusions
Patients with a history of endogenous hypercortisolism in present remission show widespread changes of white matter integrity in the brain, with abnormalities in the integrity of the uncinate fasciculus being related to the severity of depressive symptoms, suggesting persistent structural effects of hypercortisolism.

Introduction

Cushing's disease is caused by an adrenocorticotropic hormone (ACTH) producing pituitary adenoma, which in turn causes hypercortisolism (Nieman and Ilias, 2005). Hypercortisolism is associated with severe physical and psychological symptoms and cognitive impairments (Forget et al., 2000; Nieman and Ilias, 2005; Newell-Price et al., 2006; Leon-Carrion et al., 2009; Michaud et al., 2009). Patients with Cushing's disease are treated by undergoing surgery, in some cases followed by postoperative radiotherapy and/or hydrocortisone substitution, dependent on the outcome of the surgery. Remission occurs with the re-instatement of normalized basal and ACTH-stimulated cortisol values. Although the clinical picture improves substantially after the successful treatment of hypercortisolism (Starkman et al., 1986), various symptoms remain present during long-term remission. Compared to healthy controls, patients treated for Cushing's disease demonstrated more cognitive impairment (Dorn and Cerrone, 2000; Forget et al., 2002; Merke et al., 2005; Tiemensma et al., 2010b; Resmini et al., 2012), more quality of life impairments (van Aken et al., 2005; Tiemensma et al., 2011), a higher prevalence of psychopathology (e.g. affective disorders and apathy; (Tiemensma et al., 2010a), and maladaptive personality traits (Tiemensma et al., 2010a). These persistent symptoms following transient hypercortisolism are not well understood.

Neuroimaging studies investigating brain characteristics related to (a history of) Cushing's disease are scarce, and the few studies examining structural brain characteristics have mainly focused on gray matter volumes. Hypercortisolism was found to be associated with smaller hippocampal volumes and overall brain atrophy (Starkman et al., 1992; Simmons et al., 2000; Bourdeau et al., 2002). After an early successful abrogation of hypercortisolism, hippocampal volume increased and emotional and cognitive functioning improved (Starkman et al., 1999; Bourdeau et al., 2002; Starkman et al., 2003; Hook et al., 2007; Toffanin et al., 2011). Patients with remission of Cushing's disease demonstrated no differences in hippocampal volume compared to controls (Starkman et al., 1999; Resmini et al., 2012; Andela et al., 2013). However, smaller gray matter volumes in the anterior cingulate cortex (ACC) and larger gray matter volumes of the left posterior lobe of the cerebellum have been shown in patients with remission of Cushing's disease (Andela et al., 2013). Interestingly, both the hippocampus and ACC are involved in the functional neurocircuitry of stress (Dedovic et al., 2009), with subregions of the ACC having an inhibitory effect on various limbic structures (Phelps et al., 2004; Baumann and Turpin, 2010). Disturbances in this inhibitory function resulted in the dysregulation

of emotion and impaired cognition in affective disorders (Phan et al., 2005; Shin and Liberzon, 2010). In this light, studying the structural connectivity between these brain regions in patients with long-term remission of hypercortisolism could give further insight into the pathophysiology of persistent psychological symptoms and cognitive impairment.

White matter integrity has never been studied in Cushing's disease, and the relationship between white matter and elevated cortisol levels has only been studied in animal models (Alonso, 2000; Miyata et al., 2011; Willette et al., 2012). These studies showed an association between the prolonged exposure to elevated corticosteroid levels and the inhibition of the proliferation of oligodendrocyte precursors throughout the white matter. Oligodendrocytes play a major role in the process of remyelination, and thus white matter integrity (Alonso, 2000; Miyata et al., 2011). In addition, studies examining white matter in stress-related psychiatric disorders (depressive disorders, anxiety disorders and posttraumatic stress disorder) are of interest because these disorders are often accompanied by an unbalanced hypothalamic–pituitary–adrenal axis, resulting in increased levels of cortisol, as well as psychiatric symptoms similar to those reported by Cushing's disease patients. Stress-related psychiatric disorders have been related to reduced white matter integrity in mainly the corpus callosum, the cingulum and the uncinate fasciculus (Villarreal et al., 2004; Eluvathingal et al., 2006; Jackowski et al., 2008; Sexton et al., 2009; Cullen et al., 2010; Kieseppa et al., 2010; Schuff et al., 2011).

In the present study we examined white matter integrity by measuring fractional anisotropy (FA) in patients with long-term remission of Cushing's disease and matched healthy controls. FA reflects the degree of diffusion directionality and is a sensitive marker of the tissue microstructural organization (Hasan et al., 2004; Alexander et al., 2007). Lower FA values are associated with decreased white matter integrity. However, as FA is a non-specific marker for white matter integrity, it gives no information about the structural properties underlying abnormalities in white matter tissue. In order to interpret the differences in FA, the mean diffusivity (MD) and the tensor regional eigenvalues were also assessed. Decreased diffusion along the principal direction of the fiber (axial diffusivity; AD) indicates axonal loss (Budde et al., 2009), while increased diffusion perpendicular to the principal direction of the fiber (Radial Diffusivity; RD) indicates demyelination (Song et al., 2002; Song et al., 2005; Alexander et al., 2007). In addition, an increase in overall water diffusion in all directions (MD) is also an indication for demyelination (Horsfield and Jones, 2002), but could be caused by the presence of edema as well (Alexander et al., 2007).

Taking into account the previously identified gray matter abnormalities and the findings of studies on stress-related disorders, we hypothesized lower FA values in patients with long-term remission of Cushing's disease in a region-of-interest (ROI) including the corpus callosum, the bilateral cingulate cingulum, the bilateral hippocampal cingulum, and the bilateral uncinate fasciculus. In addition, we hypothesized that white matter abnormalities correlate with psychological and cognitive functioning, as well as duration of remission, disease duration and clinical severity. Furthermore, we performed an explorative whole brain analysis to detect possible changes in FA values in white matter outside our a priori defined regions of interest. Finally, to assess the nature of the white matter abnormalities indicated by differences in FA, post-hoc analyses are performed on the AD, RD, and MD.

Methods

Subjects

All patients with remission of Cushing's disease of pituitary origin monitored at our institute (n = 49) and between 18 and 60 years of age, were invited by letter and those who did not respond were contacted by phone. The response rate was 96%, 31 patients were willing to participate and were screened for eligibility. 6 patients were excluded due to one of the following exclusion criteria: a (history of) drug- or alcohol abuse, neurological problems, contraindications for undergoing a magnetic resonance imaging (MRI) scan and left-handedness. Healthy controls were pair-wise matched to the patients on the variables gender, age, and education. They were recruited by advertisements in grocery stores and via the Internet. In addition to the general exclusion criteria of the study (a history of) psychiatric disorders was an exclusion criterion for the control group.

A total of 25 patients with remission of Cushing's disease and 25 matched healthy controls were included in this study. In the data analysis process, we decided to further exclude the data of 3 patients and their matched controls due to insufficient quality of the diffusion tensor imaging (DTI) data, resulting in a final sample of 22 patients and 22 matched controls.

The diagnosis of Cushing's disease had been confirmed in all patients, following previously described criteria (Tiemensma et al., 2010b). Some patients remained glucocorticoid dependent after surgery and were substituted with hydrocortisone (on average 20 mg/day, divided into three dosages). The estimated duration of disease was determined through patients' history by looking for the earliest physical/somatic signs. Duration of remission was calculated from the date of

curative transsphenoidal surgery, or in case of persistent disease, from the date of normalization of biochemical tests after postoperative radiotherapy. Demographics and patient characteristics are reported in Table 1. Written informed consent was obtained from all participants prior to the clinical assessment and the MRI-scan session. The medical ethical committee of the Leiden University Medical Center approved the study protocol.

Education

The education level was classified following the Dutch education system, which is comparable to the International Standard Classification of Education (ISCED). Low = primary education (elementary school) and lower secondary education (preparatory secondary education); intermediate = higher secondary education (higher general continued education, pre-university secondary education) and post-secondary education (intermediate vocational education); high= tertiary education (higher professional education, university).

Study procedure

We scheduled a single study visit that consisted of approximately two hours for MRI scanning (60 min), an interview for the evaluation of the clinical data and the assessment of psychological and cognitive functioning. After the scan session, participants were asked to complete several self-rating questionnaires at home for the assessment of psychopathology and cognitive functioning and to return them within a week.

Assessment of psychopathology and cognitive functioning

Presence and severity of depressive symptoms were evaluated using the Montgomery–Åsberg Depression Rating Scale (MADRS; (Montgomery and Asberg, 1979; Snaith et al., 1986), which was the only interviewer rated scale, and the Inventory of Depression Symptomatology (IDS; (Rush et al., 1996). Anxiety was evaluated using the Beck Anxiety Inventory (BAI; (Beck et al., 1988) and the Fear Questionnaire (FQ; (Marks and Mathews, 1979). Apathy and irritability were assessed using the Apathy Scale (AS) and the Irritability Scale (IS), respectively (Starkstein et al., 2001; Chatterjee et al., 2005). The Cognitive Failures Questionnaire (CFQ) was used to assess failures in perception, memory, and motor function (Broadbent et al., 1982).

Table 1. Demographics of the total sample and clinical characteristics of the patients with long-term remission of Cushing's disease

Characteristics	Patients with long-term remission of Cushing's disease (n = 22)	Matched Healthy Controls (n = 22)
Gender (male/female)	4 / 18	4 / 18
Age (years), mean (S.D.)	44.42 (7.33)	46.42 (7.30)
Education, n (%)		
Low	5 (22.7 %)	5 (22.7 %)
Intermediate	11 (50 %)	10 (45.5 %)
High	6 (27.3 %)	7 (31.8 %)
Surgery, n (%)		
Transsphenoidal adenomectomy	22 (100 %)	
Bilateral Adrenalectomy	2 (9.1 %)	
Radiotherapy, n (%)	5 (22.7 %)	
Disease duration (years), mean (S.D.)	6.73 (5.39)	
Duration of remission (years), mean (S.D.)	11.87 (8.49)	
Estimated age on onset (years), mean (S.D.)	25.81 (9.04)	
Hypopituitarism, n (%)		
Any axis	13 (59.1 %)	
GH	9 (40.9 %)	
LH/FSH	8 (36.4 %)	
TSH	9 (40.9 %)	
ADH	2 (9.1 %)	
Hydrocortisone substitution	12 (54.5 %)	
Clinical severity index (CSI), mean (S.D.)		
Active phase, total	8.05 (1.96)	
Remission phase, total	2.59 (1.50)	

Cushing's syndrome Severity Index (CSI)

To assess clinical severity, the Cushing's syndrome Severity Index (CSI; (Sonino et al., 2000) was used for current severity of symptoms and to retrospectively estimate (clinical) severity at the time of active disease. The CSI contains eight clinical features and can be scored on a 3-point scale, ranging from 0 to 2. A higher total score on the CSI indicates greater severity, with a range of 0–16. The information necessary for completing this index was derived from clinical history and medical files. Two raters, who reached consensus on each feature in case of discrepancy, scored the CSI. For the active phase, the CSI was scored retrospectively. The current score was evaluated based on the last yearly evaluation. The total score of the active phase and the total score of the remission phase were used in the analyses.

Data acquisition and preprocessing

DTI data were collected using a Philips 3.0 T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) with a 32-channel SENSE (sensitivity encoding) head coil. A single-shot echo-planar imaging sequence was used with the following scan parameters: repetition time = 6250 ms, echo time = 69 ms, flip angle = 90°, b-factor = 1000 s/mm2, voxel dimensions = 2 mm isotropic, number of slices = 60, and no slice gap. DTI data were acquired along 32 directions, together with a baseline image having no diffusion weighting (b = 0). Total DTI scanning time was ~ 8 min. Collected DTI data were preprocessed and analyzed using the Oxford Centre for Functional MRI of the Brain (FMRIB) software library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) version 5.0.2. First, DTI data were corrected for distortion and motion artifacts induced by eddy currents or by simple head motions, using affine registration of each diffusion weighted image to the b = 0 reference image. Next, non-brain tissue was removed using the Brain Extraction Tool. Following, in order to generate individual FA maps for each participant, the diffusion tensor model was fitted to each voxel using FMRIB's diffusion toolbox.

Whole brain TBSS

Tractal based spatial statistics (TBSS) version 1.2 was used for voxelwise analysis of the preprocessed FA data. First, individual FA images were aligned to the FMRIB58_FA standard-space image using nonlinear registration. Next, the mean FA image was generated and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the entire group. The mean FA skeleton was then thresholded at a FA value of ≥ 0.4 to exclude peripheral tracts and minimize partial voluming. Finally, each participant's aligned FA images were projected onto the mean FA skeleton, and the resulting data were fed into voxelwise permutation-based analysis.

Region of interest TBSS

To test for regional specific FA alterations, we first implemented a ROI-based TBSS. A binary mask encompassing the bilateral cingulate cingulum, the bilateral hippocampal cingulum, the corpus callosum, and the bilateral uncinate fasciculus was created as a ROI using the Johns Hopkins University (JHU) White Matter Atlas provided by FSL (Mori, 2005). The mask was then applied to the mean FA skeleton in order to include only voxels comprised in the mean FA skeleton. This confines the statistical analysis exclusively to voxels from the center of the tract, thereby minimizing anatomic inter-subject variability, registration errors, and partial voluming. The resulting study-specific ROI mask, consisting of 12,357 voxels, was used for voxelwise permutation-based ROI analysis.

Statistical analysis

Using FSL's Randomise Tool, permutation-based inferences with threshold-free cluster enhancement (TFCE) were carried out for voxelwise analysis of FA data (Smith and Nichols, 2009). In both the ROI analysis and the whole brain analysis 5000 random permutations were generated to build up the null distribution of the cluster size statistic, while testing the following contrasts: 1) controls < patients and 2) controls > patients. Age, gender and education (demeaned across groups) were included in the analysis as nuisance regressors to correct for between group variances. The resulting statistical maps were corrected for multiple comparisons ($p < 0.05$, TFCE corrected), and the JHU (John Hopkins University) White Matter and Juelich Histological atlases were used to label clusters with significant FA alterations.

Post-hoc analyses

In the patient group, the association between FA abnormalities and psychological and clinical characteristics was examined using a voxel-wise correlation approach. Clinical characteristics (disease duration in years, duration of remission in years, and CSI scores), and scores on psychological measures found to be significantly different between the patients and the controls (MADRS and AS scores), were fed into FSL's Randomise Tool along with the FA values of the voxels within regions of significant group differences resulting from the ROI analysis.

A mask was created of the voxels that were found to differ significantly between groups on FA resulting from the exploratory whole brain analysis. Along with this mask, information on each individuals' AD (the 1st eigenvalue), RD (the average of the 2nd and 3rd eigenvalues), and MD was fed into FSL's Randomise Tool using permutation-based inferences with TFCE.

Results

Psychometric data

At the day of the MRI scan, patients were asked about a lifetime history of psychiatric disorders. None of the patients reported any. Because of the subjective nature of this information, we also inspected their medical records and found no indications for the presence of psychiatric disorders prior to the onset of Cushing's disease.

The psychometric data are reported in Table 2. To correct for multiple comparisons we applied a Bonferroni correction and adjusted the level of significance to $p < .005$. Comparisons on the psychometric data between patients with long-term

remission of Cushing's disease and the matched healthy controls showed significant differences on the MADRS (p <.001) and on the AS (p=.003). No further significant differences were found between patients and controls. Within the patient group we found no significant correlation between age and age of onset (p=.510), and age and disease duration (p=.057).

Table 2. Symptom severity scores patients with long-term remission of Cushing's disease versus matched healthy controls

	Patients with long-term remission of Cushing's disease (n = 22)	**Matched healthy controls** (n= 22)	*P* value
Montgomery–Åsberg Depression Rating Scale (MADRS), mean (S.D.)	6.09 (5.71)	1.45 (1.84)	**.000**[a]
Inventory depression scale (IDS), mean (S.D.)	45.67 (12.99)	36.14 (6.10)	.020[a]
Beck Anxiety inventory (BAI), mean (S.D)	27.95 (5.65)	24.18 (3.20)	.020[a]
Fear Questionnaire (FQ), mean (S.D.)	23.76 (16.60)	14.36 (10.04)	.033[b]
Agoraphobia subscale, mean (S.D.)	4.90 (6.26)	2.82 (3.43)	.495[a]
Blood injury phobia subscale, mean (S.D.)	6.71 (8.75)	3.55 (4.28)	.334[a]
Social phobia subscale, mean (S.D.)	12.14 (7.88)	8.00 (4.97)	.048[b]
Irritability scale (IS), mean (S.D.)	11.76 (9.17)	8.73 (6.22)	.342[a]
Apathy scale (AS), mean (S.D.)	13.62 (6.70)	8.23 (3.77)	**.003**[b]
Cognitive failure questionnaire, mean (S.D.)	35.19 (14.57)	29.36 (8.68)	.123[b]
Motion parameters in millimeters			
Absolute displacement, mean (S.D.)	1.67 (.367)	1.54 (.345)	.078[a]
Relative displacement, mean (S.D.)	.640 (.082)	.597 (.111)	.146[b]

[a] = Mann-Whitney U test;[b] = Independent sample t-test; S.D. = Standard deviation. Due to multiple comparisons, level of significance was adjusted to $p < .005$ using Bonferroni correction.

TBSS analysis

Motion parameters (Table 2) did not differ significantly between groups for both absolute displacement (p=.078) and relative displacement (p=.146). The TBSS ROI analysis showed significant reductions (p <.05, TFCE corrected) of FA values in patients compared to controls in 8394 voxels of the 12,357 voxels that comprised the ROI study specific mask (67.9%). Regions that were found to have smaller FA values in patients are the corpus callosum, the bilateral cingulate cingulum, and the bilateral uncinate fasciculus (Fig. 1). No significant differences in FA values were observed in the bilateral hippocampal cingulum.

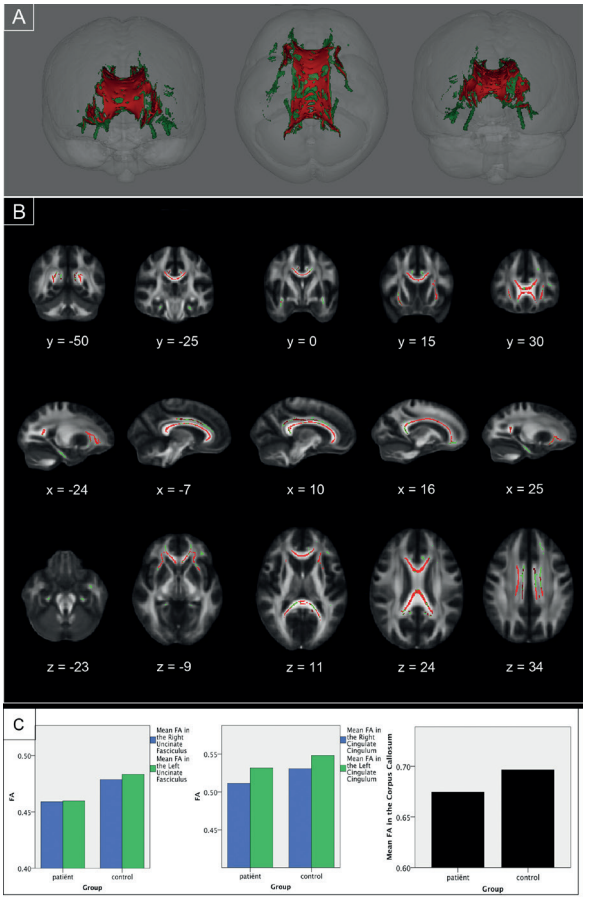
Using a voxel-wise correlation approach, we examined the association between the observed smaller FA values, and psychological measurements and clinical characteristics in the patient group. This was conducted within the patient group by correlating the FA values of the voxels that were observed in the between-group

difference with the scores on psychological measures. Because patients scored significantly higher on the MADRS and the AS, these were tested in addition to disease duration, duration of remission, and CSI scores (active phase and remission phase). To correct for multiple comparisons we applied a Bonferroni correction and adjusted the significance level to $p < .0083$. MADRS scores in the patient group correlated negatively with FA values in the left uncinate fasciculus (Fig. 2). We found no significant correlations in the patient group between FA values and AS scores, disease duration, duration of remission, and CSI scores.

The exploratory whole brain analysis showed multiple reductions of FA values ($p < .05$, TFCE corrected) in various white matter tracts throughout the brain in patients compared to controls (Fig. 3). 31,343 voxels of the 56,976 voxels that comprised the study-specific whole brain mask were found to have significantly smaller FA values in patients (55%). Lowering the threshold ($p < .1$, TFCE corrected) resulted in the same pattern of reductions although slightly expanded. Tracts that were not found to differ significantly include: the inferior parts of the brainstem, the white matter in the bilateral cerebellum, the bilateral hippocampal cingulum, the left inferior fronto-occipital fasciculus, and parts of the bilateral superior longitudinal fasciculus.

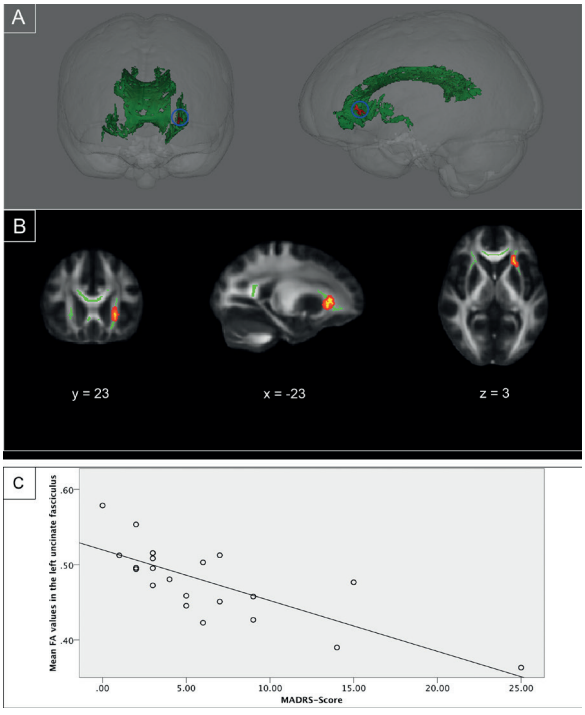
Post-hoc analyses of the AD, RD, and MD in the voxels that showed decreased FA between groups revealed a significant increase ($p < .05$, TFCE corrected) of RD and MD in the patient group compared to controls. No significant differences were found between groups in AD.

Figure 1. Region-of-interest analysis results



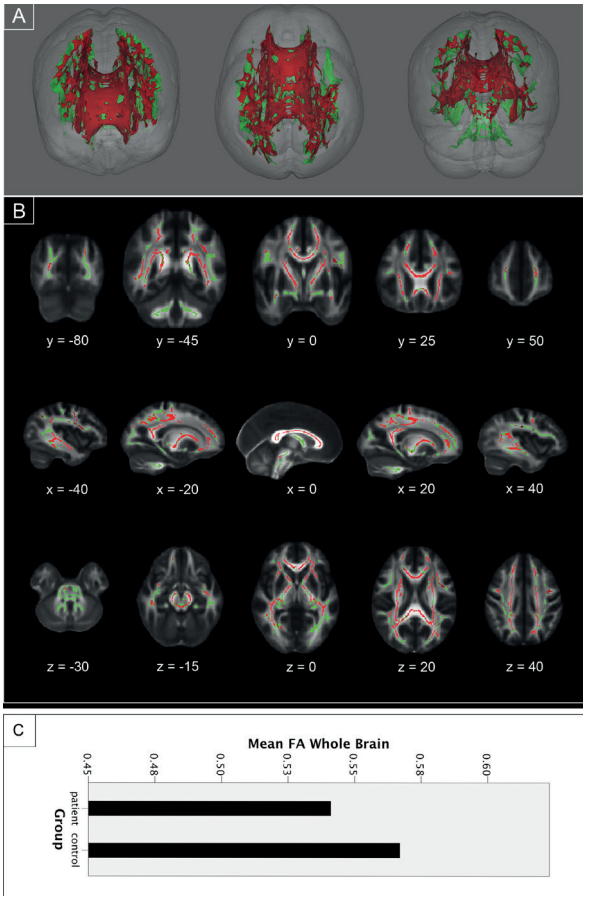
Region-of-interest analysis results. (A) Three-dimensional renderings showing (from left to right) anterior, top, and posterior views of the white matter skeleton (green) of the bilateral cingulate cingulum, the bilateral hippocampal cingulum, the corpus callosum, and the bilateral uncinate fasciculus. Superimposed in red are the regions in which FA values are significantly smaller in patients with long-term remission of Cushing's disease compared to matched healthy controls. (B) Coronal, sagittal and transversal axial sections of the white matter skeleton (green; 12,357 voxels) superimposed on the FMRIB58_FA_1mm standard brain (gray). Depicted in red are the regions in which FA values are significantly smaller in patients with long-term remission of Cushing's disease compared to matched healthy controls (8394 voxels). All TBSS results are corrected for multiple comparisons ($p < 0.05$, TFCE corrected), and the axial images are in radiological convention (the right side of the image corresponds with the left hemisphere of the brain and vice-versa). (C) Plots depicting the mean FA value per group in the significant ROIs. From left to right, the first plot shows FA values in the right uncinate fasciculus (blue) and left uncinate fasciculus (green). The second plot shows FA values in the right cingulate cingulum (blue) and left cingulate cingulum (green). The third plot shows the mean FA values in the corpus callosum.

Figure 2. Voxel-wise correlation between MADRS scores and FA values in patients with long-term remission of Cushing's disease



(A) Three-dimensional renderings showing (from left to right) anterior and left side views of the white matter skeleton (green) of areas that differed between groups in the ROI analysis. FA values in the left uncinate fasciculus correlated negatively with MADRS scores ($p < .0083$) in the patients with long-term remission of Cushing's disease (red). (B) Coronal, sagittal and transversal axial sections of the white matter skeleton (green) superimposed on the FMRIB58_FA_1mm standard brain (gray). FA values in the left uncinate fasciculus correlated negatively with MADRS scores ($p < .0083$) in the patients with long-term remission of Cushing's disease (yellow). For better visibility, the results are thickened using the "tbss-fill" command (red). The axial images are in radiological convention (the right side of the image corresponds with the left hemisphere of the brain and vice-versa). (C) A scatterplot showing the negative correlation between the mean FA in the left uncinate fasciculus and the scores on the MADRS in the patient group.

Figure 3. Exploratory whole brain analysis results



Exploratory whole brain analysis results. Whole brain TBSS results. (A) Three-dimensional renderings showing (from left to right) anterior, top, and posterior views of the white matter skeleton (green). Superimposed in red are the regions in which FA values are significantly smaller in patients with long-term remission of Cushing's disease compared to matched healthy controls. (B) Coronal, sagittal and transversal axial sections of the white matter skeleton (green; 56,976 voxels) superimposed on the FMRIB58_FA_1mm standard brain (gray). Depicted in red are the regions in which FA values are significantly smaller in patients with long-term remission of Cushing's disease compared to matched healthy controls (31,343 voxels). All TBSS results are corrected for multiple comparisons ($p < 0.05$, TFCE corrected), and the axial images are in radiological convention (the right side of the image corresponds with the left hemisphere of the brain and vice-versa). (C) A plot of the mean FA value per group in the significant effect found in the whole brain analysis.

Discussion

Using a ROI approach, we found reduced white matter integrity in patients compared to controls in the corpus callosum, the bilateral cingulate cingulum, and the bilateral uncinate fasciculus, but not in the bilateral hippocampal cingulum. The explorative whole brain analysis showed widespread reductions of white matter integrity throughout the brain. In the examined regions, we observed increased RD and MD, as well as no differences in AD. Furthermore, we found that FA values in the left uncinate fasciculus correlated with the severity of depressive symptoms in the patients.

Our ROI analysis showed decreased FA in most of the hypothesized regions in patients. However, the exploratory whole brain analysis showed reductions of FA values in nearly all white matter tracts throughout the brain. This leads to the idea that a general, white matter-affecting mechanism underlies the observed differences between patients and controls. Due to the cross-sectional design of this study no causal conclusions can be drawn about the relation between FA reductions and the reported psychopathology (i.e. depressive symptoms, apathy). However, since all patients had a history of hypercortisolism, we suggest that prolonged exposure to high levels of cortisol has directly or indirectly affected the white matter tissue in patients with Cushing's disease. Data from animal studies seem to support this suggestion. White matter mRNA expression (likely reflecting oligodendrocytes responses) responds strongly and acutely to corticosterone treatment (van Gemert et al., 2006). Prolonged exposure to corticosteroids was associated with the inhibition of proliferation of oligodendrocyte precursors throughout the white matter (Alonso, 2000). A more direct link with the lower FA values that we observed in patients, is the finding that corticosterone in mice can lead to an increased distance between nerve fibers in fiber tracts as a consequence of direct glucocorticoid receptor activation in oligodendrocytes leading to increased branching of these cells and perhaps lower levels of myelination. This is in line with our findings of increases in RD, which has been related to demyelination and dysmyelination (Song et al., 2005; Alexander et al., 2007). The increased MD we found in the patient group is also an indication for decreased demyelination (Horsfield and Jones, 2002), but could also indicate the presence of edema (Alexander et al., 2007).

