Serotonin, cortisol, and stress-related psychopathology

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Chapter 1

General Introduction

INTRODUCTION

The concept of stress was first introduced by Hans Selye who described stress as a "non specific response of the body to any demand placed upon it" (1). Stress may also be described as any environmental challenge, either internal or external, that disturbs the maintenance of homeostasis (2). Thus, the term "stress" can be used in two ways; either to identify events or circumstances that are perceived adversely (stressors) or the state induced by such events or circumstances (the stress response). The stress response is primarily a normal physiological response that allows the organism to respond to its environment and enhance the probability of survival (3;4). Stress may become problematic when the individual perceives a discrepancy between the demands of a situation and the resources of the person's biological, psychological or social systems. In this situation the stressor may have become too severe for the person to cope with.

Stress has been implicated in the etiology of many psychiatric disorders, the most common stress-related disorder is major depressive disorder (MDD). It has been reported by several authors that a causal relationship between stressful life events or chronic daily hassles and the onset of first episodes of MDD occurs (5-8). However, stressful events do not automatically lead to psychopathology, indicating that not the stressor itself, but the individual sensitivity to stress is crucial or rather the interaction between stress and vulnerability (9-11). Individual characteristics such as resiliency, influence the degree of the individual's vulnerability to stress, thereby mediating the relation between stressful events and stress-related psychopathology (12). This vulnerability is likely to be determined by genetic factors, but also by psychosocial and biological factors. Thus, a genetic predisposition in combination with environmental stressors are probably necessary to induce the disorder (13). Two important biological systems that have been implicated in the etiology of stress-related psychiatric disorders, such as major depression, are the HPA-axis and the serotonergic system. The focus of this thesis will be based on the interaction of these systems in the vulnerability to stress and depression.

The stress response

A sudden stressor enhances sympathetic activation and release of adrenaline and noradrenaline into the bloodstream and increases heart rate and blood pressure by stimulation of α and β adrenergic receptors in the heart muscle and vessel walls. A delayed reaction leads to behavioral changes, altered immunologic and autonomic function and activation of the HPA-axis, resulting in increased release of cortisol/corticosterone from the adrenal glands (1;14). Normally, these stress responses gradually fade following repeated exposures of the same stressor, indicating adaptive capability of the organism (15-18). More intense persistent and uncontrollable forms of threat or distress may lead to maladaptive responses (19).

HPA-axis

The HPA-axis system receives input from stressors via the cerebral cortex in the paraventricular nucleus of the hypothalamus (PVN). Consequently, Corticotropin Releasing Hormone (CRH), is released by the PVN neurons into the hypophyseal portal bloodstream. CRH stimulates the production of adreno-corticotropin releasing hormone (ACTH) in the pituitary, which is released in the bloodstream. In turn ACTH stimulates the synthesis and release of glucocorticoids (cortisol in humans and corticosterone in rodents) by the adrenal cortex into the systemic circulation (see figure 1).

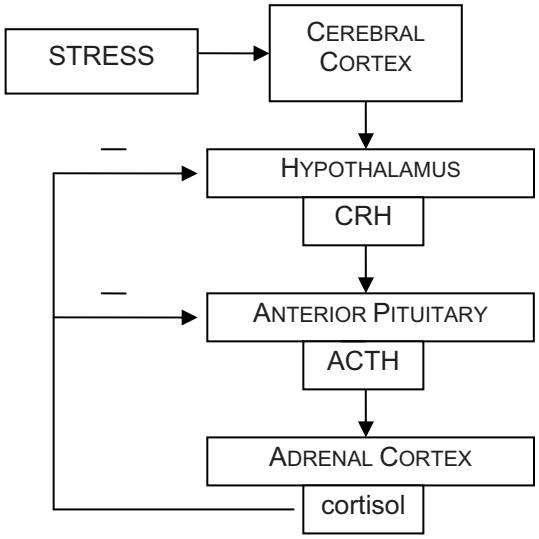

Figure 1: Schematic depiction of the HPA-axis system, including negative feedback mechanisms.

