Substance P and the neurokinin 1 receptor

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Chapter 1

Introduction

An affective disorder can have a major impact on the life of patients and their environment. Not surprisingly, the underlying neurobiology has been the topic of a large number of research projects. However, in spite of the efforts made in the past decades, the mechanisms and factors that play a role in developing affective disorders are far from clear. Moreover, pharmacological treatment of affective disorders is still far from ideal. There is thus a need for better understanding and improved treatment of these disorders.

Affective disorders can roughly be divided in mood and anxiety disorders. According to the diagnostic and statistical manual (DSM-IV-TR) of the American psychiatric association, mood disorders have a disturbance in mood (either depressed or the loss of interest or pleasure in nearly all activities) as the predominant feature. They are subdivided into depressive disorders, bipolar disorders and mood disorders connected with a general medical condition or substance induced. Anxiety disorders are as heterogeneous a group as mood disorders, with (increased) anxiety as the predominant feature. Examples are generalized anxiety disorder (GAD), panic disorder (PD), obsessive-compulsive disorder (OCD), phobias and stress disorders.

The prevalence of affective disorders is higher in females than in males, suggesting that estrogen could have an influence on the susceptibility of developing affective disorder, however the notion that women are more inclined to seek professional help is likely to play a role too. In general three types of treatment are available: Antidepressants are considered to be the first choice treatment with the majority of mood and anxiety disorders. Benzodiazepines are also used for treating certain dimensions of anxiety, but sedation and withdrawal problems have diminished their popularity. In addition several types of psychotherapy are available for the treatment of affective disorders.

Clinical depression affects about 16% of the population on at least one occasion in their lives (Bland, 1997). This is an average number, however, and much higher prevalence rates have been reported in females, but also in some western countries. In Australia for instance, one in four women and one in six men will suffer from depression. The mean age of onset, from a number of studies, is in the late 20s. About twice as many females as males report or receive treatment for clinical depression. Though this imbalance is decreasing over the course of recent history; this difference seems to completely disappear after the age of 50 - 55, when most females have passed the end of menopause. Clinical depression is currently the leading cause of disability in the US as well as in other countries, and it is expected to become the second leading cause of disability worldwide (after heart disease) by the year 2020, according to the World Health Organization (Murray & Lopez, 1997). This prognosis is even more alarming considering the outcome of a meta-analysis of clinical studies performed with the 6 most prescribed antidepressants in the USA versus placebo, suggesting that antidepressant treatment was only marginally better than placebo (1-3 points improvement on the Hamilton Depression scale) (Khan *et al.*, 2002)

Over the past 50 years several antidepressant therapies have been developed to treat clinical depression. The first effective antidepressants were based on serendipitous findings with the tuberculosis drug iproniazide and belonged to the group of monoamine oxidase inhibitors (MAOi's). These compounds inhibit the enzyme responsible for the oxidative degradation of monoamines, resulting in increased extracellular levels of monoaminergic neurotransmitters. Occasionally MAOi treatment could lead to hypertensive crisis especially with people of advanced age. Next, the tricyclic antidepressants (TCAs) were discovered. These compounds inhibit the transporters responsible for the reuptake of monoamines, also leading to increased extracellular levels of monoamines. However, due to their 'dirty' pharmacological profile, treatment was accompanied with a considerable number of side effects. Cleaning up their pharmacological profile has led to compounds with a more benign side effect profile, such as the selective serotonin re-uptake inhibitors (SSRIs) and the atypical antidepressant mirtazapine. In contrast to certain benzodiazepines, all monoamine-based antidepressants have in common that their onset of action is around 3 to 6 weeks after the start of the treatment, whereas some side effects may occur immediately. Since a number of patients do not tolerate these side effects, they may be inclined to abandon pharmacotherapy prematurely. Furthermore, clinical studies indicate that 30-40 % of the patients do not experience any improvement of their depression after treatment, and in reality this percentage may be even higher. Unfortunately, for this group of patients no alternative treatments exists (Moncrieff, 2008).

One could argue that more effective antidepressants can be developed as soon as the mechanisms underlying depression are unraveled. However, despite the progress of neurobiology in the last 50 years, our knowledge of the pathological processes in affective disorders remains poor. The brain is a very complex organ protected by the blood-brain-barrier (BBB) and direct neurobiological assessment is difficult because invasive techniques are normally not allowed in humans. Measurement of neurotransmitters and their metabolites in cerebrospinal fluid (CSF), serotonin uptake and receptor binding of blood cells and neuro-endocrine strategies have been used as an alternative. However, these approaches must be considered as relatively crude and indirect ways to obtain information from the normal and pathological brain. Yet, based on clinical and preclinical research, several hypotheses of depression have been developed.

