Stressed-out stress systems: dysregulated stress-systems in the pathophysiology of stress-related disorders

Bauduin, S.E.E.C.

Chapter 1

Stressed-out stress systems

Dysregulated stress-systems in the pathophysiology of stress-related disorders

"It's not stress that kills us, it is our reaction to it."
– Hans Selye

General Introduction

What is stress?
The term 'stress' was first coined by endocrinologist Hans Selye in order to describe the "nonspecific response of the body to any demand"[1]. The first study with regard to this phenomenon that Selye published in 1936 under the title: "A Syndrome Produced by Diverse Nocuous Agents" identified a "typical syndrome", of which the symptoms "are independent of the nature of the damaging agent of the pharmacological type of drug employed, and represent rather a response to damage as such". This syndrome presented if the organism was severely damaged by diverse, nonspecific, nocuous agents such as surgical injury, trauma to the spinal cord leading to spinal shock, exposure to cold, excessive muscular exercise, or intoxications with sublethal doses of various drugs (for example, formaldehyde, morphine, adrenaline, and atropine). Since then the term 'stress' has been further elaborated upon, and differentiation has been made between somatic stress as described by Selye on the one hand, and psychological stress on the other hand. It is on psychological stress where focus of this thesis will lie. Psychological stress has been defined in a variety of ways. One of the more globally accepted definitions is "a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being"[2], referring to the processes that are thought to contribute to the onset and maintenance of several stress-related disorders.

By defining 'stress' as such, the term seems to carry a negative connotation. However, the stress response is essential as it allows for adaption and survival. Throughout our lives, we are presented with a variety of challenges, ranging from daily hassles to severe traumatic events. Our stress-response enables us to respond to a stressor as quickly and efficiently as possible in order to speedily return our bodies to a homeostatic state, although the intra-individual variation in this response is large.

The American Psychological Association (APA) divides stress into two different major types or forms, namely acute stress and chronic stress. These stress types are characterized in a different way in terms of characteristics, duration, symptoms, and treatment approaches. Acute stress is the most common and frequent form of stress. This form of stress usually characterizes itself as brief, and is often caused by reactive thinking, stemming from "the demands and pressures of the recent past

and anticipated demands and pressures of the near future"[3]. Chronic stress is a long-term form of stress. This form of stress is characterized by feelings of hopelessness and despair3.

In the case of chronic stress, where stress systems are no longer only stressed, but stressed-out[4], the repeated exposure to a stressor, numerous stressors, or exposure to a severe acute stressor can result in alterations in psychological and neurobiological processes. Upon persistence of these reactive psychoneurobiological processes, a variety of stress-related psychiatric disorders, such as mood, anxiety, and/or somatic symptom disorders, but also somatic disorders, may develop in vulnerable individuals. As a result, poor coping strategies can develop such as substance abuse, maladaptive avoidance techniques, or social withdrawal[5].

Stress-systems

When an individual experiences stress, whether it be acute or long-term, stress-systems are activated, setting a cascade of biological and psychological processes in motion. The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), specifically the sympathetic nervous system that triggers the acute flight, fight, or freeze response, are the two major systems that respond to stress in humans and most mammals[6].

The HPA axis

In an uncontrollable, challenging, or threatening situation, the HPA axis, the primary driver of the endocrine stress response, is activated. The hypothalamus synthesizes and secretes corticotropin-releasing factor (CRF), causing the pituitary gland to release adrenocorticotropic hormone (ACTH) into the blood stream, stimulating the adrenal cortex to produce and secrete the glucocorticoid (GC) cortisol (also known as one of the main stress hormones). This leads to increased concentrations of free cortisol circulating in the body. As these levels rise, CRF release is blocked, leading to a decline in ACTH levels, and in turn, a decline in cortisol levels, extinguishing the stress response and returning the body back to its homeostatic state. This process is referred to as the negative feedback loop[7,8]. In reaction to acute stress, cortisol enables a person to react by suppressing the immune function, mobilizing stored energy, and facilitating several processes of the central nervous system, such as learning and memory. However, chronically increased levels of CRF or cortisol also come paired with numerous deleterious effects, such as cognitive disturbances, depressed mood, anxiety, immune destabilization[9,10], and increased risk of cardiovascular disease, diabetes, and stroke[11].

GCs affect both the nervous and immune systems. GCs bind to intracellular,

mineralocorticoid (MR-), and glucocorticoid (GR-) receptors. GC action in the brain is mediated by the two types of corticosteroid receptors, the MR[12] and the GR[13,14]. However, they differ in their distribution throughout the brain GRs are located widely throughout the brain, whereas MRs are located predominately in the limbic brain areas, specifically in the hippocampus and the amygdala[15]. MR affinity to cortisol is 10-fold higher than GR affinity, and MRs have been found to mediate the effect of cortisol on the regulation of initial stress reactions[16-22]. Imbalances in the MR-/GR-mediated signaling pathways that develop under conditions of chronic stress can increase susceptibility to stress-related disorder and diseases[23,24]. Loss of functioning and expression of the MR in the limbic brain reduces the MR-mediated inhibition of the HPA-axis, leading to higher levels of cortisol in the brain[24]. Therefore, the hypothesis has been offered that MR activation in the limbic brain could have potential as an anti-depressant strategy[25-27].

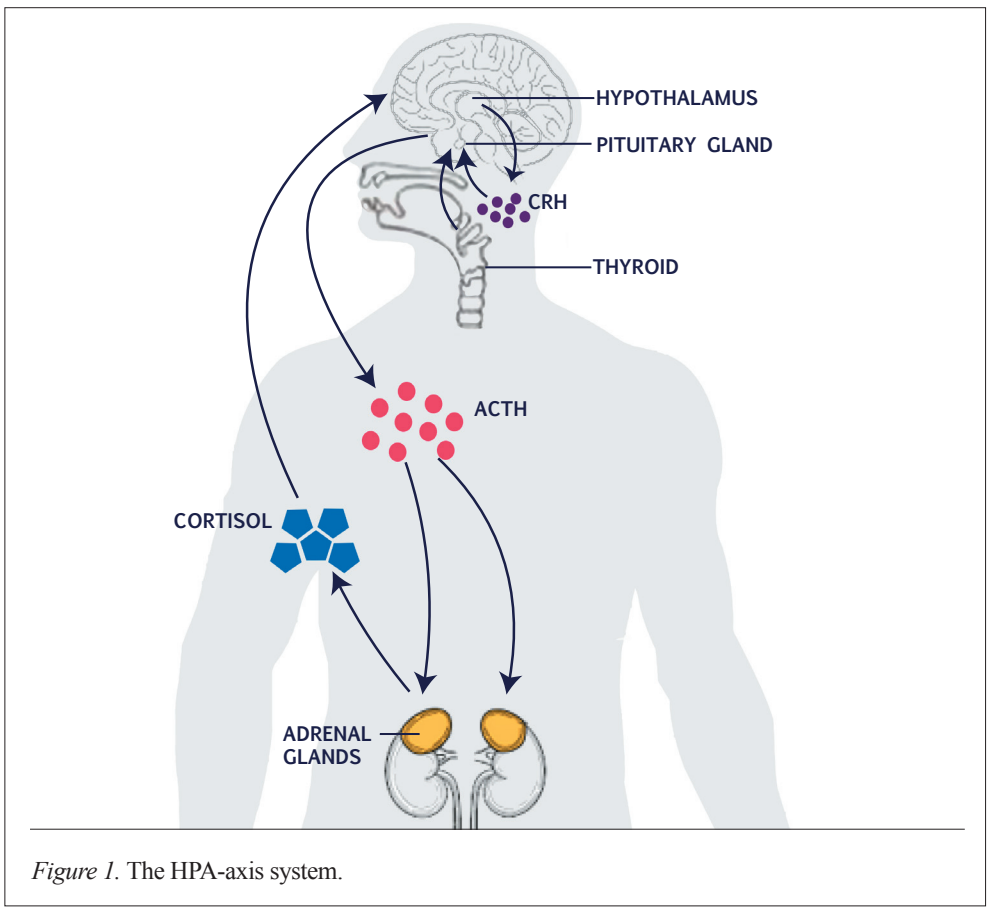

Figure 1. The HPA-axis system.

