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

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Chapter 9

General discussion

The aim of this thesis was to further unravel the role of 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.

Two main hypotheses were posited at the beginning of this thesis. The first was that sAA, unlike salivary cortisol, can differentiate between certain stress-related disorders. The second was that exposure to high levels of endogenous cortisol over a long period of time, as is the case in Cushing's disease, will result in persisting abnormalities of certain brain regions and circuits. In the following sections the results from our studies are discussed and integrated alongside contemporary study findings where possible.

Section I: General implications with regard to sAA

In **Chapter 2** we explored 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 by investigating diurnal salivary alpha amylase (sAA) and salivary cortisol levels in patients with mood-, anxiety-, and symptom somatic (MAS)-disorders and healthy controls (HCs). Although an ample amount of research has been conducted with regard to diurnal salivary cortisol (sC), few naturalistic studies have investigated diurnal sAA levels in patients with MAS-disorders to date. Our hypotheses were that MDD patients would show the same diurnal sAA pattern as HCs, however, we expected the morning sAA levels to be higher in the MDD patient group compared to both the HC and other MAS-disorders groups. Furthermore, we expected to find an elevation of sAA in the AUCg in both the patient groups in comparison to HCs. Seven saliva samples were collected over the course of 24 hours in a naturalistic setting (i.e. at awakening on day 1, 30 minutes after awakening, 45 minutes after awakening, 1 hour after awakening, at 10:00 p.m., 11:00 p.m., and at awakening at day 2). As expected and in line with previous research, sC was able to differentiate between stress-related psychiatric disorders on the one hand, and HCs on the other hand, but not between the stress-related disorders. Our main results with regard to sAA showed that sAA levels at awakening in the MDD group were higher than those in the other MAS-disorder patient group and in the HC group on both day 1 and day 2, a novel finding and in line with one of our hypotheses. In contrast to our second hypothesis, we did not find an elevated AUCg in the MDD and other MAS-disorders patient groups compared to the HCs. Furthermore, we found that the mean sAA levels at awakening to be positively associated with the brief symptom inventory (BSI) depression subscale scores, indicating that sAA levels are higher in those with more severe depression. Whereas two previous studies with depressed patients and HCs have found evidence that sAA is able to differentiate between these two groups at awakening and in the morning, respectively[1,2], our findings provide the first scientific evidence for the differentiating quality of naturalistic sAA levels at awakening in patients with MDD in comparison to both HCs as well as patients with other MAS-disorders.

In light of the findings in Chapter 2, we further investigated the possible role of sympathetic nervous system (SNS) activity in stress-related disorders in **Chapter 3**. In line with the goals of the DSM-5 and National Institute of Mental Health Research Domain Criteria (RDoC), which aims at identifying new ways of classifying psychiatric disorders based on dimensions of neurobiological measures and observable behaviour, a transdiagnostic dimensional approach was applied. We examined the

relationship between sAA and social withdrawal (SW), which has been posited to be a more stable endophenotype that is more closely connected to biological pathways than psychiatric disorders are[3,4]. Previously, evidence has been found supporting the likelihood of a temporal relationship starting with SW, leading to subsequent depression. Furthermore, increased SW has been found to be a mediating variable in the relationship between salivary cortisol and depression. We were interested in exploring possible associations between SNS activity and SW, and hypothesized that higher sAA levels would be positively associated with higher levels of SW. We included sAA en sC samples from patients with MAS-disorders HCs, and ordered them along the dimension of three SW subscales (i.e. the BSI social withdrawal subscale[5], the SF-36 social withdrawal subscale[6], and the DAPP-SF social avoidance subscale[7]). We did not find any associations between sAA and the SW subscales, however several associations were found between the BSI social withdrawal subscale and sC.
Furthermore, increased SW has been found to be a mediating variable in the relationship between sC and depression. We were therefore interested to explore whether SW was a mediating variable in the relationship between sAA and depression, however although we found further evidence for the aforementioned relationship between sC and depression, we did not find any evidence for this relationship with regard to sAA. Therefore, our findings do not support our hypothesis that SNS activation is involved in SW, though it may also be the case that the measurement instruments that were used in this study were not sensitive enough to measure the complex SW construct. On the other hand, we were able to replicate earlier associations between salivary cortisol and SW, although the effect sizes were small. In the future, better validated measurement instruments of SW (such as the World Health Organization Disability Assessment Schedule 2.0 social withdrawal scale[8]), should be employed to further explore these possible associations. Moreover, future studies should also attempt to differentiate between state and trait characteristics of SW[9].