In non-stressful situations, secretion of glucocorticoids follows a circadian pattern, peaking in the early morning hours and steadily declining during the rest of the day. In periods of acute stress, the CRH pulse amplitude in the hypothalamus increases, which results in increased release of ACTH and glucocorticoids into the bloodstream within 10 -30 minutes (20). The enhanced secretion of glucocorticoids leads to mobilization of stored energy, suppression of immune function and facilitation of many processes in the central nervous system (CNS) such as memory and learning (21).

Overshoot of HPA-axis activity is kept within borders by feedback mechanisms responding to variations of glucocorticoid hormone levels. Glucocorticoids suppress the transcription of genes coding for CRH and ACTH via binding to glucocorticoid receptors (GR) or mineralocorticoid receptors (MR) at various stages of the HPA-axis (22). Due to MR's higher affinity for corticosteroids, they are almost fully occupied under normal conditions, while GR are not. However, during stress glucocorticoid levels increase leading to additional occupation of GR (23;24). The balance between occupation of GR and MR is delicate and any

form of dysregulation may increase neuronal vulnerability to stress and adversely influence the stress response (25).

HPA-axis dysregulation and depression

A disturbed function of the hypothalamic-pituitary-adrenal (HPA)-axis is one of the most consistent neurobiological findings in MDD (26;27). In about 50% of the patients suffering from a major depressive episode increased HPA-axis activity has been reported by elevated cortisol levels in plasma, urine and cerebrospinal fluid, as well as enlarged pituitary and adrenal glands (28-31). Atypical depression on the other hand seems to be associated with low HPA-axis activity (32;33). In addition, the circadian rhythm of cortisol secretion is often attenuated in depression (34;35), which might be due to inadequate feedback regulation of ACTH and cortisol (28;36;37). The altered feedback could be the result of increased cortisol levels in blood, but it has also been proposed that feedback is overruled by an abnormally high CRH secretion (27;38). The course of depressive episodes might also be related to HPA-axis dysfunction because persisting impaired feedback constitutes an enhanced risk for relapse or recurrence of depression, while normalization of such feedback mechanisms predicts recovery from depression (39-42).

In animals corticosterone treatment reduced the stress response (43-45) and facilitated habituation by altering the behavior that was not longer necessary (46). Thus, HPA-axis activity appears to play an important role in the etiology and progression of depression through its effect on stress-responsivity.

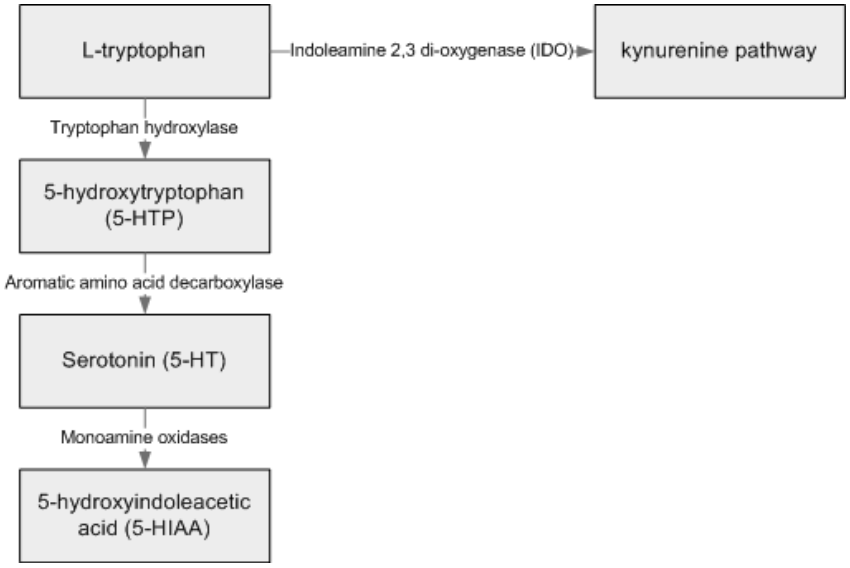

Figure 2:: The main metabolic pathways of tryptophan and serotonin

The central serotonin system

Serotonergic cell bodies with ascending projections to cortical and limbic areas are predominantly housed in the midbrain dorsal and median raphe nuclei. The neurotransmitter serotonin (5-HT) is unable to pass the blood-brain barrier, and has to be synthesized locally from the precursor molecule L- tryptophan (TRP) (figure 2). Cerebral availability of TRP is the rate limiting step in the synthesis of 5-HT. TRP is an essential amino acid which has to compete with other large neutral amino acids for transport through the blood-brain barrier. Therefore, TRP depletion through dietary restriction is an effective method to substantially reduce both cerebral TRP (47;48) and 5-HT levels (49;50).