Under normal circumstances, cortisol follows a distinct diurnal pattern that has been well documented. Typically, cortisol concentration increases upon awakening and slowly declines throughout the day. Upon awakening, a peak in cortisol production is typical, which is referred to as the cortisol awakening response (CAR; the variation in cortisol concentration occurring in the first hour after awakening, with cortisol typically peaking approximately 30 minutes after awakening)[28,29]. Generally, cortisol levels are low at the end of the day towards night, which is indicative of basal HPA axis activity (see Figure 2).

As cortisol is a low weight lipophile molecule, it can diffuse passively across the blood brain barrier. Cortisol can be measured in bodily fluids such as blood, urine, and saliva. Measuring cortisol in saliva is one of the most preferred methods to date, as it is able to measure the amount unbound free cortisol (i.e. the biologically active form), in contrast to the bound form in, for example, blood. Furthermore, saliva sampling is non-invasive and can be obtained by respondents themselves at home, and under normal, stress-free conditions. Additionally, this saliva sampling method makes it possible for respondents to measure their cortisol levels over the course of the day, including the CAR, therefore enabling respondents to capture the dynamics of their HPA axis activity more accurately.

A number of decades ago, salivary cortisol was thought to be a promising biomarker specific to depression. However, ample research has found salivary cortisol levels to be altered in many stress-related disorders. To begin, studies investigating basal cortisol levels in psychiatric disorders have rendered equivocal results. Many studies have found evidence of hypersecretion of cortisol in depressed and anxious patients[30,32], although long-term stress has also been found to lead to a downregulation or exhaustion of the HPA-axis, and less hypercortisolemia[31]. Studies specifically investigating the salivary CAR in this patient population have shown a high level of variability. A number of studies have found similar morning salivary cortisol concentrations in depressed patients and healthy controls [(e.g.32)], although other studies found a blunted cortisol response in patients with major depressive disorder (MDD) in comparison to healthy controls[33,34]. Again, other studies have found an increased CAR for both area under the curve with respect to the ground (AUCg) and area under the curve with respect to the increase (AUCi) variables in MDD patients in comparison to healthy controls [(e.g.31,35-37)]. Two earlier studies conducted with MDD patients and healthy controls found (partially) increased evening cortisol levels in depressed subjects[37,38]. Finally, several studies have identified diminished negative feedback in depressed patients compared to healthy controls after a low-dose dexamethasone-suppression test (DST)[31,33-37]. Studies examining HPA activity in patients with generalized anxiety disorder (GAD)

have reported normal[39-41] to increased cortisol levels[42] and normal suppression[43] to more non-suppression following a DST[44]. In sum, these findings provide evidence for differences in HPA stress-axis regulation in patients with mood- and/or anxiety disorders on the one hand and healthy controls on the other, however highlight the inability of salivary cortisol to distinguish between these disorders. With regard to the research conducted concerning the HPA-axis in this thesis, depression and anxiety will be the stress-related disorders predominately focused on. Further research with regard to HPA-axis activity will be conducted using Cushing's disease as a naturalistic model for the effects of long-term exposure to high amounts of endogenous cortisol (see section: The HPA axis: prolonged exposure to endogenous cortisol for further detail).

The ANS

The ANS is a component of the peripheral nervous system. This system plays a crucial role in both the manifestation and maintenance of stress-related symptoms and biological stress processes45. The ANS consists of two systems, the parasympathetic nervous system (PNS), responsible for the body's rest and digest response, and the sympathetic nervous system (SNS), responsible for the fight or flight response. When the SNS is activated, it signals the adrenal glands to release hormones (i.e. adrenalin (epinephrine) and cortisol). As with HPA-axis activation, the SNS response is fairly sudden in order to prepare the body to respond to an emergency situation, an acute stress situation, or short-term stressors.

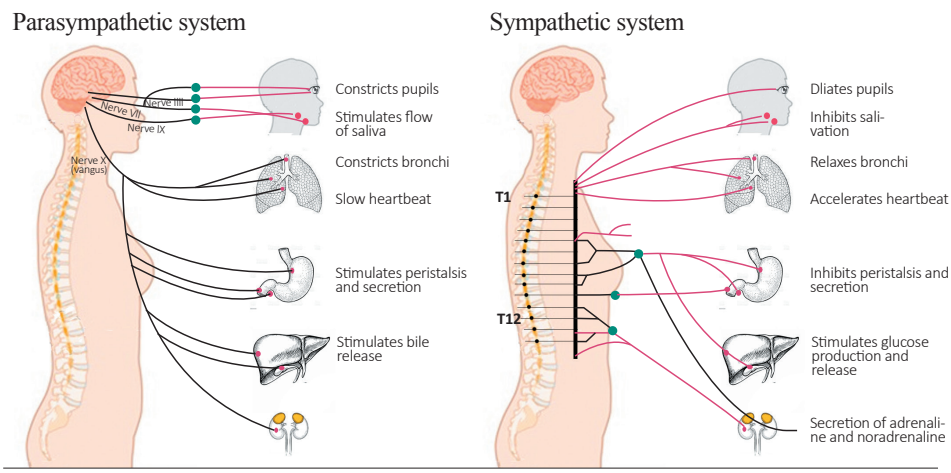

Figure 2. The autonomic nervous system.

Once the crisis is over, the body typically returns to a homeostatic state. This recovery is facilitated by the PNS, which generally has opposing effects to the SNS. Several parameters serve as indices for ANS activity, such as plasma levels of catecholamines, heart rate, heart rate variability, and blood pressure.

A lesser known parameter for ANS activation: salivary alpha amylase

The enzyme sAA has been studied less extensively than salivary cortisol. In contrast to the small cortisol molecule, sAA is a long-chain macromolecule protein, that is secreted in the oral cavity by the salivary gland upon beta-adrenergic stimulation, is a relatively new candidate marker of autonomic nervous system (ANS) functioning and reactivity, accounting for 40 to 50% of salivary protein[46-48]. It is strongly conserved in evolutionary history and can be found in other animals, insects, plants, and bacteria[49].

Research into the oral microbiome has found that particular strains of oral bacteria are adapted in order to break down starch, and have a unique ability to capture sAA, with which they feed themselves[49]. This mechanism is only activated when the regular diet includes starch, that has been found to be essential to survival[50]. There is evidence that carbohydrate intake increases sAA response in individuals whose ancestors consumed starch-rich diets. For this reason, people of Southern European ancestry experience larger sAA increases in sAA levels in comparison to people of Northern European ancestry[51].

sAA plays an important role in the first step of starch digestion in the oral cavity as it catalyzes (breaks down) large long-chain carbohydrate starch molecules into dextrin, glucose and maltose by cleaving alpha-1,4-glucosidic bond[52,53]. Starch is further digested in the small intestine by pancreatic alpha amylase, an enzyme similar to sAA that is produced by the exocrine pancreas and released into the duodenum. Both pancreatic and salivary amylase isoforms are also present in serum with an approximately even proportion[54]. In contrast to the diurnal profile of salivary cortisol, studies with healthy controls have demonstrated that sAA presents an opposing, distinct diurnal profile (see Figure 3), with lower levels in the morning and higher levels in the early evening[45,46].

