Feelings of restlessness, hopelessness, irritability and fatigue, difficulties concentrating and sleep disturbances. These are but a few of the symptoms that characterize stress-related affective disorders as anxiety and depression. These psychiatric disorders can be very draining on those who have the disease as well as their families. Victims of these disorders often experience difficulties functioning in various aspects of their lives, such as work, school, family life, etc. As a result, these disorders have been described as the leading cause of disability worldwide. In this regard it is thus important to pursue improved medications, alternative treatments, causes and eventual cures for such disorders. Unfortunately however, in recent decades little progress has been seen with respect to the development of novel therapeutic (antidepressant) agents.

Interestingly, these psychiatric disorders have been characterized by a marked gender-related prevalence. In fact epidemiological studies across a number of cultures consistently show that beginning at puberty, depression and anxiety disorders are two to three times more common in women than in men. Although this is perhaps the reason why the female sex has often been termed “neurotic”, this is not necessarily fair. In the search for new targets, an interesting avenue of approach thus lies in elucidating the fundamental differences between male and female psychophysiology. Scientists have long known of the anatomical differences between the sexes, but only recently have they begun to uncover significant biological and physiological differences between the sexes. Sex differences have been found everywhere ranging from bone matter composition and pain experience to drug metabolism and the rate of neurotransmitter synthesis in the brain. Naturally, gender differences may thus also be reflected in the circuitry underlying pathology and thus potential therapies. In view of these observations, it is therefore remarkable that until recently women were often excluded from clinical trials of new psychotropic drugs, while most of the preclinical studies on the efficacy of new compounds and their neurochemical modes of action were conducted with male animals. The primary reason for this lack of equal representation lies in concerns associated with the menstrual cycle, which may complicate results of experimental trials.

With regard to future development of new pharmacotherapy, this viewpoint is unfortunate however since insight into new therapeutic targets requires a better understanding of the pathology; and this holds true for both genders if we are to gain a full understanding of these disorders. Elucidating the pathophysiology however is realized in part through investigating current medications’ modes of action, while most of the currently prevailing theories of antidepressants’ modes of action are based solely on the results obtained with experiments with male rats. Research progression thus calls for a paradigm shift, which replaces male-oriented investigation with that of dual-gender comparative analyse.
Fortunately today, women’s health has gained widespread attention in popular magazines, television shows and the wellness industry. John Gray’s “Men are from Mars Women are from Venus” is a testimony to the media interest and acknowledgment of the role of the sexes throughout society. Research in biological psychiatry and psychopharmacology is now starting to focus more on the analysis of sex influences. Discovering why these gender differences in risk exist has in fact become one of the most intriguing and important questions in psychiatric research today: intriguing because a deeper understanding of why women are more likely than men to experience anxiety and depression will provide insight into the pathophysiology of these syndromes and important because such knowledge will improve our ability to design interventions that treat and/or prevent these illnesses.

Oftentimes however psychiatric disorders like depression can develop or exacerbate as a result of stress in the form of stressful life events (loss of a job, spouse etc), particularly when severe or long lasting. As a matter of fact, preclinical studies reveal that chronic stress exposure in rats induces marked degeneration of brain structures responsible for regulating mood and emotions; findings that are also in line with neuroimaging results observed in depressed patients. Chronic stress, may thus contribute directly to the development of neuronal dysfunctions and psychopathology. Although considerable progress has been made in unraveling the neurobiological substrates of the acute stress response, which can facilitate consolidation of new memories and promote cognitive processes, it is thus ironic that chronic stress-induced neurochemical changes are still poorly understood. Also contrary to expectation is the fact that most research on stress-related neuronal abnormalities (like psychotropic drugs) have been conducted in males since the genders differ considerably in their sensitivity to stress and related disorders, making it questionable if the mechanisms of stress response regulation, neuronal function, dysfunction, pathology and pharmacotherapy apply equally to both sexes. Therefore the experiments described in this thesis were designed to elucidate the concept of stress and simulate chronic stress-induced impairments so that these questions may be addressed. In order to do so, male and female rats were given daily sessions of footshock stress for a period of 21 days after which stress and gender comparative interactions were investigated.