Taken together, our studies in **Chapters 2 and 3** are innovative in several ways. First, they further assessed diurnal sAA in more detail than previous research conducted to date. Second, we found that sAA levels, specifically those at awakening, have the potential of differentiating between MDD patients and patients with other MAS-disorders, a novel finding. Third, we found that it is unlikely for SNS activation to be involved in SW. However, further research is necessary to determine if, and if so, to what extent, our current findings can be replicated. As the studies conducted in this thesis were of a cross-sectional nature, no conclusions can be made regarding

the temporal directions and causal pathways. Furthermore, these studies made use of a number of saliva samples over the course of the day, whereas collecting multiple samples over the course of the day for a longer period of time will likely yield more significant insights into its role in the disease course. This would aid in new insights as to whether sAA could be seen as a risk marker (i.e. neurological or biological traits indicating a predisposition towards developing a disease, but not part of the causal chain), or risk factor (implicating elevated sAA levels as a causal factor in the development of a stress-related disorder), the latter of which is unlikely. Importantly, our studies were again able to highlight the advantages of measuring ANS and HPA-axis activation in saliva. Specifically, saliva sampling is a relatively inexpensive, non-invasive (thus painless) sampling method that can be performed in one's natural habitat and under normal conditions, which makes it possible to collect multiple saliva samples in a stress-free environment. In sum, our findings add to the increasing body of evidence indicating that the (inter-)relations between ANS activation and stress-related disorders warrants further exploration.

Section II: General implications with regard to Cushing's disease

The second aim of this thesis was to further explore the possibly persistent brain abnormalities in patients with long-term remitted Cushing's disease likely due to the long-term exposure to cortisol. This is important for several reasons. The first is the clinical importance to this patient population itself. As mentioned previously, patients that are in remission of Cushing's disease often experience persistent deficits within certain cognitive and psychiatric domains[10-12], even after several years of biochemical curation (i.e. cortisol levels that lie within the normal range). This phenomenon clearly warrants further investigation. Second, study findings are of importance for other patients suffering from autoimmune diseases who are prescribed immunosuppressive drugs (i.e., glucocorticoids). Excessive use of glucocorticoids can induce Cushing's syndrome, a syndrome that is similar to Cushing's disease, but where the cause is of an exogenous origin, instead of endogenous (i.e. an adenoma on the pituitary gland or an adrenal gland tumor). Remitted Cushing's syndrome patients have also reported to experience similar and persistent deficits in the same domains as those reported by patients with remitted Cushing's disease. Further research investigating the possible long-term side-effects of dosages regarding augmented glucocorticoid therapy on the brain and its functioning could yield additional important insights. Finally, earlier research has posited that Cushing's disease might be a suitable naturalistic model to explore the (possibly lasting) effects of endogenous cortisol overexposure on the brain[86]. Patients with other psychiatric stress-related disorders also experience elevations in

cortisol levels over longer periods of time, although to a lesser extent. By comparing brain abnormalities found in patients with remitted Cushing's disease with brain abnormalities found in patients with other (remitted) stress-related psychiatric disorders we can further explore the validity of this proposed model.