After synthesis, 5-HT is stored into small vesicles in the axon terminal. Upon release, 5-HT activates both pre- and postsynaptical receptors. Until now, 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes have been identified, which are now assigned to one of seven families 5-HT$_{1-7}$ (51). Neuronal release of 5-HT is controlled by 5-HT$_{1A}$ autoreceptors in the raphe area and presynaptic 5-HT$_{1B}$ receptors in axon terminal areas (52;53). Most other 5-HT receptor subtypes primarily function as postsynaptic receptors. Extracellular serotonin is taken up into the cells via the serotonin carrier (5-HTT) and either reused or degraded by monoamine oxidase A (MAOA) into 5-hydroxyindoleacetic acid (5-HIAA) (figure 3.)

5-HT has a variety of physiological and behavioral functions, among others mood regulation (54;55). Abnormal cerebral serotonin function has been associated with depression, anxiety, aggression and poor impulse control (56).

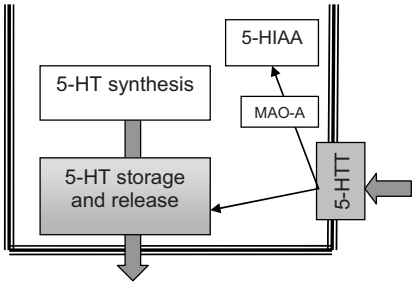

Figure 3: Serotonin (5-HT) synthesis, reuptake and degradation in the presynaptic neuron

Serotonin dysfunction and psychiatric disorders

Normal 5-HT function is important in the control of sleep, wakefulness, feeding behavior, the control of sensory transmission, mood and a wide range of behavior (55). Changes in 5-HT function have been associated with changes in behavior. Abnormalities in mood and behavior have been attributed to 5-HT hypofunction. For instance, low 5-HT function has been thought to cause depression. This finding is explained by the observation that

depletion of monoamine stores in the brain by reserpine induced depressive symptoms, while increasing extracellular monoamine levels in the brain appeared to be effective in several forms of depression (57). Indeed, un-medicated depressed patients have shown reduced metabolism of 5-HT in several areas of the brain (e.g. raphe nuclei and hippocampus), reduced levels of 5-HIAA in the cerebrospinal fluid, reduced whole blood platelet 5-HT content, and reduced plasma TRP levels (58-61). Reduced 5-HT function might be a consequence of increased sensitivity of 5-HT receptor subtypes involved in negative feedback, leading to a reduced turnover of 5-HT. Manipulation of brain 5-HT levels, e.g. by using acute TRP depletion, may induce decreased mood. However, this is observed mainly in vulnerable subgroups, such as patients successfully treated with 5-HT-specific antidepressants and in healthy subjects with a family history of major depressive disorder (MDD) (62-65). Studies have shown that 5-HT manipulations also were associated with increased impulsivity and aggression (66-68). Persistently low 5-HT and TRP levels in animals have been associated with increased reactivity and aggression (69;70), but also with impaired stress-coping and habituation in some (71-74), but not all studies (75;76).

Interactions between the HPA-axis and serotonin system

Stress and increased HPA-axis activity are both involved in serotonin function (55;77-80). In animals it has been shown that liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase) activity was enhanced by dexamethason administration, that resulted in 20% decrease in tryptophan availability and 10% decrease in brain 5-HT concentrations (81). Also in rats undergoing immobilization stress, a 20% decrease in plasma tryptophan levels was observed (82). In addition, CRH infusion in the cerebrospinal fluid increases serotonergic neuronal activity in the dorsal and median raphe nuclei (83;84), while long-term administration of corticosterone reduces post-synaptic 5-HT_{1A} receptor mRNA expression as well as 5-HT_{1A} receptor binding (85;86). In humans, administration of dexamethasone resulted in reduced plasma TRP levels according to the majority of studies (78).