There are different dimensions that are necessary to understand the impact of stress on an animal’s physiology, ranging from systemic endocrinological functions to its state of neuroplasticity or the ability of the brain to shape or mold itself by expansion or contraction of neuronal processes due to injury or adverse circumstances. Notably, this ability of the brain to adapt is also reflected at multiple levels; cellular and molecular, including the functional state of emotional brain structures, as well as proper regulation of signal transduction, gene transcription and protein expression. Fortunately the resemblance between many chronic stress induced impairments seen in laboratory animals and psychiatrically ill patients is striking:
Systemic level and neuroendocrine aspects

Depression is often associated with hypercortisolemia, persistently elevated levels of cortisol, the stress hormone secreted by the adrenal glands. Similarly, studies with chronically stressed rats also reveal increased concentrations in the blood of corticosterone (the equivalent of cortisol in humans). This finding has been attributed to an overactivity of the hypothalamus-pituitary adrenal or HPA axis. This is the system that initiates activation of the stress response and without which the body is unable to exert a proper adaptive response in the face of threat or danger. Acute activation of this system can be important for the survival of an animal as it prompts the body for the well known “fight or flight” response. During this process, the autonomic nervous system is stimulated to prepare for escape or a confrontation; heart beat rises, blood pressure increases, catecholamines such as adrenaline are released as well as glucocorticoids. Although short-term activation can be beneficial for an organism, long term stimulation of this system due to prolonged stress exposure can lead to HPA axis hyperactivity. Subsequently excessive release of glucocorticoids can have detrimental effects in the long run however.

Glucocorticoids are the mediators of the stress response and through specific receptors, located in key regulatory brain structures, they modulate feedback regulation so that the response can be terminated when the stressor is eliminated. When chronically stimulated however the feedback becomes disturbed leading to potentially injurious effects on the body’s homeostasis or balance. This in turn has been reported to be a major contributing factor to the atrophy or degeneration that occurs in the prefrontal cortex (hypofrontality) and hippocampus (dendritic atrophy). Depressed patients reveal reduced prefrontal cortex blood flow and metabolism while chronically stressed rats have revealed reduced dendritic branching in the hippocampus. Proper functioning of these regions is important, not only due to their regulatory role in instructing HPA axis activity, but also because of their role in regulating mood and emotional responses. Given their interconnections with other limbic structures, insults to these regions can thus contribute to a dysregulated stress response, as well as disturbed neurotransmission, often associated with affective illnesses as depression.

The studies described in part I of this thesis provide evidence to illustrate the physiological and neuronal effects of stress in rats of both gender. Chapter 2 describes results ranging from body weight gain and adrenal morphology to corticosteroid and catecholamine plasma concentrations and glucocorticoid receptor densities. These findings not only validate the impact of this stress paradigm on both sexes, they also confirm an effect of stress on HPA axis activity. Interestingly however, although the results suggest an injurious net effect of stress leading to prolonged HPA axis activity in both genders, the data also demonstrate distinct gender differences in physiology and regulation of this basic system. Several brain structures implicated in modulating HPA axis activity that were investigated with markers of neuronal activity (FOS) revealed that the brain circuits preferentially affected by stress is largely dependent upon the sex of the animals.
Cellular level and signal transduction

**Part II** of this thesis discusses how chronic stress exposure affected various members of second messenger cascades involved in the transduction of neurotrophin signals, particularly brain derived neurotrophic factor (BDNF). *Neurotrophins* are growth factors sometimes seen as a type of “elixir” for the brain. They are essential for the development and survival of neurons and become especially important in a compromised brain, to counter damage induced for instance by chronic stress. Chronic stress as well as depression have in fact been associated with reduced levels of this protein as well as phosphorylated CREB, the transcription factor responsible for its expression, while local infusion of BDNF into the brain has been associated with antidepressant-like effects. Likewise, antidepressants have indeed been reported to raise levels of this protein and its signaling member pCREB. Chapter 3 describes immunohistochemical evidence for stress effects on selected MAPK/CREB cascade members (ERK1/2, pCREB, PP2B). These results, seen first in males, provide indirect indications of impaired BDNF transcription and thus decreased neuroplasticity in prefrontal regions of the brain. To determine whether these changes were stress and/or gender specific, chapter 4 describes the results found in the female counterpart. Interestingly, males appeared more susceptible to this type of insult since females showed little or no disturbance of this cascade.