In humans, direct effects of exposure to extreme levels of cortisol on white matter integrity have never been studied. However, Johansson et al. (2012) found that

long-standing psychological distress in midlife increases the risk of white matter lesions (Johansson et al., 2012). Additionally, it has been found that a specific genotype (ER22/23EK) related to glucocorticoid resistance is associated with lower presence and progression of white matter lesions in dementia (van Rossum et al., 2008). These results and the findings from our study, support the hypothesis of prolonged elevated cortisol levels affecting white matter in the brain in a non-localized manner. Longitudinal research should elucidate the mechanisms of the lasting effects of extreme levels of cortisol on white matter integrity.

Our data may also point at a neural substrate for the persistence of affective symptomatology in patients with Cushing's disease after treatment for hypercortisolism (Tiemensma et al., 2010a). Using a voxel-wise correlation approach, we found an association between depressive symptom severity, as measured on the MADRS, and decreases in FA values in the left uncinate fasciculus in patients. Recent studies in patients with depressive disorder also found decreased FA values in the uncinate fasciculus (Taylor et al., 2007; Carballedo et al., 2012). The uncinate fasciculus passes from the rostral part of the temporal lobe (lateral to the amygdala) and the insula, through the orbital and medial frontal cortices (Kier et al., 2004; Schmahmann et al., 2008). This white matter bundle effectively connects the limbic system with the frontal regions of the brain and is an important connection in the emotion regulation and stress network (Ghashghaei and Barbas, 2002).

In a previous study from our group, it was demonstrated that patients with long-term remission of Cushing's disease demonstrate subtle cognitive impairments (Tiemensma et al., 2010b). In the present study no differences were found using the CFQ. Although the CFQ is a validated and commonly used questionnaire, it is not equal to extensive neuropsychological testing that would provide a more accurate assessment of cognitive functioning. It might be that the CFQ was not sensitive enough to detect these subtle impairments. 12 patients remained hydrocortisone dependent after treatment for Cushing's disease. We did not find differences in FA values between patient with and without hydrocortisone substitution (data not shown), suggesting that our results were not driven by hydrocortisone dependence. Due to the gradual onset of the a-specific symptoms of Cushing's disease, and the high prevalence of psychiatric symptomatology in Cushing's disease it is difficult to make a clear distinction between Cushing's related psychiatric symptoms and independently developed psychiatric symptoms. Therefore, it is possible that psychiatric symptomatology in the patients developed independently or prior to the onset of Cushing's disease.

The data presented in this study provide a further perspective towards a detailed phenotyping of patients after the treatment of Cushing's disease, who often report persisting psychological symptomatology and cognitive impairment. It is tempting to speculate that our findings, to a certain extent, could also apply to patients with chronic or recurrent forms of highly prevalent stress-related disorders such as depression, and, in addition, to patients treated with exogenous corticosteroids that are commonly prescribed to suppress the immune system (Brown and Suppes, 1998).

This study is the first to demonstrate that white matter integrity is affected in patients treated for Cushing's disease, despite long-term remission of cortisol excess. More research is needed to elucidate the role of cortisol in affecting white matter tissue integrity and the cellular mechanisms that underlie this process. Furthermore, it should be clarified as to how abnormalities in structural connectivity interact with potential differences in functional connectivity and how this interaction relates to the psychological symptomatology observed in patients with remission of Cushing's disease.

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Chapter 5

Resting-State functional connectivity in patients with long-term remission of Cushing's disease

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Abstract

Background

Glucocorticoid disturbance can be a cause of psychiatric symptoms. Cushing's disease represents a unique model for examining the effects of prolonged exposure to high levels of endogenous cortisol on the human brain as well as for examining the relation between these effects and psychiatric symptomatology. This study aimed to investigate resting-state functional connectivity (RSFC) of the limbic network, the default mode network (DMN), and the executive control network in patients with long-term remission of Cushing's disease.

Methods

RSFC of these three networks of interest was compared between patients in remission of Cushing's disease (n = 24; 4 male, mean age = 44.96 years) and matched healthy controls (n = 24; 4 male, mean age = 46.5 years), using probabilistic independent component analysis to extract the networks and a dual regression method to compare both groups. Psychological and cognitive functioning was assessed with validated questionnaires and interviews.

Results

In comparison with controls, patients with remission of Cushing's disease showed an increased RSFC between the limbic network and the subgenual subregion of the anterior cingulate cortex (ACC) as well as an increased RSFC of the DMN in the left lateral occipital cortex. However, these findings were not associated with psychiatric symptoms in the patient group.

Conclusions

Our data indicate that previous exposure to hypercortisolism is related to persisting changes in brain function.

Introduction

In various stress-related psychiatric disorders such as major depressive disorder and posttraumatic stress disorder (PTSD), alterations in the activity of the hypothalamic–pituitary–adrenal axis (HPA axis) are present, with hyperre¬sponsivity or chronic activation of the HPA axis often resulting in increased levels of cortisol (Carroll et al., 1976; Schlechte et al., 1986; Young and Breslau, 2004; Inslicht et al., 2006; Carroll et al., 2007; Friedman et al., 2007; Steudte et al., 2011). Animals chronically exposed to increased levels of cortico¬sterone display an anxiodepressive-like phenotype, providing a neuroendocrine animal model for stress-related disorders (David et al., 2009; Darcet et al., 2014). However, extrapola¬tion of findings of this animal model to humans may not always be appropriate, for example when studying human brain activation patterns during specific challenges or resting state.

Cushing's disease represents a unique human model for examining the effects of prolonged exposure to increased levels of endogenous cortisol on brain structure and func¬tion, and the relation between these effects and psychiatric symptomatology. In Cushing's disease, hypercortisolism is caused by an adrenocorticotropic hormone (ACTH) produc¬ing pituitary adenoma, stimulating the adrenal glands to continuously release cortisol. In patients with Cushing's disease, a variety of psychiatric symptoms can be induced by hypercortisolism. Major depressive disorder is the most common psychiatric disorder seen in Cushing's disease patients, but comorbidity also includes anxiety, mania, and cognitive dysfunction (Sonino and Fava, 2001). The majority of these symptoms are also seen in stress-related psychiatric disorders, suggesting a common underlying mechanism. After successful biochemical treatment of Cushing's disease, psychiatric symptoms decrease, but patients still show cogni¬tive impairment (Dorn and Cerrone, 2000; Forget et al., 2002; Merke et al., 2005; Tiemensma et al., 2010b; Resmini et al., 2012), decreased quality of life (Tiemensma et al., 2011), and a higher prevalence of affective disorders and apathy (Tiemensma et al., 2010a) compared with healthy controls. These findings suggest that hypercortisolism may cause per-sisting changes in the brain.

Effects of hypercortisolism on brain structure include a reduction of hippocampal volume (Starkman et al., 1992; Simmons et al., 2000; Bourdeau et al., 2002) that is reversible after successful abrogation of the hypercortisolism (Starkman et al., 1999; Bourdeau et al., 2002). In addition, our group found reduced anterior cingulate cortex (ACC) gray matter volumes and increased gray matter volumes in the cerebellum in patients with remission of Cushing's disease (Andela et al., 2013),

as well as widespread reductions of white matter integrity (van der Werff et al., 2014). Remarkably, similar patterns of reduced hippocampal volume (Gurvits et al., 1996; Bremner et al., 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004; Kitayama et al., 2005; Colla et al., 2007), reversibility of hippocampal atrophy after treatment (Vermetten et al., 2003; Nordanskog et al., 2010; Boldrini et al., 2013; Tendolkar et al., 2013), and reductions of ACC volumes (Yamasue et al., 2003; Kasai et al., 2008; Thomaes et al., 2010) have been found in stress-related psychiatric disorders.

The effects of hypercortisolism on brain functional con¬nectivity have yet to be investigated. One important network that should be investigated in light of hypercortisolism is the limbic network, consisting of the hypothalamus, hippocam¬pus, amygdala, insula, and parts of the nucleus accumbens (Maclean, 1952; Janes et al., 2012). This network is involved in emotional processing and regulation, as well as the encoding of memories. In addition, the components of this network are responsible for the initial stress response and activation of the HPA axis, with subsequent release of corti¬cotropin-releasing hormone (CRH), followed by ACTH that, in turn, stimulates the adrenal glands to secrete cortisol (Shin and Liberzon, 2010). Exposure to severe stress has been related to altered functional connectivity of various compo¬nents of the limbic network (Admon et al., 2009; Jin et al., 2013; Brown et al., 2014). The network has consistently been found in other resting-state analysis using an ICA approach (Veer et al., 2010; Laird et al., 2011; Janes et al., 2012). However, naming of the network has been less consistent, with studies using the terms limbic network (Janes et al., 2012), medial temporal network (Veer et al., 2010; Laird et al., 2011), and amygdala–hippocampus complex (Veer et al., 2010) to indicate the same network. Another network of interest is the default mode network (DMN). This network consists of the precuneus, the posterior cingulate cortex, the medial prefrontal cortex, and parts of the parietal cortex, and is involved in the retrieval and manipulation of episodic memories and semantic knowledge, self-referential proces¬sing, and prospective memory (Raichle et al., 2001; Fox et al., 2005). Alterations in DMN connectivity have been found in stress-related disorders (Bluhm et al., 2009a; Bluhm et al., 2009b; Zhu et al., 2012). A third network of interest is the executive control network, consisting of the bilateral dorsolateral prefrontal cortex (PFC), ventrolateral PFC, dorsomedial PFC, and lateral parietal cortices (Beckmann et al., 2005; Seeley et al., 2007). This network is involved in attention demands, working memory, and cognitive control, and is relevant because of the cognitive impairment in patients with long-term remission of Cushing's disease.

The aim of this study was to examine alterations in resting-state functional connectivity (RSFC) in patients with long-term remitted Cushing's disease. We anticipated to find aberrant RSFC with the limbic network, the DMN, and the executive control network in these patients compared with matched healthy controls.

Methods

Sample Description

All patients with long-term remission of Cushing's disease monitored in a unique cohort at the Leiden University Medical Center (n = 49) and between 18 and 60 years of age, were invited by letter and those who did not respond were contacted by phone. The response rate was 96%. Thirty-one patients were willing to participate and were screened for eligibility. Six patients were excluded because of one of the following exclusion criteria: a (history of) drug or alcohol abuse, neurological problems, contraindications for undergoing a magnetic resonance imaging (MRI) scan and left-handedness. Healthy controls were pair-wise matched to the patients based on gender, age, and education and recruited by advertisements in grocery stores and via Internet. In addition to the general exclusion criteria of the study, a history or presence of a psychiatric disorder was an exclusion criterion for the control group. At the day of the MRI scan, patients were asked again about a lifetime history of psychiatric disorders. One patient reported a history of major depressive disorder before the suspected onset of the Cushing's disease.

A total of 25 patients with remission of Cushing's disease and 25 matched healthy controls were included in this study. The MRI scanning session of one patient was aborted prema¬turely before the resting-state scan was acquired. Therefore, we excluded data from this patient and the matched healthy control from the current study, resulting in a total sample size of 24 patients and 24 controls. The diagnosis of active Cushing's disease had been confirmed in all patients, follow¬ing previously described criteria (Tiemensma et al., 2010b). All patients were treated with transsphenoidal surgery. Thirteen patients remained glucocorticoid dependent after surgery and were substituted with hydrocortisone (on average 20 mg/day, divided into three dosages). Remission of the Cushing's disease was confirmed in the 11 other patients. The estimated duration of disease was determined through patients' history by looking for the earliest physical/somatic signs. Duration of remission was calculated from the date of curative transsphenoidal surgery, or in case of persistent disease, from the date of normalization of biochemical tests after

postoperative radiotherapy. Demographics and patient characteristics are reported in Table 1. Written informed consent was obtained from all participants before the clinical assessment and the MRI scan session. The medical ethical committee of the Leiden University Medical Center approved the study protocol.

Table 1. Demographics of the total sample and clinical characteristics of the patients with long-term remission of Cushing's disease

Characteristics	**Patients with long-term remission of Cushing's disease** (n = 24)	**Matched Healthy Controls** (n = 24)
Gender (male/female)	4 / 20	4 / 20
Age (years), mean (S.D.)	44.96 (7.52)	46.5 (7.06)
Education, n (%)		
Low	6 (25 %)	6 (25 %)
Intermediate	11 (45.8 %)	10 (41.7 %)
High	7 (29.2)	8 (33.3 %)
Surgery, n (%)		
Transsphenoidal adenomectomy	24 (100 %)	
Bilateral Adrenalectomy	2 (8.3 %)	
Radiotherapy, n (%)	6 (25 %)	
Disease duration (years), mean (S.D.)	8.30 (8.12)	
Duration of remission (years), mean (S.D.)	10.92 (8.36)	
Hypopituitarism, n (%)		
Any axis	14 (58.3 %)	
GH	10 (41.7 %)	
LH/FSH	9 (37.5 %)	
TSH	10 (41.7 %)	
ADH	3 (12.5 %)	
Hydrocortisone substitution	13 (54.2 %)	
Clinical severity index (CSI), mean (S.D.)		
Active phase, total	8.08 (2.02)	
Remission phase, total	2.50 (1.53)	

S.D. = Standard deviation.

Clinical Data Acquisition

Presence and severity of depressive symptoms were evalu¬ated using the Montgomery–Åsberg depression rating scale (MADRS; (Montgomery and Asberg, 1979) and the Inventory of Depression Symptomatology (IDS; (Rush et al., 1996). Anxiety was evaluated using the Beck Anxiety Inventory (BAI; (Beck et al., 1988) and the Fear Questionnaire (FQ; (Marks and Mathews, 1979). Apathy and irritability were

assessed using the Apathy Scale (AS; (Starkstein et al., 2001) and the Irritability Scale (IS; (Chatterjee et al., 2005), respec¬tively. The Cognitive Failures Questionnaire (CFQ; (Broadbent et al., 1982) was used to assess cognitive functioning. The Cushing's syndrome Severity Index (CSI; (Sonino et al., 2000) was used to assess current severity of symptoms and to retrospectively estimate (clinical) severity at the time of active disease.

The MADRS was assessed before scanning on the scanning day. For all other questionnaires, the time between the scan and filling out the questionnaires was on average 1.4 ± 3.2 days (range 0–19). This broad range was mainly caused by one outlier of 19 days, whereas all other participants filled out questionnaires within 1 week. Moreover, 83% of the participants filled out questionnaires within 1 day after scanning.

MRI Data Acquisition

Imaging data were acquired on a Philips 3T magnetic resonance imaging system (software version 3.2.1; Philips Healthcare, Best, The Netherlands). A SENSE-32 channel head coil was used for radio frequency transmission and reception. Beforehand, the participants were instructed to lie as still as possible, with their eyes closed and without falling asleep. After completion of the scan, all participants confirmed not having fallen asleep. Resting-state fMRI (RS-¬fMRI) data were acquired using T2*-weighted gradient-echo echo-planar imaging with the following scan parameters: 200 whole-brain volumes, repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, flip angle = 80°, 38 axial slices, matrix size = 80 • 80, voxel size = 2.75 • 2.75 • 2.75 mm, slice gap = 0.275, scan duration = 449 s.

A high-resolution anatomical image (T1-weighted se¬quence; TR = 9.8 ms, TE = 4.6 ms, 140 axial slices, matrix size = 256 • 256, voxel size 1.17 • 1.17 • 1.2 mm, no slice gap, scan duration = 296 s), and a high-resolution T2*-weighted gradient echo EPI scan (TR = 2200 ms, TE = 30 ms, flip angle = 80°, 84 axial slices, matrix size = 112 • 112, voxel size = 1.96 • 1.96 • 2 mm, no slice gap, scan duration = 46.2 s) were acquired for registration to standard space.

A neuroradiologist, blinded to the status of the subjects, examined all anatomical images. Apart from incidental age-related white matter hyperintensities and minor effects of the posttranssphenoidal surgery in the perisellar area, no other macroscopic abnormalities were observed in the patients and controls.

Data Preprocessing

All MRI data were processed using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; version 5.0.6; (Smith et al., 2004). Non-brain tissue removal was applied to the high-resolution T1-weighted image and the high-resolution EPI image. The preprocessing of the RS-fMRI was carried out using FMRI Expert Analysis Tool (FEAT). Motion correction was applied to the RS-fMRI data along with non-brain tissue removal, spatial smoothing using a 6-mm full-width at half-maximum (FWHM) Gaussian kernel, grand-mean intensity normalization of the entire 4D data set by a single multi¬plicative factor and high-pass temporal filtering (Gaussian ¬weighted least-squares straight line fitting, 0.01 Hz cutoff).

The RS-fMRI data of each participant were then registered to their respective high-resolution EPI images. The high-resolution EPI image was registered to the T1-weighted image, and the T1-weighted image to MNI-152 standard space image (T1-weighted standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada), with a resampled resolution of 4 mm.

Extracting Resting-State Networks

Analysis was carried out using Probabilistic Independent Component Analysis (PICA; (Beckmann and Smith, 2004) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components). We opted to use this method over a seed-based approach, because our hypotheses were aimed at investigating connectivity with networks rather than with more localized areas. In addition, PICA gives us the advantage to distinguish physiological signals from noise, as they are separated in different com¬ponents. This technique also enables us to omit global signal regression that is known to induce negative connectivity between networks (Murphy et al., 2009). Default group PICA processing steps were applied to the individual preprocessed and normalized data sets: masking of non-brain voxels, voxel-wise de-meaning of the data, and normalization of the voxel-wise variance based on all data sets. Subsequently, the preprocessed data were concatenated in time to create a single 4D data set that was then projected into a 20-dimen¬sional subspace using principal component analysis. The observations were decomposed into 20 sets of independent vectors that describe signal variation across the temporal (time courses) and spatial (maps) domains by optimizing for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen, 1999). We chose to use 20 independent components to reach the same balance between the amount of clustering and splitting as previous studies applying the same techniques (Smith et al., 2009). The resulting estimated component maps were divided by the

SD of the residual noise and thresholded at a probability threshold of p>0.5 (ie, an equal weight is placed on false positives and false negatives) by fitting a mixture model to the histogram of intensity values.

Statistical Analyses

The set of spatial maps from the group-average analysis was used to generate subject-specific versions of the spatial maps, and associated time courses, using dual regression (Filippini et al., 2009). In short, for each subject, the group-average set of spatial maps was regressed as spatial regressors in a multiple regression into the preprocessed individual 4D resampled data set. This resulted in a subject-specific time course for each component separately. Next, those time courses were regressed as temporal regressors in a multiple regression into the same preprocessed individual 4D data set, resulting in a set of subject-specific spatial maps, one for each of the 20 components. In addition, subject-specific z-maps for each of the components were constructed through nor¬malization of the subject-specific spatial maps by the residual within-subject noise.

Upon visual inspection, we selected three components based on spatial similarity to functional networks described before: the limbic network (Shin and Liberzon, 2010), the DMN (Raichle et al., 2001; Beckmann and Smith, 2004; Fox et al., 2005), and the executive control network (Seeley et al., 2007). We segmented the 1 mm MNI-152 standard brain into gray matter, white matter, and cerebrospinal fluid (CSF) and created a mask comprising gray and white matter only. This mask was then registered to an isotropic resolution of 4 mm and applied to the study-specific mask in order to exclude voxels containing CSF from our analyses.

The subject-specific spatial maps of each of these three components along with the study-specific gray and white matter mask were fed into FSL's Randomise tool. The groups were compared using a general linear model (GLM) including age and level of education as confound regressors. In addition, for each subject, gray matter density maps were derived from the anatomical scans using FSL. Previously, Cushing's disease-related gray matter abnormalities were reported in the same sample (Andela et al., 2013). Therefore, to control for structural abnormalities possibly confounding differences in functional connectivity and to correct for the effects of possible misregistration (Oakes et al., 2007), information about gray matter values of each subject was included as a voxel-wise confound regressor in the GLM. Between-and within-group effects were tested using per-mutation-based (5000 permutations) nonparametric testing. To control for family-wise error we

applied threshold-free cluster enhancement (TFCE; (Smith and Nichols, 2009), and statistical maps were thresholded at p<0.05.

Results

Psychopathology Scores

The psychometric data are reported in Table 2. To correct for multiple comparisons we applied a Bonferroni correction and adjusted the level of significance to p<0.005. Compar¬isons of the psychometric data between patients with long-term remission of Cushing's disease and the matched healthy controls showed significant differences on the MADRS (p<0.001) and AS (p = 0.001). The following psychometric data showed uncorrected differences between groups: IDS (p = 0.008), BAI (p = 0.010), CFQ (p = 0.028), and FQ (p = 0.006). The only subscale of the FQ that showed an uncorrected difference between groups was the social phobia subscale (p = 0.012).

Table 2. Symptom severity scores of patients with long-term remission of Cushing's disease versus matched healthy controls

	Patients with long-term remission of Cushing's disease (n = 24)	**Matched healthy controls** (n = 24)	*P* value
Montgomery–Åsberg Depression Rating Scale (MADRS), mean (S.D.)	6.37 (5.59)	1.42 (1.79)	**<.001**[a **]
Inventory of Depression Symptomatology (IDS), mean (S.D.)	47.00 (13.23)	36.29 (5.85)	**.008**[a *]
Beck Anxiety Inventory (BAI), mean (S.D)	28.48 (5.79)	24.33 (3.16)	**.010**[a *]
Fear Questionnaire (FQ), mean (S.D.)	25.52 (17.00)	13.92 (9.72)	**.006**[b *]
Agoraphobia subscale, mean (S.D.)	6.35 (7.98)	2.67 (3.33)	.209[a]
Blood injury phobia subscale, mean (S.D.)	6.48 (8.40)	3.46 (4.13)	.270[a]
Social phobia subscale, mean (S.D.)	12.70 (7.77)	7.79 (4.82)	**.012**[b *]
Irritability scale (IS), mean (S.D.)	12.13 (8.91)	8.54 (6.02)	.176[a]
Apathy scale (AS), mean (S.D.)	13.91 (6.50)	8.13 (3.80)	**.001**[b **]
Cognitive failure questionnaire, mean (S.D.)	37.39 (16.55)	28.58 (9.10)	**.028**[b *]
Motion parameters in millimeters			
Absolute Displacement in mm, mean (S.D.)	0.29 (0.14)	0.35 (0.22)	.315[b]
Relative Displacement in mm, mean (S.D.)	0.12 (0.06)	0.12 (0.05)	.725[b]

[a] = Mann-Whitney U test; [b] = Independent sample t-test; S.D. = Standard deviation.
* = $p < .05$, uncorrected
** = P < .005, Bonferroni corrected

Resting-State Functional Connectivity

Motion parameters (Table 2) did not differ significantly between groups for both absolute displacement (t = − 1.017, p = 0.315) and relative displacement (t = 0.355, p = 0.725). The three networks of interest were identified among the 20 components resulting from the PICA (Figure 1). These three networks have been consistently

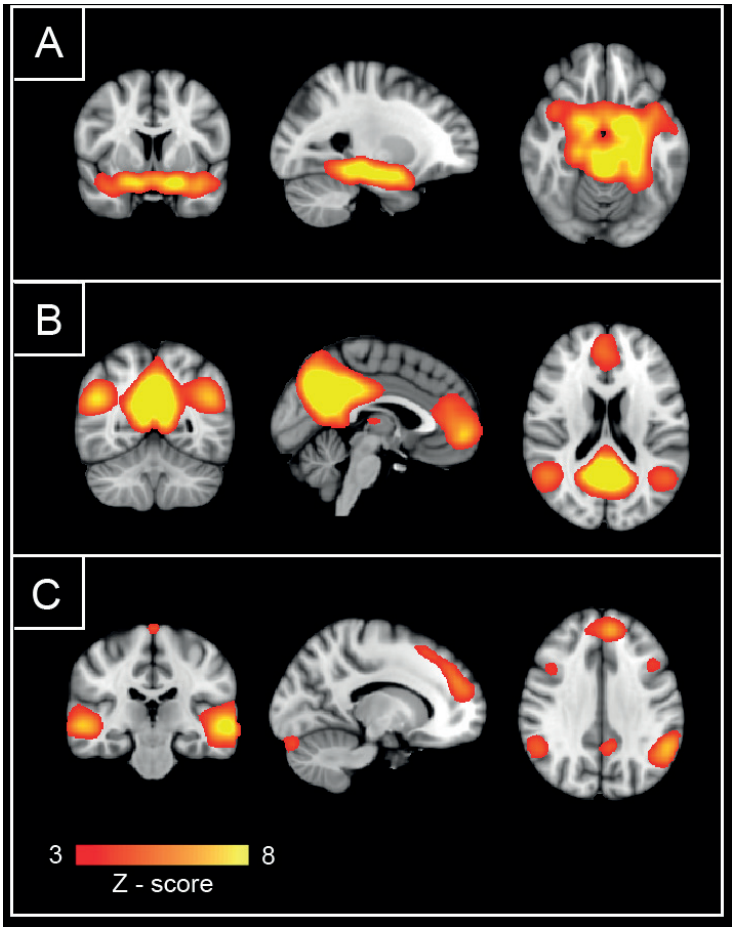
present across subjects and over time using PICA in previous studies (Beckmann et al., 2005; Veer et al., 2010). All three functional networks were present in both the patient group and healthy control group. Between-group differences in RSFC for each network were examined by comparing the following contrasts: (1) controls < patients and (2) controls > patients. These comparisons revealed a between-group difference in RSFC between the limbic network and the subgenual subregion of the ACC ($p<0.05$, TFCE corrected; Figure 2a). To determine whether this effect was driven by a decrease in negative connectivity or an increase in positive connectivity, mean individual z-scores for this region were extracted from the subject-specific z-maps of the limbic network component.

Comparison of the mean z-scores of both groups showed an increase in positive connectivity of the limbic network with the sub¬genual ACC in patients with Cushing's disease in remission compared with healthy controls (Figure 2b). To determine whether the strength of RSFC between the limbic network and the subgenual ACC was associated with psychopathol¬ogy scores of the patients with long-term remission of Cushing's disease, we performed linear regression analyses with adjustment for age and education. The psychopathology scores reported in Table 2, as well as the active and the remission subscales of the CSI, and the disease and remission duration in years did not show a significant association with the strength of the RSFC between the limbic network and the subgenual ACC in patients with long-term remission of Cushing's disease (for all: $p>0.1$). Furthermore, the found effect could not be related to treatment with hydrocortisone, as there was no significant difference in z-scores between the 13 patients who were treated and those who were not ($t = 0.396$; $p = 0.696$).

Analyses of between-group differences in RSFC with the DMN revealed aberrant connectivity with a small cluster located in the superior division of the left lateral occipital cortex ($p<0.05$, TFCE corrected; Figure 3a). Examination of the individual z-scores showed positive connectivity of this area with the DMN in the patients and negative connectivity in the healthy control group (Figure 3b). Linear regression analyses with adjustment for age and education did not show a significant association between individual z-scores and the symptom scores of the behavioral measurements as well as the subscales of the CSI and the disease and remission duration in the patients with long-term remission of Cushing's disease. In addition, there was no difference in z-scores between patients with and without hydrocortisone treatment ($t = 0.063$; $p = 0.950$). Contrasts testing the between-group differences in RSFC of the executive control network did not show any effects. Omitting the voxel-wise gray matter covariate from the statistical model did not

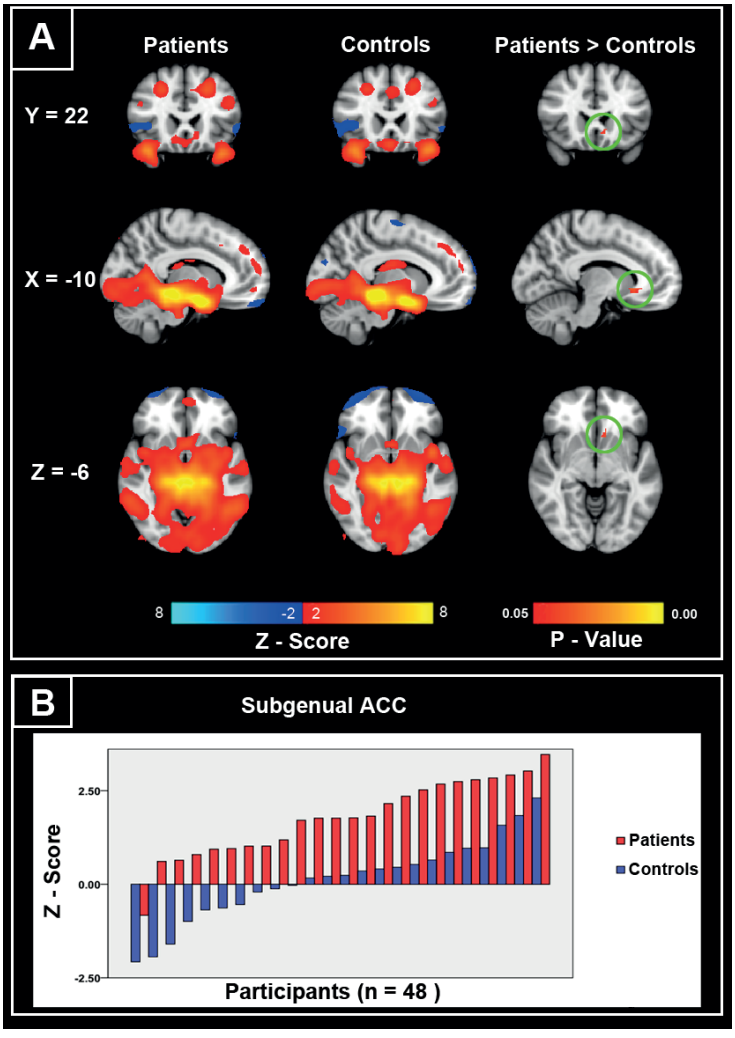
change the functional connectivity results as described in the previous section. This indicates that the altered RSFC within the three networks is unlikely to be related to macroscopic (ie, MRI observable) gray matter abnormalities.