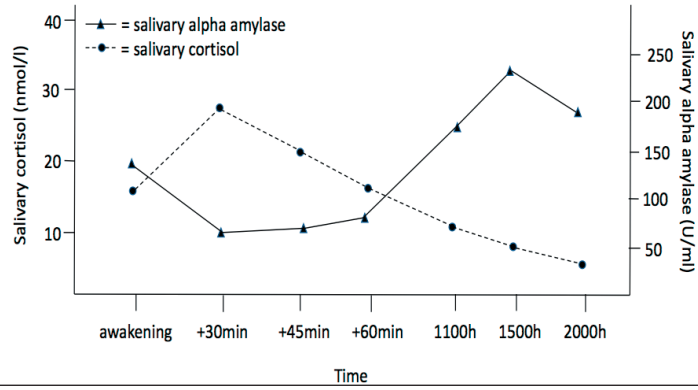

Figure 3. An example of the diurnal profiles of sAA and salivary cortisol for illustration purposes.

This thesis aims to further unravel the role of the stress systems in the pathophysiology of stress-related psychiatric disorders, by exploring elements of regulation and dysregulation of the two major stress systems (i.e. the ANS and the HPA-axis), and their relation with psychological and psychiatric symptoms.

Salivary alpha-amylase in stress-related disorders

Studies with healthy controls have shown that sAA is highly sensitive to acute stress-related changes, increasing under psychosocial stress tasks administered in the afternoon (i.e. Trier Social Stress Test) [(e.g.47,55,56)]. These elevations of sAA levels have been found to be indicative of increased autonomic activity and have been found to occur in response to neurotransmitter stimulation[57,58]. The enzymatic activity and quantity of sAA have been found to vary between individuals under environmental factors, such as stress levels, but also circadian rhythms[59,60]. Within the mood-, anxiety-, and symptom somatic disorder (MAS)-patient population, interventional studies using psychosocial stress tasks in the afternoon (between 12:00 and 5:00 p.m.) have provided evidence that sAA levels in patients with MAS-disorders increase more than those of healthy controls [(e.g. 61-64)].

Five previous observational studies have been conducted with the MAS-patient group. Two were conducted in a laboratory setting and found low baseline sAA levels in MDD patients in comparison to healthy controls in the morning[65], and elevated sAA levels in the afternoon in current MDD patients in comparison to remitted MDD patients and healthy controls[66]. Three studies were conducted in a naturalistic setting. One study found that patients with generalized social anxiety disorder (gSAD) had increased sAA levels in the area under the curve with respect

to the ground (AUCg) on day 1 and at 4:00 p.m. on day 2[67]. The two other studies were conducted with (remitted) MDD patients and found (i) elevated levels of sAA in MDD patients using tricyclic antidepressants (but not SSRIs), in comparison to the controls and remitted MDD patients in the late evening (between 10:00 and 11:00 p.m.)[68], and (ii) higher salivary cortisol and sAA levels in the morning, afternoon, and evening sample times in MDD patients in comparison to healthy controls, although this study was largely underpowered[69].

Based on these findings, it seemed that sAA levels in the late evening do not differentiate between MDD patients who do not use tricyclic antidepressants, remitted MDD patients, and healthy controls. However, sAA levels may be able to differentiate between MDD patients and healthy controls more effectively at other time points throughout the day (i.e. in the morning, afternoon, and in the evening based on the Booij et al.[69] study). Still, as sAA levels were also found to be elevated in gSAD patients at certain time points, sAA may not be able to adequately differentiate between MDD patients and patients with other MAS-disorders at every time point. Furthermore, certain factors have been found to influence sAA levels and should be considered as potential confounders in epidemiological studies. For example, there is evidence that carbohydrate intake increases sAA response in individuals whose ancestors consumed starch-rich diets. Thus, those of Southern European ancestry experienced a larger increase in sAA levels and vice versa[70]. Furthermore, a study conducted in a sample of healthy participants (N = 487), found that sAA levels were also influenced by age and alcohol use[68].

In sum, several gaps in the literature remain with regard to sAA research within psychiatric patient populations. One important aspect that is currently lacking is the mapping of naturalistic diurnal sAA levels in patients with MAS-disorders in comparison to healthy controls. Chapter 2 of this thesis will explore this, taking important characteristics as mentioned above (i.e. ancestry, age, and alcohol use) into account.

Salivary alpha amylase and social withdrawal

Social withdrawal (SW) has recently been defined as "an umbrella term referring to an individual's voluntary self-isolation from familiar and/or unfamiliar others through the consistent display of solitary behaviors such as shyness, spending excessive time alone, and avoiding peer interation"[71]. SW has been identified an early symptom of several stress-related psychiatric disorders [(e.g. 72,73)], and hypothesized to be partially due to long-term activation of biological stress systems. As such, and in line with the National Institute of Mental Health Research Domain Criteria (RDoC) project aimed at identifying new ways of classifying psychiatric disorders

based on dimensions of neurobiological measures and observable behavior, SW has been posited to be related to a more stable endophenotype that is more closely connected to biological pathways than psychiatric disorders are[74,75]. Increased SW can lead to poor social functioning and social isolation, and can in turn cause feelings of loneliness. Loneliness prevalence in European countries has been found to range from 10% in the West and North to 55% in the East[76].

Low levels of SW have been found to be positively related to longevity, physical-, psychological-, and emotional well-being[77,78], whereas high SW has been associated with severe detrimental health outcomes, such as depression[79,80], adverse coronary condition rates[81-84], alcoholism[83], increased mortality rates[81-83], increased suicidality[85,86], and Alzheimer's disease[87]. Furthermore, associations between SW and alterations in hypothalamic-pituitary-adrenocortical (HPA) axis activity have been found[88,89]. Thus, it seems that the dimension of SW may aid in linking overlapping biological underpinnings across several conditions[75,76]. The identification of dimensional behavioral phenotypes across disorders may help to deepen our understanding of the neurobiology involved and complements the approach to incorporate dimensional measures as in the DSM-5 system.

As mentioned above, previous research has found associations between SW and HPA-axis activation[89-94], although certain findings were not consistent with this[76,94]. Evidence has also been found supporting the likelihood of a temporal relationship starting with SW and leading to subsequent depression[79]. Furthermore, increased SW has been found to be a mediating variable in the relationship between salivary cortisol and depression, although this study did not adjust for numerous influential covariates (i.e. adjusted only for gender, age, and cortisol concentration)[95]. However, the interrelationships between sAA, SW, and depression have not yet been explored, and based on the previous research regarding HPA-axis activation and SW, it is reasonable to hypothesize there may be an association with SNS activity and SW. These interrelationships will be further explored transdiagnostically in a population of patients with MAS-disorders and healthy controls in Chapter 3 of this thesis, using a dimensional approach in line with the RDoC guidelines in order to determine whether SW could offer a new way of classifying certain stress-related psychiatric disorders. Also, the interrelationships between sAA, social withdrawal, and depression will be investigated in this Chapter.