Molecular level and gene expression

**Part III** of this thesis describes chronic stress effects on a more molecular level. While stress effects on limbic structure activity can be achieved through differential protein expression, the latter can in turn be dependent upon transcriptional activity and gene expression. To investigate this aspect, a technique called cDNA microarrays were used to establish gene expression profiles of circa 1200 genes following chronic stress. The most interesting finding described in chapter 5 was a strong gender dependent aspect underlying regulation of transcriptional activity and patterns of neuronal activity. Compared to females, males showed a strong upregulation of genes in response to stress. These microarrays were then further investigated to identify genes most strongly affected and most likely responsible for this gender-distinctive result. A group of genes encoding for synaptic vesicle associated proteins appeared particularly affected, and were further investigated based on their role in regulating synaptic plasticity, which requires both neurotrophins and synaptic activity. Loss of either can lead to cognitive impairments since new information cannot be properly stored or recalled later. The results obtained by microarray analysis were corroborated by RT-PCR and chapter 6 describes the results which were in line with gene expression data; males, but not females, indicated increased expression of several of these proteins (synaptophysin, synaptotagmin, synapsin I,II). Although we can only speculate about the meaning of these findings, it is evident that stress clearly has a gender-specific effect on synaptic function. It is important however to learn to recognize and
understand synaptic dysfunction following stress since this may decrease efficient neurotransmission and even neurotrophin production, which eventually precedes overt neurodegeneration seen in psychiatric disorders as depression.

**Implications for pharmacotherapy**

Since we saw strong effects of stress and gender on neuronal function and dysfunction, an interesting and logical question would regard the implications of these factors for drug application and development. Chapters 2-6 provide indications to support 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. Development of most new antidepressants however is based on the monoaminergic theory of depression.

To explore this final possibility, antidepressants of different mechanisms of action were investigated in chapter 7 of **part IV**. The results reveal striking gender differences with regard to antidepressant effects on systemic tryptophan levels, the precursor for serotonin, and neurogenesis, the birth of new neurons. Although males showed decreased tryptophan levels, neurogenesis/proliferation was unaffected (or slightly raised) by antidepressants; this was in contrast to females that showed increased tryptophan levels but considerably reduced neurogenesis. This effect was also common to all antidepressants (citalopram, tianeptine and reboxetine), despite their mechanisms of action. In contrast to the effects seen on signal transduction and (synaptic protein) gene expression in males, it appears women are more prone to stress induced effects on neurogenesis and survival of brain cells. The results confirm once again the importance of considering gender distinctions on all levels of stress research. More importantly however, these gender differences evidently also extend onto the application of antidepressants.

To summarize the above, it seems that no matter where we look, stress exerts strong effects on the physiology and psychophysiology of an organism. Although beneficial in the short run it is not surprising that long term stress can disturb the natural equilibrium state of an animal, given it's effects on the extended range of parameters (systemic, endocrinological, cellular, molecular; see figure below). The most interesting observation however was that females consistently showed an opposite or different effect than males. With regard to the question of which sex is more or less susceptible to stress, it appears this answer is thus less clear-cut than we would hope. While stress seems to affect transcriptional activity and cellular signal transduction in males, females appear more vulnerable to the negative stress effects on cell birth and/or survival. The manner in which HPA axis activity is affected in males and females is a testimony to this gender-differential regulation, since both sexes seem to indicate prolonged HPA axis overactivity, albeit in a sex-specific manner. The question we should therefore focus on is not which sex is more vulnerable, but how do the sexes differ in their vulnerabilities. It is more insight into this matter that may ultimately lead to way to new and improved drug development that considers sex specific neural circuits implicated in stress response regulation.
Perhaps one day we can even speak of customized drug design, targeted to meet gender specific disturbances.

Figure 1.
Although the neuronal effects of stress stand central to this investigation, the data illustrate that stress also exerts interregulatory effects on multiple levels of an organism’s physiology (and vice versa).