INTRODUCTION

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Stress and the Stress Response

Stress, a response to aversive stimuli, is a concept that is difficult to define since its interpretation tends to vary across individual disciplines. In 1976, Hans Selye, a pioneer in addressing general principles of physiology and pathophysiology in the exploration of stress, defined stress as “the nonspecific response of the body to any demand placed upon it” 4. He emphasized the role of an integrated response of multiple systems rather than isolated reflexes. Exposure to threat or hostile conditions, for instance results in a series of coordinated responses referred to as the “stress response” and is composed of altered behavior, immunologic and autonomic function and the secretion of multiple hormones including adrenocorticotropin hormone (ACTH), cortisol/corticosterone, and adrenal catecholamines. These coordinated responses are organized to enhance the probability of survival and stressors can thus be defined as intrinsic or extrinsic forces, that endanger or are perceived to endanger the survival of an organism 5. Nevertheless, not all states of stress are necessarily noxious or negative. Mild, brief (“acute”) and controllable challenges or eustress could be perceived as pleasant or exciting stimuli and could be a positive input for the emotional and intellectual development, while the more intense, persistent (“chronic”) and uncontrollable situations of threat, or distress may lead to maladaptive responses 4,6. In general stressors can be grouped into three broad categories: (1) psychological stressors based on a learned response to the threat of an impending adverse conditions (fear, anxiety or exposure to a novel or uncontrollable environment) (2) stressors that consist of a physical stimulus and have a strong psychological component (pain or immobilization) (3) stressors which challenge cardiovascular homeostasis (hemorrhage, exercise or heat).

Throughout the experiments described in this thesis, use was made of repeated foot-shock application to serve as a chronic stressor. This model allows simultaneous investigation of the first two stressor categories, namely an aversive physical condition with a strong psychological component. As such, the term stress in this thesis will generally refer to distress, like most clinical and scientific contexts, unless otherwise defined. The emphasis was placed on cumulative and persistent exertion of the stress response since this can lead to overreaction of the stress system, a situation described primarily as the stress syndrome 7 and widely recognized as an important trigger in the expression of various psychiatric syndromes, such as major depression and anxiety disorders.

The principal components of the adaptive response to stress are the sympahto-adrenergic-noradrenergic (SAN) and the limbic-hypothalomo-pituitary-adrenal (L-HPA) systems. The SAN system implies the biosynthesis and release of adrenaline and noradrenaline, regulated respectively by the sympathetic division of the autonomic nervous system (ANS), and the locus coeruleus (LC), in the CNS. The L-HPA system involves limbic structures, such as the prefrontal cortex and hippocampus, in association with the HPA axis, and their respective interconnections. Both systems also participate in their mutual positive regulation, so that activation of one also involves activation of the other 4. In addition, the
Stress system also includes other brain areas involved in implementation of the appropriate adaptive response 9. Although it goes beyond the scope of this thesis to describe in detail each contributing component, a selection of most relevant issues to the understanding of the data presented here is highlighted in the following paragraphs.

Stress and the limbic system

Multiple brain structures are involved in the organization of responses to aversive or stressful stimuli. Among them are the hypothalamus, hippocampus, amygdala, cingulate and prefrontal cortices, hindbrain regions such as the brainstem catecholamine cell body groups (in the nucleus of the tractus solitarius, ventrolateral medulla and locus coeruleus), the parabrachial nucleus, cuneiform nucleus and dorsal raphe nucleus 5. Figure 1 summarizes a few of the well-characterized brain structures and neural circuits involved in the adaptive response to stress. Most sensory inputs or environmental stimuli, playing as external stressors are perceived by specific sensory receptor systems, which convey information to their respective sensory areas of the thalamus. This structure functions as a relay station so that sensory information concerning individual stimuli is then transmitted to the amygdala and sensory cortex 10,11. The sensory cortex in turn communicates either directly or via the hippocampus with the lateral amygdala through the perirhinal cortex 12-14. The amygdala is composed of several nuclei, each of which performs a different function. The lateral and basolateral nuclei of the amygdala funnel and integrate sensory input from the thalamus and cognitive information from the cortex and hippocampus, while the central amygdaloid nucleus is involved in behavioral, autonomic, and endocrine responses 5. The amygdala also innervates and is innervated by the dorsal raphe nucleus and catecholaminergic nuclei located in the brainstem, which in turn innervate corticotropin releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN) 15,16. The PVN is a particularly essential structure for proper stress response modulation as it plays a pivotal role in the adaptive response to stressors through regulation of HPA axis activity described below.

Figure 1.