Figure 1. Resting-state networks of interest



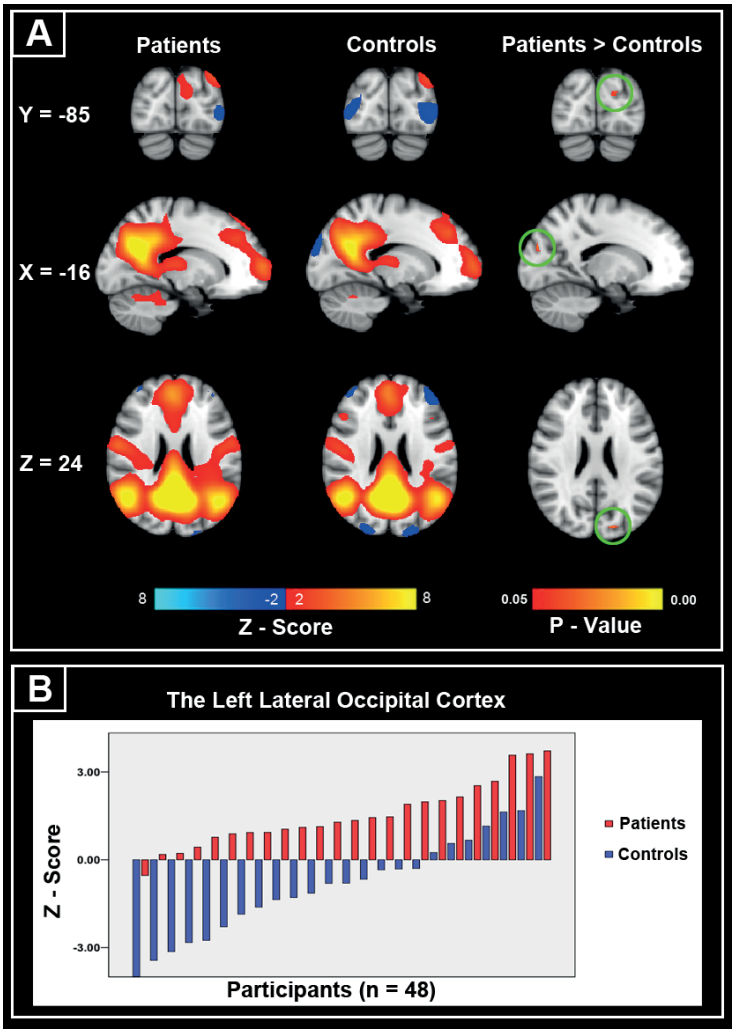
Resting-state networks of interest. (A) The limbic network. (B) The default mode network. (C) The executive control network. Images are z-statistics, overlaid on the MNI-152 1 mm standard brain. The left hemisphere of the brain corresponds to the right side of the coronal and transversal images.

Figure 2. Resting-state functional connectivity of the limbic network



Resting-state functional connectivity of the limbic network. (A) Depicted here are the within-group mean z-scores and the between-group effect for the limbic network. Images representing the statistics are overlaid on the MNI-152 1 mm standard brain. The between-group effect is displayed as p-statistics, TFCE corrected for family-wise error ($p<0.05$). The between-group effect shows increased RSFC between the limbic network and the subgenual ACC. (B) Distribution of the mean individual z-scores within the subgenual ACC. Depicted in red are the patients with long-term remission of Cushing's disease, depicted in blue are the matched healthy controls, and both are sorted from smallest to highest z-score.

Figure 3. Resting-state functional connectivity of the default mode network



Resting-state functional connectivity of the default mode network. (A) Depicted here are the within-group mean z-scores and between-group effect for the default mode network. Images representing the statistics are overlaid on the MNI-152 1 mm standard brain. The between-group effect is displayed as p-statistics, TFCE corrected for family-wise error ($p<0.05$). The between-group effect shows increased RSFC between the default mode network and a small cluster located in the superior division of the left lateral occipital cortex. (B) Distribution of the mean individual z-scores within the superior division of the left lateral occipital cortex. Depicted in red are the patients with long-term remission of Cushing's disease, depicted in blue are the matched healthy controls, and both are sorted from smallest to highest z-score.

Discussion

We examined RSFC in patients with long-term remission of Cushing's disease and hypothesized to find aberrant RSFC of three networks of interest including the limbic network, the DMN, and the executive control network in patients with long-term remission of Cushing's disease compared with matched healthy controls. Our hypothesis was confirmed for both the limbic network and the DMN, but not for the executive control network.

In patients we found an increase in RSFC between the limbic network and the subgenual ACC and an increase in RSFC between the DMN and a small cluster located in the superior division of the left lateral occipital cortex. Animal studies suggest that the subgenual ACC is a target site for the negative feedback effects of glucocorticoids on stress-induced HPA axis activity. Damage to this location leads to a diminished ability to regulate and inhibit HPA axis activity (Diorio et al., 1993). This has also been observed in humans, in whom behavioral studies showed that the subgenual ACC function is involved in emotion regulation (Bush et al., 2000; Shin et al., 2000; Phillips et al., 2003a; Quirk and Beer, 2006). The subgenual ACC exerts top-down inhibitory control over subcortical structures, most importantly the amygdala (Kim et al., 2011). Failing to exert this inhibitory control could lead to the disturbed emotion regulation seen in stress-related psychiatric disorders (Phillips et al., 2003b; Johnstone et al., 2007; Patel et al., 2012). Therefore, the subgenual ACC is the target site for some specific forms of treatment for stress-related disorders. Deep brain stimulation of the subgenual ACC is currently used as a last resort treatment for treatment-resistant depression (Mayberg et al., 2005; Johansen-Berg et al., 2008; Lozano et al., 2008). Furthermore, subgenual ACC activity has been found to change under influence of fluoxetine use (Mayberg et al., 2000), an antidepressant that is used as a treatment for both PTSD and depression. These studies suggest that targeting sub¬genual ACC function is an effective treatment element for stress-related psychiatric disorders.

Our finding of increased RSFC connectivity between the limbic network and the subgenual ACC in patients with long-term remission of Cushing's disease suggests that these changes in coupling are established under the influence of hypercortisolism. This hypothesis is further supported by a study by Veer et al (2012) who found that RSFC connectivity between the amygdala (a key component of the limbic network) and the subgenual ACC is reactive to endogenous cortisol (Veer et al., 2012). In addition, our findings indicate that elevated RSFC between the

subgenual ACC and key regions of the limbic network (like amygdala and insula) in depression (Connolly et al., 2013) and PTSD (Gilboa et al., 2004; Brown et al., 2014) could be an effect of exposure to high levels of cortisol often accompanying these stress-related disorders.

It is well known that optimal HPA axis reactivity is crucial to make correct adaptive responses in challenging situations but, once out of balance, can also be related to vulnerability or the emergence of psychiatric symptoms. We did not find an association of any of the behavioral measurements with the strength of the connectivity between the limbic network and the subgenual ACC. As our study was designed to test group differences, our study could have been underpowered to test for this type of associations. Therefore, it remains unknown whether these increases in connectivity reflect or underlie psychiatric symptoms, or more adaptive behavior. Recently, the latter has been suggested for increased RSFC between the hippocampus and the ventromedial prefrontal cortex in a group of trauma-exposed combat paramedics (Admon et al., 2013).

We also found an increase in RSFC in the patient group between the DMN and a small cluster located in the superior division of the left lateral occipital cortex. This area is situated adjacent to parietal regions that are part of the DMN. Therefore, an interpretation could be that the DMN of the patients recruits larger areas compared with healthy controls. This may indicate a less efficient use of the DMN in patients with long-term remission of Cushing's disease.

Contrary to our expectations, the executive control network did not show differences in RSFC between the patients and controls. However, the observed cognitive impairment still present in the patients with Cushing's disease in remission was subtle, and the cognitive demands during resting state are low because of the absence of specific goal-oriented tasks. Consequently, differences in functional activity might only be expressed when demands on cognition are high (ie, during tasks). Task-related fMRI studies with a high cognitive load could be used to investigate this, as these tasks also show aberrant activity patterns in the executive control network in patients with depression and PTSD (Wang et al., 2008; Aizenstein et al., 2009; Daniels et al., 2010).

Limitations of our study consist of the cross-sectional design of our study that does not permit any causal conclusions to be drawn from the data. Therefore, we cannot rule out that the found between-group differences preceded the onset of the

Cushing's disease. It is also possible that the difference in connectivity is an effect of the depres¬sive symptomatology in the patient group that developed independent of the exposure to hypercortisolism. However, we did not find a correlation between depression severity and the strength of connectivity, making it less likely that the difference in connectivity purely reflects psychopathology. Another limitation is that we did not correct for the number of networks investigated in this study, thereby giving the study an explorative nature. Finally, we have not applied global signal regression that is known to induce negative con¬nectivity between networks (Murphy et al., 2009). However, this also means our method is less sensitive toward detecting negative connectivity in the data.

In summary, findings from neuroendocrine animal models for stress-related disorders using rodents with chronically elevated levels of corticosterone may have limited applic¬ability to human brain function during challenges or resting state. Cushing's disease represents a unique model for investigating effects of prolonged hypercortisolism on the human brain. Earlier studies using Cushing's disease to investigate the long-term effects of hypercortisolism on the structure of the brain showed smaller gray matter volumes of the ACC, increased gray matter volumes of the cerebellum, and reduced structural connectivity to be rather persistent (Andela et al., 2013; van der Werff et al., 2014), whereas normalization of cortisol levels reversed hippocampal atrophy following treatment of Cushing's disease (Starkman et al., 1999). Our study is the first to suggest that exposure to hypercortisolism may lead to persistent changes in brain functional connectivity as well. Our finding of increased RSFC between the limbic network and the subgenual ACC is similar to the results of studies performed in patients with stress-related disorders, suggesting that these changes in RSFC in these stress-related disorders are established under the influence of increased levels of cortisol.

Future studies in the effects of stress or cortisol on RSFC should focus on connectivity between the limbic network (or specific key regions of this network) and the subgenual ACC in order to replicate this finding. This could also be achieved by conducting a targeted approach using the subgenual ACC as seed to investigate connectivity differences. Longitudinally designed studies should be performed to investigate the causal effects of hypercortisolism on RSFC. In addition, task-related fMRI paradigms could contribute to investigate changes in brain activity during specific demands.

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Chapter 6

Neuroimaging resilience to stress: a review

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Abstract

There is a high degree of intra-individual variation in how individuals respond to stress. This becomes evident when exploring the development of posttraumatic symptoms or stress-related disorders after exposure to trauma. Whether or not an individual develops posttraumatic symptoms after experiencing a traumatic event is partly dependent on a person's resilience. Resilience can be broadly defined as the dynamic process encompassing positive adaptation within the context of significant adversity. Even though research into the neurobiological basis of resilience is still in its early stages, these insights can have important implications for the prevention and treatment of stress-related disorders. Neuroimaging studies contribute to our knowledge of intra-individual variability in resilience and the development of posttraumatic symptoms or other stress-related disorders. This review provides an overview of neuroimaging findings related to resilience. Structural, resting-state, and task-related neuroimaging results associated with resilience are discussed. There are a limited number of studies available and neuroimaging research of resilience is still in its infancy. The available studies point at brain circuitries involved in stress and emotion regulation, with more efficient processing and regulation associated with resilience.

Introduction

The lifetime prevalence of exposure to severe traumatic events in the United States ranges between 51.2 and 60.7% (Kessler et al., 1995). Individuals who have been exposed to a trauma can develop stress-related psychopathologies, such as depression, anxiety disorders, or posttraumatic stress disorder (PTSD; (Egeland, 1993; Schnyder et al., 2001; Kilpatrick et al., 2003; Bryant et al., 2011). These disorders are a major cause of long-term disability. The United States National Comorbidity Survey found that ~7.8% of the population in the US develops a PTSD at least once in their lives, with women having a higher chance than men (Kessler et al., 1995; Mota et al., 2012). In patients with an anxiety disorder, a higher number of episodes of major depressive disorder (MDD) have been reported in patients with a history of trauma compared to those without the experience of a traumatic event (Zlotnick et al., 1997). Notably, not every individual develops posttraumatic symptoms after experiencing a traumatic event. Many people will experience symptoms of an acute stress disorder, but only a minority will develop PTSD or other affective psychopathologies. Major life-events are also known to be precipitating factors for other psychiatric disorders, and again many individuals will show symptoms of psychological distress, but only a relative minority will develop a disorder like MDD or an anxiety disorder. Whether or not an individual develops posttraumatic symptoms or another stress-related disorder after experiencing a traumatic event can be considered from a summative view, i.e., as being accounted for as a balance between positive and negative influences, affecting most people in the same way or to the same degree (Rutter, 2006), but also from a more dynamic and interactive view. According to this view, both vulnerability and resilience in the context of a specific stressor are higher-order, multidimensional phenomena spanning an individual's biological and psychological profile, developmental history, previous (traumatic) experiences, active choices, social context, current environment, social support, and timing of the traumatic event (Charney, 2004; Feder et al., 2009; Cicchetti, 2010; Holman et al., 2011). Importantly, there is not one universally accepted definition of resilience. Resilience is often more broadly defined as a dynamic process encompassing positive adaptation within the context of significant adversity, and also, from a more psychobiological standpoint, as short- and long-term responses that reduce allostatic load (Charney, 2004; Curtis and Cicchetti, 2007). This definition would differentiate resilience from the concept of resistance. Stress resistance prevents the experience of negative consequences of stressor exposure, whereas stress resilience requires one to experience the negative consequences of stressor exposure in order to demonstrate facilitated

recovery from that experience (Fleshner et al., 2011). Moreover, in some individuals the experience of negative effects in response to stressors or adversity may also lead to a decreased vulnerability later in life through a "steeling" or inoculation effect (Rutter, 2012).

Research into psychological factors contributing to resilience is longstanding, and has identified factors such as emotional flexibility, locus of control, social problem solving, and cognitive skills, and several others [for a review see (Curtis and Cicchetti, 2003)]. More recently, studies have begun to examine biological factors in resilience and their interplay with psychological and environmental factors. In humans, cross-sectional studies have focused on neuroendocrine and neural markers, while animal models are providing complementing experimental data on behavioral, neural, molecular, and hormonal basis of resilience. Animal data show that in resilient animals there is an absence of the key molecular abnormalities found in susceptible individuals, but also distinct epigenetic and cellular adaptations in response to stressors in various neurotransmitter systems and brain areas (Fleshner et al., 2011; Russo et al., 2012). Insight into biological factors underpinning resilience to stress may open new avenues for prevention and treatment of stress-related disorders (Charney, 2004). In addition, these insights could prove useful in the selection and training of professions known to have a higher risk of trauma exposure (i.e., military personnel, police officers, first responders). Over the past decades neuroimaging has become an increasingly important tool to study neural correlates of adaptive and non-adaptive behavior. Furthermore, neuroimaging allows studying the associations of these neural correlates with other biological factors as well as their interaction with environmental and psychological factors. In addition, neuroimaging facilitates examining the psychobiological mechanisms that underlie these interactions (Meyer-Lindenberg and Tost, 2012).

To investigate neurobiological correlates of resilience to psychological trauma in humans, one would ideally study a group of individuals at baseline, before exposure to trauma, and then assess these individuals repeatedly after exposure to trauma. Those who would have developed sustained symptomatology would then be compared to those who remained symptom-free or only had transient symptoms.

For the sake of this neuroimaging review, we will consider trauma-exposed, non-PTSD (TENP) individuals as resilient subjects. We are aware that defining resilience as the absence of PTSD symptoms after the experience of a traumatic event does not fully cover the multi-dimensional, dynamic nature of the construct of resilience.

The term resilience as used throughout our review will therefore be more reflective of the capacity of an individual to avoid negative social, psychological and biological consequences, and cognitive impacts of extreme stress that would otherwise compromise their psychological or physical well-being (Russo et al., 2012). In the present review we will focus on the neuroimaging of resilience to especially severe stressful experiences, such as combat-related trauma, sexual abuse, and sustaining severe injuries through accidents. We will first briefly introduce the most widely used neuroimaging approaches to date. Subsequently, we discuss the brain circuitry of stress and present a review of the available neuroimaging literature on resilience in humans up until 2012.

Neuroimaging Methods

The rapid growth of modern neuroimaging techniques enables us to study both structure and function of the human brain in great detail. Additionally, it allows us to examine the influence of specific biological or specific environmental factors on brain functioning (Meyer-Lindenberg and Tost, 2012). Nowadays, magnetic resonance imaging (MRI) methods are the most widely used tools to examine brain structure and function in living humans, because of the low risks involved, the non-invasive nature of the technique, and the high quality of the obtained images. MRI techniques can be used to localize neuropathological abnormalities or to determine the size or shape of various structures in the brain (Pitman et al., 2001). The MRI-based diffusion tensor imaging (DTI) method can be used to examine white matter tracts. Functional neuroimaging, i.e., dynamic (indirect) measurements of brain activity during rest or during a cognitive, emotional, or pharmacological challenge, can be assessed using functional MRI (fMRI). With fMRI, changes are measured in regional cerebral blood flow based on changes in the concentration of the blood oxygenation level. A relative estimate of the level of activity within a given region of interest can be derived from this blood oxygenation level-dependent (BOLD) signal (Pitman et al., 2001; Huettel et al., 2009). Brain activity and blood flow can also be measured with functional neuroimaging methods that use radioactive ligands such as positron-emission tomography (PET) or single photon-emission computed tomography (SPECT). PET can use radioactive labeled water, oxygen or glucose (Bremner, 2007a; Townsend, 2008). In addition, PET and SPECT methods can use radioactive ligands to visualize biochemical elements such as transporters or receptors for certain neurotransmitters.

Neurocircuitry of Stress

Converging data from animal studies, lesion studies in humans and neuroimaging research in healthy controls and patient populations, point at the involvement of specific brain structures and circuitries in the generation of emotional, cognitive, and behavioral responses to stressors and the subsequent regulation of these responses. Key structures involved in this neurocircuitry are the amygdala, insula, hypothalamus, hippocampus, and cortical structures like the medial prefrontal cortex (mPFC) and the anterior cingulate cortex (ACC; (Dedovic et al., 2009)

The amygdala is located in the medial temporal lobes of the brain. It is involved in the encoding and consolidation of emotional memory of events (Bremner, 2007b) and regulates part of the fear response by activating the hypothalamic-pituitary-adrenal axis that releases hormones involved in the stress response. The amygdala can also increase the startle response via connections with the pons in the midbrain and is involved in the modulation of the autonomic nervous system via the hypothalamus (Davis, 1992; Jovanovic and Ressler, 2010). Amygdala activation has been reported in response to positive stimuli as well, suggesting a broad involvement of this structure in emotional arousal (Shin and Liberzon, 2010). Lesions in the amygdala of both humans and rodents have shown that the amygdala is also involved in the elimination of the fear response and emotional behavior (Zola-Morgan et al., 1991; Funayama et al., 2001). The insula is involved in high-level cognitive control and attentional processes (Menon and Uddin, 2010). Additionally, together with the hippocampus, the insula plays a role in processing the context of a potential threat (Gilbertson et al., 2002; Feder et al., 2009). The hippocampus not only has an important role in declarative memory, but is probably also a key-regulator of the hypothalamic-pituitary-adrenal axis activation (Bremner, 2007b). Furthermore, having a small hippocampus could diminish the neuroendocrine regulation, leading to a stronger emotional or hormonal stress response (Gilbertson et al., 2002; Lyons et al., 2007). Animal studies have shown that hippocampal lesions or genetically smaller hippocampi lead to a stronger conditioned fear response and alterations in fear-mediated responses (Phillips and LeDoux, 1992).

The prefrontal cortex (PFC) receives somatosensory, visual and auditory inputs and underlies many cognitive skills. Areas in the PFC have an inhibitory effect on the amygdala (Phelps et al., 2004; Baumann and Turpin, 2010), and the modulation of emotional responsiveness by the PFC through inhibition of the amygdala is supported by lesion studies (Morgan and LeDoux, 1999; Milad and Quirk, 2002).

The PFC encompasses many structures, among which the ACC. The ACC is divided into subregions including the dorsal ACC (dACC), the rostral ACC (rACC), and the subgenual ACC (sgACC). Hypofunction of the PFC, the rACC and the sgACC, and hyperactivity of the amygdala and dACC was found to be related to dysregulation of emotion in anxiety or mood disorders (Phan et al., 2005; Shin and Liberzon, 2010). Animal studies have shown that enhanced activation of the infralimbic PFC, the rodent analog of the rACC and sgACC, inhibits the fear response. The ventral-rostral area of the ACC is involved in the processing of emotionally relevant stimuli, while the dorsal-caudal region is more relevant for non-emotional cognitive tasks (Shin et al., 2005; Mohanty et al., 2007; Shin et al., 2007). However, recent studies seem to indicate that both subregions contribute to emotional processing, with ventral-rostral portions of the ACC and the mPFC involved in regulation (Etkin et al., 2011).

With respect to studying stress and resilience to stress, animal studies allow more freedom in manipulating stress responsiveness compared to human research. Interestingly, interventions designed to decrease stress responsiveness by forcing repeated application and selection of the most successful coping strategies have been found to increase the volume of the ventral mPFC (Lyons et al., 2002; Katz et al., 2009), and increase neurogenesis of the hippocampus (Lyons et al., 2010a). In addition, Delgado et al. showed that an inward displacement of the ventral part of the right hippocampus was specific for resilience to stress in rats (Delgado y Palacios et al., 2011). These findings suggest a firm relationship between successful application of coping strategies and these key structures in the neurocircuitry of stress in animals. For more animal literature on resilience to stress see (Lupien et al., 2009; Lyons et al., 2010b; Franklin et al., 2012) (Figure 1).

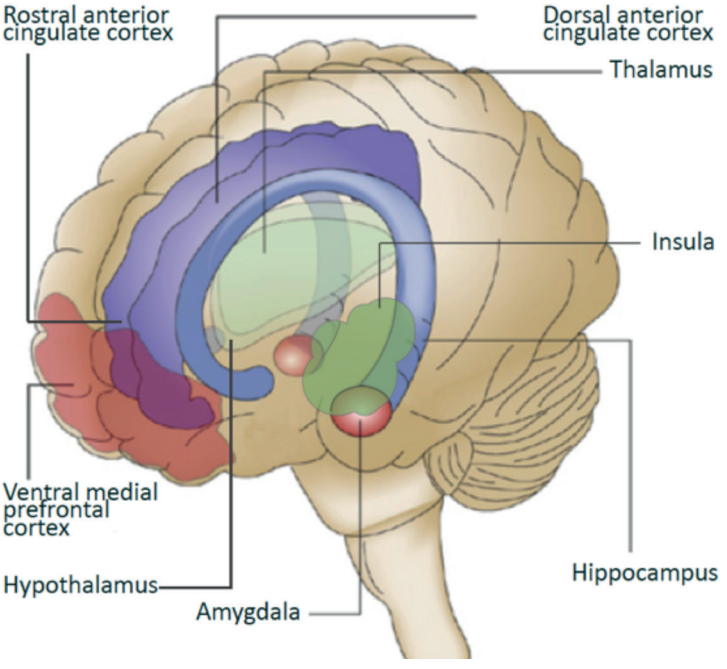
Review

For the current review we conducted a Pubmed search up till 2012, using key terms including: “resilience,” “vulnerability,” “neuroimaging,” “PET,” “SPECT,” “MRI,” “posttraumatic,” “anxiety” “depression” “affective” “stress,” “trauma.” Bibliographies were also reviewed for further citations. We limited our search to studies in humans and in English. Papers were selected based on relevance with a focus on studies in PTSD, but studies on MDD were also included. We also included electronic publications ahead of print.

We will first discuss studies examining structural neural correlates of resilience to stress, followed by studies examining functional neural correlates of resilience

to stress, and studies in which the neural correlates of personality factors known to be involved in resilience, i.e., trait-resilience, were taken into account. Table 1 presents the studies that allowed examining resilience, i.e., examined stress-resilient subjects, subjects with psychopathology after stress, and healthy controls without both trauma exposure and PTSD. Because studies in PTSD have informed hypotheses on the neural circuitry involved in resilience, findings from studies in PTSD (comparing PTSD individuals with TENP individuals) will be briefly presented in each section before discussing the results of studies focusing on resilience.

Figure 1. Brain regions involved in resilience to stress



Depicted in this figure are brain regions often linked to resilience to stress. Adapted from (Schloesser et al., 2008).

Table 1. An overview of neuroimaging studies specifically examining resilience by using comparisons between three group: (1) a PTSD group, (2) a TENP group and (3) a healthy control group without both trauma exposure and PTSD.

Three-group studies

Reference	n PTSD / TENP / HC	Protocol	Findings specific to resilience
Woon, 2009	121 / 77 / 116	Structural	No resilient specific findings in amygdala volume
Fennema-Notestine, 2002	11 / 11 / 17	Structural	Smaller frontal and occipital gray matter volumes
Britton, 2005	16 / 15 / 14	Trauma-script	Decrease in amygdala activation
Liberzon, 1999	14 / 11 / 14	Trauma-related sounds	No resilient specific findings
Blair, 2011	14 / 15 / 19	Affective Stroop task	Increased activation in medial prefrontal regions during top-down control
New, 2009	14 / 14 / 14	Emotion regulation	Increased activation in medial prefrontal regions during top-down control
Falconer, 2008	23 / 17 / 23	Go/No-Go inhibition task	No resilient specific findings

Twin studies

Reference	n PTSD Exposed/non-exposed twins	 Non-PTSD Exposed/non-exposed twins	Protocol	Findings specific to resilience
Shin, 2011	12 / 12	14 / 14	Multi-source interference task	Decreased dorsal anterior cingulate activation
Gilbertson, 2002	17 / 17	23 / 23	Structural	Increased hippocampus volume
Shin, 2009	14 / 14	19 / 19	Resting-state	Decreased dorsal anterior cingulate activation
May, 2004	20 / 23	23 / 24	Structural	Decreased cavum septum pellucidum size
Kasai, 2008	18 / 18	23 / 23	Structural	Decreased density in right hippocampus, pregenual ACC, bilateral insulae

HC = Healthy Controls, PTSD = posttraumatic stress disorder, TENP = Trauma-exposed non-PTSD

Structural Neuroimaging of Resilience

We could only identify a very limited number of MRI studies explicitly designed to explore resilience by focusing on its structural correlates. Many studies did not include a healthy, non-exposed control group, which is needed in a cross-sectional design to establish whether differences between exposed groups are related to vulnerability or resilience. Remarkably, there appear to be no studies on resilience using methods to assess white matter integrity or connectivity, such as DTI, or methods to examine structural aspects other than volume or gray matter density, i.e., shape analysis or cortical thickness. Studies usually report on structural aspects of the hippocampus, amygdala, and ACC.

Hippocampus

One of the core symptoms of PTSD is reliving the traumatic event. With the hippocampus being central in declarative memory, it could be hypothesized that variations in the structure of the hippocampus could contribute to resilience (Bremner, 2007b). Importantly, the hippocampus is also a key-regulator of the hypothalamic-pituitary-adrenal axis activation in response to stressors (Bremner, 2007b). The hippocampus has frequently been studied in PTSD and several studies examining TENP and PTSD subjects found larger volumes of the hippocampus in the TENP subjects (Gurvits et al., 1996; Bremner et al., 2003; Lindauer et al., 2004b; Kitayama et al., 2005; Kasai et al., 2008; Felmingham et al., 2009; Morey et al., 2012), but others did not report this finding (Lindauer et al., 2005; Freeman et al., 2006; Rogers et al., 2009). Smaller hippocampal volumes have also been described in MDD and in healthy "at risk" subjects with a history of depression (Campbell et al., 2004; Videbech and Ravnkilde, 2004).