1.1 Hypotheses of affective disorders

1.1.1 The monoamine hypothesis

The monoamine hypothesis simply states that depression is caused by a deficiency of brain monoaminergic activity, which can be treated by drugs that increase this

activity (Schildkraut, 1965). The hypothesis is supported by the following observations:

- *The majority of antidepressants increase the extracellular levels of monoamines in the brain, either via inhibition of monoaminergic metabolism (DELAY et al., 1952) or through blockade of monoamine reuptake into the nerve terminal (Wolf et al., 1985)*
- *Reserpine, an antihypertensive drug, which induces a depletion of monoamine stores, is capable of inducing symptoms of depression (Goodwin & Bunney, Jr., 1971).*
- *Patients successfully treated with SSRI's fall into remission on a low tryptophan (an essential amino acid and precursor of serotonin) diet (Spillmann et al., 2001).*

The monoamine hypothesis was very attractive because of its simplicity, yet it appeared unable to offer an explanation for a number of clinical observations.

First, the large number of side effects of MAOi's and TCAs has prompted pharmaceutical companies to develop more specific monoamines reuptake inhibitors. Combinations of inhibitors for serotonin, noradrenaline and dopamine reuptake were also developed. However, focusing on increased monoamine levels has not led to considerable improvement of clinical efficacy. Second, the monoamine hypothesis fails to explain why a number of drugs that also increase brain monoaminergic activity, such as cocaine, amphetamine and fenfluramine are devoid of antidepressant efficacy. Third, a relatively large group of patients does not respond to monoamine-related antidepressant treatment. Finally, most antidepressants increase monoamine levels within hours after administration, but the therapeutic response is delayed for several weeks (Baldessarini, 1989).

The latter has led to adaptations of the monoamine hypothesis stating that sustained increased levels of monoamines are necessary to induce desensitization of auto- and heteroreceptors, eventually leading to the antidepressant effect (Blier *et al.*, 1987). These auto- and heteroreceptors regulate monoaminergic activity and it has therefore been proposed that the process of desensitization coincides within the time course of the therapeutic antidepressant response. Conversely, resensitization of such receptors might offer an explanation for the relapse of depressive symptoms after discontinuation of antidepressant treatment. Changes in sensitivity of receptors for all three monoamines have been subject to research, however most attention has been paid to the 5-HT_{1A} serotonin autoreceptor. As

the monoamine receptors have been extensively described elsewhere, only a number of important monoamine receptors will shortly be discussed here.

The ß-adrenoreceptor (ß-AR) may have been the first monoamine receptor which has been functionally investigated in relation to chronic antidepressant treatment (Banerjee *et al.*, 1977). It appeared that a variety of antidepressant treatments, including TCAs, MAOIs and repeated electroconvulsive shock therapy down-regulated ß-AR density in limbic brain regions, including cerebral cortex and hippocampus (Vetulani & Sulser, 1975; Sugrue, 1983). Moreover, nearly all antidepressants diminished the ability of ß-ARs to stimulate adenylate cyclase-mediated cAMP production in these regions (Vetulani & Sulser, 1975; Charney *et al.*, 1981). The time needed to establish the changes in receptor density and functionality was consistent with the time course of the therapeutic antidepressant response.

The α_2-adrenoreceptor subtype has also been studied in relation to major depression. It has been reported that α_2-adrenergic autoreceptors are hyperactive in major depression (Garcia-Sevilla *et al.*, 1999), which may lead to a decrease of both noradrenergic and serotonergic neurotransmission. TCAs have been shown to desensitise α_2-adrenergic autoreceptors, and may thus be capable of normalizing the alleged disrupted monoaminergic transmission in depression (Mateo *et al.*, 2001).

Preclinical evidence regarding 5-HT receptor subtypes is not very consistent. In fact desensitization has only been convincingly shown for the 5-HT_{1A} autoreceptor, however not all antidepressants are capable of desensitizing this receptor following chronic treatment (Hjorth & Auerbach, 1994).