Schematic representation of the neural circuits involved in the adaptive response to stress. Sensory receptors perceive sensory input and convey the information to their respective sensory thalamus, primary sensory cortices and higher order cortices. Basic sensory information is simultaneously transmitted to the amygdala from these structures particularly the prefrontal cortex (through transitional cortices and the hippocampus). These limbic structures regulate activation of both neural and neuroendocrine responses. The neural component involves the NA system represented by the locus coeruleus and the adrenergic system, through the lateral hypothalamus, which activates the SNS and the adrenal medulla. The neuroendocrine component involves activation of the PVN, the pituitary and the adrenal cortex, with the consequent release of CRH, ACTH and glucocorticoids.
Stress and the HPA axis

Living organisms survive by maintaining a complex dynamic equilibrium or homeostasis that is constantly challenged by intrinsic or extrinsic stressors. These stressors set in motion responses aimed at preserving homeostasis, including activation of the HPA axis, which in turn initiates the hormonal cascade illustrated in figure 2. In short, the HPA system receives and integrates various inputs indicative of stress that converge into the PVN. CRH, synthesized by PVN neurons, is then released into the hypophysial portal blood and reaches the anterior pituitary where it regulates the transcription of the proopiomelanocortin (POMC) gene, a common precursor for adrenocorticotropic hormone (ACTH) and related peptides, and stimulates the release of ACTH into the bloodstream. ACTH then stimulates the biosynthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) by cells of the adrenal cortex into the systemic circulation.

HPA axis regulation

Glucocorticoids are the final effectors of the HPA axis and they play a key regulator role in the neuroendocrine control of the HPA-axis and on the termination of the stress response by exerting negative feedback at several sites to inhibit their own release. At the pituitary level, glucocorticoids exert direct effects on the ACTH precursor POMC, influencing gene transcription, POMC mRNA levels and subsequent ACTH peptide stores. This occurs through glucocorticoids-receptor complex binding, translocation to the nucleus and binding to DNA sites. Studies have also shown that glucocorticoids interact with the CRH receptors in the anterior pituitary, acutely inhibiting the binding of CRH to its receptors and chronically decreasing CRH receptor numbers. Such direct effects of glucocorticoids on CRH receptors may account for some of the inhibitory action of glucocorticoids on ACTH release. In addition to pituitary sites of action, glucocorticoids also act at various brain sites to modulate HPA axis activity including hypothalamic and some supra-hypothalamic structures.

Glucocorticoid effects are mediated by two receptor subtypes: the mineralocorticoid receptor (MR) that has a higher affinity for corticosterone and the glucocorticoid receptor (GR) that possesses a lower affinity for corticosterone. Consequently, MRs are predominantly occupied at lower, basal corticosterone levels and GRs become occupied when corticosterone levels are high, such as during stress. MRs are found in some brain areas such as the hippocampus, whereas GRs have a widespread distribution and are abundantly expressed in several brain regions involved in the stress response, including the frontal cortex, hippocampus and hypothalamic paraventricular nucleus (PVN). These receptors act as ligand-dependent-transcription factors and upon glucocorticoid binding, these receptors undergo conformational changes to facilitate their subsequent binding to DNA. The hormone-receptor complex may thereby regulate the expression of various target genes, either through activation or deactivation.
In a formulation that may be of relevance to stress-induced disorders as depression, Sapolsky and colleagues have proposed the glucocorticoid cascade hypothesis, a model that describes the effects of chronic stress on hippocampal neurons. According to this model, repeated stress or chronic glucocorticoid administration, downregulates hippocampal steroid receptors, but not hypothalamic or pituitary receptors. Animals with downregulated hippocampal glucocorticoid receptors exhibit delays in the turnoff of the glucocorticoid response to stress and demonstrate decreased sensitivity to glucocorticoid fast feedback. This decrease in glucocorticoid receptors and insensitivity to negative feedback is thought to lead to prolonged hypercortisolism, resulting in atrophy of hippocampal neurons and further glucocorticoid hypersecretion. Mechanisms such as these will be discussed in the following paragraphs, but serve here to illustrate how HPA system activity may be regulated through a variety of possibilities.

Interestingly however, rat and human studies provide evidence that the stress response is also sexually dimorphic and that gonadal steroids play an important role in modulating the HPA axis, acting particularly on sensitivity to glucocorticoid negative feedback through effects on brain CRH systems, pituitary responsiveness to CRH or glucocorticoid receptors. This concept, discussed in the next paragraph may provide a better understanding of the machinery underlying observations that women have an increased vulnerability to stress and related disorders as depression.

**Figure 2** The stress response system
Considering gender dichotomy in the stress response.

Being male or female is an important individual contribution to mental and physical health. With regard to psychiatry, this perspective calls for careful consideration of sex-based differences in stress susceptibility and sex-based differences in the manifestation and prevalence of various psychiatric disorders. Ovarian steroids are known to contribute to a wide variety of neurochemical effects, which produce gender differences in multiple processes such as cognition, emotional regulation, affective style, pain sensitivity and psychopathology. Evidently, reproductive as well as non-reproductive dimorphisms exist between males and females and an intriguing possibility thus holds that non-reproductive gender related affective dissimilarities might underlie the increased prevalence rates of women compared to men for stress-related disorders such as post-traumatic stress disorder, major depression and other anxiety disorders. Although multiple factors could account for this differential and contribute to the increased vulnerability in women, understanding the impact of stressors is central to understanding the development of stress disorders.