In **Chapter 4**, we built further upon structural and functional abnormalities found to be associated with Cushing's syndrome as reported in the elaborate review by Andela and colleagues[13]. Specifically, we evaluated the newest findings with regard to the gray- and white- matter structural abnormalities that have been found in patients with active Cushing's disease and Cushing's syndrome, and also the extent of reversibility of these abnormalities. With regard to the gray matter structural abnormalities, we found volume reductions of the hippocampus and a prefrontal region involving the medial frontal gyrus (MFG) and the anterior cingulate cortex (ACC). Regarding reversibility of these abnormalities, hippocampal volume was found to be partially reversible, whereas the alterations in the MFG and ACC seem to be more persistent. (Regions of) the ACC have been found to be critically involved in cognitive control, cognitive processing of anxiety and fear, emotional functioning, and reward-based decision making[e.g.14]. Therefore, it seems likely that damage to this region may lead to reductions in motivation, spontaneity, problem-solving capacity, and increased apathy, domains that have often also been found to be impaired in patients with stress-related disorders. This may explain part of the cognitive and psychiatric symptoms commonly observed both in active and remitted CD patients.

In this review, two further important aspects were emphasized. The first is that the alterations in the ACC seem to be a persistent effect of exposure to hypercorticolism, indicating that this may be part of the pathophysiological pathway that keeps the persisting reported symptomology intact. The second is that there is a lack of well-designed studies that use advanced neuroimaging methods and analysis techniques, as well as a lack of studies that investigate the possible underlying microbiological processes of Cushing's disease. We highlighted that MRI studies alone can offer only part of the insights necessary, but that by converging MRI data with other data modalities our knowledge regarding these processes could be extended. Specifically, we posited that by combining data from the Allen Human Brain Atlas, a multi-modal atlas mapping gene expression across the healthy human brain[15], further insights into these mechanisms could be attained. For example, possible genes that interact with hypercorticolism, and thus may influence the structural changes that have been identified in the brain, may be uncovered.

In light of the abovementioned lack of well-designed studies using advanced

neuroimaging methods and analysis techniques, we further investigated the ACC by examining the cortical thickness and cortical surface area of remitted Cushing's disease patients and their matched HCs in **Chapter 5**. Specifically, we investigated cortical thickness and cortical surface area separately in long-term remitted CD patients, and age-, gender-, and education matched HCs. In line with the Andela et al.[13] results, we identified the ACC as region of interest (ROI), followed by an exploratory whole-brain analysis. In line with findings from a previous meta-analysis and a systematic review with MDD patients[16,17], we hypothesized that long-term remitted CD patients would have thinner ACC cortices in comparison to HCs. Our three most important findings were (i) that remitted Cushing's disease patients had reduced cortical thickness of numerous cortical areas, amongst which the left caudal ACC, (ii) that no differences were found in cortical surface area, and (iii) that the cortical thickness of the left caudal ACC was inversely associated with and disease duration. Taken together, these findings again highlight the importance of further exploring the ACC, and also offer evidence that the length of exposure to cortisol leads to cortical thinning of this brain area. Importantly, these results highlight the added value of examining cortical thickness and cortical surface area separately, instead of as a whole. Finally, it is also of importance to branch beyond the use of single data modalities such as MRI to study this disease, as insights into, for example, the possible underlying microbiological processes that lead to these seemingly persistent alterations in this, and perhaps other stress-related disorder patient populations cannot be identified using MRI data alone.

In **Chapter 6**, we explored the ACC from a resilience perspective in an attempt to identify neural correlates of resilience against traumatic experiences. As police officers are often first-responders, they are more likely to experience traumatic events in comparison to other occupational groups[18]. However, no evidence has been reported that police officers experience higher rates of psychopathology in comparison to occupations that are deemed as less high-risk. Police officers thus seem particularly resilient.