Conversely, serotonin reportedly influences several components of the HPA-axis (28;30;31;42;87). For example, CRH release is regulated through serotonergic neurons from the raphe nuclei that form synapses on the CRH containing neurons in the PVN (88). The serotonin precursors 5-hydroxytryptophan (5-HTP) and TRP, as well as 5-HT_{1A} agonists stimulate ACTH and cortisol release, possibly via impaired suppression of the HPA-axis by cortisol and ACTH (89). Acute tryptophan depletion has also been shown to increase cortisol levels in patients with seasonal affective disorder and attenuates cortisol levels after a stressful task in subjects with a family history of depression, indicating multiple and complex interactions between 5-HT and the HPA-axis (78;90). It is also noteworthy that acute and chronic treatment with serotonergic drugs may have opposite effects on HPA-axis function, possibly as a result of adaptations within the serotonin system. For instance, acute administration of antidepressants stimulates the release of ACTH and corticosteroids (91;92), while long-term treatment suppresses HPA-axis activity (41;93-95).

Genetic polymorphisms

A genetic polymorphism of the serotonin transporter (5-HTT) is involved in increased vulnerability to stress and depression (96-99). The serotonin transporter is a Na^+ dependent transporter and contains 12 putative membrane-spanning regions (100). 5-HTT is expressed in various cell types including neurons, blood platelets, pulmonary endothelial cells, smooth muscle cells, and intestinal mucosal cells (101-104). Originally, a long (L) and a short (S) version were described in the 5-HTT promoter region (5-HTTLPR), but recently an adenine>guanine (A>G) single nucleotide polymorphism (SNP) is described within the L allel. The alleles are currently described as S, L_G and L_A (105). The S and L_G alleles are associated with lower expression of the transporter protein relative to the L_A allel (105). The S-variant has been described to be associated with many neuropsychiatric disorders (106).

Antidepressant drugs

The first generations of antidepressant drugs, the tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs) were discovered by serendipity. TCAs inhibit the reuptake of the monoamines norepinephrine (noradrenaline) and serotonin, but they also have strong cholinergic and histaminergic effects. Despite their efficacy, especially in severe clinical depression, TCAs have now been replaced by new generations of safer drugs with a more benign side effect profile. MAOIs block the enzymes, that are partially responsible for the degradation of the neurotransmitters dopamine, serotonin, and norepinephrine (noradrenaline). Because of potentially fatal interactions with certain foods (tyramine effect) and drugs, treatment with MAOIs are nowadays restricted to cases where other antidepressant medication is not effective. Depressive symptoms may also improve after supplementation with serotonin precursors such as TRP and 5-HTP (107). The selective serotonin reuptake inhibitors (SSRIs) are currently the golden standard of antidepressant treatment. These compounds selectively inhibit the reuptake of 5-HT into the presynaptic neuron and are believed to increase the concentration of 5-HT in the synapse, but this opinion is not shared by everyone (108). The most selective (racemic) SSRI currently available is citalopram (109). Recently, it was found that long-term citalopram administration via osmotic mini-pumps markedly depletes intracerebral 5-HT stores in rats (110). Newer classes of antidepressants consist of specific serotonin-norepinephrine reuptake inhibitors (SNRIs) such as venlafaxin, specific noradrenaline reuptake inhibitors (SNaRIs) such as reboxetine and atypical antidepressants such as mirtazapine.

Antidepressant discontinuation syndromes

Abrupt termination or abrupt dose-reduction of antidepressant treatment may lead to discontinuation symptoms. Discontinuation symptoms include affective symptoms (irritability, anxiety/agitation, low mood), sleep disturbances, general somatic symptoms and gastrointestinal symptoms (111-114). A conclusive explanation for the antidepressant discontinuation syndrome does not yet exist, but it has been speculated that a temporal deficiency of synaptic 5-HT is involved. One would expect that the rapidly decreasing SSRI

concentrations result in increased uptake of 5-HT and consequently decreased extracellular 5-HT levels. Arguably, this will lead to decreased autoreceptor inhibition. However, because chronic SSRI treatment also desensitizes 5-HT_{1A} autoreceptors and its function may not be restored within several days, it is difficult to predict what the short-term consequences are for 5-HT synthesis and release. Interestingly, several symptoms of the SSRI discontinuation syndrome, e.g. hyperarousal and irritability resemble those reported with patients featuring low circulating tryptophan levels (115;116).