Since the HPA axis represents the final effector in the modulation of the stress response, a large number of clinical and preclinical studies have attempted to define a direct link between sex-related differences in key elements in this system and the higher female susceptibility to stress-related pathology. Differences in HPA axis regulation may account for the differential response to stress observed between males and females, since the latter demonstrate a more robust HPA axis response to stress consisting of a faster onset of glucocorticoid secretion and a faster elevation of plasma adrenal steroid levels. Apparently, a steeper rise in circulating stress hormones seems to be necessary to elicit the faster glucocorticoid-mediated feedback inhibition needed in females. The mechanisms underlying the latter however are unclear although there is evidence that ovarian hormones are at least partly responsible for this sexual dimorphism.

The HPA axis and the female gonadal system are closely intertwined and exhibit a complex bi-directional relationship. A partial estrogen response element for instance has been found on the promoter of the CRH gene. This not only implicates the CRH gene and thus the HPA axis as an important target of gonadal steroids, but also represents a potential mechanism by which estrogen may enhance stress responsiveness, increase glucocorticoid levels and yield greater HPA axis resistance to glucocorticoid-mediated feedback inhibition. Accordingly estrogen has also been shown to delay ACTH and glucocorticoid shutoff. Like estrogen, progesterone also appears to be involved in the differential modulation of stress response. It has been shown for instance, that progesterone binds faster to GR than cortisol and that they bind to different sites. Furthermore female rats have been shown to have a greater number of hippocampal GRs than males, which seems to be modulated by progesterone. Although most progesterone-induced effects on the HPA axis are mediated by GR binding, its affinity for MR has also been shown in a range similar to dexamethasone. Despite the fact that estrogen and progesterone have been shown to exert protective effects against hypercortisolemia, they also seem to antagonize
glucocorticoid-mediated terminating action, perhaps delaying recovery from deleterious effects of stress. Taken together they may account for the greater overall reaction seen in females and help explain gender-related differences in response to stress and related disorders.

Dysregulation & implication for psychopathology

After prolonged and intensive stress exposure the HPA system becomes dysregulated, leading to impaired negative feedback of the HPA axis, persistent activation of this system and consequential increase in circulating glucocorticoids. Normal HPA axis activity is hence altered during chronic stress, resulting in sustained increase of plasma corticosterone or cortisol levels. Since adaptive responses are meant to be acute, limited by specific characteristics of the stressor, an essential component of adaptation is thus protection of the organism against overreaction of this system. To maintain glucocorticoids within physiological ranges, the HPA axis is controlled by multiple negative-feedback loops, mediated mainly by the steroids themselves. If however the organism is unable to terminate the stress response at the end of exposure or if chronic and unavoidable stressful situations persist, then the sustained adaptive responses may lead to pathophysiological changes produced by dysregulation of the stress syndrome. These changes may in turn develop into various types of disorders such as anxiety disorders or major depression. In this regard a significant association between stress and depression is now well-documented and the parallels between some aspects of the stress response and severe depression are striking. Hypercortisolism, for instance represents one of its most consistent biological markers. Furthermore clinical and preclinical studies reveal that chronic exposure to stressful events is strongly associated with the development of depressive symptoms.

Stress and Depression

As opposed to most diseases of other organ systems, diagnosis of depression is not based on objective diagnostic tests, but rather on a highly variable set of symptoms. Accordingly, depression may be better viewed as a heterogeneous syndrome rather than a single disease, as it is comprised of numerous diseases with distinct causes and pathophysilogies. Attempts have been made to establish subtypes of depression defined by certain sets of symptoms, yet these subtypes are based solely on symptomatic differences. For the sake of clarity throughout this thesis it must thus be noted that there is as yet no clear consensus regarding their different underlying disease states. The following paragraphs will describe underlying circuitry and dysregulation generally attributed to this syndrome, without distinction for the possible subtypes unless otherwise specified.
Neural circuitry of depression

As previously described, many brain regions have been implicated in regulating emotions. It is unfortunate however, that our understanding of neural circuitry underlying normal mood is still rudimentary, since mood abnormalities are the hallmark of depression. It is likely however that many brain regions mediate the diverse symptoms of depression and depressed patients exhibit distinct pathological changes in various selective brain regions. These changes are observed in limbic (hippocampus, basal ganglia and amygdala) and cortical brain regions implicated in the affective and cognitive impairments observed in depression. Accordingly, brain-imaging studies also indicate impaired cerebral blood flow and glucose metabolism in limbic and cortical structures. In fact, functional imaging studies reveal that prefrontal cortical, ventral striatal and hippocampal volume is decreased in patients with depression. The emerging picture from these clinical studies is that cellular loss and volume decrease is associated with depression.

Although it cannot be excluded that depression also has a genetic component, it is well acknowledged that neuroendocrine changes and stressful events can also lead to a depressive episode and as previously stated, several studies indicate that a subset of depressed patients have glucocorticoid hypersecretion or HPA axis hyperactivity. In line with the cellular loss, patients with HPA hyperactivity also exhibit distinct reductions in hippocampal volume.