In order to explore the biological underpinnings of this phenomenon, we included a sample of Dutch police officers and police academy recruits, and categorized them by means of a three-group design (i.e. trauma and psychopathology, trauma and no psychopathology, and recruits with neither trauma nor psychopathology). Previous studies have found smaller hippocampal size to be associated with stress-related disorders[e.g.19-21], active Cushing's disease, and Cushing's syndrome[22,23] on the one hand, and increased hippocampal size to be related to resilience[24,25] on the other

hand. It has therefore been posited that increased hippocampal size may be a biomarker of resilience. However, a voxel-based morphometry analysis in this same population did not find any differences in the hippocampus between the three groups included in this study (i.e. vulnerable', 'resilient', and 'controls')[26]. In addition to the studies conducted in patients with stress-related disorders that have found the ACC to be involved in higher cognitive processes and emotion regulation, studies have also found associations between the ACC and psychopathology[27,28]. Based on these findings and those in a more recent systematic review, we hypothesized that the ACC may be associated with resilience in this patient population in a similar way, as this area has been found to be thicker in many resilient populations[29]. We investigated cortical thickness and surface area separately in this patient population using the ACC as region of interest (ROI), followed by a whole-brain analysis. In contrast to our hypotheses, we did not find any differences in cortical thickness or cortical surface area between the resilient group and the other two groups in both the ROI or in the whole brain analyses. It was a surprising finding that the vulnerable group did not show any brain alterations in comparison to the resilient group specifically, as we would at least expect the ACC in the vulnerable patient population to be altered, in line with the previous findings in the remitted Cushing's disease population. However, perhaps the cortisol exposure and disease duration in the vulnerable patient population were less extensive than in the remitted Cushing's disease patient group, as even the vulnerable group included in this study were previously preselected on certain resilience-specific criteria in order to be admitted to the police academy. These findings suggest that either there are no resilience specific correlates regarding cortical thickness and cortical surface area, or that the sample included in this study showed insufficient variability on reliance to test this hypothesis.

To briefly recap, previous research has identified several domains which seem to remain impaired in patients with (long-term) remitted Cushing's disease. Amongst others, persisting deficits have been found in emotional and executive functioning[12,30-33], psychopathological morbidity[30-32], as well as a reduced quality of life[34]. An important skill necessary to function in daily life is the cognitive skill of planning. In **Chapter 7**, we therefore explored whether cognitive planning and executive functioning differs between the same remitted Cushing's disease patient population and their matched HCs as those in Chapter 6. Earlier studies examining the cognitive and executive functioning of patients with remitted CD using standard neuropsychological tests have found seemingly lasting impairments within both of these domains. We further explored these deficits using the fMRI Tower of London

(ToL) task, an often-used task to measure possible neurobiological alterations in brain activity patterns regarding cognitive planning and executive functioning[22]. We hypothesized that cognitive planning in the remitted Cushing's disease patient group would be impaired, and that this patient population would display increased activation in the ACC compared to their age-, gender-, and education matched HCs. We asked the participants to complete the ToL task in a 3T-MRI scanner. No differences were found in cognitive planning or in ACC activation compared to the matched HCs. However, the exploratory whole-brain analysis identified the over-recruitment of several brain regions associated with higher cognitive processes on most trial steps in comparison to HCs. This highlights the importance of conducting an exploratory whole-brain analysis alongside the apriori defined hypothesis driven ROI analysis. In sum, although we found differences in brain activation, we did not find any evidence for pervasive cognitive impairments in remitted Cushing's patients regarding visuospatial planning and executive functioning as measured on the ToL task, demonstrating brain flexibility upon recovery of Cushing's disease to a certain extent. Our findings suggest that the over-recruitment of a number of brain regions is necessary for remitted Cushing's patients to successfully complete the ToL task at the same level that matched HCs do. Taken together, although several studies using different measurement instruments than the ToL have identified impairments within the domain of visuospatial planning in similar patient populations [(e.g.35)], our findings suggest that certain visuospatial impairments may improve in remitted Cushing's disease patients.