Because a non-exposed healthy control group was not included in these studies, it is not possible to distinguish whether the differences in hippocampal volume should be attributed to resilience in the TENP group or to the presence of psychopathology or vulnerability in the PTSD group. Controversy exists on the origin of structural differences of the hippocampus in relation to exposure to severe stress. Based on animal studies it has been hypothesized that exposure to traumatic events may damage neurons and inhibit neurogenesis in the hippocampus, showing that the hippocampus is sensitive to the effects of stress (Sapolsky et al., 1990; Bremner et al., 1999b; Golub et al., 2011). This suggests that a smaller hippocampus is a consequence of neurobiological changes associated with extreme or chronic stress. In line with this, smaller left hippocampal volumes were found in women with MDD

who have experienced chronic maltreatment in their childhood compared with women with MDD and without childhood maltreatment (Vythilingam et al., 2002). In addition, a decrease in hippocampal volume in MDD has been found to be associated with the duration of depressive episodes (Sheline et al., 1999; MacQueen et al., 2003). In PTSD, psychopharmacological treatment of symptoms has been associated with increases in hippocampal volumes (Vermetten et al., 2003). However, a study in which hippocampal volumes were larger in TENP subjects compared to PTSD subjects, showed that this difference did not change after the PTSD was effectively treated with psychotherapy, suggesting smaller hippocampal volumes to be either a residue or scar, caused by the experience of the trauma, or a factor related to vulnerability, pre-existing the traumatic event (Lindauer et al., 2005). Apart from considerations on how psychotherapy and psychopharmacological treatment influence the brain, an alternative explanation could be that larger hippocampal volume in the TENP is linked to resilience.

To directly address the important controversy, Gilbertson et al. (2002) in an elegant design examined hippocampal volume in a group of PTSD patients and their non-exposed twins, and in combat-related TENP subjects and their non-exposed twins (Gilbertson et al., 2002). It was possible to correct for childhood abuse and alcohol use, factors known to influence hippocampal volume. The authors found smaller hippocampal volumes in both PTSD patients (n = 17) and their non-traumatized co-twins (n = 17) compared to TENP subjects (n = 23) and their non-traumatized co-twins (n = 23). In addition, severity of PTSD symptomatology in patients was negatively correlated with the hippocampal volume of both the patients and their trauma-unexposed identical co-twins, suggesting smaller hippocampal volume to be a familial risk factor for developing stress-related psychopathologies. The TENP subjects showed no differences in hippocampal volumes compared to their non-exposed twins. As there were no non-exposed twin pairs in this study, it could not be examined whether the hippocampal volume of the resilient subjects and their co-twins was perhaps larger than average and a potential familial resilience factor. Another, smaller study examined morphometry of the mesial temporal lobe area in adult female victims of intimate partner violence with (n = 11) and without (n = 11) PTSD, and in non-victimized controls (n = 17) (Fennema-Notestine et al., 2002). There were no differences in hippocampal volumes among the three groups. Interestingly, the authors found smaller overall frontal and occipital gray matter volumes in the resilient (violence exposed non-PTSD) subjects, but the interpretation of the findings is difficult because of the small sample and the presence of childhood emotional abuse.

In conclusion, the available data suggest that smaller hippocampal volumes might be the result of exposure to severe stress and perhaps also a vulnerability factor, but it is not clear whether an increased volume is associated with resilience.

Amygdala

Structural MRI studies in PTSD have also examined structural changes of the amygdala, with some studies reporting a larger amygdala volume in TENP subjects compared to PTSD subjects (Rogers et al., 2009; Morey et al., 2012), while others did not (Gurvits et al., 1996; Bonne et al., 2001; Lindauer et al., 2004b; Kuo et al., 2012).

A recent meta-analytic study, using data from nine different studies in adults, compared the amygdala volumes in PTSD patients (n = 121), TENP individuals (n = 77), and non-trauma exposed healthy controls (n = 116) (Woon and Hedges, 2009). The authors found a larger right amygdala vs. left amygdala in all three groups, which is consistent with a previously conducted study (Pedraza et al., 2004). This suggests that in both PTSD and in resilience to trauma the asymmetry in volume of the amygdala is preserved. In addition, this meta-analysis found no significant differences between amygdala volume in PTSD patients relative to TENP and trauma-unexposed healthy controls (Woon and Hedges, 2009). These results suggest that although the amygdala has a key role in the neurocircuitry of stress, the volume of the amygdala is not associated with influence on vulnerability or resilience toward developing psychopathology after a traumatic event. However, a single case-control study with comparable group sizes to those of the meta-analysis (n = 99 PTSD patients; n = 101 TENP individuals) found larger left and right amygdalae in the TENP individuals. Evidence of the association between the structure of the amygdala and resilience as well as PTSD symptomatology is currently inconsistent and inconclusive (Morey et al., 2012).

Anterior Cingulate Cortex / Prefrontal Cortex

Studies comparing PTSD subjects with TENP subjects found smaller volumes and lower gray matter density of the ACC in PTSD subjects (Rauch et al., 2003; Woodward et al., 2006; Kasai et al., 2008). More specifically, a smaller volume of the rACC (Rauch et al., 2003; Kasai et al., 2008) and subcallosal cortex was found (Rauch et al., 2003).

Smaller gray matter volumes of the ACC and mPFC have also been found in MDD patients compared to healthy controls, with one of the few longitudinal studies showing loss of volume in MDD in these areas during depressive episodes (Frodl et

al., 2008). Similarly, a twin study in subjects with PTSD showed that the gray matter volume reductions were not present in non-PTSD co-twins, suggesting that the reductions are the consequence of the exposure to stress, rather than a possible familial vulnerability factor (Kasai et al., 2008). Furthermore, May et al. (2004) showed in a twin study design a significant reduction of cavum septum pellucidum size in TENP individuals and their co-twins compared to PTSD individuals and their co-twins (May et al., 2004). Increases in the size of the cavum septum pellucidum is linked to impaired limbic development (Raine et al., 2010).

Interestingly, a recent structural neuroimaging study on resilience to MDD examined volumes of the hippocampus, several prefrontal areas, and the basal ganglia in healthy adults without any family history of MDD (n = 64), "resilient" healthy individuals with a family history of MDD (n = 30), and participants with a current diagnosis of MDD (n = 33). A smaller right hippocampal volume, which in PTSD putatively reflects a genetic risk factor, was found in the resilient healthy subjects with a family history of MDD. However, the resilient individuals also showed increased white matter volumes of the right dorsal mPFC as compared to the two other groups. The authors interpreted this as a potential correlate of resilience to stress, possibly linked to the regulatory functions of this region (Amico et al., 2011). To examine the hypothesized modulatory function of emotional responsiveness by the mPFC, Milad et al. (2005) subjected healthy individuals (n = 14) to trials of presented pictures of virtual lights followed by an electric shock. In the extinction phase, participants were presented the virtual light without the electric shock. The next day only the virtual lights were presented again and skin conductance was registered as a measure of fear extinction. They found that greater extinction memory (lower skin conductance) was associated with an increased thickness of the ventral mPFC (Milad et al., 2005). These results led the authors to suggest that the size of the ventral mPFC might explain individual differences in the ability to modulate fear. A potential relationship between the ventral mPFC and modulation of emotion responsiveness is further supported by animal studies (Lyons et al., 2002; Katz et al., 2009). These studies used the early handling paradigm [subjecting pups to short periods of separation from their mother during the first week(s) of life] or social separation in order to decrease stress responsiveness and increase successful application of various coping strategies. Animals subjected to this paradigm were found to have an increased volume of ventral mPFC. Other data in rodents show that in animals resilient to certain stress paradigms the expression of certain genes in glutamatergic neurons in the mPFC increases, suggesting increased neuronal activation. This also suggests that the complexity of neuronal architecture in the mPFC increases, which may be mechanisms underlying volume changes (Russo et al., 2012).

Structural Connectivity

Studies examining structural connectivity in PTSD have shown abnormalities of structural integrity of cingulate regions, the cingulum bundle and/or the amygdala, and other frontal regions [for a review see (Ayling et al., 2012)]. We did not identify studies in which resilience was or could be explored in humans. In monkeys, the recent study by Katz et al. (2009) not only found increased white matter volumes, but also increased myelination in the mPFC after stress inoculation. This could be the substrate for the decreased stress responsiveness observed in these monkeys, given the role of the ventral mPFC in emotion and arousal regulation (Katz et al., 2009).

Taken into account the limited available structural imaging data from both human and animal studies, the findings most consistently indicate that the (ventral) mPFC volume and structure are associated with resilience, with volumetric and structural alterations reflecting or even underlying increased emotion regulation capacities. For two other key-structures in the stress and emotion circuitry, the hippocampus and the amygdala, the available data suggests associations of structure with vulnerability, but not clearly with resilience.

Functional Neuroimaging

Given the concept of resilience as being a dynamic process, encompassing positive adaptation within the context of significant adversity, studies examining functional correlates of resilience are clearly of importance. One approach is to examine the spontaneous brain activity and its temporal and spatial connectivity in the absence of externally presented tasks or stimuli, so-called resting-state fMRI. Older studies have used PET and SPECT methods; more recently there is an increasing use of resting-state fMRI approaches. With resting-state fMRI several functional networks have been detected, including the default mode network, thought to be involved in autobiographic memory and self-referential processing. With respect to studying resilience, resting-state fMRI can also be applied during anticipation and recovery of stress.

A more widely used functional neuroimaging approach is to study the correlates of brain activity and connectivity while subjects have to engage in a specific emotional or cognitive task, as opposed to the situation in resting-state imaging. Emotional tasks may involve paradigms with specific stimuli associated with a previous stressful

or traumatic event, such as trauma scripts to study emotion processing related to the specific event, or with emotional stimuli not related to a previous event to study general emotion processing. In addition, it is also possible to induce psychological and social stress before scanning.

Resting-State Studies

We did not identify PET or SPECT studies in which resilience could be explored, i.e., including a TENP group, a psychopathology and a healthy control group. PET and SPECT studies have found increased amygdala activity at rest in PTSD subjects, with one twin study reporting increased resting metabolic activity as a familial risk factor for PTSD (Chung et al., 2006; Shin et al., 2009).

Only a few resting-state fMRI studies have been performed in PTSD so far, and they seem to point at the importance of resting-state connectivity of different areas and networks involved in self-processing and fear conditioning with an amygdala/ACC circuitry. In a small, but very interesting prospective resting-state fMRI study, Lanius et al. (2010) examined the relationship between connectivity of the default mode network and severity of concurrent and prospective PTSD symptoms in 11 acutely traumatized subjects recruited from emergency departments (Lanius et al., 2010). Participants were assessed at 2, 6, 12, and 36 weeks postaccident and scanning took place at week 6 or 12. A seed-based approach with a seed in the posterior cingulate cortex (PCC)/precuneus region was used. The PCC/precuneus region is implicated in autobiographical memory processes and self-processing operations and a key region in the default mode network (Greicius et al., 2003). Connectivity of this region with the perigenual ACC and the right amygdala was positively correlated with current PTSD symptomatology, whereas the connectivity with the right amygdala predicted symptoms 6 weeks subsequently. The authors interpreted their results as reflecting an increased trauma-related input from amygdala and perigenual ACC circuitry into the default mode network, which could lead to disturbed aspects of self-processing. Less resting-state connectivity between the insula and the right amygdala was shown in a combat-related TENP group compared to PTSD subjects (Rabinak et al., 2011). The insula and amygdala have been shown to be connected during fear conditioning and a reduced resting-state connectivity may underlie less exaggerated fear responses, less persistence of traumatic memories and proneness to affective disorders (Etkin and Wager, 2007).

In another, quite large study comparing TENP (n = 72) and PTSD subjects (n = 54) recruited from earthquake survivors, the resting-state connectivity of the thalamus

was examined. The thalamus is connected to nearly all areas in the cortex and acts as a relay between subcortical areas and the cerebral cortex. The TENP group showed decreased positive connectivity between the thalamus and bilateral inferior and left middle frontal gyri, left inferior parietal lobule, and right precuneus. An increased positive functional connectivity between the thalamus and right medial frontal gyrus and left rACC was also found in this group (Yin et al., 2011).

Of interest for the more dynamic concept of resilience, some resting-state fMRI studies in healthy subjects have aimed to identify patterns of adaptive recovery to laboratory-induced stress. Clearly, this is relevant for elucidating brain mechanisms underlying resilience and vulnerability, as models for stress-related psychopathology usually postulate a loss of the adaptive recovery. Resting-state fMRI seems particularly suited to examine these recovery processes, because brain activity recovery patterns are not disturbed by tasks demands. In a resting-state study in healthy participants, Van Marle et al. investigated poststress amygdala-centered connectivity patterns in order to characterize the aftermath of acute, experimentally induced stress in healthy humans. The investigators recorded resting-state fMRI in 26 female participants immediately following a period of moderate psychological stress induced by means of aversive (vs. emotionally neutral) movie watching. The authors found a prolonged activation in an amygdala-connectivity network after the moderate stress, thought to reflect an extended state of hypervigilance that promotes sustained salience and mnemonic processing after stress (van Marle et al., 2010).

In another resting-state fMRI study in healthy subjects Veer et al. (2011) examined resting-state functional connectivity during the recovery period after experimentally induced social stress. Forty participants were randomly assigned to the social stress condition or the non-stressful control condition. Resting-state fMRI scans were acquired 60 min after these conditions. In the stressed subjects resting-state fMRI showed an increase in connectivity between the amygdala and the mPFC and between the amygdala and the (PCC)/precuneus region (Veer et al., 2011). The authors interpreted this as showing the top-down inhibitory control by the mPFC and the stress-induced facilitation of self-evaluative processes, involving the default mode network, after or during salient experiences. Both processes can be considered key-elements of the behavioral homeostasis after stress, and this paradigm might be interesting to study adaptive responses in resilient subjects or prospectively.

Task-Related Functional Studies

As is the case for most other imaging approaches described in this review, task-related functional neuroimaging studies explicitly studying resilience, i.e., using the previously mentioned design with three groups, are scarce. More task-related data are available from studies comparing PTSD subjects with TENP subjects. With disturbed regulation of (emotions evoked by) traumatic memories being a core symptom of PTSD and thought central to its pathophysiology, task-related functional studies typically have employed traumatic memory retrieval scripts to examine alterations in the regulation of emotions induced by traumatic memories. A growing line of research is studying the regulation of non-traumatic induced emotions, focusing on more general emotion regulation capacities in PTSD and TENP. Finally, some recent studies have taken hypervigilance, another core symptom of PTSD in which attention cannot be diverted, as a starting point and studied top-down attentional control systems.

Studies in PTSD vs. Non-PTSD Controls

Several functional task-related neuroimaging studies have examined brain activity in PTSD compared to TENP or healthy control subjects [for an extensive review see: (Hughes and Shin, 2011)]. The most consistent findings in these studies with regard to findings in TENP subjects are an increased activity of the ventral mPFC and rACC, and a relatively lower activity of the amygdala and the dACC as compared to the PTSD subjects during exposure to emotion evocative stimuli. Studies have shown a negative correlation between the increased mPFC and decreased amygdala activity, in line with the regulatory function of mPFC regions over the amygdala. As mentioned above, the first line of research discussed here has used traumatic memory retrieval paradigms, in which subjects are exposed to trauma-related stimuli, such as pictures, sounds, or individual-specific scripts (Liberzon et al., 1999).

Seminal work was done by the group of Bremner et al. (1999a,b) who exposed both Vietnam combat veterans and sexually abused women with and without the diagnosis of PTSD to memories of their trauma during PET scanning and found differences in activity of several brain areas between TENP subjects and PTSD subjects (Bremner et al., 1999a; Bremner et al., 1999b). Differences were found in areas involved in emotion regulation, notably in inhibition of the amygdala. In both groups of TENP subjects (war and sexual abuse), an increase in blood flow in the medial prefrontal area, including the subcallosal gyrus, middle temporal gyrus, and right rACC, compared to PTSD patients was found. However, the TENP subjects also showed decreased activity in areas not typically involved in emotion regulation like

the PCC, inferior parietal cortex, lingual gyrus, and left precentral gyrus in the motor cortex. In the sexually abused TENP group, there was also increased blood flow in the right hippocampus, inferior fusiform gyrus, supramarginal gyrus, and visual association cortex relative to women with PTSD. This possibly suggests that there may be specific correlates of resilience or vulnerability for specific types of trauma (Bremner et al., 1999a).

More recent studies have predominantly used fMRI paradigms. A study in police officers using traumatic memory retrieval scripts showed an increased activity of the medial frontal gyrus during exposure to trauma scripts in TENP subjects as compared to PTSD subjects (Lindauer et al., 2004a). Traumatic memory retrieval was also used as an fMRI paradigm to examine a group of PTSD and TENP police officers who had experienced the same trauma, but with a (small) subgroup of the PTSD subjects receiving psychotherapy. After therapy symptom scores of this treatment group were similar to those of the TENP group. Subsequent analysis of the scans showed a pattern of increased activity of the mPFC and reduction of amygdala activity during traumatic memory retrieval in the therapy group, comparable to that in the TENP police officers, suggesting a key role for increased emotion regulation capacities in both resilient individuals as well as by therapy (Peres et al., 2011).

Another script-driven fMRI study showed an association between less re-experiencing and less dissociation on the one hand, and activation of the inferior frontal gyrus on the other hand (Hopper et al., 2007). This area was found to be significantly more activated in TENP subjects. Other traumatic script-driven fMRI studies have shown a greater activity of the thalamus region in TENP compared to PTSD patients (Lanius et al., 2001; Lanius et al., 2005), which might be interpreted as more efficient information processing capacities in TENP subjects.

Another line of neuroimaging studies in PTSD and TENP subjects examined whether alterations of more general emotion processing capacities are present in PTSD and used paradigms with stimuli unrelated to the specific trauma. Tasks aimed on more general emotion processing included among others pictures of emotional faces (Rauch et al., 2000; Shin et al., 2005), memory tasks with pictures or words unrelated to trauma (Shin et al., 2004; Phan et al., 2006), the emotional counting Stroop task (Shin et al., 2001), or a multi-source interference task (Shin et al., 2011). These studies on more general emotion processing did indeed reveal a similar brain activity pattern in TENP subjects as the studies using trauma-related stimuli, with increased activity of the rACC and ventral mPFC, decreased activity of the amygdala,

increased activity of the hippocampus, and a decrease in PCC activity as compared to PTSD subjects. This suggests that more general emotional information processing capacities are involved in vulnerability or resilience.

Functional Studies Involving a Three-Group Design with PTSD Subjects, TENP Subjects, and Healthy Non-Exposed Controls.

Studies comparing PTSD subjects with TENP point at a central role of emotion regulation brain capacities in the adaptive response to trauma, but the lack of a non-exposed healthy control group does not allow any firm conclusion about whether alterations are specific for resilience or vulnerability. A small number of functional imaging studies have employed task-paradigms and included PTSD, TENP, and a non-exposed healthy control group. Britton et al. performed PET scanning during script driven imagery of emotionally evocative and neutral events in combat-related TENP subjects (n = 15), combat-related PTSD subjects (n = 16), and healthy controls (n = 14) (Britton et al., 2005). Emotionally evocative events included general highly stressful events as well as specific traumatic events. PTSD subjects did not show changes in amygdala activity over conditions, but showed deactivation of the rACC during stressful scripts. Healthy controls showed activation of the amygdala and deactivation of the ventral mPFC during the stressful condition, while the resilient subjects showed the same deactivation of the ventral mPFC. Importantly, they also showed a specific pattern of deactivation of the amygdala during imagery of emotionally evocative events. This can be interpreted as a resilience specific mechanism. However, another study using a similar design with three groups found no patterns of amygdala activity that were specific for the TENP group. Liberzon et al. (1999) used [99mTc]HMPAO SPECT to examine combat-exposed PTSD subjects (n = 14), combat-related TENP subjects (n = 11), and a group of healthy controls (n = 14) and exposed them to white noise and combat noises (Liberzon et al., 1999). Both the TENP group and healthy controls showed less amygdala activation than the PTSD subjects.

Most recent studies with a three-group design have used fMRI paradigms. As the ability to exercise voluntary control over emotional responses was found to be linked to better functioning and emotion regulation in healthy volunteers, and as discussed above, patients with PTSD show less activity in the emotion regulation circuitry when confronted with challenging negative stimuli, several researchers have hypothesized that the capacity to voluntarily or automatically regulate emotions may be a resilience factor (Charney, 2004; Yehuda et al., 2006; New et al., 2009). To directly examine this hypothesis, New et al. (2009) investigated

deliberate regulation of emotion in PTSD, TENP, and healthy control groups of 14 women exposed to sexual assault (New et al., 2009). Emotionally neutral and negative pictures were presented, with the negative pictures being not related specifically to sexual assault. The participants had to focus specifically on the deliberate modification (up and down regulating) of emotional responses to the stimuli. Contrary to the general regulation hypothesis, both TENP subjects and PTSD subjects were less capable of downregulation responses to negative stimuli and showed less activity in the lateral PFC compared to healthy controls. However, the TENP subjects were more successful in upregulating their responses to negative stimuli, which was associated with increased activity in the dACC compared to both the PTSD group and the control group. Interestingly, the personality trait "optimism" was significantly correlated with the intensity of ACC activation during voluntary upregulation in TENP subjects compared to both PTSD subjects and healthy controls. This interesting preliminary result suggests that specifically the ability to deliberately engage cognitive-emotional strategies to extinguish negative emotional responses and the functional brain correlates are associated with resilience (New et al., 2009).

A last group of studies has focused on another core symptom of PTSD, hypervigilance, i.e., the increased attentional bias to environmental threat associated cues and the decreased possibility to focus on other stimuli. Previous work in healthy controls showed that emotional attention involves amygdala priming of representations in the temporal cortex, while the involvement of top-down attentional control systems is needed to divert attention toward task-relevant stimuli and weaken (emotional) responding to (emotional) distracters (Vythilingam et al., 2007).

The few neuroimaging studies that have examined top-down attentional control in PTSD vs. TENP subject or controls did find some alterations, but also for this domain it remains unclear whether it concerns a general deficit or a specific deficit within the context of emotional distracters.

Some studies with a three-group design have tried to address this issue. Falconer et al. (2008) used a Go/No-Go fMRI task to measure inhibitory control of non-emotional stimuli in 23 PTSD patients, 17 TENP individuals, and 23 healthy controls. PTSD subjects showed deficiencies in the recruitment of right inferior frontal cortex and the ventral PFC during inhibitory control. However, there were no activation patterns specific to the resilient TENP individuals (Falconer et al., 2008). A recent twin fMRI study by Shin et al. examined whether functional task-related abnormalities of the ACC, after exposure to severe stress, are acquired characteristics or represent

a familial risk. They studied combat-exposed PTSD subjects, their non-exposed co-twins (12 pairs) and combat-related TENP subjects and their co-twins (14 pairs). Subjects performed a cognitive attentional task, the multi-source interference task. Vietnam combat veterans in the TENP group and their identical co-twins showed less task-related dACC activity as compared to Vietnam combat veterans with PTSD and their identical co-twins during the interference task. The dACC activity in the non-exposed twins predicted the severity of the symptomatology in the PTSD subjects (Shin et al., 2011). The results suggest that hyperresponsivity of dACC is a familial risk factor for PTSD. The relative hyporesponsivity in the TENP subjects and their co-twins could be a familial resilience factor.

Recently, Blair et al. (2013) performed an fMRI study on attentional control in 15 TENP individuals, 14 patients with PTSD, and 19 healthy controls. They also explicitly specified a hypothesis on the pattern in the resilient group, expecting resilient subjects to show superior recruitment of regions involved in top-down emotional attention relative to the other two groups during the task performance. Subjects performed the affective number Stroop task, with positive, negative, and emotional pictures selected from the international affective picture system presented as emotional distractors. The PTSD group showed deficiencies in the recruitment of lateral regions of superior and inferior frontal cortex, corresponding with the findings of Falconer et al. (2008), but also a deficiency of recruitment of the parietal cortex that appeared only in the presence of negative distracters (Blair et al., 2013). As hypothesized, the resilient subjects showed an enhanced ability to recruit regions involved in top-down attentional emotional control when compared to the matched healthy controls and the PTSD subjects. Taken together, these studies suggest that deficiencies in the recruitment of especially inferior frontal regions during top-down attentional control in general are specific to PTSD, but resilience specific activity patterns are only present during top-down control of emotional attention.

Trait Resilience

Research has shown that specific personality characteristics contribute to vulnerability and resilience to stress. The Big Five model of personality traits is a widely used model in which individual differences in personality are described in five overall personality factors: neuroticism (also referred to as absence of emotional stability), extraversion, openness, agreeableness, and conscientiousness (Friborg et al., 2005). Studies have shown that neuroticism is a risk factor for the development

of PTSD (Breslau et al., 1991; Nakaya et al., 2006). A resilient personality profile was found to consist of low neuroticism, high extraversion, and conscientiousness, but also high scores on openness and agreeableness (Friborg et al., 2005; Campbell-Sills et al., 2006). High trait resilience also coincides with a construct with high scores on optimism, low neuroticism, and behavioral activation sensitivity (Block and Kremen, 1996).

Sub-facets of the Big Five personality traits that are thought to contribute to resilience are high self-esteem, internal locus of control, flexibility in thinking, sense of meaning, and problem-solving skills (Bryant et al., 2011; Daniels et al., 2012). High levels of these traits, together with a fast physiological recovery have been shown to enhance recovery from experimentally induced stress (Tugade and Fredrickson, 2004). Posttraumatic adjustment could also be enhanced by sub-facets of the personality characteristics that include hardiness, believing in having an influence on one's surroundings and also the ability to learn from both positive and negative experiences (Daniels et al., 2012). Furthermore, individuals with high scores on conscientiousness, extraversion and agreeableness are more likely to have a secure and stable environment with supportive social relationships, which also contributes to successful adaptation to stress (Friborg et al., 2005; Daniels et al., 2012).

We identified one study that has examined neural correlates of resilient personality traits, focussing on arousal regulating capacities in healthy volunteers. Waugh et al. (2008) studied the functional neural correlates of trait resilience during anticipation, but also during recovery from threat (Waugh et al., 2008). They operationalized emotional resilience as the flexible and appropriate use of emotional resources. In their event-related fMRI design, healthy participants viewed "threat" cues signaling the possibility of either viewing an aversive picture or a neutral picture, and "non-threat" cues, signaling the viewing of only a neutral picture. High-trait resilient participants exhibited less early and less prolonged insula activity to the neutral pictures shown after a "threat" cue than low-trait resilient participants, indicating quicker and more appropriate adaptation to the neutral stimulus by the high resilient subjects.
In a very interesting small study Daniels et al. (2012) prospectively investigated the neural correlates mediating the relationship between trait resilience and the recovery from a traumatic event. They used a convenience sub-sample of 12 acutely traumatized subjects, derived from a larger sample of 70 acutely traumatized subjects recruited at an emergency department, and fulfilling the DSM-IV PTSD criterion A. Subjects were followed-up for several months to monitor the development of PTSD

symptomatology. Trait resilience was assessed with the Connor-Davidson resilience scale (CD-RISC) (Connor and Davidson, 2003). Trait resilience was found to predict a better outcome throughout the first 3 months of follow-up. A trauma script-driven symptom provocation fMRI paradigm with neutral and trauma scripts was used to investigate neural correlates of trait resilience two to four months posttrauma. For imagery of the traumatic vs. the neutral event, CD-RISC scores showed a positive correlation with activity in the right inferior and middle frontal gyrus and the right thalamus. As these regions are known to be involved in arousal regulation and emotional reappraisal, the findings can be interpreted as pointing toward the broader concept of emotion regulation as the mediator between trait resilience and posttraumatic adjustment (Daniels et al., 2012).

Discussion

Whereas research into psychological factors contributing to resilience is longstanding, only more recently studies have begun to examine biological factors in resilience in humans and their interplay with psychological and environmental factors. Insight into biological factors underpinning resilience to stress may open new avenues for prevention and treatment of stress-related disorders (Charney, 2004). Neuroimaging has become an increasingly important tool to study neural correlates of behavior and to elucidate the role of neural mechanisms in the interaction between genes and environment (Meyer-Lindenberg and Tost, 2012). In a seminal review paper in 2004, Charney stated that with the recent advances it would be possible to create more comprehensive psychobiological models of the ordinary magic of resilience (Charney, 2004). Based on our present review we have to conclude that neuroimaging of resilience is still in its early stages, with only a limited number of studies allowing to specifically examine functional or structural brain characteristics that may contribute to resilience.

Based on findings in studies comparing stress-related psychopathologies, especially PTSD, with healthy controls, the neural circuitry of resilience is usually postulated to overlap with the brain circuitry involved in emotion and stress regulation. Data from imaging studies comparing TENP subjects with PTSD subjects do indeed find important differences in structural and functional characteristics of emotion regulating brain circuitries, putatively underlying or reflecting increased emotion regulation capacities in TENP subjects. By and large, this pattern is also found in the few studies in which subjects with PTSD, TENP subjects and healthy, non-exposed control subjects are compared. Structural studies point at increased gray matter

volumes in structures such as the hippocampus, the ventral mPFC, and the rACC and sgACC. Subsequently, functional studies show increased activity in these structures during tasks using emotion evocative stimuli, such as the traumatic script-driven paradigm. The ventral mPFC, rACC, and sgACC exert top-down control over the amygdala and the stress system, which is putatively more efficient or increased in resilience. In addition, the hippocampus is known to be involved in the processing of traumatic experiences, but also in regulation of the stress system. The mPFC also processes traumatic memories and is involved in the regulation of extinction learning and the modulation of fear responses.