Given these observations, depression research in animal models is therefore focused on understanding the changes induced in limbic and cortical brain structures by different stressors and glucocorticoids. Likewise, the focus in this thesis lies primarily in the prefrontal cortex and hippocampus, given their crucial roles in terminating the stress response, and the fact that they are particularly susceptible to the structural impairments induced by stress. Although their stress-induced changes may not explain all affective symptoms of depression, they might provide a cellular basis for understanding the structural and neuronal dysfunctions in these structures as well as other regions associated with stress and stress-related disorders as depression. Distinct cellular mechanisms have already been proposed to underlie some stress-induced pathological impairments and are discussed in the following paragraphs.

Hypotheses of depression and neuronal dysfunction

Unfortunately, a major impediment in depression research is the lack of validated animal models. This is in part attributable to the above-mentioned heterogeneous aspect of depression, as it constitutes diverse syndromes while varying subtypes are likely to have distinct causes and pathophysiologies. This being the case, valid animal models are needed to ascertain distinctions of these disorders. However, such animal models can only be developed once we have gained a full understanding of this disorder; thereby creating a catch twenty-two. As a result, all available animal models of depression rely on one of
two principles: (1) actions of known antidepressants or (2) responses to stress. With regard to antidepressant research, various neurotransmitter systems have been investigated with respect to their role in CNS pathophysiology and possibly origin and development of depression. This in turn has given rise to different aminergic hypotheses, commonly referred to as the “monoaminergic theory of depression.” The pathological effects of stress on HPA axis activity and crucial regulating structures as the hippocampus however have contributed to another recent hypothesis that proposes a role for neuronal plasticity in the etiology of depression and its treatment. Specific mechanisms responsible for mediating the underlying neuronal dysfunction of each theory are discussed below.

Monoamine theory

For a long time the “monoamine hypothesis of depression” provided the starting point for investigators attempting to explain antidepressant action and depression pathophysiology. The main assumption of this hypothesis holds that depression is due to a deficiency in the neurotransmission mediated by the biogenic amines, e.g., serotonin (5-HT), noradrenaline (NA), and dopamine (DA), and that antidepressants work by increasing the availability of the amines and neurotransmission in the brain. Early research focused on changes in neurotransmitter concentrations and receptor levels, but the results of these studies were at odds with the observation that the therapeutic effects of antidepressants requires chronic administration while the inhibition of serotonin and noradrenaline reuptake occurs immediately. Sequential evolution of this hypothesis hence yielded the monoamine receptor hypothesis, which proposes that the drug-induced increase in monoaminergic neurotransmission causes changes in sensitization state of monoamine receptors, which may explain both the effects and the delayed onset of action of these drugs.

Although each of the above-mentioned monoamines represents a putative target, the role of serotonin is perhaps the most studied in stress and depression, given the major therapeutic advance in psychopharmacology provided by selective serotonin reuptake inhibitors (SSRIs). The serotonergic hypothesis of depression postulates that a deficient serotonergic neurotransmission in the CNS may account for a higher vulnerability to this disorder. Hence, the role of serotonin in the pathophysiology of depression has long been investigated, giving rise and supporting this hypothesis. At the molecular level, serotonergic neurotransmission is regulated by its rapid removal from the synaptic cleft, primarily through its re-uptake into the presynaptic terminals by the serotonin transporter. This process exerts control on the effective concentration of the neurotransmitter at the synaptic cleft, and its availability for the interaction with both pre- and postsynaptic receptors. The therapeutic effect of antidepressants that enhance serotonergic neurotransmission such as tricyclics and SSRIs are in line with this theory. SSRIs, often the treatment of choice for depression, marked a milestone in neuropsychopharmacology and rational drug design. Nevertheless, given various drawbacks inherent to this theory (see “Pharma-
cotherapy”), new approaches to understanding depression focus on the regulation of key signaling pathways involved in cellular survival and plasticity.

Evidence linking stress, depression and antidepressant action suggest that depression may result from an impairment of neurons to make appropriate adaptations and/or synaptic connections. The following paragraph describes the cellular mechanisms that may underlie the structural impairments observed in the brains of animals used in models of depression or stress exposure as well as in patients with depression.

**Neuroplasticity theory**

**Dysregulation of the HPA axis and hippocampus**

As mentioned afore, hyperactive HPA axis activity and consequent hypercortisolemia is one of the most consistent findings seen in depressed subjects. Glucocorticoids have been shown to affect various areas in the CNS, particularly the hippocampus, where MRs and a particularly high density of GRs have been found. When normal glucocorticoid secretion is altered, leading to increased glucocorticoid levels, this may result in down-regulation of hippocampal GRs. This potentially adaptive response observed in neural tissue, is apparently directed to counteract an excessive concentration of glucocorticoids, but may lead to altered negative feed-back mechanisms, resulting in increased circulating cortisol levels. This elevation may persist, even after termination of the original stimulus that gave rise to it, and could then result in degenerative changes in the hippocampus. Hippocampal alteration produced by prolonged and excessive cortisol levels, with a consequently impaired negative feedback loop, could at this stage account for the inability of glucocorticoids to regulate their own secretion during chronic stress. These observations have given rise to the hypothesis that links depression with an alteration of the L-HPA system, particularly focusing on the down-regulation of GRs at hippocampal and hypothalamic levels, with the resulting hypercortisolism. In accordance, antidepressants could thus act through improvement of GR function, thereby leading to normalization of the HPA axis.