Finally, as mentioned previously in Chapter 4, MRI studies alone cannot offer enough insight into the underlying biological processes that may lead to the observed alterations regarding atrophy and white matter integrity in the ACC of remitted Cushing's disease patients. In **Chapter 8** we therefore combined our high-resolution MRI scans with Allen Human Brain Atlas (AHBA) whole genome mRNA expression data[15] to further explore the possible microbiological processes underlying CD. As this was an exploratory analysis, we did not specify any hypotheses a priori. First, our differential gene expression analysis found that the majority of the differentially expressed genes identified were immune signaling genes, and that of these genes, the underexpressed genes were often enriched for functionalities largely involving immune-signaling. Interestingly, the top ten most underexpressed genes found in our study have all been previously associated with Alzheimer's disease in a variety of different ways[37-45]. Our most important finding was the underrepresentation of deactivated microglia and oligodendrocytes in the ACC, which may explain the persevering alterations found in the ACCs of remitted Cushing's disease patients,

as well as the (possibly related) cognitive impairments. Specifically, deactivated microglia have been found to release a number of anti-inflammatory cytokines, participate in neuroprotection[46], matrix deposition, and tissue remodeling[47], whereas oligodendrocytes are largely responsible for the remyelination process[48-49], and damage to oligodendrocytes has been found to lead to mental or physical disability[50]. In support of the found underrepresentation of oligodendrocytes, previous research has found prolonged exposure to corticosteroids to be associated with the inhibition of oligodendrocyte precursor proliferation throughout the white brain matter[85], and widespread reductions of white matter integrity throughout the brain of remitted Cushing's disease patients[86]. Although we did not study the ACCs of remitted Cushing's disease patients directly in this study, our findings denote differences in basal gene expression, and indicate which genes are less likely to be part of this initial vulnerability.

As mentioned previously, earlier research has postulated that Cushing's disease is a suitable naturalistic model to explore the (possibly lasting) effects of endogenous cortisol overexposure on the brain. Although subtler, patients with stress-related disorders also experience elevations in cortisol levels over longer periods of time. Additionally, there is an overlap in the psychiatric symptomatology that both (remitted) Cushing's disease patients and patients with other psychiatric stress-related disorders present. In order to further explore this model, we assessed whether we could identify any overlap in brain abnormalities between those found in our remitted Cushing's disease patient population and those found in patients with other stress-related psychiatric disorders.

To date, ample previous research has highlighted associations between structural brain abnormalities in patients with other stress-related disorders[e.g.51-61], and to a lesser extent in remitted patients with other stress-related disorders[e.g.55-57,61]. Longitudinal studies in these patient populations are scarce[e.g.61]. Although overlap was found between certain brain areas (e.g. areas that have been implicated in executive and emotional functioning) in the current stress-disorder patient population[e.g.51-60], the alterations identified in the remitted Cushing's patients were much more widespread. Less overlap was found between the remitted stress-disorder patients61 and the remitted Cushing's disease patients. As the levels of endogenous cortisol are much higher in patients with Cushing's disease compared to patients with other stress-related disorders, it seems that this excessive exposure leads to more persistent and widespread brain alterations upon remission than is the case with other stress-related psychiatric disorders. However, it is important to

denote that the patient populations included in the aforementioned studies were patients that generally did not report high symptom severity levels.
With regard to fMRI studies, we identified a previous study that investigated the neural correlates of the ToL task with the same paradigm as used in our study in out-patients with depression and anxiety[36]. They found that patients with a current moderate or severe depression had increased dorsolateral prefrontal cortex activation as a function of increasing task load, whereas patients with current mild or remitted depression, current anxiety disorder(s) (such as generalized anxiety disorder and/or panic disorder and/or social anxiety disorder) did not, in comparison to HCs. Thus, in line with the structural study findings, it seems that prolonged exposure of endogenous cortisol on the brain in the vast amounts as is the case with Cushing's disease leads to more widespread alterations that seem to persist after disease remission, as seen in the over-recruitment of certain brain regions in the remitted Cushing's patient population on the ToL task.