Various subregions of the ACC have been found to be involved in emotion regulation, among others through inhibition of the amygdala. In line with this a decreased reactivity of the amygdala together with an increased rACC activity was found in several studies in TENP subjects. The association between resilience and increased regulation of amygdala activation is further supported by a study showing that symptoms of PTSD were very low in combat veterans with unilateral damage in the ventral mPFC or amygdala (Koenigs et al., 2008). It is also thought that adaptive processes after trauma exposure may occur through functional interactions between the mPFC, ACC, and amygdala (Osuch et al., 2008). In line with the findings in humans, a non-human primate study demonstrated alterations of volume and myelination of the ventral mPFC in animal showing reduced stress responsiveness after an inoculation paradigm (Katz et al., 2009). It should be noted that although the majority of the available studies seems to point at the neurocircuitry involved in aspects of emotion and arousal regulation, studies examining functional connectivity do suggest that in resilience, the connectivity of an amygdala-prefrontal network with several other functional networks, such as the default mode network or the salience network, also plays a role.

Another area of neuroimaging research from which insight into possible neural mechanisms underlying resilience may be derived, is that of the neural correlates of personality traits known to facilitate the adaptation to severely stressful situations. This is particularly the case for trait-resilience, a meta-construct involving traits like low neuroticism, high extraversion, and conscientiousness, with several of these traits known to be linked to the characteristics of the neurocircuitry involved in emotion and stress regulation. The few existing neuroimaging studies examining high vs. low-trait resilient subjects found that high trait resilient subjects are characterized by a brain pattern reflecting more efficient arousal modulation and emotional reappraisal. These patterns overlap with the areas and circuitries that

were identified in TENP subjects, again suggesting that the broader concept of emotional control and its neural substrate may indeed be pivotal in resilience.

As resilience is probably best conceptualized as a dynamic, context-dependent phenomenon it could be hypothesized that some of the elements specific for resilience only develop during or after the experience of a traumatic event. However, as most available research in humans is cross-sectional, no causal conclusions can be drawn on the temporal order of trauma exposure and brain changes observed in resilient individuals. Moreover, as in the studies conducted so far resilience has been most frequently defined as the absence of PTSD, although data on symptomatology of other trauma-related disorders were gathered in the majority of the discussed studies, the strictness of the exclusion criteria varied between studies. Therefore, it cannot be excluded that the TENP individuals did suffer, to some extent, from (subclinical) depressive or anxiety symptomatology or substance abuse. Hence, it remains uncertain whether the presented findings are specific for the absence of PTSD psychopathology, or for the absence of psychopathology after exposure to trauma altogether.

Given the current state of the art of neuroimaging of resilience as laid out by this review several avenues to gain further insight into the neural mechanisms involved in resilience can be chosen. Ideally, the neural correlates of resilience should be studied longitudinally. Neurobiological and other variables potentially related to resilience would be measured before and after an individual has been exposed to a severe stressor, after which key variables are assessed over time among individuals who develop trauma-related psychopathology, individuals who do not develop psychopathology, and a control group that has not been exposed to trauma. This would allow the identification of baseline “predictors” of resilience as well as that of potential mediators on different levels, i.e., psychological, (epi)genetic, biochemical and neural, and examine their interaction. A homogeneous cohort of subjects for such a longitudinal study is probably most easily recruited amongst first responders or military personnel, who usually are already assessed extensively before active duty. A caveat is that these populations may consist of a selection of resilient individuals. Such a design would also allow to further examine patterns of resilience based on trajectories of psychological complaints after exposure. In addition, this design would enable examination of the temporal stability of neural correlates of resilience (as assessed at baseline) and their malleability by exposure to a severe stressor.

A first approach could be to focus on the structural neural correlates of resilience.

Based on our present review of the structural neuroimaging research of resilience, we would postulate resilience to be associated with alterations of gray matter volume and structure of especially the (ventral) mPFC before the exposure to severe trauma. In addition to studying gray matter volume with both region of interest (i.e., mPFC) and whole brain approaches, we suggest to subsequently examine both shape and corticol thickness of the brain. Examining structural connectivity by means of DTI scans would also be an important approach for the structural neuroimaging of resilience. Taking into account the neurocircuitry of stress, the findings in PTSD as well as those in animal studies, the structural connectivity of white matter tracts that connect limbic structures with the ventral PFC and subregions of the ACC would be a clear focus. We would hypothesize increased structural connectivity between these areas, underlying increased emotion regulation capacities, in resilient individuals.

A second approach would be to focus on the functional neural correlates of resilience and their temporal characteristics. Based on our review we believe more general emotion and arousal regulation capacities to underlie resilience, with resilient subjects being especially more capable in upregulating their emotions and having top-down control over emotional attention. However, so far only explicit emotion regulation was examined, and the role of automatic emotion regulation capacities, which may have a stronger neural correlate, has not yet been assessed with functional neuroimaging. In addition to emotion regulation paradigms, we think that a broader design should incorporate novel neuroimaging task paradigms that have not been used to examine functional neural correlates of resilience yet. One would be to visualize the neural activity during acute, non-trauma-related stress, using a paradigm such as the Montreal imaging stress task (Dedovic et al., 2005). We would postulate that during this acute social stress paradigm, resilient individuals exert more control over their limbic system by increasing activity of especially their ventral PFC and rACC. In addition to studying neural characteristics of resilience during stress, neural characteristics of resilience during anticipation, and recovery of stress should be incorporated in the same design. This will allow us to get a complete oversight of neural activity patterns used by resilient individuals in order to process stress over time, from anticipation pre-stress up until recovery during the aftermath of stress. Waugh et al. (2008) already showed patterns of brain activity specific for trait resilient individuals during anticipation and recovery of threat (Waugh et al., 2008). Subsequently, some recent resting-state studies have, as we discussed in the present review, focused on the immediate adaptive recovery after acute (social) stress. This adaptive recovery, which may be a key element in a more dynamic concept of resilience, was not only associated with involvement of

emotion regulation circuitry, but also with that of circuits involved in self-referential processing (Lanius et al., 2010; Veer et al., 2011). Furthermore, based on research from other domains, it can be hypothesized that other brain circuits (e.g., underlying social affiliation, reward dependence, but also higher order cognitive skills) are also involved in resilience and should be investigated in concert (Charney, 2004).

Two other avenues to elucidate neural mechanisms in resilience can be considered as stand-alone cross-sectional designs, but also as baseline assessment in a larger scale, longitudinal pre-post exposure design as described above. One would be to further examine neural correlates of trait resilience, a concept probably directly linked to resilience to psychotrauma. This would theoretically require no special study populations, but so far only a few studies have investigated the structural and functional neural correlates of trait resilience. Another avenue would be to examine the functional and structural effects of stress inoculation or "steeling" paradigms in humans, building on the work in non-human primates. This could not only shed more light on changes in neural mechanisms underlying increased resilience, but may also identify potential neural predictors of response to inoculation. An interesting question would be whether inoculation paradigms in adults result in (inoculation) specific or more general adaptations of especially functional neural mechanisms. We are not aware of any neuroimaging studies that have focused on the effects of inoculation in humans. Finally, we believe that studies examining the functional and structural correlates of resilience could be enriched by including other neurobiological measures, such as measures for autonomic nervous system reactivity, and by examining the genetic influences on brain structure and function.

In conclusion, several years after the seminal review of Charney (2004), neuroimaging of resilience still seems to be in its infancy, but is expected to benefit from the increasing interest in the "positive" concept of resilience and may be informed by neuroimaging approaches already more widely used in affective disorders and normal behavior, and state-of-the art strategies such as neuroimaging approaches for complex gene-environment interactions (Charney, 2004; Meyer-Lindenberg and Tost, 2012)

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Chapter 7

Resilience to childhood maltreatment is associated with increased resting-state functional connectivity of the salience network with the lingual gyrus.

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Abstract

Background
The experience of childhood maltreatment is related to an increased risk of developing a variety of psychiatric disorders, as well as a change in the structure of the brain. However, not much is known about the neurobiological basis of resilience to childhood maltreatment. This study aims to identify resting-state functional connectivity (RSFC) patterns specific for resilience to childhood maltreatment, focusing on the default mode and salience network and networks seeded from the amygdala and left dorsomedial prefrontal cortex.

Methods
Resting-state functional MRI scans were obtained in 33 individuals. Seeds in the bilateral amygdala, the dorsal anterior cingulate cortex (dACC), the posterior cingulate cortex and the left dorsomedial prefrontal cortex were defined and used to examine whether resilient individuals differed from vulnerable individuals and healthy controls in RSFC with other brain regions.

Results
Within the salience network, the resilient group was associated with increased RSFC between the left dACC and a region containing the bilateral lingual gyrus and the occipital fusiform gyrus compared to both the vulnerable group and the healthy controls.

Conclusions
In this study, we found RSFC patterns specific for resilient individuals. Regions that are implicated are related on a functional level to declarative memory and the processing of emotional stimuli.

Introduction

With estimates of almost 10% of all children living in the U.S. being subjected to child maltreatment, prevalence of child maltreatment is high (U.S. Department of Health and Human Services, 2009). The most common types of child maltreatment are emotional neglect, emotional abuse, physical abuse and sexual abuse. Exposure to child maltreatment has been related to adverse effects like disturbances of mental health that can persist well into adulthood. Associations have been found between childhood maltreatment and an increased risk of a variety of DSM-IV axis I and axis II mental disorders, including mood, anxiety, substance abuse and impulse control disorders (MacMillan et al., 2001; Afifi et al., 2008; Scott et al., 2010; Afifi et al., 2011). In addition, it has been found that childhood maltreatment has an effect on the morphology of the left medial prefrontal cortex (mPFC; (van Harmelen et al., 2010) and on connectivity in the resting brain (van der Werff et al., 2013a). While the experience of childhood maltreatment increases the chance of developing psychopathology in some individuals, others will not develop symptoms or will return to a stable level of functioning very soon after the adverse life events have ended. These individuals are usually described as being resilient.

Resilience can be understood as 'a dynamic process encompassing positive adaptation within the context of significant adversity' (Luthar et al., 2000). Protective psychological factors found to be associated with the facilitation of resilience after childhood maltreatment include highly developed cognitive skills, high self-esteem, internal locus of control, external attribution of blame, ego-control and (extra)familial support (Cicchetti and Rogosch, 1997; Cicchetti, 2010). In contrast to the studies on protective psychological factors, studies on the neurobiological mechanisms involved in resilience are still very scarce. The available data suggests the involvement of neurocircuitry involved in stress reactivity and emotion processing, such as the limbic network. Key brain regions in this circuitry are the amygdala, the hippocampus and parts of the medial prefrontal cortex (Shin and Liberzon, 2010). The amygdala is associated with memory consolidation of emotional experiences and the acquisition of fear responses (LeDoux, 2000). It is reciprocally connected to the hippocampus, which plays an important role in declarative memory (memories that can be consciously recalled such as facts and knowledge; (Bremner, 2007). In this limbic network, the medial prefrontal cortex has a more regulating function and plays a role in the inhibition of fear responses and modulating emotional responsiveness (Veer et al., 2011). In line with a central role for the limbic network are the findings of New et al (2009). In this functional MRI (fMRI) study, the authors

hypothesized that resilient individuals process their emotions differently than non-resilient individuals. Using an explicit emotion regulation task, they indeed found that during deliberate regulation of emotional responses, regions of the prefrontal cortex showed more activation in resilient individuals compared to vulnerable individuals and healthy controls (New et al., 2009).

It has been demonstrated that fMRI during resting-state (i.e. in the absence of externally controlled tasks, stimuli or instructions) can reliably measure coherent low-frequency fluctuations in regional brain activity, allowing the monitoring of activation and functional connectivity in the resting brain (Fox and Raichle, 2007). With resting-state fMRI a number of functional networks have been consistently identified. Three networks that are of interest when studying the neurobiological basis of resilience are the previously discussed limbic network, but also the default mode network (DMN) and the salience network. The DMN contains the precuneus, the posterior cingulate cortex, medial prefrontal cortex, and parts of the parietal cortex (Raichle et al., 2001). The DMN is thought to be associated with the retrieval and manipulation of episodic memories and with semantic knowledge, self-referential processing and prospective memory (Raichle et al., 2001; Buckner et al., 2008; Kim, 2012). Hence, this network may be relevant for a better understanding of the neurobiology of resilience, as intrusive memories of traumatic events and decreases in self-esteem are typically reported in vulnerable individuals after the experience of a traumatic event. In line with this, alterations in DMN functioning have also been related to depressive symptoms and the presence of stress-related psychopathologies (Kluetsch et al., 2012; Marchetti et al., 2012; Schwindt et al., 2012; Whitfield-Gabrieli and Ford, 2012).

The salience network, containing the bilateral anterior insula and bilateral dorsal anterior cingulate cortex (dACC), is important in assessing the relevance of internal and external stimuli in order to guide behavior (Seeley et al., 2007). Experiencing traumatic events like childhood maltreatment has been associated with the development of a cognitive bias toward unpleasant stimuli that indicate potential threat (McNally et al., 2000). Recently, a study indeed revealed differences in resting-state functional connectivity (RSFC) in both the limbic and salience network in a group of adults with a history of childhood emotional maltreatment (van der Werff et al., 2013a). Importantly, RSFC of the limbic, DMN and salience networks in resilient individuals may provide more insight in the neural mechanisms involved in resilience, but this has not been investigated yet.

Therefore, in the present study we aim to study RSFC in individuals resilient to childhood maltreatment, comparing them with matched healthy controls without a history of childhood maltreatment and with individuals with a history of childhood maltreatment and psychopathology. We use a 'seed'-based approach in which an in the literature previously described key anatomical structure of a specific network is used as a starting point for the RSFC, i.e. seed. Using seeds enables us to identify specific resting-state networks in a new sample, in a reliable and reproducible manner.

Given the association of the limbic network with stress responsiveness and emotion processing, and the link between emotion regulation and resilience (New et al., 2009; Shin and Liberzon, 2010), we hypothesize to find differences in RSFC within the limbic network in resilient individuals compared to non-resilient individuals and healthy controls. Furthermore, given the function of the salience network and the involvement of the ACC in a variety of anxiety disorders including posttraumatic stress disorder (PTSD; (Damsa et al., 2009) we hypothesize to find specific alterations in RSFC in resilient individuals. In addition, based on its function and findings in depression and anxiety disorder, we hypothesize alterations in RSFC in the DMN (Greicius, 2008; Veer et al., 2010). Finally, as we previously found an effect of childhood maltreatment on the structure of a region in the left mPFC, we also hypothesize alterations in RSFC in this region (van Harmelen et al., 2010)

Methods

Participants

Subjects were drawn from the large-scale longitudinal Netherlands Study of Depression and Anxiety (NESDA; (Penninx et al., 2008). They were recruited in three manners: (1) From the community through two cohorts available through prior studies, (2) through recruitment from primary care practices, (3) through recruitment from mental health organizations. To determine whether subjects had a psychiatric disorder the Composite International Diagnostic Interview (CIDI) was completed (Robins et al., 1988). Subjects scoring positive for disorders other than major depressive disorder, panic disorder, social anxiety disorder or general anxiety disorder were excluded from the study. All subjects without MRI contraindications were invited to participate in the MRI part of the study, resulting in 301 included subjects. From the 301 subjects with a resting-state fMRI scan we formed three groups. The resilient group consisted of 12 subjects who reported having experienced any type of childhood maltreatment more than once based on responses to the

Nemesis interview and scored negative on the lifetime occurrence of any of the DSM-IV axis-1 disorders. Due to having the eyes opened during the resting-state scan, data from one participant were excluded, resulting in our resilient group consisting of 11 subjects. The two other groups were pairwise matched to the first group on variables: gender, scan location, age and highest level of completed education. The vulnerable group consisted of 11 individuals reported to have experienced childhood maltreatment more than once and scored positive on any of the included DSM-IV axis-1 disorders for the last six months. This resulted in a group that was vulnerable to both depressive and anxiety disorders. The third group consisted of 11 healthy controls. Individuals in this group neither experienced childhood maltreatment (reported 'never' on each of the four categories of childhood maltreatment during the Nemesis trauma interview) nor reported any lifetime psychopathologies on the CIDI. Demographics are reported in Table 1.

To be absolutely sure none of the resilient or healthy controls had suffered from a PTSD we retrospectively administered the PTSD Symptom Scale – Interview Version (PSS-I; (Foa et al., 1993). Participants were asked how often (never, 0; a few times a month, 1; a few times a week, 2; a few times a day or continuously, 3) they had experienced each of the 17 criteria on the three subscales for PTSD as listed in DSM-IV (i.e. five items on Cluster B: re-experiencing; seven on Cluster C: avoidance/numbing and five on Cluster D: arousal) during a period of four weeks when the symptoms were the most severe. Finally, they were asked whether this was also the case during the last month and to indicate in which year of the last five years the symptoms had been the most severe. A symptom was scored as present when experienced at least a few times a week (Brewin et al., 2002).

Childhood maltreatment

Childhood maltreatment was assessed through the use of the Nemesis trauma interview (de Graaf et al., 2002). In this interview, respondents were asked whether they had experienced emotional neglect, emotional abuse, physical abuse and/or sexual abuse before the age of 16, what their relationship to the perpetrator was, and how often the childhood maltreatment had occurred (responses were recorded as: 0 = never, 1 = once, 2 = sometimes, 3 = regularly, 4 = often, or 5 = very often). To compare frequency of experienced maltreatment between the groups, we added the sum scores of how often the childhood maltreatment occurred to Table 1. Emotional neglect was described as: 'people at home didn't listen to you, your problems were ignored, you felt unable to find any attention or support from the people in your house'. Emotional abuse was described as: 'you were cursed at, unjustly punished,

your brothers and sisters were favored – but no bodily harm was done'. Physical abuse was described as: 'you were kicked, beaten with hands or other objects, or other forms of physical abuse were done to you'. Sexual abuse was described as: 'were you sexually touched against your will, or forced to touch another sexually'. Our definition of child maltreatment is in line with the definitions of both the department of health & human services of the USA and the Centers for disease control and prevention. This definition states: 'Child maltreatment involves any act or series of acts of commission or omission by a parent or other caregiver that results in harm, potential for harm or threat of harm to a child (Leeb et al., 2008).

Severity of the experienced childhood maltreatment was assessed retrospectively using the childhood trauma question-naire (CTQ; (Bernstein et al., 1997; Thombs et al., 2009)

Data acquisition

Image acquisition took place at either one of the three participating scanning locations, situated in the University Medical Centers in Leiden, Amsterdam and Groningen, using Philips 3T MR-systems (Best, The Netherlands). These systems were equipped with a SENSE-8 (Leiden University Medical Center and University Medical Center Groningen) or SENSE-6 (Academic Medical Center, Amsterdam) channel head coil, respectively. As part of a fixed imaging protocol (scan sequence: Tower of London, word encoding, T1-weigthed scan, word recognition, perception of facial expression, resting-state scan), resting-state functional MRI (RS-fMRI) data were acquired for each subject. Subjects were instructed to lie as still as possible, with their eyes closed and without falling asleep. After completion of the scan all subjects confirmed not having fallen asleep. To obtain RS-fMRI data, T2*-weighted gradient-echo echo-planar imaging was used with the following scan parameters in Amsterdam and Leiden: 200 whole-brain volumes; repetition time (TR) 2300 ms; echo time (TE) 30 ms; flip angle 80◦; 35transverse slices; no slice gap; matrix 220 mm × 220 mm; voxel size 2.3 mm × 2.3 mm; slice thickness 3 mm. The total scan duration of the RS-fMRI was 7.51 min. Scan parameters in Groningen were the same except for echo time 28 ms; 39 axial slices; voxel size 3.45 mm × 3.45 mm. For registration purposes as well as gray matter density analysis, anatomical images were acquired using a sagittal 3-dimensional gradient-echo T1-weighted sequence with the following scan parameters: repetition time (TR) 9 ms; echo time (TE) 3.5 ms; flip angle 80◦; 170 sagittal slices; no slice gap; matrix 256 ms × 256 ms; voxel size 1 mm isotropic. The total scan duration of the anatomical scan was 4.5 min. A neuroradiologist examined all anatomical images. No abnormalities were found.

Table 1. Demographic and clinical characteristics of the resilient group (1), the vulnerable group (2) and the control group (3)

	(1) Resilient group (*n*=11)	**(2) Vulnerable group** (*n*=11)	**(3) Control group** (*n*=11)	**Group (1) vs (2)** *P* value	**Group (1) and (3)** *P* value
Gender, % M/F	27.3/72.7	27.3/72.7	27.3/72.7	1.000[a]	1.000[a]
Handedness, % L/R	0/100	0/100	0/100	1.000[a]	1.000[a]
Age, Mean (SD)	40.36 (10.94)	39.73 (9.61)	40.45 (9.47)	.975[b]	.375[b]
Highest completed education, Mean (SD)	6.64 (2.29)	6.64 (1.43)	6.55 (1.64)	.634[c]	.943[c]
Current CIDI Diagnosis,					
MDD, *n*	0	3	0	<.0001[a]	1.000[a]
ANX, *n*	0	3	0	<.0001[a]	1.000[a]
CDA, *n*	0	5	0	<.0001[a]	1.000[a]
HC, *n*	11	0	11	<.0001[a]	1.000[a]
PSS-I PTSD diagnosis,	0	2	0	<.0001[a]	1.000[a]
Scan Location Amsterdam, *n*	3	3	3	1.000[a]	1.000[a]
Scan Location Leiden, *n*	6	5	5	.670[a]	.670[a]
Scan Location Groningen, *n*	2	3	3	.611[a]	.611[a]
NEO-FFI neuroticism, Mean (SD)	25.64 (4.68)	43.73 (8.39)	25.09 (4.55)	<.0001[c]	.797[c]
NEO-FFI extraversion, Mean (SD)	42.18 (5.00)	29.81 (10.12)	45.45 (5.34)	.003[b]	.153[b]
NEO-FFI openess, Mean (SD)	31.73 (6.00)	32.90 (6.24)	30.00 (4.77)	.656[b]	.464[b]
NEO-FFI agreeableness, Mean (SD)	42.45 (5.34)	42.64 (6.22)	46.56 (4.5)	.942[b]	.066[b]
NEO-FFI Conscientiousness, Mean (SD)	41.54 (3.93)	34.00 (5.66)	42.63 (5.2)	.002[b]	.585[b]
BAI at baseline, Mean (SD)	2.27 (1.79)	20.64 (10.18)	2.64 (5.89)	<.0001[c]	.151[c]
BAI at scanning, Mean (SD)	3.00 (2.63)	14.45 (9.94)	2.00 (2.76)	<.0001[c]	.282[c]
MADRS at scanning, Mean (SD)	1.30 (1.70)	13.55 (6.01)	.82 (1.54)	<.0001[c]	.512[c]
IDS at scanning, Mean (SD)	5.50 (3.14)	24.91 (14.21)	3.82 (4.64)	<.0001[c]	.085[c]
# of negative life events	5.18 (2.04)	6.36 (2.11)	4.18 (1.89)	.686[b]	.638[b]
Trauma Type,					
Emotional Neglect, *n*	11	11	0	1.000[a]	<.0001[a]
Emotional Abuse, *n*	5	5	0	1.000[a]	<.0001[a]
Physical Abuse, *n*	3	4	0	.647[a]	<.0001[a]
Sexual Abuse, *n*	1	2	0	.534[a]	<.0001[a]
Sum score frequency of maltreatment, mean (SD)	6.27 (5.10)	6.27 (3.10)	0	.606[c]	<.0001[c]
CTQ, Mean (SD)	46.09 (17.26)	45.40 (8.88)	29.90 (4.38)	.654[c]	.001[c]

ANX = anxiety disorder; BAI = Beck Anxiety Inventory; CDA = comorbid major depressive disorder and anxiety disorder; CIDI = Composite International Diagnostic Interview; CTQ = Childhood trauma questionnaire; IDS = Inventory of depressive symptomatology; MADRS = Montgomery-Åsberg Depression Rating Scale; PSS-I = Posttraumatic stress disorder Symptom Scale – Interview Version; PTSD = posttraumatic stress disorder

[a] = Chi-Square Test.

[b] = Independent Sample t-test.

[c] = Mann-Whitney U Test

Data Preprocessing

The RS-fMRI images were preprocessed using FEAT (FMRI Expert Analysis Tool) version 5.90, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; (Smith et al., 2004). Non-brain tissue removal was applied to the structural images. Motion correction was applied (subject movement >3 mm in any direction, resulted in exclusion of the data from further analysis) to the RS-fMRI data along with non-brain tissue removal, spatial smoothing using 6 mm full-width at half-maximum Gaussian kernel, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, 0.01 Hz cut-off). RS-fMRI data were registered to the high-resolution structural image (T1) and subsequently the T1 image was registered to the 2 mm isotropic MNI-152 (T1 standardbrain average over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) images. The resulting transformation matrices derived from these registration steps were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

Statistical analysis

Resting-state data were analyzed using seed-based correlations identifying three networks of interest: the limbic network, the DMN and the salience network. The following seed regions of interest were selected: bilateral amygdala for the limbic network, bilateral dACC for the salience network (Margulies et al., 2007) and posterior cingulate cortex (PCC) for the DMN (Fox et al., 2005). The bilateral amygdala seeds were created in standard space using the Harvard-Oxford Subcortical Structural Probability maps. Timecourses of fMRI signal in these locations are known to correlate strongly with the timecourses of fMRI signal in the other constituents of their network, thus allowing the replicable identification of the network. Post hoc, this was checked by examining the main effects of the seeds. In addition to the seeds, a mask was created for the structural abnormality earlier identified in individuals reporting childhood emotional maltreatment located in the left mPFC (van Harmelen et al., 2010), as well as a white matter mask and a cerebrospinal fluid (CSF) mask. MNI coordinates for each of the seeds are reported in Table 2.

Around every coordinate a 4 mm sphere was created. These spheres were then transformed to native space using the inverse transformation matrices obtained during registration in the preprocessing phase. Spatially averaged time series were extracted for each seed and each subject. A time series was also extracted for the global signal. For each subject and for each network separately, a multiple regression

Table 2. Networks of interest

Network	Constituents	Function	Seed region	MNI Coordinates		
				x	y	z
Limbic network	Amygdala, insula, hypothalamus, hippocampus, mPFC	Stress reactivity and emotion processing	Left Amygdala	-20	-6	-16
			Right Amygdala	26	-2	-18
Salience Network	Bilateral anterior insula dACC	Assessing the relevance of internal and external stimuli in order to guide behavior	Left dACC	-6	18	28
			Right dACC	6	18	28
Default Mode Network	Precuneus, PCC, mPFC, and medial, lateral and inferior parietal cortex	Retrieval and manipulation of episodic memories and semantic knowledge, self-referential processing and prospective memory	PCC	-2	-36	37
Left mPFC			Left mPFC	-11	23	40
Confound Regressors			Left WM	-24	26	18
			Right WM	24	26	18
			Left CSF	-4	4	14
			Right CSF	4	4	14

This table describes each of the studied networks, the regions involved in the networks, the function of the networks, the chosen seed regions to probe the networks and the MNI coordinates of the chosen seeds. MNI = Montreal Neurological institute; dACC = dorsal anterior cingulated cortex; PCC = posterior cingulated cortex; mPFC = medial prefrontal cortex; CSF = cerebrospinal fluid; WM = white matter.

analysis was performed using the general linear model (GLM) as implemented in FSL (Smith et al., 2004). The timecourses that were extracted from the voxels in all of our seed regions were entered as a regressor in a GLM for each network separately. Nine nuisance regressors were included in the model: signal from the white matter, CSF signal, and the global signal, as well as six motion parameters (three translations and three rotations). The global signal was included to reduce artifacts associated with physiological signal sources (i.e. cardiac and respiratory; (Raichle et al., 2001; Birn et al., 2006). After reslicing the resulting individual correlation maps and their corresponding within-subject variance maps into 2 mm isotropic MNI space, they were entered into a higher level within and between groups mixed effects analysis (one- and two-sample t-test).

For each subject, gray matter density maps were derived from the anatomical scans. Participants in this study were drawn from the same sample (the NESDA) as the subjects used to investigate the structural abnormalities of childhood emotional maltreatment (van Harmelen et al., 2010). In this study differences were found in adults who experienced childhood emotional maltreatment when compared to adults who did not experience childhood emotional maltreatment. Therefore, in order to control for structural differences possibly confounding differences in functional connectivity and to correct for the effects of possible misregistration (Oakes et al., 2007), information about gray matter density of each subject was included as a voxelwise confound regressor. To achieve this, information about each voxel's gray-matter partial volume was directly fed into the GLM. Groups were compared using the GLM including age and scan location as additional covariates in each comparison. Cluster correction was applied in all group analyses with an initial cluster forming threshold of $Z > 2.3$ and a corrected $p < .05$.