Another mechanism however by which antidepressants may mediate their effects is by triggering cellular mechanisms to counteract the structural impairments that are induced by stress as reflected by the volumetric changes associated with the prefrontal cortex and hippocampus of depressed patients. In adult rodents, both chronic stress and glucocorticoid administration have been shown to result in distinct remodeling of the apical dendrites of hippocampal CA3 pyramidal neurons, which is manifested as a decrease in both number and length of apical dendrites. Given the complexity of stress-induced disorders however it is unlikely that disturbed dendritic morphology alone will fully explain the loss of volume seen in depression. Besides the changes in dendritic remodeling, stress and stress hormones also decrease the ongoing rate of cell birth and cell proliferation in the dentate gyrus granule cell layer of adult hippocampus by an undetermined mechanism.
Although the latter will be described in the following paragraphs, the above does suggest that dendritic restructuring as well as decreased cell-survival and neurogenesis may provide the cellular basis for stress impairments. In turn antidepressants might correct such impairments by targeting these features. In line with this theory, results of a chronic tianeptine study have shown reversal of stress-induced impairments and reduced hippocampal volumes.

Neurotrophic hypothesis

The neurotrophic hypothesis associates the origin and development of depression with a stress-induced decrease of neurotrophic factors and proposes a role for these agents in its treatment. Neurotrophic factors were first characterized for regulating neural growth and differentiation during development, but are now known to be potent regulators of plasticity and survival of adult neurons and glia. Complementary to the pathological effects on the hippocampus described above, the neurotrophic hypothesis of depression states that a deficiency in neurotrophic support may contribute to hippocampal pathology during the development of depression, and that reversal of this deficiency by antidepressant treatments may contribute to the resolution of depressive symptoms.

Work on this hypothesis has focused primarily on brain-derived neurotrophic factor (BDNF), one of the most prevalent neurotrophic factors in the adult brain. Both acute and chronic stress decrease BDNF expression levels in the dentate gyrus and pyramidal cell layer of the hippocampus in rodents. This reduction appears to be mediated in part through stress-induced glucocorticoids and partly through other mechanisms, such as increased serotonergic transmission following stress. Conversely chronic (and not acute) administration of virtually all classes of antidepressants increase BDNF expression in these regions while preventing stress-induced decreases in BDNF levels. Antidepressants’ ability to increase hippocampal BDNF levels has also been shown in human studies. Altered expression of BDNF in the hippocampus as well as the prefrontal cortex has been proposed as an important factor mediating atrophy and neuronal cell death associated with depression. As BDNF is reported to enhance long-term potentiation and other forms of synaptic plasticity in the hippocampus, increased BDNF levels induced by antidepressants may promote hippocampal function. Furthermore, the time required for BDNF levels to gradually rise and exert their neurotrophic effects, might explain why antidepressant response is delayed.

The neurotrophic hypothesis predicts that agents that promote BDNF function might be clinically effective antidepressants, but since no such compounds are currently available, another approach may lie in earlier intervention in this process. Antidepressant induction of BDNF is mediated largely via intracellular signaling cascades. Neurotrophins bind to tyrosine kinase (Trk) receptors and activate intracellular pathways such as the mitogen activated protein kinase (MAPK) or extracellular signal-regulated protein kinase (ERK) cascade. Neurotrophin-dependent MAPK plays a major role in mediating the extracellu-
lar signals to the nucleus and once activated, MAPK phosphorylates cAMP response element binding protein (CREB), which then binds to a specific response element called cAMP response element (CRE) of the BDNF gene and enhances transcription. A post-mortem study found decreased levels of ERK activity and expression both in hippocampus and cerebral cortex of depressed suicide subjects, providing additional evidence for neurotrophin dysregulation in different brain regions in stress-related disorders. Stress and stress-induced glucocorticoids can directly interfere with this process through binding of the glucocorticoid-GR complex to CREB, thereby preventing its phosphorylation and blocking the expression of target genes such as BDNF. Conversely, regulation of the MAPK/CREB signaling cascade may represent a putative target to promote a palliative effect. In fact, virtually all major classes of antidepressants increase levels of CREB expression and function in several brain regions including the hippocampus. Furthermore, increased CREB activity in the hippocampal dentate gyrus achieved through CREB encoding viral vector injections directly into this brain region has also been shown to exert antidepressant-like effects in animal models of depression.