The HPA axis: prolonged exposure to endogenous cortisol

As mentioned previously, chronic stress, the repeated exposure to a stressor, numerous stressors, or exposure to a severe acute stressor can result in alterations in psychological and neurobiological processes. However, these processes can also be set in motion by Cushing's syndrome (CS). CS is caused by excess cortisol in the body, regardless of the cause. In Cushing's disease (CD), the stressor causing the

activation of these reactive processes has an endogenous origin. Specifically, CD is an endocrine disorder caused by a benign tumor (i.e., adenoma) located on the pituitary that produces adrenocorticotropic hormone (ACTH), in turn stimulating the release of GCs by the adrenal cortex[96]. In individuals without CD, an increase in GCs will trigger a negative feedback loop, inhibiting the release of ACTH. However, the ACTH-producing tumor in CD is insensitive to this inhibition, therefore, the system is unable to regulate itself, resulting in increased levels of GCs or hypercortisolism. CD typically displays several physical manifestations, including hypertension, abnormal fat distribution, thin skin sensitive to bruising, muscle weakness, osteoporosis, hirsutism, and gonadal dysfunction. Alongside these physical symptoms, patients can also display a wide variety of psychiatric symptoms, including emotional instability, cognitive impairments, psychosis, apathy, anxiety, and depression[96-98]. These symptoms are indicative of the effects of Cushing's disease on the central nervous system (CNS). CD provides a unique human model with which to investigate the effects of prolonged exposure to vast amounts of endogenous cortisol on the brain, and also to investigate the associations between these effects, and psychiatric- and clinical symptomatology.

The association between hypercortisolism and CNS damage was first described in 1952 by Trethowan and Cobb[99]. Their findings were based on autopsy reports in which they found enlarged ventricles and a decrease in brain weight in patients with Cushing's syndrome. These findings were further validated by the first in-vivo study conducted by Momose et al.[100], using pneumoencephalography. They found high incidences of atrophy in both cerebral and cerebellar regions in patients with Cushing's disease. Since then, several studies have investigated the effects of long-term exposure to endogenous cortisol on the brain in patients with (remitted) CD and in 2015, Andela et al.[101] wrote an elaborate systematic review of the findings of studies on structural and functional abnormalities. The main findings reported that the large amounts of endogenous GCs seemed to lead to profound effects on the brain, specifically on grey matter volumes of the ACC and the cerebellum, widespread reductions of white matter integrity, and alterations in specific neuronal metabolites in the bilateral hippocampus. However, since that time, there have been several relevant publications within this area of research, indicating that a review of the newest findings may be helpful in identifying current knowledge gaps. Chapter 4 of this thesis will explore these newest findings in more detail.

Cortical thickness and surface area of the brain

The cerebral cortex is the outer covering of the surfaces of the cerebral hemispheres. It is folded into peaks (i.e. gyri) and grooves (i.e. sulci). Cortical thickness represents the combined thickness of all cerebral cortex layers with the average human cortical thickness over the whole brain being approximately 2.5 to 3 mm[102]. Although

interpersonal variation of cortical thickness is present, an abnormally thin or thick cortex could be associated with changes in gray matter that correlate with specific neurological conditions and neuropathologies[103]. Cortical surface area likely reflects folding and gyrification, which both depend on division of progenitor cells in the periventricular area during embryogenesis[104].

Several studies have reported a reduction in cortical thickness in patients with stress-related disorders (for example, generalized and social anxiety disorder[105,106], bipolar disorder[107,108], and major depressive disorder[109,110]). Two studies investigated cortical thickness in Cushing's syndrome (CS) patients and healthy controls: the first found no differences in cortical thickness[111], and the second reported increased cortical thickness in the lateral orbitofrontal and superior frontal cortex in children with CS in compared to HCs, however this study did not adjust for multiple comparisons[112]. Furthermore, studies have found loss of brain volume in CS patients (for example, in the hippocampus, bicaudate, and third ventricle), which were found to be partially reversible upon biochemical remission[113-115].

Previous analyses conducted in a cohort of long-term remitted Cushing's disease (CD) patients have revealed reductions in white matter integrity throughout the brain in addition to altered resting-state connectivity between the limbic system and the subgenual anterior cingulate cortex (ACC) in comparison to healthy controls[116,117]. Furthermore, in this same patient population a voxel-based morphology study found reductions of ACC volumes[118]. As subregions of the ACC are considered critical in cognitive control, emotional functioning and reward-based decision making; damage to this region may lead to reductions in motivation, spontaneity, and problem-solving capacity, as well as increased apathy and verbalization[119-121]. These findings suggest that alterations in structure and connectivity in the brain, and in particular the ACC, may explain part of the cognitive and psychiatric symptoms commonly observed both in active and remitted CD patients.

There are two frequently used measures for gray matter analysis. The first is cortical thickness, which is indicative of neuron and glia size, number, and arrangement in specific cortical regions[122-124]. The second is cortical surface area, which is related to the number of columns in a region of interest[123,125]. These measures together constitute gray matter volume, however separately, they provide more detailed information on changes in cortical structures. For this reason, cortical thickness and surface area have been suggested to be of more etiological relevance than gray matter volume alone[126,127]. Examining the gray matter volume by means of cortical thickness and cortical surface area in the remitted CD patient population separately may therefore yield more specific insights into the seemingly lasting impairments of

CD on the brain. This will be explored in Chapter 5 of this thesis. In this study, the ACC will be identified as the region of interest (ROI), as previous research has identified this region as altered in both structure and resting-state connectivity. This will then be followed by an exploratory whole-brain analysis, after which the associations with psychiatric- and clinical symptomatology will be explored if appropriate.

As mentioned previously, research has shown that patients with (remitted) Cushing's disease often suffer from stress-related psychopathology, such as depression and anxiety. In Chapter 6 we would like to explore whether we can identify possible resilience-specific correlates in cortical thickness and cortical surface area, and their correlations with psychometric measures. We will explore this by means of a three-group design consisting of one group of 'resilient' participants (i.e. Dutch police officers that have been exposed to trauma and do not present any psychopathology), one group of 'vulnerable' participants (i.e. Dutch police officers that have been exposed to trauma and present with psychopathology), and a control group (i.e. Dutch recruits from the police academy with no trauma exposure and no psychopathology). Previous studies have found (parts of) the ACC to be altered in patients with trauma-related psychopathology[e.g. 128-130]. For this reason, the ACC will be identified as the ROI in this study, followed by an exploratory whole-brain analysis. If appropriate, associations between brain regions with psychiatric- and clinical symptomatology will be explored.

Cognitive planning and executive functioning in remitted CD patients

As denoted earlier, several studies have found (persistent) impairments of cognitive function in the (remitted) CD patient population. Previous studies have examined cognitive functioning by means of standard neuropsychological testing in active CD patients[131,132], as well as in remitted patients after a follow-up period of up to 18 months[133]. These studies found that cognitive and executive functioning (i.e., psychomotor functioning, visuoconceptual tracking, processing speed, auditory attention, auditory working memory, verbal fluency, reading speed, and brief attention) remains impaired in remitted CD patients.

An important cognitive function necessary to lead a functional life is the cognitive skill of planning. Cognitive planning encompasses the neurological processes that are involved with the strategy formulation, coordination, evaluation, and selection of a thought sequence, and the necessary actions that are needed in order to achieve that goal[134]. Reductions of these cognitive abilities in patients with remitted CD may lead to lasting effects on planning abilities, in turn effecting one's daily functionality, psychological state, and quality of life. Examining possible neurobiological alterations in brain activity patterns regarding cognitive planning and executive functioning

within the remitted CD patient population could provide further objective insights into these possibly lasting impairments within this cognitive domain. A task that is often used to detect alterations in brain activation with regard to cognitive planning and executive function is the Tower of London (ToL) task[135]. In Chapter 7 of this thesis we will explore the cognitive planning and executive functioning of remitted CD patients using the visuo-spatial ToL task, again using the ACC as ROI, followed by a whole brain analysis, and, if appropriate, further investigating associations with psychiatric- and clinical symptomatology.

Further exploration of the anterior cingulate cortex in remitted Cushing's disease patients
Although current treatment strategies abrogate excessive cortisol signaling and offer substantial alleviation of several associated symptoms, as mentioned earlier, certain debilitating psychological symptoms often persist in remitted CD patients, even after long-term remission. (f)MRI studies conducted with this patient population have found alterations in both brain structure and connectivity, in which the ACC has often been implicated. This region has therefore has been pinpointed as a region where further exploration could lead to considerable insights with regard to these observed alterations.