EXPERIMENTAL APPROACH

Animal models

An adequate animal model must meet three important validation criteria (117). First, symptoms associated with the clinical disorder must be shared or mimicked by the animal model (face validity). Second, etiology or the alleged pathological mechanism must be comparable in patients and the animal model (construct validity). Finally, effective pharmacological treatment of the disorder must also diminish "symptoms" in the animal model (predictive validity). Currently used rodent models of depression which best meet these criteria are based on epidemiological data indicating that stress plays a crucial role in the induction of depressive episodes (117). Hence, it was decided to use a design that enables to measure the alleged changes in stress susceptibility following manipulations of the HPA-axis and the serotonergic system. In this study the immobilization stress (10 min/day) animal model was used because it does not cause physical pain to the animals. Arguably this will minimize the neurochemical and behavioral confounds inherent of stressors that produce pain. Brief daily sessions of immobilization are sufficient to provoke marked behavioral changes and elevations in HPA-axis activity (118-122). Reactivity and adaptation to stress were measured with the response to acoustic startling stimuli (123;124). The acoustic startle response (ASR) is a fast, involuntary, defensive muscular response elicited by a sudden, intense acoustic stimulus (125). The ASR is mediated by a relatively simple neuronal circuit consisting of the auditory nerve, the ventral cochlear nucleus, the lateral nucleus of the lateral lemniscus, the caudal pontine reticular nucleus (PnC), spinal interneurons and spinal motorneurons (125) (figure 4). The ASR can be elicited by the same stimuli across species (126). The magnitude of the response can be enhanced by fear, anxiety, stress or certain drugs (118;127;128). This may result from activation of the amygdala which projects directly to the PnC neurons (129). The magnitude of the ASR can be reduced by drugs, positive affect, or habituation (124;130;131, review: 123). Habituation is a reduced response during repeated exposure to a stressor. This is considered to be a very early form of learning, indicating the capability of an organism to adapt to stress (132).

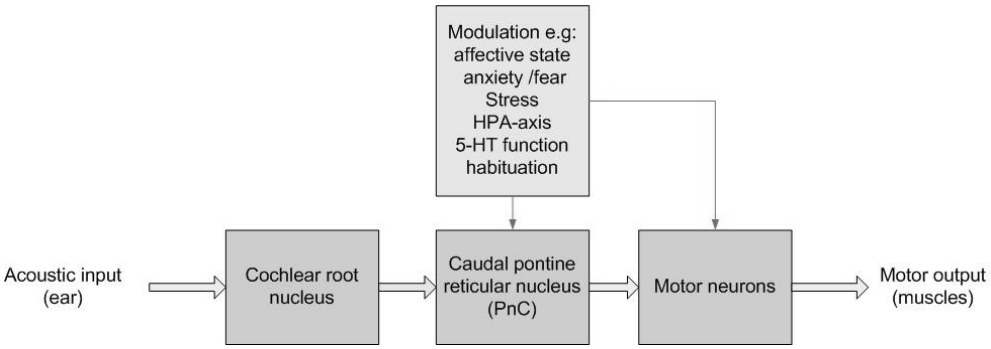

Figure 4: Simplified version of the acoustic startle pathway. Adapted from Koch et al. 1999

Human studies

In humans it is not ethical to experimentally lower plasma TRP levels for more than a few hours. Alternatively, the consequences of persistently decreased TRP levels may be studied in a naturalistic model, as presented by somatic patients suffering from a carcinoid tumor or patients that are treated with interferon-α (133). Briefly, carcinoids are neuro-endocrine malignancies that produce large amounts of 5-HT, thereby consuming up to 60% of the body supplies of TRP, thereby depleting plasma TRP considerably (134). Most carcinoids originate in the gut and this peripherally produced 5-HT cannot cross the blood-brain barrier. The excessive TRP consumption by the carcinoid tumor may thus result in a decreased availability of TRP in the brain and hence adversely influence central 5-HT functioning. Plasma TRP is also depleted in patients treated with interferon-alpha (IFN-α). IFN-α is a cytokine that is used in the treatment of viral hepatitis and certain cancers (135). Through induction of the enzyme indoleamine 2,3-dioxygenase (IDO), it also stimulates the degradation of TRP via de kynurenine pathway, which may also lead to impaired central 5-HT function (136-141). Accordingly, patients with carcinoid tumors or IFN-α treatment may be of help to study the effects of chronic TRP depletion on mood and behavior in a population that is not biased by psychiatric diagnoses.