Taken together, observations regarding this neurotrophic hypothesis combined with the monoaminergic theory as well as structural and morphological effects of the brain associated with chronic stress or depression have provided a prolific starting point for the development of a novel theory on the cellular basis of depression. The theory, as proposed by Jacobs, focused on the important role of serotonergic activity in both depression and the regulation of hippocampal neurogenesis. The theory, further developed by Duman and coworkers however, sees the CREB/BDNF signaling cascade as the bridging link between dendritic restructuring, decreased neuronal survival, and decreased adult hippocampal neurogenesis in depression. This hypothesis, which places the potential role of neurogenesis in the context of many other cellular and biochemical alterations in depression, is discussed below.

Neurogenesis hypothesis

So far, the theories suggested to explain stress-induced changes observed in prefrontocortical and hippocampal areas, are based primarily on glucocorticoid-mediated cell loss, atrophy, remodeling or compromised neuroplasticity. A failing regulation of adult hippocampal neurogenesis however might also contribute to this volume loss, at least in part. Adult hippocampal neurogenesis was first described by Altman and Das in 1965 and has undergone several rediscoveries since then. Recently a broader interest in this phenomenon has been sparked by reports of its possible implication in chronic stress associated impairments as well as antidepressants actions. Adult hippocampal neurogenesis is subject to complex regulation, and numerous factors affecting several different levels of regulation have been described. Stress however may represent one of the key factors that initiate cellular dysfunctions by down-regulating neurogenesis. This effect could be mediated through elevated glucocorticoid as well as NMDA-
receptor activation since both are also involved in other hippocampal damage following repeated or chronic stress. Conversely, recent evidence demonstrates that chronic administration of different classes of antidepressants increases the proliferation as well as number of newly formed neurons in the hippocampus. As this effect is not seen in response to other non-antidepressant psychotropic drugs, this suggests a high degree of specificity to the effects of antidepressants.

Given the role of the cAMP-CREB cascade in antidepressant action, preliminary evidence also complements its involvement in hippocampal neurogenesis. Transgenic mice expressing a dominant negative mutant of CREB show a significant decrease in the survival of newly formed hippocampal neurons, while in vitro studies have shown that activation of the cAMP cascade or the presence of BDNF in the surrounding environment increases the differentiation and survival of neurons. These results suggest that up-regulation of CREB and BDNF by antidepressants could increase the differentiation and survival of hippocampal neurons in vivo. Although the precise significance of hippocampal neurogenesis is yet to be fully elucidated, it provides a putative cellular basis for the decreased hippocampal volume observed in depressed subjects. Interestingly, cellular proliferation has also been shown to occur in the prefrontal and temporal cortex of adult rodents and primates, so in line with the observations in depression, these findings suggest that cortical cell loss could also result from decreased cell proliferation in this region.

Pharmacotherapy

Antidepressants can be effective agents for the treatment of depression and have been used clinically for more than 50 years. A list of therapeutic manipulations currently used to treat depressed patients is provided in table 1. Prior to SSRIs, almost all psychotropic medications were the result of chance observations. TCAs for instance were the result of an unsuccessful attempt to improve the antipsychotic effectiveness of phenothiazines, while MAOIs came from a failed attempt to develop effective antitubercular medications. SSRIs however were a rationally designed class of psychotropic medications which hence launched a new era in psychotropic drug development. After imipramine, the development of subsequent SSRIs occurred over a relatively short period. Eventually five SSRIs (citalopram by Lundbeck, fluvoxamine by Solvay, fluoxetine by Lilly, paroxetine by SmithKline-Beecham and sertraline by Pfizer) were launched by several companies in many countries worldwide, indicative of the shift from a chance dependent discovery process to one of rational drug development.
Introduction

Unfortunately, after this milestone discovery, development of newer drugs has seen little progress. The following years consisted mostly of optimization of older drugs (table 2). The reason for this, being the fact that to date no clear consensus has been reached regarding the precise molecular and cellular mechanisms of action of these drugs.

<table>
<thead>
<tr>
<th>TABLE 1. THERAPEUTIC MANIPULATIONS USED TO TREAT DEPRESSION</th>
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<tbody>
<tr>
<td>1. <strong>Antidepressants:</strong></td>
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<tr>
<td>Tricyclic antidepressants (TCA)</td>
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<tr>
<td>Serotonin selective reuptake inhibitors (SSRI)</td>
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<tr>
<td>Noradrenaline selective reuptake inhibitors (NRI)</td>
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<tr>
<td>Serotonin and noradrenaline reuptake inhibitor (SNRI)</td>
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<tr>
<td>Reversible inhibitor of monoamine oxidase type A (RIMA)</td>
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<td>2. <strong>Other manipulations:</strong></td>
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<tr>
<td>Electroconvulsive therapy (ECT)</td>
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<tr>
<td>Transcranial magnetic stimulation (TMS)</td>
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<td>Vagus nerve simulation (VNS)</td>
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<table>
<thead>
<tr>
<th>TABLE 2. ANTIDEPRESSANT DEVELOPMENT</th>
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<tbody>
<tr>
<td>1. 1950s       Accidental discovery of leading drugs</td>
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<tr>
<td>Iproniazide (MAOI) and imipramine (TCA)</td>
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<tr>
<td>Synaptic pharmacology as a therapeutic target</td>
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<tr>
<td>Proposal of the “monoamine hypothesis”</td>
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<tr>
<td>2. 1970s - 1980s Optimization of older drugs</td>
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<tr>
<td>Newer antidepressants (SSRI, NRI, SNRI, RIMA etc)</td>
</tr>
<tr>
<td>3. 2000s       Strategic drug development and rational drug design</td>
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<tr>
<td>Discovery novel therapeutic targets</td>
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</table>
Although the therapeutic action of antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction pathways, given the clinical efficacy of these drugs, intensive investigation has failed to find conclusive affirmation of a primary dysfunction in specific monoaminergic systems in depressed subjects. Moreover, the following major issues have not been addressed by the monoamine hypothesis:

- **Efficacy.** Clinical trials have shown antidepressants to be efficient in approximately 60% of depressed subjects. Besides higher tolerability and reduced side effects however, development of newer antidepressants since introduction of the first tricyclic agents, has failed to yield enhanced efficacy compared to these older drugs.

- **Selectivity.** Whereas SSRIs, NRIs and SNRIs clearly act through stimulation of serotonergic and noradrenergic systems, there is still some uncertainty regarding the specificity of these compounds. In fact, various lines of evidence have indicated that following long-term administration, selectivity of these drugs dissipates such that numerous neurotransmitter systems and brain structures are affected, some of which are not even directly linked to the drugs’ pharmacological profile. This yields the interesting possibility that this delayed non-specific action rather than its high specificity might underlie antidepressants’ therapeutic effects.

- **Mode of action.** Another indication that reduced monoamine levels are not the sole mechanism underlying depression and antidepressants is provided by the efficacy of antidepressants that do not potentiate monoaminergic activity. Alternative mechanisms include enhancing serotonin reuptake (tianeptine) or modulating activity of selective enzymes and/or transcription factors that are not directly linked to monoamine metabolism or signaling transduction pathways (lithium and valproate).

- **Delayed therapeutic action.** Although many antidepressants acutely regulate monoaminergic signal transduction, resulting in a significant increase in synaptic concentrations of the monoamines, noradrenaline and serotonin, there is a several-week latency before onset of clinical effects of these drugs.

- **Monoamine depletion studies.** Experimental monoamine depletion exacerbates depressive symptoms in depressed subjects that successfully respond to SSRIs or NRIs, but fails to induce similar negative effects in medication-free symptomatic patients or healthy controls. This suggests that monoaminergic dysfunctions may play a crucial role in depression and antidepressive action, but is unlikely to represent the primary cause.

Undoubtedly the monoamine theory has some merit, and while it may play a crucial role, given these inconsistencies it clearly does not act alone to fully explain the mechanisms underlying depression or antidepressant action. Despite another catch twenty-two which holds that a full understanding of stress-related pathology is required for the development of novel efficacious pharmacotherapy, it cannot be denied that currently used medications are providing substantial evidence for interesting additional targets. As outlined in the previous paragraphs, the neuroplasticity theory of depression provides plausible targets of drug-induced adaptive neuronal changes. Furthermore, common effects of traditional antidepressant treatment and alternative therapy such as electroconvulsive...
therapy (ECT) and transcranial magnetic stimulation (TMS) in combination with stress studies, are a testimony to the effects on neural plasticity that underlie stress-induced impairments and are specific to all forms of antidepressant therapy.

In summary of the alternative candidates, Hyman and Nestler proposed an “initiation and adaptation” model to describe the drug-induced neural plasticity associated with the long-term actions of antidepressants in the brain. Although the underlying detailed mechanisms are of yet unknown, the common effects of alternative therapy such as ECT and TMS and antidepressants on connectivity and synaptic plasticity in the dentate gyrus are likely to relate to affective functions of depression \(^{135}\). Furthermore, data also demonstrates that chronic ECS induces sprouting of the granule cell mossy fiber pathways in the hippocampus \(^{136}\). The therapeutic effect of antidepressants and alternative therapies could result from either indirect regulation of other non-aminergic neuronal signal transduction systems or regulation of gene transcription following chronic treatment, (possibly explaining the often seen delayed therapeutic response). Indeed, antidepressants selectively affect certain immediate early genes and transcription factors such as c-fos \(^{137,138}\) and Arc (activity regulated cytoskeleton associated protein) \(^{139}\). Alterations in the cAMP second messenger system or functional proteins related to neural plasticity such as CREB and BDNF, further suggest that changes in gene expression may also have a role in the mechanisms underlying pathology and antidepressant action. Identification and quantification of altered gene expression associated with the latter can thus pave the way for the discovery of novel molecular markers useful in the diagnosis and treatment of depression.