Previously, the hypothesis has been posed that the intrinsic impairments and alterations in connectivity and/or biochemistry of certain brain regions may have caused the structural differences observed in remitted CD patients, specifically in the ACC[101]. Unfortunately, MRI studies alone cannot offer sufficient insight into these underlying biological processes. However, such underlying biological processes could be further explored by combining information obtained from high resolution MRI scans with whole genome mRNA expression data. This data is openly available in the Allen Human Brain Atlas (AHBA), a multi-modal atlas mapping gene expression across the healthy human brain[135]. Exploring the potential mechanisms through which the structure of the ACC changes when exposed to prolonged endogenous cortisol excess by linking information derived from high resolution MRI scans with gene expression data derived from the AHBA, could offer more insights into the potential mechanisms through which these persistent alterations occur. This will be explored in Chapter 8 of this thesis.

Finally, Chapter 9 will summarize the empirical findings reported in this thesis. These findings will then be discussed and integrated into our current knowledge, focusing not only on how these findings can help us advance, but also on their implications.

In sum, this thesis aims to further unravel the role of the stress systems in the pathophysiology of stress-related psychiatric disorders, by exploring elements of

regulation and dysregulation of the two major stress systems (i.e. the ANS and the HPA-axis), and their relation with psychological and psychiatric symptoms. The two main hypotheses are that (i) sAA can differentiate between certain stress-related disorders, and (ii) that brain abnormalities in patients with remitted Cushing's disease will partially overlap with the brain abnormalities found in patients with stress-related disorders further increasing the validity of using Cushing's disease as a naturalistic model for the effects of long-term exposure of cortisol on the brain.

References

1. Selye, H., & Fortier, C. (1950). Adaptive reaction to stress. *Psychosomatic medicine*.
2. Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer publishing company. (135)
3. American Psychological Association. (2011). Stress: The different kinds of stress. http://www.apa.org/helpcenter/stress-kinds.aspx
4. McEwen, B. S. (2005). Stressed or stressed out: what is the difference?. *Journal of Psychiatry and Neuroscience, 30*(5), 315-318.
5. Connor-Smith, J. K., & Flachsbart, C. (2007). Relations between personality and coping: a meta-analysis. *Journal of personality and social psychology, 93*(6), 1080.
6. Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature reviews neuroscience, 10(*6), 397-409.
7. Keller-Wood, M. E., & Dallman, M. F. (1984). Corticosteroid inhibition of ACTH secretion. *Endocrine reviews, 5*(1), 1-24.
8. Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience, 8*(4), 383.
9. Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implicatons for therapy. *Journal of affective disorders, 62(*1-2), 77-91.
10. Bateman, A., Singh, A., Kral, T., & Solomon, S. (1989). The immune-hypothalamic-pituitary-adrenal axis. *Endocrine reviews, 10*(1), 92-112.
11. Rosmond, R. A., & Björntorp, P. (2000). The hypothalamic–pituitary–adrenal axis activity as a predictor of cardiovascular disease, type 2 diabetes and stroke. *Journal of internal medicine, 247*(2), 188-197.
12. McEwen BS, Weiss JM, Schwartz LS. Selective retention of corticosterone by limbic structures in rat brain. Nature. 1968;220:911-912.
13. De Kloet ER, Reul JMHM. Feedback action and tonic influence of corticosteroids on brain function: A concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology. 1987;12:83-105. DOI: 10.1016/0306-4530(87)90040-0
14. Meijer OC, De Lange ECM, Breimer DD, De Boer AG, Workel JO, De Kloet ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology. 1998;139:1789-1793. DOI:10.1210/endo.139.4.5917
15. Reul, J. M. H. M., & Kloet, E. D. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology, 117*(6), 2505-2511.
16. De Kloet, E. R., Wallach, G., & McEwen, B. S. (1975). Differences in Corticosterone and Dexamethasone Binding to Rat Brain and Pituitary 1. *Endocrinology, 96*, 598-609.
17. De Kloet, E. R., & Reul, J. M. H. M. (1987). Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. *Psychoneuroendocrinology, 12*, 83-105.
18. Rupprecht, R., Reul, J. M., van Steensel, B., Spengler, D., Söder, M., Berning, B., ... & Damm, K. (1993). Pharmacological and functional characterization of human mineralocorticoid and glucocorticoid receptor ligands. *European Journal of Pharmacology: Molecular Pharmacology, 247*, 145-154.
19. K., & Kellner, M. (2007). Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology, 32*, 232-238.
20. Otte, C., Wingenfeld, K., Kuehl, L. K., Kaczmarczyk, M., Richter, S., Quante, A., ... & Hinkelmann, K. (2015). Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. *Neuropsychopharmacology, 40*(2), 386-393.

21. Joëls, M., Karst, H., DeRijk, R., & de Kloet, E. R. (2008). The coming out of the brain mineralocorticoid receptor. Trends in neurosciences, 31, 1-7.
22. Hermans, E. J., Henckens, M. J., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. Trends in Neurosciences, 37, 304-314.
23 Berardelli, R., Karamouzis, I., D'Angelo, V., Zichi, C., Fussotto, B., Giordano, R., ... & Arvat, E. (2013). Role of mineralocorticoid receptors on the hypothalamus–pituitary–adrenal axis in humans. Endocrine, 43, 51-58.
24. Arp, J. M., ter Horst, J. P., Kanatsou, S., Fernández, G., Joëls, M., Krugers, H. J., & Oitzl, M. S. (2014). Mineralocorticoid receptors guide spatial and stimulus-response learning in mice. P*loS one, 9,* e86236.
25. De Kloet, E. R., & Meijer, O. C. (2019). MR/GR Signaling in the Brain during the Stress Response. *Aldosterone-Miner. Recept Cell Biol. Transl. Med.*
26. Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology, 23, 477-501.
27. Klok, M. D., Irurzun-Lafitte, A., Turner, J., Lakke, E., Huitinga, I., Muller, C., Zitman, F., De Kloet, E., & De Rijk., R. (2011b). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of Psychiatric Research, 45*, 871-878.
28. Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., ... & Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life sciences, 61(*26), 2539-2549.
29. Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening?. *Psychoneuroendocrinology, 32*(4), 358-366.
30. Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *The British Journal of Psychiatry, 177*(6), 499-504.
31. Wardenaar, K. J., Vreeburg, S. A., van Veen, T., Giltay, E. J., Veen, G., Penninx, B. W., & Zitman, F. G. (2011). Dimensions of depression and anxiety and the hypothalamo-pituitary-adrenal axis. *Biological psychiatry, 69*(4), 366-373.
32. Strickland, P. L., Deakin, J. W., Percival, C., Dixon, J., Gater, R. A., & Goldberg, D. P. (2002). Bio-social origins of depression in the community: Interactions between social adversity, cortisol and serotonin neurotransmission. *The British Journal of Psychiatry, 180*(2), 168-173.
33. Burke, H. M., Fernald, L. C., Gertler, P. J., & Adler, N. E. (2005). Depressive symptoms are associated with blunted cortisol stress responses in very low-income women. *Psychosomatic Medicine, 67*(2), 211-216.
34. Stetler, C., & Miller, G. E. (2005). Blunted cortisol response to awakening in mild to moderate depression: regulatory influences of sleep patterns and social contacts. *Journal of abnormal psychology, 114*(4), 697.
35. Bhagwagar, Z., Hafizi, S., & Cowen, P. J. (2005). Increased salivary cortisol after waking in depression. *Psychopharmacology, 182*(1), 54-57.
36. Pruessner, M., Hellhammer, D. H., Pruessner, J. C., & Lupien, S. J. (2003a). Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. *Psychosomatic medicine, 65*(1), 92-99.
37. Vreeburg, S. A., Hoogendijk, W. J., van Pelt, J., DeRijk, R. H., Verhagen, J. C., van Dyck, R., ... & Penninx, B. W. (2009). Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Archives of general psychiatry, 66*(6), 617-626.
38. Young, E. A., Haskett, R. F., Grunhaus, L., Pande, A., Weinberg, V. M., Watson, S. J., & Akil, H. (1994). Increased evening activation of the hypothalamic-pituitary-adrenal axis in depressed patients. *Archives of General Psychiatry, 51*(9), 701-707.