Some diagnostic criteria

Psychiatric disorders are diagnosed according to the consensus criteria stated by the *Diagnostic and Statistical Manual of Mental Disorders* Version IV, 4th edition (DSM-IV; American Psychiatric Association, 1994). Diagnosis usually consists of on or two key symptoms and additional symptoms. Additionally, often a threshold period and exclusion criteria are described. In scientific research psychiatric disorders are usually described in terms of scores on questionnaires, which contrasts the normal clinical practice. Such questionnaires are either interview based, e.g. Hamilton Depression Rating Scale (HDRS) or self-reported, e.g. Symptom Check List (SCL-90) or Beck Depression Inventory (BDI). They have the advantage of standardization and the ability to quantify severity of pathology. It is,

however, difficult to diagnose a psychiatric disorder according to DSM criteria, since important items such as key symptoms, different subtypes of the disorder or the threshold period are mostly not described as such in the (self-report) rating-scales. Self-report rating scales are mostly sensitive (0.85), but less specific (0.75) (142). Ideally, a psychiatric diagnosis should be made using a diagnostic interview, while follow-up or severity is being measured with a rating-scale.

AIM OF THE THESIS

The focus of this thesis will be on two neurobiological systems purportedly involved in the etiology of MDD, namely the hypothalamic-pituitary-adrenal (HPA) axis and the central serotonin (5-HT) system. The first theory proposes a predominant role of HPA- axis dysfunction in stress-coping and depression (143). The second theory postulates that a disturbed 5-HT- neurotransmission influences coping with stress (75;76) and is involved in the pathogenesis and pathophysiology of affective disorders (11;97;144;145).

The aim of the current thesis is to investigate the interaction of the HPA-axis and the serotonergic system in vulnerability to and coping with stress in rats, and to relate naturalistic alterations in these systems to psychopathology in humans.

OUTLINE

Part I: Animal studies

In the chapters on animal studies, we studied the effects of experimentally induced alterations in HPA-axis activity and serotonergic functioning on stress-reactivity and adaptation in rats. In **chapter 2**, we describe the effect of a cerebral depletion of serotonin, induced by a low tryptophan diet, on reactivity and adaptation to stress, measured as response to acoustic stimuli with or without preceding immobilization stress. **Chapter 3** presents the pattern of the acoustic startle response of rats that are fed with a tryptophan deficient diet and treated with specific serotonin receptor agonists. In **chapter 4,** we describe the effect of abrupt cessation of the SSRI citalopram, on serotonin metabolism response and habituation to acoustic startling stimuli. In the last chapter on animal studies (**chapter 5**) we describe whether a dysregulated HPA-axis achieved by corticosterone pellet implantation alters the sensitivity or habituation of rats to stress.

Part II: Clinical studies

In the clinical studies, we tried to relate alterations in the serotonergic system and HPA-axis to sensitivity to stress and psychopathology. In **chapter 6** we described the 24h cortisol excretion in 25 patients suffering from a carcinoid tumor. A carcinoid tumor is a neuro-endocrine tumor that produces serotonin, thereby using 60% of the body supplies of its precursor tryptophan, resulting in depletion of peripheral tryptophan. In **chapter 7** the

psychopathology of patients with tryptophan catabolism due to interferon-alpha treatment is described. In **chapter 8** the effect of the serotonin transporter length polymorphisms in the sensitivity to chronic low tryptophan is described. In the last research chapter (**chapter 9**) we describe the relation between HPA-axis feedback function, and the course of major depressive disorder. HPA-axis feedback function is assessed by lymphocyte glucocorticoid receptor (GR) function. Because the metabolism and gene expression of lymphocytes show remarkable similarities with cells in the brain, lymphocytes might be used as a neural probe. The severity of the depression is measured by Hamilton depression interviews. The patients are severe depressive inpatients who are all treated with antidepressant drugs that target the serotonergic system.

Finally, in **chapter 10**, we summarize the results and discuss the results of the experiments in a broader context.