**SCOPE OF THE THESIS**

**Multilevel analysis**

If it is generally accepted that stress may induce, exacerbate or contribute to the development of psychiatric disorders as depression and that the stress response differs between males and females, then mechanisms of neuronal function, dysfunction, pathology and pharmacotherapy may not apply to both sexes equally. In this regard the chapters presented in this thesis describe the results of several experiments designed to elucidate the concept of stress, investigate the effect of gender and highlight the implications of such stress-gender interactions with respect to pharmacotherapy. As shown in figure 3 there are three dimensions necessary to understand neuronal plasticity implicated in psychopathology and long-term actions of antidepressants, ranging from gene transcription to higher brain functions.
Outline of the thesis

Inherent to an organism’s (neuro)physiology, information transfer of sensory input and subsequent adaptive responses are communicated through neuronal structures and require proper regulation of signal transduction as well as transcription. Naturally, their complex interactions yield considerable overlap, as one cannot function without the other. Nevertheless, the chapters in this thesis have been grouped according to how the results pertain to these three respective categories:

Systemic level and neuroendocrine aspects

The studies described in this thesis provide evidence to illustrate the physiological and neuronal effects of stress in male and female rats. The results presented in chapter 2 entitled “Repeated stress impairs HPA axis regulation in rats: Indications for differential gender-dependent mechanisms” describe the effects of chronic stress exposure on various parameters of the HPA axis and the autonomic nervous system. The results, ranging from body weight gain to adrenal morphology and steroid plasma concentrations and glucocorticoid receptor densities, serve largely to validate the impact of this stress paradigm by suggesting similar injurious effects of stress leading to prolonged HPA axis activity in both genders. Interestingly however chapter 2 also demonstrates distinct gender differences in physiology and regulation of this basic system. Several brain structures implicated in modulation of HPA axis activity are also investigated using c-fos expression as a marker of neuronal activity.
Cellular level and signal transduction

The second part of this thesis addresses the impact of chronic stress exposure on various correlates of second messenger signal transduction cascades implicated in the neurotrophic pathway. Chapter 3 entitled “Molecular correlates of impaired prefrontal plasticity in response to chronic stress” presents the immunohistochemical effects of this stress paradigm on selected MAPK/CREB cascade members. These results, originally seen in males provide indirect indications of impaired BDNF transcription. To ascertain whether these changes were stress and/or gender specific, chapter 4 entitled “Reduced CREB phosphorylation and calcineurin content characterize the response to chronic stress in male rats: Indications for sex-dependent neuroplasticity changes” describes the results as they compare to the female counterpart.

Molecular level and transcriptional activity

Chapters 5 and 6 respectively entitled “Immunohistochemical changes induced by repeated footshock stress: Revelations of gender-based differences” and “Chronic stress effects on synaptic-vesicle associated protein expression: Indications for region/gender-dependent regulation” describe the results of chronic stress exposure on a molecular level. Chapter 5 describes gender-dependent altered regulation of transcriptional activity using cDNA gene expression analysis. Candidate genes identified as the most strongly stress-affected and putatively responsible for yielding a gender-distinctive result are provided in addition to neuronal limbic activity analysis. Chapter 6 in turn illustrates an extended investigation of synaptic vesicle associated proteins using RT-PCR to corroborate identification with microarray analysis derived from chapter 5.

Implications for pharmacotherapy

Given the stress and gender dependent regulatory effects observed in chapters 2-6, on neuronal function and dysfunction, a subsequently logical and interesting question arose regarding implications of the latter for pharmacotherapeutic application. Whereas the previous chapters provide indications to affirm association of the HPA axis dysfunction hypothesis (chapter 2) as well as impaired neuronal plasticity (chapters 3, 4, 5), synaptic plasticity (chapter 6) and indirectly the neurotrophic theory, most antidepressant development is based on the monoaminergic theory of depression. With regard to this possibility, antidepressants of different mechanisms of action were investigated in chapter 7 entitled “Impact of long-term antidepressant treatment on chronic stress induced reduction of neurogenesis in adult rats: Revelations of sex/drug specific regulation.” This chapter confirms once again the importance of considering gender distinctions on all levels of stress and antidepressant research as it corroborates significant stress/gender interactions regarding stress and antidepressant effects on neurogenesis, thereby providing insight into the final hypothesis of depression.
With the data presented in this thesis, an attempt was made to perform a broad coverage analysis with multi-level examination of stress-induced impairments based on current knowledge and leading hypotheses. Although not a novelty, the gender aspect was additionally incorporated to examine how the latter fits in with current ideas, available data and more importantly, the efficacy of pharmacotherapy. It must be noted that each chapter gave rise to interesting questions, calling in turn for additional studies. Nevertheless, a choice was made to start with the creation of a broad overview, upon which further investigations could be based. Although the studies presented in this thesis only scratch the surface of an ocean of underlying mechanisms, the concluding chapter provides a retrospective discussion of all the presented data and offers an, at times perhaps bold, attempt to integrate the given hypotheses with the inclusion of both genders and the consequences thereof.
Sex-related differences: do they matter?

J. Affect. Disord.

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