39. Chaudieu, I., Beluche, I., Norton, J., Boulenger, J. P., Ritchie, K., & Ancelin, M. L. (2008). Abnormal reactions to environmental stress in elderly persons with anxiety disorders: evidence from a population study of diurnal cortisol changes. *Journal of Affective Disorders, 106*(3), 307-313.
40. Gerra, G., Zaimovic, A., Zambelli, U., Timpano, M., Reali, N., Bernasconi, S., & Brambilla, F. (2000). Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. *Neuropsychobiology, 42*(2), 82-92.
41. Rosenbaum, A. H., Schatzberg, A. F., Jost III, F. A., Cross, P. D., Wells, L. A., Jiang, N. S., & Maruta, T. (1983). Urinary free cortisol levels in anxiety. *Psychosomatics, 24*(9), 835-837.
42. Mantella, R. C., Butters, M. A., Amico, J. A., Mazumdar, S., Rollman, B. L., Begley, A. E., ... & Lenze, E. J. (2008). Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. *Psychoneuroendocrinology, 33*(6), 773-781.
43. Tiller, J. W. G., Biddle, N., Maguire, K. P., & Davies, B. M. (1988). The dexamethasone suppression test and plasma dexamethasone in generalized anxiety disorder. *Biological psychiatry, 23*(3), 261-270.
44. Okasha, A., Bishry, Z., Khalil, A. H., Darwish, T. A., El Dawla, A. S., & Shohdy, A. (1994). Panic Disorder. *The British Journal of Psychiatry, 164*(6), 818-825.
45. Chatterton Jr, R. T., Vogelsong, K. M., Lu, Y. C., Ellman, A. B., & Hudgens, G. A. (1996). Salivary α-amylase as a measure of endogenous adrenergic activity. *Clinical physiology, 16*(4), 433-448.
46. Ali, N., & Nater, U. M. (2020). Salivary alpha-amylase as a biomarker of stress in behavioral medicine. *International journal of behavioral medicine, 27*(3), 337-342.
47. Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity. *Ann NY Acad Sci, 1032*(1), 258-263.
48. van Stegeren, A., Rohleder, N., Everaerd, W., & Wolf, O. T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology, 31*(1), 137-141.
49. Janeček, Š. (1994). Sequence similarities and evolutionary relationships of microbial, plant and animal α-amylases. *European journal of biochemistry, 224*(2), 519-524.
50. Yates, J. A. F., Velsko, I. M., Aron, F., Posth, C., Hofman, C. A., Austin, R. M., ... & Warinner, C. (2021). The evolution and changing ecology of the African hominid oral microbiome. *Proceedings of the National Academy of Sciences, 118*(20).
51. Perry, G.H., et al., *Diet and the evolution of human amylase gene copy number variation.* Nat Genet, 2007. 39(10): p. 1256-60.
52. Schumacher, S., Kirschbaum, C., Fydrich, T., & Ströhle, A. (2013). Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?—A review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology, 38*(6), 729-743.
53. Des Gachons, C. P., & Breslin, P. A. (2016). Salivary amylase: digestion and metabolic syndrome. *Current diabetes reports, 16*(10), 1-7.
54. Freitas, D., & Le Feunteun, S. (2019). Inhibitory effect of black tea, lemon juice, and other beverages on salivary and pancreatic amylases: What impact on bread starch digestion? A dynamic in vitro study. *Food chemistry, 297,* 124885.
55. Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81.
56. Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology, 55*(3), 333-342.
57. Garrett, J. R., Ekström, J., & Anderson, L. C. (1999). Effects of autonomic nerve stimulations on salivary parenchyma and protein secretion. *Neural mechanisms of salivary gland secretion, 11*, 59-79.

58. Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M., & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. *Psychoneuroendocrinology, 31*(1), 49-58.
59. Granger, D. A., Kivlighan, K. T., El-Sheikh, M. O. N. A., Gordis, E. B., & Stroud, L. R. (2007). Salivary α-amylase in biobehavioral research: recent developments and applications. *Annals of the New York Academy of sciences, 1098*(1), 122-144.
60. Chatterton Jr, R. T., Vogelsong, K. M., Lu, Y. C., Ellman, A. B., & Hudgens, G. A. (1996). Salivary α-amylase as a measure of endogenous adrenergic activity. *Clinical physiology, 16*(4), 433-448.
61. Tamura, A., Maruyama, Y., Ishitobi, Y., Kawano, A., Ando, T., Ikeda, R., ... & Akiyoshi, J. (2013). Salivary alpha-amylase and cortisol responsiveness following electrical stimulation stress in patients with the generalized type of social anxiety disorder. *Pharmacopsychiatry, 46*(07), 225-260.
62. Tanaka, Y., Ishitobi, Y., Maruyama, Y., Kawano, A., Ando, T., Okamoto, S., ... & Akiyoshi, J. (2012). Salivary alpha-amylase and cortisol responsiveness following electrical stimulation stress in major depressive disorder patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 36*(2), 220-224.
63. Tanaka, Y., Ishitobi, Y., Maruyama, Y., Kawano, A., Ando, T., Imanaga, J., ... & Akiyoshi, J. (2012). Salivary alpha-amylase and cortisol responsiveness following electrical stimulation stress in panic disorder patients. *Neuroscience research,* 73(1), 80-84.
64. Wei, K., Xue, H. L., Guan, Y. H., Zuo, C. T., Ge, J. J., Zhang, H. Y., ... & Du, Y. J. (2016). Analysis of glucose metabolism of 18F-FDG in major depression patients using PET imaging: Correlation of salivary cortisol and α-amylase. *Neuroscience letters, 629*, 52-57.
65. Cubała, W. J., & Landowski, J. (2014). Low baseline salivary alpha-amylase in drug-naïve patients with short-illness-duration first episode major depressive disorder. *Journal of affective disorders, 157*, 14-17.
66. Ishitobi, Y., Akiyoshi, J., Tanaka, Y., Ando, T., Okamoto, S., Kanehisa, M., ... & Kodama, K. (2010). Elevated salivary α-amylase and cortisol levels in unremitted and remitted depressed patients. *International journal of psychiatry in clinical practice, 14*(4), 268-273.
67. Van Veen, J. F., Van Vliet, I. M., DeRijk, R. H., Van Pelt, J., Mertens, B., & Zitman, F. G. (2008). Elevated alpha-amylase but not cortisol in generalized social anxiety disorder. *Psychoneuroendocrinology, 33*(10), 1313-1321.
68. Veen, G., Giltay, E. J., Licht, C. M., Vreeburg, S. A., Cobbaert, C. M., Penninx, B. W., & Zitman, F. G. (2013). Evening salivary alpha-amylase, major depressive disorder, and antidepressant use in the Netherlands Study of Depression and Anxiety (NESDA). *Psychiatry research, 208*(1), 41-46.
69. Booij, S. H., Bos, E. H., Bouwmans, M. E., van Faassen, M., Kema, I. P., Oldehinkel, A. J., & de Jonge, P. (2015). Cortisol and α-amylase secretion patterns between and within depressed and non-depressed individuals. *PloS one, 10*(7), e0131002.
70. Perry, G.H., et al., *Diet and the evolution of human amylase gene copy number variation. Nat Genet, 2007. 39*(10): p. 1256-60.
71. Barzeva, S. A., Meeus, W. H., & Oldehinkel, A. J. (2019). Social withdrawal in adolescence and early adulthood: Measurement issues, normative development, and distinct trajectories. *Journal of abnormal child psychology, 47*(5), 865-879.
72. Saris, I. M. J., Aghajani, M., van der Werff, S. J. A., van der Wee, N. J. A., & Penninx, B. W. J. H. (2017). Social functioning in patients with depressive and anxiety disorders. *Acta Psychiatrica Scandinavica, 136*(4), 352-361.
73. Wen, M., Hawkley, L. C., & Cacioppo, J. T. (2006). Objective and perceived neighborhood environment, individual SES and psychosocial factors, and self-rated health: an analysis of older adults in Cook County, Illinois. *Soc Sci Med, 63*(10), 2575-2590. doi:10.1016/j.socscimed.2006.06.025