Results

Psychometric data

In line with our expectancies the resilient group displayed significantly less symptoms than the vulnerable group based on the symptoms questionnaires (Beck Anxiety Inventory, Montgomery-Åsberg Depression Rating Scale and Inventory of Depressive Symptomatology). The resilient group did not differ on these questionnaires from the control group. When comparing the scores on the Neuroticism subscale of the Neuroticism Extraversion Openness Five Factor Inventory, the resilient group scored significantly lower on neuroticism compared to the vulnerable group, and higher on the extraversion and conscientiousness subscales. However, the resilient group did

not differ in scores on any of the subscales compared to the healthy control group. Importantly, type, frequency, and severity of childhood maltreatment did not differ between the resilient and vulnerable groups. Psychometric data are reported in Table 1.

Resting-state functional connectivity

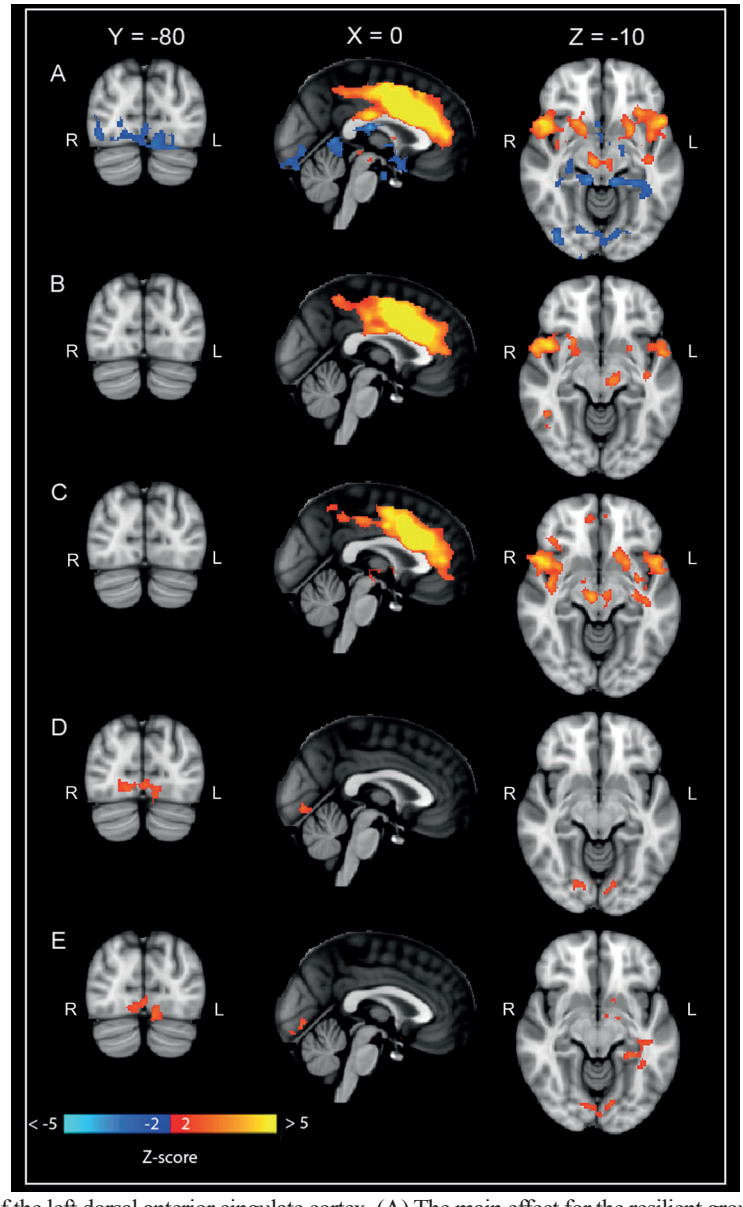
Analysis of the main effects of the seeds in each of the three groups showed connectivity between the seed chosen for the specific networks and other structures known to be constituents of these networks. In other words, our seeds probed the networks in a correct manner. Analysis of the left dACC seed (the seed in the salience network) revealed an increase in negative connectivity with a region including the lingual gyrus and the occipital fusiform gyrus in the resilient group when compared to the healthy controls. A similar increase in negative connectivity was found in these regions in the resilient group when compared to the non-resilient group (Figure 1). This means that, in the resilient group relative to both the vulnerable group and healthy controls, activation of the dACC is more strongly associated with deactivation of the lingual gyrus and the occipital fusiform gyrus and vice versa.

Discussion

The aim of this study was to investigate differences in RSFC associated with resilience to childhood maltreatment. We hypothesized alterations in RSFC associated with resilience from limbic network, salience network and DMN seeds and from a medial prefrontal region previously shown to be morphologically altered in a group of individuals who had been exposed to childhood maltreatment (van Harmelen et al., 2010). Analysis of the connectivity of the salience network seeds (the bilateral dorsal anterior cingulate cortex seeds) showed increased negative connectivity between the left dorsal anterior cingulate cortex seed and the bilateral lingual gyrus and occipital fusiform gyrus in the resilient group when compared to our vulnerable group, as well as when compared to the control.

Task related neuroimaging studies have shown that the lingual gyrus is associated with verbal declarative memory and identification of emotional facial expressions (Bremner et al., 2004; Kitada et al., 2010). In psychiatric studies, the lingual gyrus has been associated with trauma-related psychopathology (i.e. PTSD (Yin et al., 2011). It has also been shown that patients with PTSD display deficits in verbal declarative memory (Bremner et al., 2004). In addition, during exposure to traumatic stimuli, PTSD patients show an increase in activation of areas involved in memory and

Figure 1. Resting-state functional connectivity of the left dorsal anterior cingulate cortex



RSFC of the left dorsal anterior cingulate cortex. (A) The main effect for the resilient group. (B) The main effect for the vulnerable group. (C) The main effect for the control group. (D) The difference between the resilient group and the vulnerable group. (E) The difference between the resilient group and the control group. Images are z-statistics, overlaid on the MNI-152 1 mm standard brain. The left hemisphere of the brain corresponds to the right side of the images. MNI coordinates displayed at the top of the figure correspond with the coordinates of the displayed slices underneath the coordinates.

visuospatial processing, including the lingual gyrus, whereas non-PTSD trauma exposed individuals show a decrease of activation in the same regions (Bremner et al., 1999). However, due to the design of these studies it remains unclear whether these effects are to be attributed to the presence of psychopathology in PTSD patients or to the presence of resiliency factors in non-PTSD trauma exposed individuals. Our finding of increased RSFC between the left dACC and a region of the brain including the bilateral occipital fusiform gyrus and lingual gyrus in both our group comparisons, leads us to believe this might be specific for resilience. The difference in RSFC between the salience network (with its function to identify possibly harmful stimuli) and the bilateral occipital fusiform gyrus and lingual gyrus might reflect an increased ability of resilient individuals to identify and encode harmful experiences in verbal declarative memory. As impairment of this ability has often been related to the development of trauma-related disorders (Bremner et al., 2004; Samuelson, 2011), it could very well be that an increased ability facilitates resilience. This suggestion fits the dual representation theory, which states that in order to successfully process a trauma, the memories must be fully processed by the verbally accessible memory system, a system comparable to verbal declarative memory (Brewin et al., 1996). On a more psychological level, it has already been suggested that declarative memories can promote resilience through the capacity to evoke soothing emotional responses, which is a useful adaptive coping mechanism (Davis, 2001).

An alternative explanation may be based on the role of the lingual gyrus in the identification of emotional facial stimuli (Kitada et al., 2010). The salience network has a function that is closely related: assessing the relevance of internal and external stimuli. The association in RSFC between these brain regions in the resilience group could therefore be well explained on a functional level. As mentioned, our resting-state data were acquired as part of a fixed imaging protocol immediately after a facial expression task (van Tol et al., 2011). In this task a variety of faces with different emotions were presented and participants had to identify the gender. Previously, it was shown that childhood maltreatment is associated with enhanced amygdala reactivity to emotional (van Harmelen et al., 2013). It could be that processing of the stimuli during the facial expression task influenced our measure of RSFC in a different manner in the resilience group, compared to the vulnerable group and the healthy controls.

To the best of our knowledge, no previous study has been conducted that investigated RSFC patterns related to resilience. What is presently known about the neurological

mechanisms of resilience is often derived from studies comparing patients with trauma-related psychopathology with traumatized non-psychopathology individuals (for a review see: (van der Werff et al., 2013b). As these studies typically do not include healthy individuals without trauma exposure as a third group, it is not possible to determine whether group differences between trauma-exposed psychopathology and trauma-exposed non-psychopathology subjects are due to aberrant brain structures or functions in the patient group or to resilient characteristics in the non-psychopathology group. By adding a healthy control group without childhood maltreatment nor psychopathology, our design facilitated investigation of resilient specific RSFC patterns. We managed to match our subjects in a pair-wised manner, in this way controlling for age, gender, scan location and highest finished educational level. Importantly, we found an effect with the same direction and in the same region of the brain in two between-groups comparisons, making a stronger case for a resilient specific effect. Finally, our study facilitates replication and further research as a seed-based region-of-interest approach was used to analyze the data.

A limitation to our study is the small sample size of 11 individuals per group. Due to this group size findings should be considered preliminary. We have used a cross sectional approach for our study, therefore it is not possible to draw cause and effect conclusions from our findings. Another potential limitation is that it is unknown whether between-group differences in heart rate variability and breathing influenced the results, as we did not monitor physiological activity in the current study. However, regressing out global signal changes has shown to at least partly filter out the effects of cardiac and respiratory fluctuations (Raichle et al., 2001; Birn et al., 2006). Childhood maltreatment and severity were measured retrospectively using self-report, therefore the presence of a recall bias cannot be ruled out. Finally, as we discussed in our introduction resilience is a dynamic and multidimensional construct. We are aware that our operationalization of resilience, following previous studies on neurobiological characteristics of resilience (New et al., 2009), might not fully capture this dynamic and multidimensional nature of resilience.

In summary, our preliminary study shows alterations in RSFC in resilient individuals in a network involved in the processing of emotional stimuli and of areas of the brain involved in declarative memory. These alterations may be related to an increased ability of resilient individuals to identify and encode harmful experiences in verbal declarative memory. Future research should replicate our preliminary findings and further explore the specific neurobiological basis of resilience.

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Chapter 8

Structural and functional brain correlates of resilience to traumatic stress in Dutch police officers.

Discovering resilience in the brain

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In Preparation

Abstract

Background

Neurobiological research has traditionally focused on vulnerability rather than on resilience to severe stress. So far, only a few neuroimaging studies examining resilience have used designs that allow disentangling of the neural correlates of resilience from those related to psychopathology or trauma-exposure alone. The aim of the present study was to identify structural brain correlates of resilience, their specific functional resting-state connectivity patterns and correlations with behavioral measures.

Methods

Multimodal MRI scanning was performed in three groups of police officers: (1) a resilient group (N = 29; trauma-exposed, no psychopathology), (2) a vulnerable group (N = 33; trauma-exposed, psychopathology), and (3) a control group (N = 19; no trauma, no psychopathology). Using whole brain and region-of-interest approaches, we examined gray matter volume, volume and shape of the hippocampus, white matter integrity, and resting-state functional connectivity using software tools from the FSL-library.

Results

In resilient police officers we found an increase in structural connectivity in a part of the corticopontine tract. We did not find specific patterns for gray matter volumes or volume and shape of the hippocampus. Areas directly adjacent to the structural connectivity results showed increased resting-state functional connectivity with the precuneus/posterior cingulate cortex region. Both structural and functional connectivity results correlated with scores for specific emotion regulation strategies.

Conclusions

Resilient police officers show a specific pattern of increased structural and functional connectivity that implies regions involved in self-processing and is correlated with the use of higher order emotion regulation strategies.

Introduction

By virtue of their profession, police officers have a higher chance of experiencing traumatic events compared to the general population. Stringent selection criteria for admission to police academies, including an extensive psychological assessment, exist to safeguard an elevated level of resilience in police officers. Moreover, training methods have been applied to further increase resilience to stress in police forces. Although in some cases experiencing traumatic events may lead to the development of trauma-related disorders like posttraumatic stress disorder (PTSD), major depressive disorders and anxiety disorders (Carlier et al., 1997; Berg et al., 2006; Maguen et al., 2009), there is no evidence that police officers suffer from more psychiatric symptomatology compared to individuals without high-risk occupations (van der Velden et al., 2013). This makes the police force an interesting group to study in light of resilience.

There has been a long and extensive research history into intrapsychological and intersocial mechanisms influencing resilience. Mechanisms involved in resilience include emotion regulation, coping styles, social support, self-efficacy, self-esteem, personality characteristics (e.g. optimism and extraversion), and mindfulness (Southwick et al., 2013). However, despite progress in neuroscience methods, research into the neurobiology of resilience to traumatic stress is still very limited. Information we do have is often based on studies that examine PTSD patients compared to trauma-exposed non-PTSD individuals (for a review see: (van der Werff et al., 2013). With this comparison, however, it remains unclear whether differences found between these two groups are to be attributed to trauma-related symptomatology in the patient group, or to the resilience in the control group. To be able to identify alterations in brain networks associated with resilience, a third group of individuals without traumatic experiences and without psychopathologies should be added to the design. As only a comparison of these three groups can elucidate the specific characteristics of the resilient individuals compared to the other two groups and thus which of the effects are specifically associated with resilience. Structural neuroimaging studies using magnetic resonance imaging (MRI) to study gray matter in trauma-exposed twins and non-trauma-exposed co-twins suggest that an increased size of the hippocampus is related to resilience (Gilbertson et al., 2002; Kasai et al., 2008). This is plausible considering the role of the hippocampus in memory consolidation and the important role of memory in trauma-related psychopathology (i.e., intrusive memories of traumatic experiences). The hippocampus is also important for stress regulation and contains high levels of

mineralocorticoid receptors making it highly sensitive to glucocorticoids, which are released during the stress response. A reduction of hippocampal volume is often seen in stress-related psychiatric disorder (Gurvits et al., 1996; Bremner et al., 2003; Campbell et al., 2004; Kitayama et al., 2005; O'Doherty et al., 2015) and as a result of hypercortisolism (Starkman et al., 1992; Starkman et al., 1999).

The role of white matter structural connectivity has not yet been studied in the context of resilience. In stress-related disorders, decreases of white matter integrity of the uncinate fasciculus (Eluvathingal et al., 2006; Cullen et al., 2010) and the cingulum bundle (Fani et al., 2012; Daniels et al., 2013) have often been found, using diffusion tensor imaging (DTI). The uncinate fasciculus connects parts of the limbic system with the medial prefrontal cortex (mPFC). The mPFC inhibits fear responses and emotional responsiveness mediated by the amygdala (Sotres-Bayon et al., 2004), a process that has been found to be disturbed in stress-related psychiatric disorders, including PTSD (Elzinga and Bremner, 2002).

The cingulum bundle connects the hippocampus and the anterior cingulate cortex. The integrity of this tract directly influences the communication between the hippocampus and the anterior cingulate cortex, two regions that show altered function in PTSD (Elzinga and Bremner, 2002; Thomaes et al., 2013). However, it remains unclear whether these decreases in white matter integrity are purely associated the PTSD symptomatology or whether an increase of white matter integrity might also be indicative of resilience.

As to the best of our knowledge no study yet has examined the gray matter volume and white matter integrity correlates of resilience to traumatic stress in a design with a resilient group, a vulnerable group and a healthy control group.
We examined gray matter volume and white matter integrity to identify specific correlates of resilience in a cohort of Dutch police officers. Based on the existing literature we hypothesized to find an increase in gray matter volume of the hippocampus in trauma-exposed police officers without a history of psychopathology (the resilient group) compared to both trauma-exposed police officers with a history of psychopathology (the vulnerable group) and trauma non-exposed recruits from the police academy without a history of psychopathology (the control group). We also hypothesized to find an increase in white matter integrity of the uncinate fasciculus and the cingulum, specific for resilient officers. In addition to these region-of-interest (ROI) analyses, we performed an explorative whole brain analysis to detect structural correlates of resilience outside these a priori defined ROI's. In

addition, we explored associations between measures of structural connectivity and measures of functional connectivity as assessed with resting-state fMRI (RS-fMRI). For a better understanding of the results, we will associate these connectivity measures with resilience-related behavioral measurements.

Materials and Methods

Subjects

Trauma-exposed executive personnel of the Dutch police were recruited through advertisements on the intranet of the Dutch police force. For optimal homogeneity across groups the non-exposed healthy control group, was recruited from the Dutch police academy. A total of 149 subjects signed up and were screened for eligibility. Exclusion criteria for all subjects were: MRI contraindications such as metal implants, heart arrhythmia, claustrophobia and possible pregnancy, a history of neurological or other medical illness with central nervous system sequelae, the use of psychotropic medications other than stable use of SSRI's or infrequent benzodiazepine use (i.e., equivalent to 2 doses of 10 mg of oxazepam 3 times per week as a maximum and refrain from use 48 hours before scanning), a history of childhood maltreatment (i.e. < 18 years), a history of psychopathology with onset before work related traumatic events, left-handedness, insufficient knowledge of the Dutch language, and smoking > 5 cigarettes a day on average. 86 subjects were invited to participate in the study. Five subjects were excluded from the study after quality checking the MRI data, due to imaging artifacts in their respective MRI scans. The resulting 81 subjects were divided into three groups based on clinical assessment. The resilient group (N = 29) consists of individuals who report having experienced traumatic events, and do not fulfill the criteria for any DSM-IV diagnoses, either current or past. The vulnerable group (N = 33) consists of individuals who report having experienced traumatic events and fulfill the criteria for one or more DSM-IV diagnoses, either current or past. Individuals in this group met the criteria for the following diagnoses at least once in their lives, after graduating from the Police Academy: major depressive disorder (n = 27), panic disorder (n = 3), agoraphobia (n = 7), specific phobia (n = 1), social phobia (n = 2), generalized anxiety disorder (n = 2), posttraumatic stress disorder (n = 14), substance abuse (n = 8). The control group (N =19) consists of trainees recruited from the police academy who report no exposure to traumatic experiences and do not meet the criteria for any DSM-IV diagnosis in the present or past. Written informed consent was obtained from all participants before the clinical assessment and the MRI scan session. The medical ethical committee of the Leiden University Medical Center approved the study protocol. This study was

designed and conducted in accordance with the principles of the declaration of Helsinki.

Behavioral Assessment

Past and current DSM-IV axis-1 psychiatric disorders were assessed using the mini-international neuropsychiatric interview (MINI)(van Vliet and de Beurs, 2007).
Severity of depressive symptoms were evaluated using the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979), and the Inventory of Depression Symptomatology (IDS) (Rush et al., 1996). Severity of anxiety symptoms was assessed using Becks Anxiety Inventory (BAI) (Beck et al., 1988). The Harvard Trauma Questionnaire (HTQ) was used to inquire trauma-related symptom severity (Mollica et al., 1992). The Connor-Davidson Resilience Scale (CD-RISC) was used to assess self-report resilience (Connor and Davidson, 2003). The Police Life Events Schedule (PLES) was used to assess the amount of exposure to work-related life events (Carlier et al., 1997). The Cognitive Emotion Regulation Questionnaire (CERQ) was used to assess cognitive coping strategies. This questionnaire consists of nine subscales which all measure a different coping strategy (Garnefski et al., 2001).

MRI Data Acquisition

Images were acquired on a Philips 3T MRI system (Philips Healthcare, Best, The Netherlands; software version 3.2.1). A SENSE-32 channel head coil was used for radio frequency transmission and reception.

For each subject four different scans were acquired including a high resolution anatomical scan, which was obtained using a sagittal three-dimensional gradient-echo T1-weighted sequence (repetition time=9.8 ms, echo time=4.6 ms, matrix size 256x256, voxel size 1.17x1.17x1.2 mm, 140 slices, scan duration 4:56 min).
DTI scans were acquired using a single-shot echo-planar imaging sequence with the following scan parameters: repetition time = 6250 ms, echo time = 70 ms, flip angle = 90°, b-factor = 1000 s/mm2, voxel dimensions = 2.07 x 2.12 x 2.10 mm, number of slices = 60, and no slice gap. DTI data were acquired along 32 directions, together with a baseline image having no diffusion weighting (b = 0). Total DTI scanning time was ~8 min.

RS-fMRI scans were acquired using T2*-weighted gradient-echo echo-planar imaging with the following scan parameters: 200 whole-brain volumes; repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, flip angle = 80°, 38 slices, matrix size =

80 x 80, voxel size = 2.75 x 2.75 x 2.75 mm, scan duration = 7:28 min). Beforehand, the participants were instructed to lie as still as possible, with their eyes closed and without falling asleep. After completion of the scan all participants confirmed not having fallen asleep.

A high-resolution T2*-weighted gradient-echo echo-planar imaging scan (TR = 2200 ms, TE = 30 ms, flip angle = 80°, 84 axial slices, matrix size = 112 x112, voxel size =1.96 x 1.96 x 2 mm, no slice gap, scan duration = 46.2 s) was acquired for registration of the functional data to standard space.
A neuroradiologist, blinded for the clinical details of the subjects, examined all high resolution anatomical scans. No macroscopic abnormalities were observed.
All MRI data were processed using FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; (Smith et al., 2004) version 5.0.6).

Diffusion tensor imaging preprocessing
DTI data were corrected for distortion and motion artifacts induced by eddy currents or by simple head motions, using affine registration of each diffusion weighted image to the b = 0 reference image. Non-brain tissue was removed using the Brain Extraction Tool. Following, in order to generate individual fractional anisotropy (FA) maps for each participant, the diffusion tensor model was fitted to each voxel using FMRIB's diffusion toolbox (FDT). Tract-based spatial statistics (TBSS) version 1.2 was used for voxelwise analysis of the preprocessed FA data. First, individual FA images were aligned to the FMRIB58_FA standard-space image using nonlinear registration. Next, the mean FA image was generated and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the entire group. The mean FA skeleton was then thresholded at a FA value of ≥0.45 to exclude peripheral tracts and minimize partial voluming. Finally, each participant's aligned FA images were projected onto the mean FA skeleton, and the resulting data were fed into voxelwise permutation-based analysis.

Resting-state fMRI preprocessing
Motion correction was applied to the RS-fMRI data along with non-brain tissue removal, spatial smoothing using a 6-mm full-width at half maximum (FWHM) Gaussian kernel, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, 0.01 Hz cut-off). The RS-fMRI data of each participant were then registered to their respective high-resolution EPI images. The high-resolution EPI image was registered to the T1-weighted image,

and the T1-weighted image to MNI-152 standard space image. Resting-state data were analyzed using seed-based correlations. The seed was chosen immediately adjacent to the results of the DTI analysis. FSL-FIRST was used to segment this seed for each participant individually. These individual masks were then transformed to functional native space using the inverse transformation matrices obtained during registration in the preprocessing phase. Spatially averaged time series were extracted for each subject. A time series was also extracted for the global signal, white matter and cerebrospinal fluid. The timecourses that were extracted from the individual registered left putamen seeds were added as a regressor in a GLM. Nine nuisance regressors were included in the model: signal from the white matter, CSF signal, and the global signal, as well as six motion parameters (three translations and three rotations). The global signal was included to reduce artifacts associated with physiological signal sources (i.e. cardiac and respiratory; (Raichle et al., 2001; Birn et al., 2006). After reslicing the resulting individual correlation maps and their corresponding within-subject variance maps into 2 mm isotropic MNI space, they were entered into a higher level within and between groups mixed effects analysis (one- and two-sample t-test).

Gray Matter analyses

Details on MRI data acquisition, MRI preprocessing and behavioral measurements are reported in the supplementary materials.

All MRI analyses were conducted in FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl; (Smith et al., 2004) version 5.0.6). To test our hypothesis that size of the hippocampus is different in resilient individuals we used FSL's Integrated Registration and Segmentation Tool (FIRST) to automatically segment both the left and right hippocampus and construct them as vertexes, which also allows comparisons of shape. This method searches through linear combinations of shape modes of variation for the most probable shape instance given the observed intensities in the T1-weighted image, based on learned models constructed from 336 subjects (Patenaude et al., 2011). To test whether there were any between-groups differences in shape of the hippocampus a 1x3 ANOVA design was modeled in the general linear model (GLM), with age and gender included as confound regressors, and tested using FSL's Randomise Tool, permutation-based (5000 permutations) non-parametric testing, correcting for multiple comparisons across space. Threshold free cluster enhancement (TFCE) was used for finding clusters in the data and threshold for significance was set on $p < 0.05$ TFCE corrected.

The explorative whole brain analysis was performed using a voxel-based

morphometry approach (VBM) implemented in FSL. First, structural images were brain extracted and gray matter-segmented (Zhang et al., 2001). The resulting gray matter partial volume images were then aligned to MNI-152 (T1 standard brain average over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) standard space, using affine registration, followed by nonlinear registration (Jenkinson et al., 2002). The resulting images of all participants were averaged to create a study-specific template, to which the native gray matter images were then nonlinearly reregistered. In order to correct for local expansion or contraction, the registered partial volume images were then modulated by dividing them by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. The Gaussian outputs a weighted average of each voxel's neighborhood, with the average weighted more toward the value of the centrally located voxels. The application of this type of smoothing reduces the noise in the data substantially. Finally, groups were compared using a GLM including age and gender as confound regressors. To investigate where the resilient group differentiated from both the vulnerable group and the control group the voxel-wise GLM tested an inversed quadratic model, using permutation-based (5000 permutations) non-parametric testing, correcting for multiple comparisons across space. TFCE was used for finding clusters in the data, with thresholds for both the ROI comparison as well as the whole brain analysis set on $p < 0.05$ corrected.

Diffusion tensor imaging analyses

To test for regional specific fractional anisotropy (FA) alterations, we first implemented a ROI-based tract-based spatial statistics (TBSS). A binary mask encompassing the bilateral uncinate fasciculus and the cingulum was created as a ROI using the Johns Hopkins University White Matter Atlas provided by FSL, with probability set to 15%-100%. The mask was then applied to the mean FA skeleton in order to include only voxels comprised in the mean FA skeleton. This confines the statistical analysis exclusively to voxels from the center of the tract, thereby minimizing anatomic inter-subject variability, registration errors, and partial voluming. The resulting study-specific ROI mask was used for voxelwise permutation-based ROI analysis.

Using FSL's Randomise Tool, permutation-based inferences with TFCE were carried out for voxelwise analysis of FA data. In both the ROI analysis and the whole brain analysis 5000 random permutations were generated to build up the null distribution of the cluster size statistic, while testing an inversed quadratic model to investigate where the resilient group differentiated from both the vulnerable group and the

control group. Age and gender were included in the analysis as confound regressors to correct for between group variances. The resulting statistical maps were corrected for multiple comparisons ($p < 0.05$, TFCE corrected).

Post-Hoc Analyses

To enable further interpretation of the TBSS results a mask was created of the voxels that were found to differ significantly between groups on FA. Along with this mask, information on each individual's axial diffusivity (the 1st eigenvalue), radial diffusivity (the average of the 2nd and 3rd eigenvalues), and mean diffusivity was fed into FSL's Randomise Tool using permutation-based inferences with TFCE.

RS-fMRI scans were used to examine whether structural connectivity specific to resilient subjects was accompanied by differences in resting-state functional connectivity (RSFC) seeded from areas adjacent to the structural connectivity results. The seed was chosen based on the results of the DTI analysis. FSL-FIRST was used to segment this seed for each participant individually.

Individual correlation maps and their corresponding within-subject variance maps were entered into a higher level within and between groups mixed effects analysis (one- and two-sample t-test). For each subject, gray matter density maps were derived from the anatomical scans. To correct for the effects of possible misregistration (Oakes et al., 2007), information about gray matter density of each subject was included as a voxelwise confound regressor. To investigate where the resilient group differentiated from both the vulnerable group and the control group GLM tested an inversed quadratic model including age and gender as confound regressors. Cluster correction was applied with an initial cluster forming threshold of $Z > 2.3$ and a corrected $p < .05$.

To assess whether any of the effects are associated with specific emotion regulation styles within the resilient group, correlation analyses were performed using the FA values for structural connectivity, the Z-scores for the RSFC, and the scores on the CD-RISC and the nine subscales of the CERQ. Correlation analyses were performed using Pearson's r or, when data violated assumptions for parametric tests, with Kendall's tau.