In sum, by studying brain characteristics in remitted Cushing's disease patients in comparison to HCs, we further explored possible pathophysiological pathways through which the long-term exposure to excessive cortisol leads to possibly lasting alterations in brain structures and brain activation patterns.

Our research offers support for the idea that long-term overexposure to high levels of cortisol, as is the case in Cushing's disease, has lasting effects on the brain. It remains unclear however, what level and duration of cortisol exposure may lead to these seemingly permanent detrimental effects as seen in remitted Cushing's disease patients.

The following section will highlight possible future directions in order to further our research.

Directions for future research
The results presented in this thesis offer support for the hypotheses (i) that sAA is a biomarker that can differentiate between certain stress-related disorders, and (ii) that exposure to high levels of endogenous cortisol over a long period of time, as is the case in Cushing's disease, will result in persisting brain abnormalities of certain brain regions. However, with regard to both hypotheses, further research is necessary.

With regard to the first hypothesis, future research should (i) replicate and refine our current findings, and (ii) use larger data collections and more advanced

sampling methods. MDD is an extremely complex and heterogeneous disorder. Stress-related disorders are caused by many pathological mechanisms that interact with each other in complex manners. Currently, we have an imperfect psychiatric classification system, and no invasive diagnostic methods. For example, it has been reported that in the DSM-IV and DSM 5 there are 227 possible ways to reach a diagnosis of MDD. However, symptom criteria are usually met in 170 different ways (i.e. one-quarter of the possible criteria combinations generally do not occur), and nine combinations have been found to account for more than 40% of the diagnoses[62]. If the qualitative differences for six of the compounds are considered (for example, differentiating hypersomnia from insomnia), this can even lead to 10,377 unique symptom profiles[63], of which 1030 unique profiles were identified in depressed patients that partook in the STAR*D study[64]. In light of this, it is important that future studies further disentangle whether the sAA levels at awakening are associated with specific symptoms or symptom profiles of MDD. This knowledge is necessary in determining whether our findings have any predictive, diagnostic, or treatment value for individual patients. Future analyses could begin, for example, by determining whether sAA elevations at awakening are associated with certain depression symptom profiles or individual symptoms in large datasets. Also, studies should also compare remitted from current MDD patients and use prospective designs to help determine whether sAA elevations are a state or rather a trait characteristic. Studies should also include participants at risk for developing a depressive disorder in order to determine whether the elevated sAA levels at awakening are present prior to disease onset or represent a 'scar'-effect, if these elevated levels should persist in remitted MDD patients.

Furthermore, future research should implement more advanced data collection and sampling methods. Since we began collecting our saliva samples in 2007, there have been several advancements in the methods used to collect these samples, further increasing sample validity and quality. For example, wearable and flexible sensors are non-invasive, safe, easy and pain-free, allowing for high quality salvia analysis[65]. These sensors offer valuable detailed insights due to their potential to provide continuous, real-time physiological information. If this is not feasible, it is preferable to use the passive drooling method, as this method has been found to minimalize potential sources of error[66], which have been associated with cotton salivettes in previous studies. Furthermore, saliva flow rate can influence salivary measures and should therefore also be considered[67], as well as mouth position during saliva collection[68]. Besides cortisol and sAA, there are many other components of saliva that may be of value to psychiatry research. Studies focusing on immunoglobulin A

(sIgA), lysozyme, melatonin, chromogranin A (CgA), and fibroblast growth factor 2 (FGF-2) have all been found to promising markers of stress, anxiety, or depression to a certain extent[69], and could also provide valuable insights.

In future studies data collection methods could also be elaborated on. Over the course of the last decade, research based on "big data" has increased exponentially. Indeed, big data can be instrumental in uncovering robust patterns in psychological and psychiatric states[70] using, for example, machine learning approaches. However, size alone does not necessarily lead to better data[71], as there are several issues that can influence the quality of data collected (e.g. issues during data collection, issues while processing data, biases, as mentioned previously)[72]. Also, fully personalized treatments or precision medicine cannot be achieved with this type of data.