74. Porcelli, S., Van Der Wee, N., van der Werff, S., Aghajani, M., Glennon, J. C., van Heukelum, S., ... & Posadas, M. (2018). Social brain, social dysfunction and social withdrawal. *Neuroscience & Biobehavioral Reviews.*
75. van der Wee, N. J., Bilderbeck, A. C., Cabello, M., Ayuso-Mateos, J. L., Saris, I. M., Giltay, E. J., ... & Porcelli, S. (2018). Working definitions, subjective and objective assessments and experimental paradigms in a study exploring social withdrawal in schizophrenia and Alzheimer's disease. *Neuroscience & Biobehavioral Reviews.*
76. Cacioppo, J. T., Hawkley, L. C., Crawford, L. E., Ernst, J. M., Burleson, M. H., Kowalewski, R. B., . . . Berntson, G. G. (2002). Loneliness and health: potential mechanisms. *Psychosom Med, 64(*3), 407-417.
77. Holt-Lunstad, J., Smith, T. B., & Layton, J. B. (2010). Social Relationships and Mortality Risk: A Meta-analytic Review. *Plos Medicine, 7*(7).
78. Uchino, B. N. (2006). Social support and health: a review of physiological processes potentially underlying links to disease outcomes. *J Behav Med, 29*(4), 377-387. doi:10.1007/s10865-006-9056-5
79. Cacioppo, J. T., Hawkley, L. C., & Thisted, R. A. (2010). Perceived social isolation makes me sad: 5-year cross-lagged analyses of loneliness and depressive symptomatology in the Chicago Health, Aging, and Social Relations Study. *Psychol Aging, 25*(2), 453-463. doi:10.1037/a0017216
80. Cacioppo, J. T., Hughes, M. E., Waite, L. J., Hawkley, L. C., & Thisted, R. A. (2006). Loneliness as a specific risk factor for depressive symptoms: cross-sectional and longitudinal analyses. *Psychology and aging, 21*(1), 140.
81. Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., & Stephenson, D. (2015). Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspect Psychol Sci, 10*(2), 227-237. doi:10.1177/1745691614568352
82. Patterson, A. C., & Veenstra, G. (2010). Loneliness and risk of mortality: A longitudinal investigation in Alameda County, California. *Social Science & Medicine, 71*(1), 181-186. doi:10.1016/j.socscimed.2010.03.024
83. Sorkin, D., Rook, K. S., & Lu, J. L. (2002). Loneliness, lack of emotional support, lack of companionship, and the likelihood of having a heart condition in an elderly sample. *Annals of Behavioral Medicine, 24*(4), 290-298. doi:Doi 10.1207/S15324796abm2404_05
84. Qualter, P., Vanhalst, J., Harris, R., Van Roekel, E., Lodder, G., Bangee, M., . . . Verhagen, M. (2015). Loneliness across the life span. *Perspect Psychol Sci, 10*(2), 250-264. doi:10.1177/1745691615568999
85. Conroy, R. W., & Smith, K. (1983). Family Loss and Hospital Suicide. *Suicide and Life-Threatening Behavior, 13*(3), 179-194.
86. Peck, A. (1983). Psychotherapy of the elderly. Case #6. *J Geriatr Psychiatry, 16*(1), 73-77.
87. Wilson, R. S., Krueger, K. R., Arnold, S. E., Schneider, J. A., Kelly, J. F., Barnes, L. L., . . . Bennett, D. A. (2007). Loneliness and risk of Alzheimer disease. *Arch Gen Psychiatry, 64*(2), 234-240. doi:10.1001/archpsyc.64.2.234
88. Adam, E. K., Hawkley, L. C., Kudielka, B. M., & Cacioppo, J. T. (2006). Day-to-day dynamics of experience--cortisol associations in a population-based sample of older adults. *Proc Natl Acad Sci U S A, 103*(45), 17058-17063. doi:10.1073/pnas.0605053103
89. Arnetz, B. B., Theorell, T., Levi, L., Kallner, A., & Eneroth, P. (1983). An experimental study of social isolation of elderly people: psychoendocrine and metabolic effects. *Psychosom Med, 45*(5), 395-406.
90. Doane, L. D., & Adam, E. K. (2010). Loneliness and cortisol: Momentary, day-to-day, and trait associations. *Psychoneuroendocrinology, 35*(3), 430-441. doi:10.1016/j.psyneuen.2009.08.005
91. Grant, N., Hamer, M., & Steptoe, A. (2009). Social isolation and stress-related cardiovascular, lipid, and cortisol responses. *Ann Behav Med, 37*(1), 29-37. doi:10.1007/s12160-009-9081-z