Table 1. Demographics and Psychometric data

	Resilient		Vulnerable		Controls			
	N		**N**		**N**		***p* 1 vs 2**	***p* 1 vs 3**
N	29		33		19			
Females/Males	10/19		8/25		11/8		.413 [a]	.143 [a]
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***p* 1 vs 2**	***p* 1 vs 3**
Age	40.24	11.84	44.24	11.19	25.16	4.63	.178 [b]	**<.001** [b]
IDS	36.10	6.97	43.67	12.55	33.37	5.70	**.008** [b]	.091 [b]
BAI	24.07	2.82	26.18	6.5	24.06	2.92	.246 [b]	.965 [b]
MADRS	1.72	2.36	5.21	7.53	.21	.713	.195 [b]	**.001** [b]
CD-RISC	98.96	12.06	92.09	14.24	102.63	9.75	**.05** [c]	.261 [c]
HTQ	34.72	5.13	43.67	14.67	33.95	5.45	**.008** [b]	.619 [b]
PLES (before outlier omission)	168.39	140.25	334.28	619.52	25.95	53.94	.534 [b]	**<.001** [b]
PLES (after outlier omission)	168.39	140.25	235.77	257.20	25.95	53.94	.671 [b]	**<.001** [b]
CERQ: Self-blame	7.65	2.74	8.52	3.29	8.16	2.32	.308 [b]	.394 [b]
CERQ: Blaming others	5.79	1.82	7.24	2.59	5.53	1.68	**.024** [b]	.703 [b]
CERQ: Acceptance	10.38	2.93	12.30	3.19	12.80	3.34	**.017** [b]	**.018** [b]
CERQ: Refocus on planning	13.69	3.71	13.81	3.18	14.47	2.80	.898 [b]	.503 [b]
CERQ: Positive refocusing	11.66	4.29	11.33	3.36	12.11	3.51	.742 [c]	.705 [c]
CERQ: Rumination	9.93	3.91	11.94	6.75	9.31	3.38	.150 [b]	.626 [b]
CERQ: Positive reappraisal	14.59	3.49	14.00	3.86	15.37	3.44	.723 [b]	.433 [b]
CERQ: Putting into perspective	11.66	4.15	11.30	3.37	13.16	3.53	.714 [c]	.201 [c]
CERQ: Catastrophizing	4.76	1.33	6.45	3.03	4.84	1.21	**.001** [b]	.767 [b]

[a] = Chi-Square test; [b] = Mann-Whitney U test; [c] = Independent Sample T-test. IDS = Inventory of depression symptomatology; BAI = Becks Anxiety Inventory; CD-RISC = Connor-Davidson Resilience Scale; HTQ = Harvard Trauma Questionnaire; MADRS = Montgomery-Asberg Depression Rating Scale; PLES = Police Life Events Schedule; CERQ = Cognitive Emotion Regulation Questionnaire. In bold are all p-values considered significant ($p < .05$).

Results

Psychometric data

Demographic and psychometric data are reported in Table 1. As expected, there was an age difference between the resilient group and the control group ($p < .001$), but not between the resilient group and the vulnerable group ($p = .178$). Furthermore, the resilient group reported higher scores on the CD-RISC compared to the vulnerable group ($p = 0.05$), but not compared to the control group ($p = .261$).

Scores on the PLES indicated that the resilient group experienced more work related life events compared to the control group ($p < .001$), but not compared to the vulnerable group ($p = .534$) validating our selection procedures. One outlier was present in the vulnerable group, reporting 3388 work-related life events. As a member of a vice squad, the subject reported over 3000 cases of exposure to adult sexual abuse; roughly one experience every day for the last 10 years. After omission of this outlier, mean scores of the resilient group and the vulnerable group on number of experienced work-related life events grew even closer ($p = .671$). The vulnerable group reported more trauma-related symptoms on the HTQ compared to the resilient group ($p = .008$), as well as higher depression scores on the IDS ($p = .008$). The average MADRS score for all the groups was below the norm for residual depressive symptoms (MADRS score < 6), with significantly lower scores in the resilient group compared to the vulnerable group ($p < 0.01$).

With respect to cognitive emotion regulation strategies measured using the CERQ, we found that the resilient group scored lower on the subscale acceptance compared to both the vulnerable group (p.017) and the control group ($p = .018$). In addition, the resilient group scored lower on blaming others ($p = .024$) and catastrophizing ($p = .001$) compared to the vulnerable group.

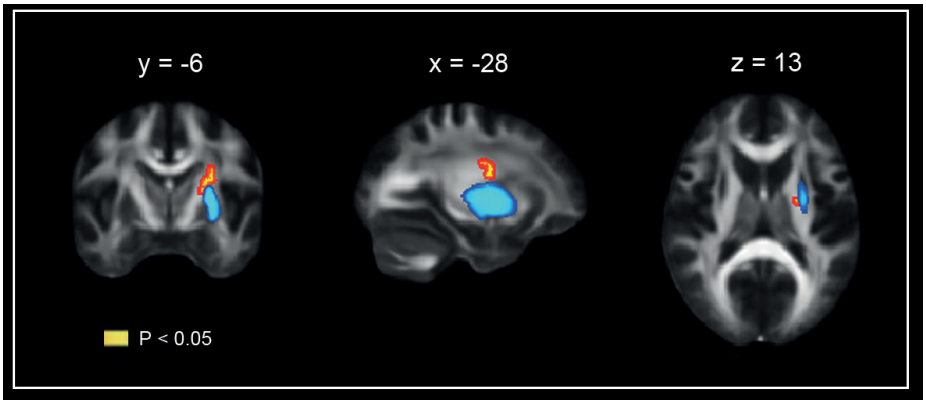
Gray Matter Structure Results

The shape analysis for both left ($p > 0.955$) and right ($p > 0.58$) hippocampus did not show significant differences in shape between groups. Information about individual volumes of the left and right hippocampus was extracted and subsequently analyzed using IBM SPSS statistics 20. A 1x3 ANCOVA test was used controlling for age and gender, both left ($p = .955$) and right ($p = .931$) hippocampus did not differ in volume between groups. The explorative whole brain VBM testing the inversed quadratic design did not show any significant results in gray matter volume with $p < 0.57$ for all voxels.

Tract-based spatial statistics

The ROI analysis of the FA values in the bilateral uncinate fasciculus and the cingulum bundle showed no significant inversed quadratic effect (for all voxels: p >.77, TFCE corrected). The explorative whole brain analyses, however, showed a significant inversed quadratic effect in FA values of the left corticopontine tract starting adjacent to the left putamen, leading up to cortical areas where it bends in the parietal direction (p <.05, TFCE corrected; Figure 1). Post-hoc analyses of the axial diffusivity, radial diffusivity, and mean diffusivity revealed that the effect in FA was driven by decreases of radial diffusivity and mean diffusivity (for both: p <.001) in the resilient group. The axial diffusivity values did not differ significantly between groups.

Figure 1. Structural connectivity specific for resilience



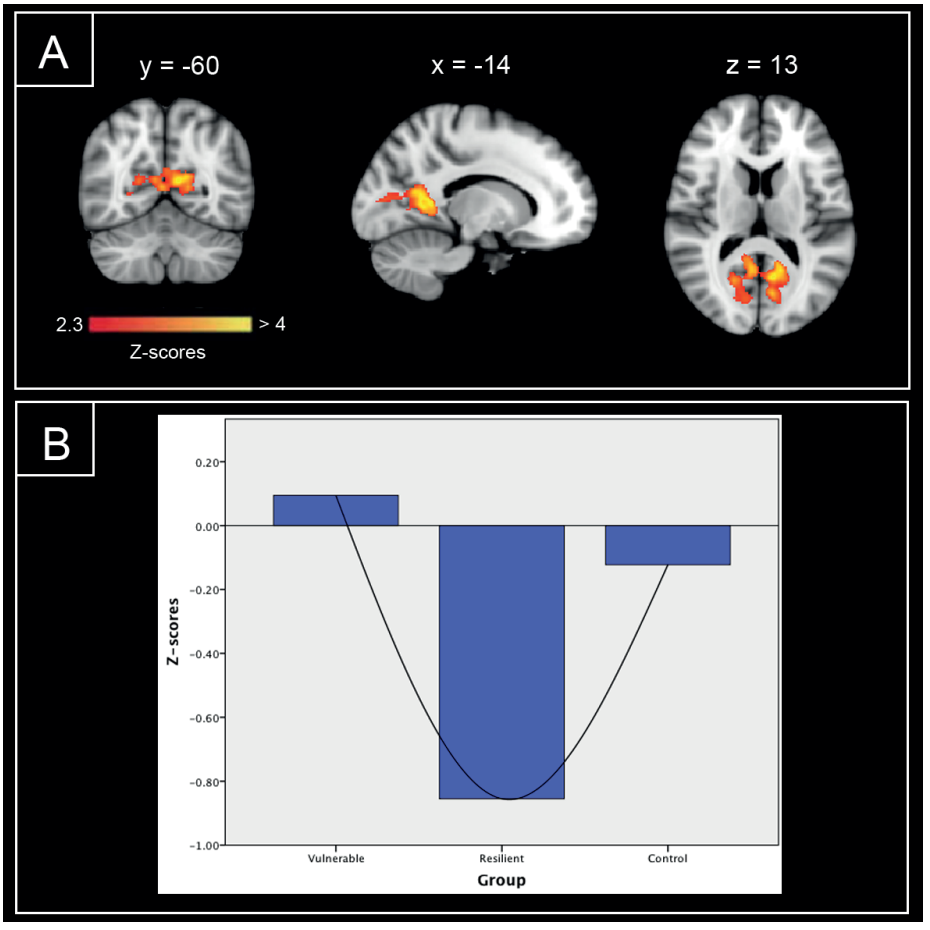
Coronal, sagittal and transversal slices of the FMRIB58_MNI_1mm standard brain (gray). Superimposed are the voxels that were found to have a significantly increased FA value in the resilient group (yellow; p <.05, TFCE corrected). For visibility reasons the tbss_fill command was used to thicken the effect (red). To further clarify the location of the effect, the left putamen is depicted (blue; Harvard-Oxford Subcortical Structural Atlas, probability: 30 -100 %). Images are in radiological convention (the right side of the image corresponds with the left hemisphere and vice versa).

Post-hoc resting-state functional connectivity analyses

Based on the location of the effect in white matter integrity, the seed of the RSFC was placed in the left putamen. Using FSL-FIRST, for each individual the left putamen was segmented and subsequently used as a seed in the RSFC analysis. We found increases in RSFC between the left putamen and an area containing the posterior cingulate cortex (PCC) and the precuneus, specific for resilient individuals (Figure 2a). Further investigation of the individual Z-scores indicating the strength

of connectivity between the left putamen seed and the PCC/precuneus revealed that this effect was driven by an increase of negative connectivity in the resilient group compared to the other groups (Figure 2b).

Figure 2. Resting-state functional connectivity with the left putamen, specific for resilience



Coronal, sagittal and transversal slices of the MNI-152 1mm standard brain (gray). Superimposed is the region that was found significant in quadratic design of the study (red/yellow), indicating the pattern in resting-state functional connectivity with the left putamen specific for the resilient group. The effect resembles z-statistics with an initial cluster-forming threshold of $z > 2.3$ and $p < .05$, corrected. Images are in radiological convention (the right side of the images corresponds with the left hemisphere and vice versa (B). The mean z-score of the area that was tested significant depicted separately for each group. The line resembles the quadratic effect.

Post-hoc correlation analyses

To assess whether FA values in the area with significantly increased FA were related to self-report resilience or emotion regulation strategies within the resilient group, the mean FA values from this specific area were extracted and exported to SPSS.

The correlation coefficients of the FA values in the corticopontine tract with the CD-RISC scores and with the nine subscales of the CERQ were examined (Table 2). To correct for multiple comparisons we applied a Bonferroni correction and adjusted the level of significance to p <.005. We found a significant correlation between the positive reappraisal subscale of the CERQ and the FA values within the resilient group (p =.004). In addition, we found a significant correlation between the Z-scores reflecting the strength of the RSFC between the left putamen seed and the PCC/ precuneus and the positive reappraisal subscale of the CERQ (p =.037)

Table 2. Intercorrelations between structural connectivity and behavioral scales in resilient individuals

	FA		
Scales	**Pearson's r**	**Kendall's tau**	***p***
CD-RISC	.346		.066
CERQ: Self-blame	.000		1.00
CERQ: Blaming others		.058	.682
CERQ: Acceptance		.327	.018 *
CERQ: Refocus on planning	.426		.021 *
CERQ: Positive refocusing	.429		.020 *
CERQ: Rumination		.186	.168
CERQ: Positive reappraisal	.513		.004 **
CERQ: Putting into perspective	.145		.453
CERQ: Catastrophizing		.054	.716

FA = Fractional anisotropy; CD-RISC = Connor-Davidson Resilience Scale; CERQ = Cognitive Emotion Regulation Questionnaire.
* = $p < .05$ (uncorrected); ** = $p < .005$ (bonferroni corrected)

Discussion

In this study we set out to investigate the gray matter structure and structural connectivity characteristics of resilience to traumatic stress in a sample of Dutch police officers. Considering that there is a clear lack of neurobiological studies focusing resilience, our hypotheses were based on previous studies on stress-related psychopathologies, such as PTSD. We hypothesized that resilient police officers would be characterized by increased volumes of the hippocampus, and an increase in white matter integrity of the uncinate fasciculus and cingulum bundle. We also performed additional explorative whole brain analyses on both volume and structural connectivity.

Using FSL-FIRST, we found no increases in volume of the hippocampus. In addition, the explorative whole brain analysis we performed using VBM showed no gray matter characteristics specific for resilience. Smaller hippocampi have been found to be associated with stress-related disorders (Gurvits et al., 1996; Bremner et al., 2003; Campbell et al., 2004), and as a result of hypercortisolism (Starkman et al., 1992; Starkman et al., 1999). However, there has been an ongoing debate on whether an increased size of the hippocampus could also be a marker of improved resilience. Our data in this particular cohort do not support such notion.

With respect to structural connectivity, we did not find increased white matter integrity of the uncinate fasciculus and cingulum in the resilient group, as we had hypothesized. However, our explorative whole brain analysis did indicate increases in white matter integrity of a part of the corticopontine tract starting adjacent to the left putamen, leading up to cortical areas where it bends in the parietal direction. The fact that these changes in white matter integrity were found in a new area that has not been reported in the PTSD literature suggests that resilience is not simply the opposite of having psychiatric symptoms, but rather an independent construct.

To be able to further interpret these findings in FA, we examined the axial diffusivity, radial diffusivity, and mean diffusivity values. We found that the increased FA in the resilient group was mostly driven by differences in radial diffusivity and mean diffusivity in the resilient group, and not by differences in axial diffusivity. This pattern of decreases in mean diffusivity and radial diffusivity is an indication for increaed myelination of the white matter tract in resilient individuals (Song et al., 2005; Alexander et al., 2007). Increased myelination promotes speed at which impulses travel along the myelinated tract, therefore increasing both structural and

functional connectivity between areas connected by these fibers (Hartline, 2008). Previous tractography studies of the putamen confirmed that tracts originating from the putamen follow the corticopontine tract in dorsal direction to the cortex, where they connect to both frontal and parietal regions (Leh et al., 2007; Jarbo and Verstynen, 2015). To investigate whether the pattern of structural connectivity in the resilient group was accompanied by similar patterns in functional connectivity, we conducted a resting-state functional connectivity analysis using the left putamen as a seed. We found increases in RSFC between the left putamen and an area including the PCC and the precuneus specific for the resilient group. The finding of negative connectivity between these two areas is in line with previous research examining RSFC using a seed in the putamen (Di Martino et al., 2008). The precuneus /PCC region is an area involved in self-referential processing, self-consciousness, and autobiographic memory (Cavanna and Trimble, 2006; Cavanna, 2007). With respect to resilience it has already been shown that psychological constructs that influence resilience include self-efficacy and self-esteem, which suggests that activity and connectivity of the precuneus / PCC region could play an important role in resilience. Additionally, this area is an important constituent of the default mode network and aberrant functioning of the default mode network has been implicated in various psychiatric disorders including depression (Sambataro et al., 2013), anxiety (Zhao et al., 2007), and PTSD (Lanius et al., 2010). The putamen is, in addition to being involved in motor skills, also involved in learning and memory. More specifically, the putamen has an active role in the acquisition (learning) and storage (memory) of stimulus-response associations (Packard and Knowlton, 2002). The increases in both structural and functional connectivity between the precuneus/PCC region and the left putamen could indicate interplay between stimulus-response learning based on experiences (including severe stressful stimuli like traumatic events) and the image an individual has of oneself. Automatic negative self-cognitions are a symptom of stress-related disorders and increased connectivity between the putamen and the PCC / precuneus area might protect an individual from acquiring such symptoms as stimulus-response learning can be more effectively influenced by higher order self-images and processing. This notion is strengthened by the results of the correlation analyses. Within the resilient group we found a significant correlation between positive reappraisal and the FA values in our found effect. Moreover, we also found a negative correlation between the strength of the RSFC between the left putamen and the precuneus/PCC region and positive reappraisal within the resilient group. Of note, positive reappraisal did not differ between groups, whereas acceptance scores were lower in the resilient group compared to the other two groups. This could indicate a proactive attitude of resilient individuals, and a drive towards a

willingness to change negative circumstances rather than accepting them.

There are some limitations to take into account. First, due to our cross-sectional design no causal conclusions can be drawn from the data. We cannot conclude whether the effects in structural and functional connectivity have always been present in the high resilient individuals, or were acquired under influence of severe stressful situations. Second, we did not find effects in the hypothesized ROI's, which were based on studies focusing on psychiatric symptomatology, but only in our whole brain analyses, giving this study an explorative nature.

To the best of our knowledge this is the first study specifically designed to investigate structural and connectivity characteristics of resilience, within a homogenous group of police officers. Future studies should include task-related fMRI paradigms, which could contribute to investigate changes in brain activity specific for resilience during the demands of externally presented tasks (i.e., during a stress reaction, or during emotion regulation). Furthermore, longitudinal designs should be implemented to enable investigation of the causal pathway of the neural correlates of resilience. We also recommend adding a non-exposed healthy control group from the general population to provide more variation, as the majority of the police officers is already high resilient compared to the general population due to selection and training.

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Chapter 9

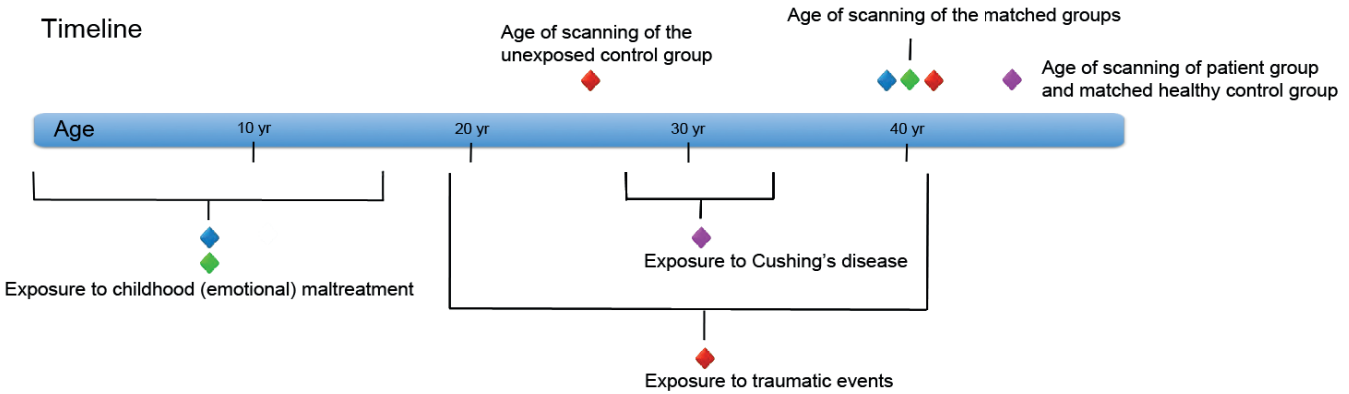
Summary and general discussion

The human body is inherently designed to be able to adapt to challenging situations. However, some experiences are so severe that they can lead to substantial and longer-lasting disturbances in an individuals' behavioral, psychological and physiological functioning. Importantly, there is a degree of inter-individual variation, as not all individuals show similar reactions to severe stress. Neuroimaging techniques can help to unravel the brain characteristics related to vulnerability and resilience to severe stress.

In the research for this dissertation several neuroimaging modalities were used to further explore the brain characteristics related to (dys)function after exposure to severe stress and after exposure to hypercortisolism, such as voxel-based morphometry and diffusion tensor imaging to study the structure of gray and white matter in the brain, and resting-state fMRI to study functional connectivity patterns. We studied brain characteristics in several groups: a group of patients in long-term remission of Cushing's disease and a group of individuals with a history of childhood emotional maltreatment to examine the effects of hypercortisolism and severe stress on the brain. In addition, we studied a group of police officers and a group of individuals with a history of childhood maltreatment to investigate the brain characteristics related to resilience to stress.

Figure 1. Summary of each Chapters findings and timeline

- Chapter 2: Childhood emotional maltreatment: Decreased RSFC between the right amygdala and the bilateral precuneus, and left insula. And between the dorsal ACC and the precuneus, and frontal parts.
- Chapter 3: A history of Cushing's disease: Decreased gray matter in the ACC and increased gray matter in the cerebellum
- Chapter 4: A history of Cushing's disease: Widespread reductions of white matter integrity
- Chapter 5: A history of Cushing's disease: Increased RSFC between the limbic network and the subgenual ACC, increased RSFC between the DMN and the left lateral occipital cortex
- Chapter 7: Resilience to childhood maltreatment: Increased RSFC between the dorsal ACC and the lingual gyrus and occipital fusiform gyrus
- Chapter 8: Resilience to traumatic stress: Increased white matter integrity of the corticopontine tract; and increased RSFC between the putamen and the precuneus.



Summary of results

Section I: Brain correlates related to severe stress exposure and their consequences
A short summary of the most important finding per Chapter is given in Figure 1, along with a timeline showing when the samples we used in each of the Chapters were exposed to stressful events or hypercortisolism and when the MRI scan was conducted.

Resting-state functional connectivity characteristics of adults with a history of childhood emotional maltreatment both with and without depression and anxiety were examined in Chapter 2, using a seed-based correlational approach. Four resting-state networks were examined: The limbic network, the salience network, the default mode network, and a network seeded by a location in the medial prefrontal cortex based on volumetric differences found in previous research (van Harmelen et al., 2010). The resting-state functional connectivity strengths of these networks were compared between a group of adults with a history of repeated emotional maltreatment during childhood (CEM; N = 44), and a control group of adults without a history of emotional maltreatment (N = 44). For the limbic network, the CEM group showed a decreased connectivity between the right amygdala and the bilateral precuneus and bilateral occipital cortex, as well as increased connectivity between the right amygdala and the other parts of the limbic network, including the putamen and the hippocampus in the left hemisphere of the brain. For the salience network, the CEM group showed decreased connectivity between the left dorsal anterior cingulate cortex seed and a region containing the angular cortex and the precuneus cortex, as well decreased connectivity between this seed and the medial prefrontal cortex. We found no differences between groups in connectivity with the default mode network and the left medial prefrontal cortex.

In Chapter 3, the effects of a history of excessive endogenous cortisol exposure resulting from Cushing's disease on gray matter volumes in the brain were investigated. Using a voxel-based morphometry method we found decreased gray matter volume in the anterior cingulate cortex, but were unable to associate gray matter volumes of this area to behavioral and cognitive functioning in the patients with long-term remission of Cushing's disease. In addition, we found an increased gray matter volume of the left posterior lobe of the cerebellum.

The structural connectivity characteristics related to a history of Cushing's disease were examined in Chapter 4, using a tract-based spatial statistics approach on

diffusion tensor imaging scans. All of our a priori hypothesized regions-of-interest showed reduced white matter integrity in the group of patients with a history of hypercortisolism. In addition, we found a relation within the patient group between depression scores and fractional anisotropy values in the left uncinate fasciculus, a white matter tract that has been implicated with major depressive disorder before. The findings of reduced white matter integrity in our regions-of-interest were put into another perspective when we found widespread reductions in white matter integrity throughout the brain, using an explorative whole brain analysis. These results indicated that effects of hypercortisolism on white matter integrity are global rather than localized.

Chapter 5 describes the resting-state functional connectivity characteristics of patients with remission of Cushing's disease. A probabilistic independent component analysis was used to extract data driven independent networks. Using visual checks, three a priori hypothesized networks were identified, which included the limbic network, the default mode network and the executive control network. An increase in resting-state functional connectivity was found in the patient group between the limbic network and the subgenual subregion of the anterior cingulate cortex, as well as an increase in connectivity between the default mode network and the left lateral occipital cortex. These findings were not associated with any of the behavioral measurements.

Section II: Brain characteristics of resilience to severe stress exposure

Chapter 6 describes the state of the art knowledge on the characteristics of brain structure and function related to resilience to stress as published until 2011. Typical regions of interest included the hippocampus, the amygdala, the anterior cingulate cortex and the prefrontal cortex. Most studies used a design that compared trauma-exposed non-PTSD (resilient) individuals with PTSD individuals. One of the most important conclusions of this review is that this design is insufficient to distinguish between characteristics related to PTSD, resilience or mere exposure. Therefore, alternative designs are described that enable disentangling the effects related to PTSD, resilience and trauma exposure, including longitudinally designed studies, and a three-group design with an additional non-trauma-exposed non-PTSD (healthy control) group.

In Chapter 7 we put into practice one of the suggested designs to study the resting-state functional connectivity characteristics of resilience to childhood maltreatment. We compared three groups (all N=11), including a resilient group of adults who

experienced childhood maltreatment without developing psychiatric disorders in later life, a vulnerable group of adults who experienced childhood maltreatment and developed a psychiatric disorder in later life, and a healthy control group of adults without a history of childhood maltreatment and without a history of psychiatric disorders. The same four networks were examined as described in Chapter 2, using a seed-based correlational approach. Increased connectivity was found between the dorsal anterior cingulate cortex seeding the salience network and the lingual gyrus and occipital fusiform gyrus in the resilient group compared to the two other groups. Examination of the other three networks did not reveal any resting-state functional connectivity results specific to the resilient group.

In Chapter 8 we examined the brain characteristics associated with resilience using a similar design but now in a group of Dutch police officers. Police officers are a highly relevant group when studying resilience duo to the frequency of which they are exposed to traumatic events. The results indicated no gray matter characteristics specific to resilience. However, we did find increased white matter integrity of a part of the corticopontine tract adjacent to the putamen, specific for the resilience group. In post-hoc analyses the white matter integrity in this tract was accompanied by increased resting-state functional connectivity between the putamen and the posterior cingulate cortex, and the precuneus. In addition, both white matter integrity and strength of resting-state functional connectivity were associated with positive reappraisal as coping style.

In the next sections the findings of our studies on vulnerability and resilience are integrated and discussed in light of findings of contemporary studies of brain characteristics of stress-related dysfunction and resilience.

Section I: The brain characteristics related to severe stress exposure and its consequences

Gray matter

Cushing's disease is an important research topic from three clinical points-of-view. The first perspective is that of the endocrinologist who treats the patients with Cushing's disease and sees these patients for checkups. After the hypercortisolism is cured patients improve substantially, but they often do not return to their normal level of functioning and most patients keep reporting symptoms despite (in some cases artificial) restoration of hormone levels (Tiemensma et al., 2010a; Tiemensma

et al., 2010b; Tiemensma et al., 2011). This phenomenon clearly warrants further investigation. The second perspective is that of the pharmacologist, as glucocorticoids are used as an immunosuppressive drug for various immune-related diseases. Excessive use of these types of medication can induce symptoms similar to those of Cushing's disease. This is called Cushing's syndrome. More insight into the persistent effects of hypercortisolism on the brain can have consequences for advised dosages and side-effect warnings. The third perspective is the behavioral point-of-view, or clinically speaking that of the psychiatrist or clinical psychologist. The HPA-axis is a hormonal system that plays a central role in many psychiatric disorders, and hypercortisolism can lead to psychiatric symptoms like depression and anxiety. Studying brain characteristics after hypercortisolism and comparing them to brain characteristics related to severe stress exposure might give an indication of the pathophysiological pathway through which increased levels of cortisol may lead to increased vulnerability for psychopathology. In this section we will attempt to integrate our findings with regard to brain characteristics of endogenous hypercortisolism (Cushing's disease) with the findings on brain characteristics related to exposure to severe exogenous stress (childhood maltreatment). It should be noted that cortisol levels in response to exogenous stressors are not as high as those seen in Cushing's disease. In addition, we did not directly compare the two groups. However, most studies in the literature do compare each of the groups with a healthy control group, so we are able to make conclusions related to the differences with the healthy population.

In Chapter 3 we found a decrease in gray matter in the anterior cingulate cortex in Cushing's disease patients. Interestingly, previous research by our group found this area was affected in adults with a history of childhood emotional maltreatment as well (van Harmelen et al., 2010), although the effect was not as large as we found in the Cushing's disease sample. Other studies support the finding of anterior cingulate cortex volume decreases in adults and adolescents with a history of childhood maltreatment (Cohen et al., 2006; Kitayama et al., 2006; Hart and Rubia, 2012). The observation that these decreases in volume are present after long-term remission of hypercortisolism or when individuals have long since passed into adulthood (as in the case of history of childhood maltreatment) indicates that these gray matter decreases are persistent. Another brain structure that is highly sensitive to exposure to excessive cortisol levels is the hippocampus (Starkman et al., 1992; Carrion et al., 2007). In our study, we did not find any abnormalities in hippocampal volume in patients with long-term remission of Cushing's disease. This is in line with previous studies, which suggest that damages to the hippocampus

can restore after hypercortisolism is normalized (Starkman et al., 1999; Bourdeau et al., 2002; Starkman et al., 2003). In line with these findings, studies into the effects of a history of childhood maltreatment on hippocampal volume report that smaller hippocampal volumes in maltreated subjects were related to present psychiatric symptoms rather than maltreatment itself (Bremner et al., 2003; Kitayama et al., 2005; Weniger et al., 2008; Hart and Rubia, 2012). Taken together, this suggests that the abnormalities in the anterior cingulate cortex represent a persistent effect of exposure to hypercortisolism and are possibly part of the pathophysiological pathway through which exposure to hypercortisolism leads to the development of psychiatric symptoms. A decrease in gray matter volume of the hippocampus, however, appears to be a temporary effect of exposure to cortisol excess and/or related to current psychiatric symptomatology. This effect is reversible after normalization of the excesses in cortisol levels.