The findings presented in this thesis were group level findings, and are therefore not directly implementable for individual patients in clinical settings. "Small data" or high-intensive data collection within an individual, coined as small as it concerns data from a single unit (for example, one person, one hospital, one clinic etc.), complements the big data approach, and offers an elegant solution to investigate on an individual level. By collecting vast amounts of detailed time series data, small data aims at matching the right intervention at the right time to a specific unit by taking all unit specific characteristics into account. Small data refers to the rigorous use of specific N-of-1 data in order to achieve detailed individual-level descriptions, predictions, and, eventually control for that one unit[73]. N-of-1 data can have very large datasets in terms of length of time series data (e.g. years), and data types (e.g. genomics, microbiomics, and metabolomics). In single patients, sequential saliva samples are more feasible that sequential blood withdrawals. Also, small data collection targets helping individuals, not transportable knowledge first, and thus has can lead to more rapid clinical consequences for an individual, and is therefore exceptionally valuable.

Finally, studying the genetic underpinnings of sAA in patients with MDD can be an alternative way to learn more about the genetics of this complex psychiatric disorder. Ultimately, this could lead to a better understanding of MDD and possibly increase its applicability as a biomarker, as it would enable us to take account of the genetic determinant of sAA in epidemiological studies. As twin studies have estimated the heritability of MDD at 30 to 40% and sAA levels have been found to be influenced by ancestry, heritability and genetics continue to be a particular field of interest[74-76]. Genome wide association studies (GWAS) can lead to a better

understanding of the genetic architecture of complex traits and can consequently aid in detecting associations between common DNA variants and human disease and disorders[77]. For example, a meta-analysis of GWAS on MDD resulted in the finding of 44 independent and significant genetic loci, supported by multiple single-nucleotide polymorphisms (SNPs)[78]. Furthermore, a recent genome-wide meta-analysis was able to identify 102 independent genetic variants associated with depression, including genes and gene pathways related to synaptic structure and neurotransmission[79]. However, this has not yet resulted in the finding of genetic variants that determine differential sAA levels. Performing a GWAS on sAA and combing this with the already available genome wide association data on depression, could unravel the genetic landscape of sAA and its connection with MDD. Such knowledge could aid studies with Mendelian Randomization designs, which may help to uncover or refute the potential effects of sAA in different diseases[80].

With regard to further Cushing's disease research, the largest current deficit is the lack of longitudinal studies, and the small and heterogenous samples included in the studies to date. Due to the cross-sectional nature of the current study designs, we remain unable to draw conclusions with regard to causality of the seeming pervasive symptomatology seen in patients with remitted Cushing's disease. Longitudinal studies (to the extent that this is possible; i.e. from the point of diagnosis onwards) using a wide array of modalities, would offer valuable insights. Besides sequential routine outcome monitoring (ROM) and salivary biomarker assessments, (f)MRI could be used to evaluate brain volume and structures involved in cognitive function, integrity of white matter, structural connectivity, as well as functional connectivity over time. This would also offer further insights regarding the neurobiological processes supporting the correction of certain brain regions upon remission of hypercorticolism. Future studies could also benefit from larger data sets. This is, of course, difficult as the estimated prevalence of endogenous Cushing's syndrome has been estimated to be 1 in 26.000, of which Cushing's disease has been found to represent more than two-thirds of all cases[81]. A possible solution for this could be to establish a rare disease working group in which data is pooled, like the working groups initiated by the Enhancing Neuro Imaging Genetics through Meta-Analysis (ENIGMA) consortium. As individual Cushing's disease trajectories show large amounts of variation, more robust datasets may aid in understanding certain frequently occurring phenomena in this patient population (e.g. why certain patients require cortisol supplementation after surgery or why certain patients redevelop a pituitary tumor).