92. Hawkley, L. C., Cole, S. W., Capitanio, J. P., Norman, G. J., & Cacioppo, J. T. (2012). Effects of social isolation on glucocorticoid regulation in social mammals. *Horm Behav, 62*(3), 314-323. doi:10.1016/j.yhbeh.2012.05.011
93. Pressman, S. D., Cohen, S., Miller, G. E., Barkin, A., Rabin, B. S., & Treanor, J. J. (2005). Loneliness, social network size, and immune response to influenza vaccination in college freshman (vol 24, pg 297, 2005). *Health Psychology, 24*(4), 348-348. doi:Doi 10.1037/0278-6133.24.4.348
94. Steptoe, A., Owen, N., Kunz-Ebrecht, S. R., & Brydon, L. (2004). Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. *Psychoneuroendocrinology, 29*(5), 593-611. doi:10.1016/S0306-4530(03)00086-6
95. Wai, S. T., & Bond, A. J. (2004). Relationship between baseline cortisol, social functioning and depression: a mediation analysis. *Psychiatry research, 126*(3), 197-201.
96. Lonser, R. R., Nieman, L., & Oldfield, E. H. (2017). Cushing's disease: pathobiology, diagnosis, and management. *Journal of neurosurgery, 126*(2), 404-417.
97. Pereira, A. M., Tiemensma, J., & Romijn, J. A. (2010). Neuropsychiatric disorders in Cushing's syndrome. *Neuroendocrinology, 92*(Suppl. 1), 65-70.
98. Pivonello, R., Simeoli, C., De Martino, M. C., Cozzolino, A., De Leo, M., Iacuaniello, D., ... & Colao, A. (2015). Neuropsychiatric disorders in Cushing's syndrome. *Frontiers in Neuroscience, 9*, 129.
99. Trethowan, W. H., & Cobb, S. (1952). Neuropsychiatric aspects of Cushing's syndrome. *AMA Archives of Neurology & Psychiatry, 67*(3), 283-309.
100. Momose KJ, Kjellberg RN & Kliman B. High incidence of cortical atrophy of the cerebral and cerebellar hemispheres in Cushing's disease. Radiology 1971 99 341-348.
101. Andela, C. D., van Haalen, F. M., Ragnarsson, O., Papakokkinou, E., van der Wee, J. A., & Pereira, A. M. (2015). Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional MRI 2 studies 3. *studies, 3*, 4.
102. Zilles, K., & Amunts, K. (1990). The human nervous system.
103. Hutton, C., De Vita, E., Ashburner, J., Deichmann, R., & Turner, R. (2008). Voxel-based cortical thickness measurements in MRI. *Neuroimage, 40*(4), 1701-1710.
104. Mensen, V. T., Wierenga, L. M., van Dijk, S., Rijks, Y., Oranje, B., Mandl, R. C., & Durston, S. (2017). Development of cortical thickness and surface area in autism spectrum disorder. *NeuroImage: Clinical, 13*, 215-222.
105. Syal, S. et al. (2012). Grey matter abnormalities in social anxiety disorder: a pilot study. *Metabolic Brain Disease, 27*:299-309.
106. Molent, C. et al. (2018). Reduced cortical thickness and increased gyrification in generalized anxiety disorder: a 3 T MRI study. *Psychological Medicine, 48*(12):2001-2010.
107. Lan, M.J. et al. (2014). Cortical thickness differences between bipolar depression and major depressive disorder. *Bipolar Disorders, 16*(4):378-388.
108. Abé, C. et al. (2016). Cortical thickness, volume and surface area in patients with bipolar disorder types I and II. *Journal of Psychiatry & Neuroscience, 41*(4):240.
109. Zhao, K. et al. (2017). Altered patterns of association between cortical thickness and subcortical volume in patients with first episode major depressive disorder: a structural MRI study. *Psychiatry Research: Neuroimaging, 260*:16-22.
110. Schmaal, L. et al. (2017). Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. *Molecular Psychiatry, 22*(6):900.
111. Crespo, I. et al. (2014). Impaired decision-making process and thinner prefrontal cortex in patients with Cushing's syndrome. *Clin Endocrinol, 81*, 826-33.
112. Tirosh, A. et al. (2020). Computerized analysis of brain MRI parameters dynamics in young patients with Cushing Syndrome–a case-control study. *The Journal of Clinical Endocrinology & Metabolism.*

113. Starkman, M. N., Giordani, B., Gebarski, S. S., Berent, S., Schork, M. A., & Schteingart, D. E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological psychiatry, 46*(12), 1595-1602.
114 Starkman MN, Giordani B, Gebarski SS, Schteingart DE (2003). Improvement in learning associated with increase in hippocampal formation volume. *Biological Psychiatry, 53*(3): 233-238.
115. Bourdeau I, Bard C, Noël B, Leclerc I, Cordeau MP, Bélair M, et al (2002). Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. *The Journal of Clinical Endocrinology & Metabolism, 87*(5):1949-1954.
116. van der Werff, S. J., Andela, C. D., Pannekoek, J. N., Meijer, O. C., van Buchem, M. A., Rombouts, S. A., ... & van der Wee, N. J. (2014). Widespread reductions of white matter integrity in patients with long-term remission of Cushing's disease. *NeuroImage: Clinical, 4*, 659-667.
117. van Der Werff, S. J., Pannekoek, J. N., Andela, C. D., Meijer, O. C., Van Buchem, M. A., Rombouts, S. A., ... & Van Der Wee, N. J. (2015). Resting-state functional connectivity in patients with long-term remission of Cushing's disease. *Neuropsychopharmacology, 40*(8), 1888-1898.
118. Andela, C. D., Van der Werff, S. J., Pannekoek, J. N., van den Berg, S. M., Meijer, O. C., van Buchem, M. A., ... & Pereira, A. M. (2013). 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. *Eur J Endocrinol, 169*(6), 811-819.
119. Tekin, S., & Cummings, J. L. (2002). Frontal–subcortical neuronal circuits and clinical neuropsychiatry: an update. *Journal of psychosomatic research, 53*(2), 647-654.
120. Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain structure and function, 213*(1-2), 93-118.
121. Harrison, P. J. (1999). The neuropathology of schizophrenia: a critical review of the data and their interpretation. *Brain, 122*(4), 593-624.
122. Narr, K. L., Bilder, R. M., Toga, A. W., Woods, R. P., Rex, D. E., Szeszko, P. R., ... & DeLuca, H. (2004). Mapping cortical thickness and gray matter concentration in first episode schizophrenia. *Cerebral cortex, 15*(6), 708-719.
123. Rakic, P., & Swaab, D. F. (1988). Defects of neuronal migration and the pathogenesis of cortical malformations. In *Progress in brain research* (Vol. 73, pp. 15-37). Elsevier.
124. Mountcastle, V. B. (1957). Modality and topographic properties of single neurons of cat's somatic sensory cortex. *Journal of neurophysiology, 20*(4), 408-434.
125. Im, K., Lee, J. M., Lyttelton, O., Kim, S. H., Evans, A. C., & Kim, S. I. (2008). Brain size and cortical structure in the adult human brain. *Cerebral Cortex, 18*(9), 2181-2191.
126. Ehrlich, S., Brauns, S., Yendiki, A., Ho, B. C., Calhoun, V., Schulz, S. C., ... & Sponheim, S. R. (2011). Associations of cortical thickness and cognition in patients with schizophrenia and healthy controls. *Schizophrenia bulletin, 38*(5), 1050-1062.
127. Ragnarsson, O., Berglund, P., Eder, D. N., & Johannsson, G. (2012). Long-term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol-producing adrenal adenoma in remission. *The Journal of Clinical Endocrinology & Metabolism, 97*(9), E1640-E1648.
128. Kasai, K., Yamasue, H., Gilbertson, M. W., Shenton, M. E., Rauch, S. L., & Pitman, R. K. (2008). Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. *Biological psychiatry, 63*(6), 550-556.
129. Woodward, S. H., Kaloupek, D. G., Streeter, C. C., Martinez, C., Schaer, M., & Eliez, S. (2006). Decreased anterior cingulate volume in combat-related PTSD. *Biological psychiatry, 59*(7), 582-587.
130. Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O., ... & Kato, N. (2003). Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *Proceedings of the National Academy of Sciences, 100*(15), 9039-9043.

131 Tiemensma, J., Kokshoorn, N. E., Biermasz, N. R., Keijser, B. J. S., Wassenaar, M. J., Middelkoop, H. A., ... & Romijn, J. A. (2010). Subtle cognitive impairments in patients with long-term cure of Cushing's disease. *The Journal of Clinical Endocrinology & Metabolism, 95*(6), 2699-2714.

132. Hook, J. N., Giordani, B., Schteingart, D. E., Guire, K., Giles, J., Ryan, K., ... & Starkman, M. N. (2007). Patterns of cognitive change over time and relationship to age following successful treatment of Cushing's disease. *Journal of the International Neuropsychological Society, 13*(1), 21-29.

133. Morris, R. G., Miotto, E. C., Feigenbaum, J. D., Bullock, P., & Polkey, C. E. (1997). Planning ability after frontal and temporal lobe lesions in humans: The effects of selection equivocation and working memory load. *Cognitive Neuropsychology, 14*, 1007-1027.

134. Shallice, T. (1982). Specific impairments of planning. Philosophical Transactions of the Royal Society of London B: *Biological Sciences, 298*, 199-209.

135. Hawrylycz, M. J., Lein, E. S., Guillozet-Bongaarts, A. L., Shen, E. H., Ng, L., Miller, J. A., ... & Abajian, C. (2012). *An anatomically comprehensive atlas of the adult human brain transcriptome. Nature, 489*(7416), 391-399.