White Matter

Studies on the effects of hypercortisolism on white matter integrity are scarce. Traditionally studies examined white matter volume using protocols similar to those being used to investigate gray matter volume. However, relatively recently the development of diffusion tensor imaging protocols has enabled the investigation of white matter integrity rather than volume. As white matter tracts are the wiring of the brain networks, the integrity of these tracts are thought to be better estimates of detrimental effects compared to white matter volumes. In Chapter 4, we found widespread reductions in white matter integrity in the patients with long-term remission of Cushing's disease, suggesting a more general effect of cortisol excess on white matter rather than localized effects. This finding is supported by animal studies finding reduced white matter integrity in multiple tracts throughout the brain as a result of early life exposure to severe stress, as well as an association with increased levels of cortisol (Howell et al., 2013). Animal studies also give an indication which microbiological processes might be involved in reducing white matter integrity as they show an association between prolonged exposure to corticosteroids and the inhibition of proliferation of oligodendrocyte precursors throughout the white matter (Alonso, 2000; van Gemert et al., 2006). Importantly, the findings of the study described in Chapter 4 have recently been replicated in a sample of active as well as remitted Cushing's syndrome patients (Pires et al., 2015).

In the context of childhood maltreatment there are few studies using diffusion tensor imaging protocols to investigate white matter integrity, and the results of the few studies that do are not univocal. Studies show reduced white matter integrity

in a wide array of white matter tracts, including the arcuate fasciculus, cingulum bundle, the fornix, the inferior longitudinal fasciculus, the corpus callosum, and the corona radiata (Choi et al., 2009; Teicher et al., 2010; Choi et al., 2012; Daniels et al., 2013). While there is a lack of clarity regarding the localization of the effects it does support the idea that Cushing's disease is associated with more general white matter affecting properties of cortisol excess, as opposed to more localized effects like we demonstrated in examination of gray matter.

With regard to the relation between behavioral symptoms and white matter integrity we found an association within the Cushing's group between depression and white matter integrity of the uncinate fasciculus, a tract connecting the limbic system with the medial prefrontal cortex areas. Reduced integrity of this tract has been a consistent finding in patients suffering from major depression disorder (Taylor et al., 2007; Cullen et al., 2010; Carballedo et al., 2012), suggesting that white matter integrity of this tract could partly underlie the development of depressive symptoms under influence of cortisol exposure.

On a more analytical note, the findings of this study provide a good example why it is important to perform a whole brain analysis supplementary to the more hypotheses driven region-of-interest analysis. If we had settled for the region-of-interest analysis, we would have confirmed our hypotheses about localization of the effects on white matter integrity, but would have missed the fact that the effects were widespread, suggesting more general white matter affecting properties of hypercortisolism instead of localized effects.

Resting-state functional connectivity

Resting-state functional connectivity between limbic areas and the medial prefrontal cortex appears to be very sensitive to stress (Urry et al., 2006; Kern et al., 2008; Henckens et al., 2010; Veer et al., 2012). In addition, aberrant connectivity patterns between these two areas have consequently been reported in stress-related disorders (Phillips et al., 2003; Drevets et al., 2008; Liberzon and Sripada, 2008; Veer et al., 2010), as well as in relation to childhood maltreatment (Herringa et al., 2013; Birn et al., 2014; Insana et al., 2015). Contrary to these findings, our results in Chapter 2 did not point at aberrant connectivity between the amygdala and the medial prefrontal cortex. An explanation could be that this pattern on functional connectivity reflects behavioral symptoms, whereas we controlled for the presence of psychopathology in our study. This also fits the model in which the medial prefrontal cortex plays an important modulatory role within the stress

system by inhibiting the amygdala (Liberzon et al., 2007). Failure to exert top-down inhibition on the amygdala can lead to sustained excessive amygdala activity, which is reflected in behavioral symptomatology.

Investigating seed-based resting-state functional connectivity from the amygdala in adults with a history of childhood maltreatment, we found decreased connectivity with bilateral precuneus and a cluster extending from the left insula to the hippocampus and putamen. Although we used another technique (probabilistic independent component analysis) to examine resting-state functional connectivity in the patients with remission of Cushing's disease, findings did not correspond. This suggests that the aberrant resting-state functional connectivity with the amygdala in adults with childhood emotional maltreatment was not modified under influence cortisol exposure.

Both the resting-state functional connectivity patterns we found in Chapter 5 and those in previous research conducted in stress-related disorders and stress exposure show patterns of aberrant connectivity between subcortical subregions of the limbic network (i.e., the amygdala and the hippocampus) and the medial prefrontal cortex. However, the direction of the findings is conflicting. Stress exposure and stress-related disorders are related to decreased connectivity between the areas, which also fits the model of top-down inhibition by the medial prefrontal cortex. However, we found increased connectivity between the limbic network and the subgenual anterior cingulate cortex. This could indicate that the human brain attempts to correct the initial suppression in functional connectivity under influence of hypercortisolism. Analogue to holding and releasing an air filled football underwater; it might be the case that once the hypercortisolism is successfully treated the suppression in functional connectivity will fade out, resulting in an overshoot of functional connectivity. Studies focusing on resting-state functional connectivity patterns of active hypercortisolism as well al more longitudinally designed studies should be able to provide more conclusive knowledge.

Future Perspectives

The results presented in the current thesis are in support of the hypothesis that individuals with a history of hypercortisolism show differences in the brain structure and function in the absence of tasks compared to a healthy control group. The next step would be to investigate the brain in directed action, using task-based fMRI. In doing so we can study brain function during tasks that are closely related to the behavioral symptoms displayed by the patients in long-term remission of Cushing's

disease. These studies give an indication about localization of affected brain function under influence of exposure to hypercortisolism. In fact, we already have taken a first step in this direction by investigating brain activity during processing of emotional stimuli (Bas-Hoogendam et al., 2015). In this study we found that patients with long-term remission of Cushing's disease showed less mPFC activation during the processing of emotional faces compared to the healthy control group.

Next, Cushing's disease should be studied in a longitudinal design to be able to draw causal conclusions about the effects of hypercortisolism on the brain. In addition, longitudinal studies could tell us something about the flexibility of the brain after correction of hypercortisolism. We already know that behavioral and cognitive functioning is improved after successfully curing the hypercortisolism. Longitudinal MRI studies will show us, which brain regions are associated with this restoration in functioning and which brain areas show more persistent changes under influence of hypercortisolism.

MRI is an instrument that allows us to investigate the human brain in vivo. However, MRI scans can only give us information about the brain on a macro level. Therefore, we still not know what happens to the brain of a living person under influence of hypercortisolism on a microbiological level. With regard to white matter integrity there is evidence from animal studies indicating that prolonged exposure to hypercortisolism affects oligodendrocyte-influenced remyelination (Alonso, 2000; Miyata et al., 2011). Animal studies on the effects of glucocorticoids on gray matter using stress-inducing paradigms found changes in hippocampal pyramidal cell morphology, and cell loss and suppression of neurogenesis in the hippocampus and parts of the medial prefrontal cortex (Uno et al., 1989; Sapolsky et al., 1990; Watanabe et al., 1992; Lambert et al., 1998; McEwen, 2008; Arnsten, 2009). Ideally, we would also want to measure these microbiological changes in humans. Progress in this area is being made by for instance the Allen Institute for Brain science. Combining information on neuroanatomy and genomics, the Allen Institute for Brain science has created gene expression maps for the human brain as well as the mouse brain. Using this information in combination with information from MRI studies like our study in Cushing's disease could give us more insight into which genes are influenced by cortisol excess and how they relate to changes in brain structure and function. The use of these Allen brain atlases could prove a pivotal in establishing an association between knowledge gained from MRI studies and localized gene expressions (Lein et al., 2007). In the studies with the patients with long-term remission of Cushing's disease, we focused on differences in brain

structure and function and subsequently attempted to explain persisting behavioral effects by examining brain characteristics. This was only possible through an intensive collaboration of various specialized departments, including psychiatry, endocrinology, and radiology. As science progresses it is insurmountable that, as we try to solve these complicated issues, collaboration between different specializations becomes more integrated in research structure.

Section II: Brain characteristics related to resilience to stress.
The main research question of this section is: What are brain characteristics of resilience to stress and how do they relate to brain characteristics associated with stress-related disorders?

Since neuroimaging studies on resilience are still very scarce (Chapter 6), it is very difficult to give a robust answer to the research question. Most studies examining resilience use knowledge gained from research into stress-related disorders as an underpinning to derive hypotheses on relevant networks in the brain (the most prominent regions are described in Chapter 6). Although this is a logical first approach, it assumes that on a neural level resilience is the opposite of having stress-related symptoms, which is not necessarily true. As a result, the majority of locations in the brain are ignored and potentially valuable information is lost. Based on the data that is currently available, it is still too early for building comprehensive models on the neural mechanisms of resilience to trauma. Due to the fact that resilience is such a dynamic and multidimensional construct, studies also vary on the operationalization they use to identify resilience. Some studies chose to define resilience as a trait, using personality characteristics and questionnaires to define resilience at a certain point in time (baseline) (Friborg et al., 2005; Campbell-Sills et al., 2006). Others define resilience as adequate or adaptive behavior during exposure to stressors, focusing on the ability to learn new adaptive coping techniques or speed of recovery (Masten and Coatsworth, 1998). Resilience is sometimes even interchanged with the concept of resistance to stress, the latter meaning that individuals show no dysregulation during exposure to a stressor at all. Lastly, some studies (including the ones presented in this thesis) define resilience by outcome, e.g., individuals who did not develop any psychiatric symptoms after the experience of a traumatic event (Gilbertson et al., 2002; Admon et al., 2013). These variations in definitions and time points of measurements complicate the integration of findings into a model for resilience.

Gray Matter

In Chapter 8 we hypothesized to find specific characteristics related to resilience in gray matter volume and shape of the hippocampus. This hypothesis was based on prior research indicating that increased hippocampal size is related to resilience (Gilbertson et al., 2002; Yehuda et al., 2007), and on the notion that the hippocampal structure is very susceptible to stress hormones (Starkman et al., 1992; Pryce, 2008). Contrary to our expectations we did not find increased hippocampal volumes in the resilient individuals. One explanation could be that the police force typically selects specific personality profiles and psychological abilities related to resilience, based on the extensive psychological testing that takes place before admittance. If these characteristics are also related to larger hippocampal volumes the selection criteria might inadvertently be biased towards selecting larger hippocampal volumes, thus taking away some of the variation that is common within the general population; a so called ceiling effect. To examine whether a ceiling effect is present in groups consisting of high-risk professionals, future designs should encompass a control group of individuals from the general population. Comparing this group to the high-risk professionals could clarify whether high-risk professionals already have a larger hippocampus compared to the general population as a result of the selection criteria.

Connectivity

The regions-of-interest we used in Chapter 8 consisted mainly of white matter tracts connecting medial prefrontal areas with subcortical areas. These areas are mainly implicated in emotion and behavioral regulation through risk and reward analysis as well as inhibitory control of prefrontal areas over the subcortical areas (Bechara et al., 2000; Roberts and Wallis, 2000; Hansel and von Kanel, 2008; Johnson et al., 2011). Proper structural connectivity between these areas is imperative for optimized functioning of these behavior and emotion regulating processes. This could also be deducted from studies showing decreased structural connectivity of these white matter tracts in stress-related disorders with accompanying deficits in emotion regulation (Taylor et al., 2007; Kawashima et al., 2009; Kim and Whalen, 2009; Phan et al., 2009; Baur et al., 2012; Zhang et al., 2012; Baur et al., 2013). The ceiling effect, due to the selection profile of our subjects, could also underpin the null-findings in white matter integrity in these a priori hypothesized regions. However, it did enable us to examine in which other regions connectivity differentiated the most resilient inside our already high-resilient group of police officers. To investigate these regions and to counter the risk of missing out potentially valuable information in other non-hypothesized areas, we used an explorative whole brain

analysis. We found that increased white matter integrity of the corticopontine tract was specific for the high-resilient group. The corticopontine tract is a white matter tract not otherwise implicated in stress-related disorders previously. Additionally, the structural connectivity differences in this area were accompanied by specific patterns of resting-state functional connectivity between a gray matter seed directly adjacent to the corticopontine effect (the putamen), and the posterior cingulate cortex and the precuneus. This pattern of findings suggests that resilience and stress-related disorders are not opposites on a neural level. Importantly, both the structural connectivity and functional connectivity findings were related to positive reappraisal strategies in resilience individuals. Positive reappraisal as a cognitive strategy to cope with stressful experiences refers to strategies that encompass giving positive meaning to experiences in terms of personal growth (Spirito et al., 1988; Carver et al., 1989; Garnefski et al., 2001). It is a higher-order cognitive process that can take place long after a stressful experience has ended, and is therefore remotely different from the more fast-paced emotion-regulation strategies that take place during stressful situations and implicate medial prefrontal and subcortical areas as well as their reciprocal connectivity. In addition, positive reappraisal can influence the image an individual has of oneself. Not surprisingly, we found cognitive reappraisal to be related to connectivity differences with the precuneus; an area involved in self-referential processing, self-consciousness, and autobiographic memory (Cavanna and Trimble, 2006; Cavanna, 2007).

The findings in Chapter 7 also support the notion that higher-order cognitive processes are important in resilience. We found increased resting-state functional connectivity between the salience network and an area encompassing the lingual gyrus and the occipital fusiform gyrus. This finding fits the dual representation theory of visual intrusions (Brewin et al., 1996; Brewin et al., 2010). Visual intrusions are a core symptom of PTSD, but are also present in depression (Kuyken and Brewin, 1994; Brewin et al., 1998; Reynolds and Brewin, 1998), panic disorder (Beck et al., 1974; Ottaviani and Beck, 1987), social anxiety (Hackmann et al., 1998; Hackmann et al., 2000), agoraphobia (Day et al., 2004), and obsessive-compulsive disorder (Speckens et al., 2007; Lipton et al., 2010). The dual representation theory explains visual intrusions in terms of memory encoding and retrieval in two memory systems: contextual memory and sensation-based memory. Integrative processing of both memory systems is necessary for normal encoding of traumatic events. When traumatic experiences are only processed in the sensation-based memory this leads to involuntary access of traumatic memories and accompanying autonomic responses, in other words: visual intrusions. According to the neural model of

memory and imagery the salience network, with its function of assessing relevance of internal and external stimuli, is part of the sensation-based memory system. The lingual gyrus and occipital fusiform gyrus, however, are involved in associating autobiographical knowledge to the encoding and retrieval of memories. (Burgess et al., 2001; Byrne et al., 2007; Brewin et al., 2010). Communication between these two areas is necessary for memories to be integrated in both the sensation-based memory system and the contextual memory system. Our results of increased functional connectivity between these areas related to resilience, indicate that an increase in connectivity between these two areas provide a protective mechanism for developing psychopathology after severe stressful experiences. Possibly this is achieved by an increased ability to successfully encode memories in both memory systems, resulting in decreased chances on experiencing intrusive images, which in turn could lead to other psychiatric symptoms and eventually psychopathology.

Future perspectives

We investigated characteristics of brain structure and resting-state functional connectivity related to resilience to trauma. Next, brain function during externally controlled tasks that elicit processes involved in resilience should be further investigated. For instance, it would be highly relevant to see how brain function in the resilient group differs from the other groups during emotion regulation, working memory performance during emotional distraction, and during (social) stress. Cortisol response to a stressor and the occurrence of certain mineralocorticoid receptor and glucocorticoid receptor influencing haplotypes should be studied in concert, since there is evidence that these haplotypes are related to resilience (Klok et al., 2011).

As explained in the previous Chapter, we suggest adding a healthy control group from the general population when studying resilience in high-risk occupations like police officers and military personnel. This would allow investigating whether some of our null-findings could be explained by a ceiling effect.

Finally, we highly recommend studying a complex construct like resilience in a longitudinal design, which allows for drawing causal conclusions. In addition, it will enable investigation of brain characteristics that predict resilient behavior in the face of trauma exposure, which may provide very valuable knowledge for establishing further selection criteria for high-risk occupations, as well as possible targets for prevention.

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Appendix

Nederlandse samenvatting
Curriculum vitae
List of publications
Dankwoord

Nederlandse samenvatting

Het menselijk lichaam is erop ingericht om zich aan te kunnen passen aan uitdagende situaties. Toch kunnen sommige ervaringen zo heftig zijn dat ze kunnen leiden tot verstoringen in gedrag en geestelijk en lichamelijk functioneren. Uit het feit dat niet alle mensen eenzelfde reactie vertonen als gevolg van een hevig stressvolle ervaring kunnen we afleiden dat er een mate van intra-individuele variatie bestaat. Neuroimaging technieken kunnen helpen om de karakteristieken van hersenstructuur en functie die gerelateerd zijn aan kwetsbaarheid en veerkracht voor ernstige stress in kaart te brengen.

Voor het in dit proefschrift gerapporteerde onderzoek zijn de volgende neuroimaging modaliteiten gebruikt om de karakteristieken van het brein gerelateerd aan (dis) functie na blootstelling aan hevige stress te bestuderen: 'Voxel-based morphometry (VBM)' is gebruikt om grijze stof volumes te bestuderen, 'diffusion tensor imaging (DTI)' is gebruikt om de integriteit van witte stof banen te bestuderen en resting-state functionele mri (rs-fMRI) is gebruikt om functionele connectiviteit te bestuderen. Om de effecten van hypercortisolisme en heftige stress op het brein te onderzoeken hebben we zowel mensen bestudeerd die in het verleden de ziekte van Cushing's hebben gehad als mensen die in de kindertijd emotionele mishandeling hebben meegemaakt. Daarnaast hebben de karakteristieken van het brein gerelateerd aan veerkracht onderzocht binnen de Nederlandse politie en in een groep mensen die in hun kindertijd regelmatig blootgesteld zijn aan mishandeling.

Blootstelling aan extreme stress, hersenstructuur en hersenfunctie
Mensen die in de kindertijd regelmatig slachtoffer zijn geweest van emotionele mishandeling hebben een verhoogde kans op de ontwikkeling van psychopathologie tijdens de volwassenheid. In hoofdstuk 2 worden de resting-state functionele connectiviteit karakteristieken van volwassenen die in hun kindertijd regelmatig emotionele mishandeling hebben meegemaakt (CEM) bestudeert. Daarvoor is gekozen voor een seed-based correlationele benadering. Deze benadering brengt de functionele connectiviteit van een a priori gekozen gebied (de seed) met de rest van het brein in kaart. De seeds in dit hoofdstuk zijn zo gekozen dat ze vier de functionele connectiviteit van vier netwerken in kaart brengen: 'het limbische netwerk', 'het salience netwerk', 'het default mode netwerk', en een netwerk dat gebaseerd is op eerdere vondsten in structurele verandering. De sterkte van de connectiviteit van de netwerken met de rest van het brein is in dit hoofdstuk vergeleken tussen twee groepen bestaande uit volwassenen met en zonder blootstelling aan herhaaldelijk emotionele mishandeling in de kindertijd. Beide

groepen bestonden uit 44 mensen. We vonden verminderde connectiviteit tussen het limbische netwerk en de bilaterale precuneus en bilaterale occipitale cortex in de CEM groep. Daarnaast vonden we toegenomen connectiviteit tussen het limbische netwerk en de linker putamen en de linker hippocampus. Connectiviteit van het salience netwerk en een gebied met de angular cortex en de precuneus was afgenomen binnen de CEM groep vergeleken met de controle groep, daarnaast was de connectiviteit met de mediale prefrontale cortex afgenomen. We vonden geen verschillen tussen de groepen in resting-state functionele connectiviteit van het default mode netwerk en het netwerk gebaseerd op eerdere bevindingen in hersenstructuur.

De ziekte van Cushing wordt gekenmerkt door een tumor op de hypofyse die dit hersendeel stimuleert tot de afgifte van ACTH. ACTH op zijn beurt stimuleert de bijnieren tot de afgifte van cortisol (het stresshormoon). Hierdoor hebben mensen met deze ziekte constant hoge niveaus van cortisol in hun lichaam. Dit gaat gepaard met een reeks van fysieke, maar ook psychische klachten. De behandeling van de ziekte van Cushing bestaat uit de verwijdering van de tumor door middel van een operatie, waarna de HPA as (het stresssysteem) weer in balans gebracht wordt. In sommige gevallen gebeurt dit natuurlijk, en in sommige gevallen wordt de balans herstelt door middel van supplementen. Alhoewel de fysieke en psychische klachten afnemen, blijven veel patiënten kampen met klachten als depressie, angst en apathie. Onze hypothese was dat dit zou kunnen komen doordat hersenstructuur en functie persistent verandert zijn onder invloed van de langdurige blootstelling aan hoge niveaus van cortisol (hypercortisolisme)

In hoofdstuk 3 worden de effecten van langdurige blootstelling aan hoge niveaus van cortisol op het brein onderzocht. We hebben hiervoor MRI scans gemaakt van 25 mensen die langere tijd in remissie zijn van de ziekte van Cushing en 25 controles die overeenkwamen wat betreft leeftijd, geslacht en niveau van opleiding. Vergeleken met de controle groep vonden we in de patiënten groep afgenomen grijze stof volumes in de antorior cingulate cortex en toegenomen grijze stof volumes in de linker cerebellum, maar konden deze veranderingen in structuur niet relateren aan gedrag of cognitie.

Binnen dezelfde patiëntengroep wordt de structurele connectiviteit (of witte stof integriteit) onderzocht in hoofdstuk 4. We vonden verminderde witte stof integriteit in alle gebieden waarover we hypotheses hadden (de uncinate fasciculus, de cingulum, en het corpus callosum). Daarnaast vonden we een relatie tussen

depressie scores binnen de patiëntengroep en witte stof integriteit van de linker uncinate fasciculus. Deze bevindingen werden in een ander perspectief gezet door de resultaten van de exploratieve analyse op het gehele brein. Met deze analyse vonden we dat de afgenomen witte stof integriteit niet beperkt was tot de eerder gevonden gebieden, maar dat de witte stof integriteit afgenomen was door het gehele brein. Deze bevinding is later gerepliceerd door Pires et al. (2015).

Hoofdstuk 5 beschrijft de karakteristieken van resting-state functionele connectiviteit van de patiënten waarbij de ziekte van Cushing langere tijd in remissie is. Eerst zijn door de data gedreven onafhankelijke componenten geïdentificeerd, waarbij er drie netwerken zijn geselecteerd voor onze resting-state functionele connectiviteit analyses: het limbische netwerk, het default mode netwerk, en het executieve controle netwerk. Binnen de patiëntengroep vonden we toegenomen connectiviteit tussen het limbische netwerk en de subgenuale gedeelte van de anterior cingulate cortex. Daarnaast vonden toegenomen connectiviteit tussen het default mode netwerk en de linker laterale occipitale cortex.
Deze studies laten zien dat stress veroorzaakt door zowel externe factoren (emotionele mishandeling tijdens de kindertijd), als door interne factoren (de ziekte van Cushing) de structuur en functie van het brein voor langere tijd kunnen veranderen en dit zou een verklaring kunnen zijn voor de toegenomen kwetsbaarheid voor psychische problematiek binnen de mensen die in het verleden slachtoffer zijn geweest van emotionele mishandeling, en de patiënten met de ziekte van Cushing in remissie.

Veerkracht voor extreme stress, hersenstructuur en hersenfunctie
Hoofdstuk 6 is een review waarin beschreven staat wat we wisten over veerkracht en hoe dit geregeld is in het brein toen we begonnen aan ons onderzoek in 2011. Locaties die met veerkracht in verband werden gebracht waren de hippocampus, de amygdala, de anterior cingulate cortex en de prefrontale cortex. De meeste studies gebruikten een design waarbij mensen die blootgesteld waren aan trauma opgedeeld waren in twee groepen die onderling vergeleken werden: een groep met PTSD en een groep zonder PTSD. Eén van de belangrijkste conclusies uit deze review was dat door dit design de meeste studies niet ingericht waren om de karakteristieken van PTSD, veerkracht en trauma exposure te ontvlechten. We beschrijven daarom een aantal suggesties voor designs die ons wel in staat stellen om de karakteristieken te ontvlechten, zoals een longitudinale studies, en een drie-groeps design waarbij een controle groep bestaande uit mensen zonder trauma blootstelling en zonder PTSD wordt toegevoegd aan het klassieke design.

In hoofdstuk 7 gebruiken we het door ons voorgestelde drie-groeps design om resting-state functionele connectiviteit van veerkracht te onderzoeken binnen een groep mensen die tijdens de kindertijd mishandeld zijn. We vergelijken hierbij drie groepen: mensen die tijdens de kindertijd mishandeld zijn en geen psychiatrische stoornissen hebben ontwikkeld in het latere leven (de veerkrachtige groep); mensen die tijdens de kindertijd mishandeld zijn en wel een psychiatrische stoornis hebben ontwikkeld in het latere leven (de kwetsbare groep); en een gezonde controle groep zonder een verleden van mishandeling en zonder psychiatrische stoornissen. Hierbij zijn dezelfde netwerken onderzocht als in hoofdstuk 2. We vonden toegenomen connectiviteit tussen het salience netwerk en de linguale gyrus en occipitale fusiforme gyrus, specifiek voor de veerkrachtige groep ten opzichte van de andere twee groepen.

Hoofdstuk 8 beschrijft de karakteristieken van hersenstructuur en functie van veerkracht binnen Nederlandse politie agenten. Agenten zijn een zeer relevante groep om veerkracht te onderzoeken gezien het hoge aantal traumatische ervaringen waaraan ze worden blootgesteld vanwege hun beroep. In deze studie hebben we drie groepen vergeleken, alle deelnemers waren werkzaam binnen de Nederlandse politie. De veerkrachtige groep bestond uit agenten die veelvuldig blootgesteld zijn aan traumatische ervaringen, en geen psychiatrische stoornissen hadden ontwikkeld; de kwetsbare groep bestond uit agenten met veelvuldige blootstelling aan traumatische ervaringen, die wel een psychiatrische stoornis hebben ontwikkeld; de controle groep bestond uit leerlingen van de politieacademie die nog niet blootgesteld zijn aan traumatische ervaringen en die nooit een psychiatrische stoornis hadden gehad. De resultaten lieten zien dat nergens in het brein grijze stof volume specifiek was voor veerkracht. We vonden echter wel dat de witte stof integriteit van een deel van de corticopontine baan ter hoogte van de linker putamen specifiek was voor veerkracht. Post-hoc analyses wezen uit dat de toegenomen witte stof integriteit van de corticopontine baan vergezeld werd door toegenomen functionele connectiviteit tussen gebieden die door deze baan verbonden worden, namelijk de putamen en de precuneus. Daarnaast waren zowel de witte stof integriteit als de functionele connectiviteit gerelateerd aan positieve herinterpretatie als coping stijl.

Het feit dat onze resultaten zich niet bevonden in gebieden die vaak worden gerelateerd aan stress-gerelateerde psychiatrische stoornissen wijst erop dat, op het niveau van hersenstructuur en functie, veerkracht niet simpelweg het tegenovergestelde is van het hebben van klachten, maar juist gezien moet worden als een onafhankelijk construct.

Curriculum Vitae

Stephanus Johannes August was born on January 18th 1985 in Pijnacker, the Netherlands. In 2003 he completed his pre-university education at the Sint Stanislas College in Delft. In 2007 he received his Bachelor's degree in Psychology at Leiden University after studying attention bias modification using dynamic emotional stimuli. In 2009, he received his Master's degree in Psychology, sub-specialization Clinical Psychology, after writing a thesis on the relation between resting-state measured with EEG and attentional bias. This study later resulted in his first publication in a scientific journal. From 2009 to 2010, Steven worked as a research assistant at Leiden University department of Clinical Psychology as well as at PsyQ Psychotrauma in The Hague, an outpatient facility specialized in treating patients with trauma-related psychiatric disorders. Research revolved mainly around attentional bias modification. In 2010, Steven started his PhD at the department of Psychiatry of the Leiden University Medical Center (LUMC). Using various magnetic resonance imaging techniques he examined the neurobiological mechanisms of resilience to stress and the persisting effects of hypercortisolism on the brain. During his PhD, he had the opportunity to visit the University of Cape Town, to work at the department of Psychiatry. From 2015, he combined the completion of his PhD with more clinical oriented activities, as he started working as a Psychologist at PsyQ Psychotrauma, The Hague. In April 2016, Steven started working as a postdoctoral researcher at the department of Psychiatry of the LUMC, where he collaborates on a European multicenter research trial aiming to provide quantitative biological measures to facilitate the discovery and development of new treatments for social and cognitive deficits in Alzheimer's disease, schizophrenia and depression. To date, he still combines research and clinical work.

List of publications

Journal Publications

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