Patients with Cushing's disease have also been found to have a globally diminished cerebral metabolism in comparison to controls[82]. Studying glucose utilization by means of positron emission tomography (PET) using the radiotracer F18-fluorodeoxyglucose (which enables the assessment of cerebral glucose metabolism) may offer insights into the psychiatric and cognitive problems often observed in this patient population. Currently, a study is investigating the possible prevention of neuropsychiatric adverse perioperative effects often caused by dexamethasone (a potent glucocorticoid activator that suppresses cortisol production, depleting its mineralocorticoid (MR) binding) in patients with brain tumors by reinstating MR activity by cotreating with hydrocortisone[83]. Based on previous study conducted in childhood leukemia patients the results of this cortisol refill approach seemed to be beneficial in reducing the occurrence of serious neuropsychological adverse effects[84]. The findings from this study will likely be hypothesis-generating, highlighting new paths towards a more advanced treatment of Cushing's disease.

Furthermore, as previously mentioned, the point when the effects of overexposure to endogenous cortisol become seemingly lasting, remains unclear. Research conducted to date in patients with other stress-related disorders have often included patients low to moderate symptom severity, possibly painting a skewed picture. Perhaps patients with more extreme forms of stress-related psychiatric symptomatology and higher cortisol levels over a longer period of time experience similar brain alterations as the (remitted) Cushing's disease population. It would therefore be of interest to transdiagnostically explore whether a certain 'tipping point', or point where brain alterations become seemingly pervasive, can be identified in patient populations suffering from more severe stress-related symptomatology. In support of this notion, one study with (remitted) MDD patients with more persistent forms of MDD (e.g. longer disease duration, repeated relapses or multiple episodes) to be associated with a greater impact on regional brain volumes[61]. A second study found higher severity of depression symptoms in MDD patients to be associated with thinner rostral anterior cingulate cortices[62]. In order to explore this, brain structure and brain function should be investigated in (remitted) MAS-disorder patients that experience(d) moderate to severe stress-related symptoms, longer duration of symptoms, multiple relapses or multiple episodes. Important in this is that the same functional tasks that have been investigated in the (remitted) Cushing's disease population are used in order to aid generalizability of the findings. In this, it may also be of interest to further explore the role of the autonomic nervous system (ANS) in these patient populations, using sAA levels as a marker for ANS activation as sAA can easily be derived from the same saliva sample as that in which salivary cortisol is localized.

Finally, in order to validate the findings from Chapter 8, further (experimental) studies using post-mortem tissue of remitted Cushing's disease or Cushing's mouse models are necessary. These findings may eventually aid in developing novel treatments for certain stress-related disorders of the brain, and specifically for Cushing's disease patients.

General conclusion

At the beginning of this thesis, two research aims were formulated. The first aim was to disentangle the role of stress systems and their possible involvement in the etiology and pathophysiology of certain stress-related psychiatric disorders (i.e. mood-, anxiety- and somatic symptom (MAS)-disorders). This was done by exploring elements of regulation and dysregulation of the two major stress systems (i.e. the ANS and the HPA-axis). In order to measure this, we used salivary alpha amylase and salivary cortisol as markers for sympathetic nervous system (SNS) - and HPA-axis -activation, respectively. Our hypothesis was that sAA, unlike salivary cortisol, would be able to differentiate between certain MAS-disorders. Our research supports this hypothesis as we found that sAA levels at awakening were able to differentiate between MDD and other MAS-disorders.

The second aim of this thesis was to investigate the possibly lasting detrimental effects of long-term exposure to excessive levels of endogenous cortisol on the brain, as is the case with Cushing's disease patients. Our results indicate that certain brain areas are affected by hypercortisolemia and do seem to recover upon disease remission, however, certain cortical areas involved in higher cognitive functioning seem to remain permanently altered.

In sum, our findings yield further insights into the etiology, pathophysiology, and neurobiology of stress-related psychiatric disorders, with which we aim to ultimately aid in the identification or refinement of early detection tools, more advanced treatments, and more successful prevention strategies.

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