Dopamine receptors have received relatively little attention in antidepressant research compared to serotonin and noradrenaline. Traditionally, dopamine research was targeted at other fields of psychiatry such as schizophrenia, obsessive-compulsive disorder and addiction but also at neurological disorders such as Parkinson's and Huntington's disease. In European countries the dopamine reuptake inhibitor bupropion is prescribed to diminish nicotine craving, however in the USA the compound is registered as an antidepressant. A recent study indicates that antidepressant efficacies of bupropion and venlafaxine, a dual serotonin and noradrenaline reuptake inhibitor, are comparable (Hewett *et al.*, 2008). Postsynaptic D_2 receptors may be hypersensitive in depression, although this idea is primarily based on behavioral studies in laboratory animals following chronic antidepressant treatment (Maj *et al.*, 1996; Spyraki & Fibiger, 1981). Typical antipsychotics such as dopamine D_2 receptor antagonists may also have antidepressant efficacy (Ruther *et al.*, 1999). Moreover, the atypical antipsychotic amisulpride was shown to have antidepressant efficacy, which was attributed to a preferential blockade of presynaptic D_3 dopamine receptors, leading to an increased dopamine release (Racagni *et al.*, 2004). Accordingly, several augmentation strategies with antipsychotics and SSRIs have been investigated,

albeit with varying success rates (Montgomery, 2008). Several compounds combining SSRI and partial dopamine D_2 agonist activity in one molecule are currently under investigation (Michael-Titus *et al.*, 2008).

The general hypothesis remains that deficiencies in dopamine, serotonin and norepinephrine underlie the pathology of major depressive disorder (Nutt, 2006). However, in spite of the adaptations made to the monoamine hypothesis and its usefulness in developing more or less effective antidepressants, it remains questionable whether this hypothesis adequately reflects the underlying pathology of affective disorders. It is clear that other hypotheses also have to be considered (Krishnan & Nestler, 2008).

1.1.2 The HPA-axis hypothesis/stress induced dysfunction

1.1.2.1 The role of Stress

The role of stress hypothalamic-pituitary-adrenal axis (HPA-axis) hyperactivity in psychiatric disorders is well established. The initiator of stress research is Hans Selye, who described stress as a syndrome produced by various noxious agents (Selye, 1936). His paper in Nature is considered to be the starting point of stress research, which has finally resulted in the elucidation of HPA-axis functioning. Seyle states that "stress is the state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biologic system" (Selye, 1985). The stress syndrome can be induced by all kind of stressors with individual vulnerability playing an important role. It is believed that prolonged exposure to stressors leads to the stress syndrome. Based on Selye's and many other observations, it is no surprise that a connection with affective disorders was made. Strong evidence over the years has linked failure to cope with stressful events to the etiology of depressive disorders (Kendler *et al.*, 1999; Hammen, 2005).

1.1.2.2 The role of the HPA-axis

The HPA-axis is the communication system that prepares the body for the so-called fight, fright and flight response. The body has to be prepared to respond to stressful situations. First, the sympathetic adrenal medullary (SAM) system is activated, which results in an increased release of adrenaline and noradrenaline from the adrenals and sympathetic nerve terminals, respectively. In the secondary phase, corticotrophin-releasing hormone (CRH) is released by the hypothalamus. CRH stimulates the excretion of adrenocorticotrophin (ACTH) from the pituitary (Bale & Vale, 2004). This hormone is released into the bloodstream and transported to the adrenals, where it stimulates the excretion of corticosteroids (cortisol in primates and corticosterone in rodents) (Charmandari *et al.*, 2005). Under normal conditions, HPA-axis activity is regulated by several negative feedback mechanisms. Briefly, ACTH exerts a negative feedback control on the

release of CRH, whereas cortisol exerts a negative feedback control on both the release of ACTH and CRH. This returns the body from the activated to a more normalized state and prevents overexposure to cortisol. In the short term cortisol is essential for the body to cope with acute stressors, however it is believed that sustained elevation of cortisol will lead to severe physiological dysfunctions (Sapolsky *et al.*, 2000). For instance, during constant activation of the pituitary and adrenals, these glands become enlarged. Moreover, the constant activation of certain target areas by cortisol may eventually lead to tissue damage; for example, in the hippocampus, where transcription of certain genes is halted by the glucocorticoid receptor (GR) and dendritic retraction occurs (Kole *et al.*, 2004; Sapolsky, 2000). Other physiological functions, like the immune responses are also impaired by a continuous activation of the HPA-axis. Some authors have even suggested that exposure to a single severe stressful event has life-long implications (Koolhaas *et al.*, 1997).

1.1.2.2.1 HPA-axis and depression

Several observations support an involvement of HPA-axis dysfunction in depression (Pariante & Lightman, 2008). A number of patients experiencing depression excrete significantly more cortisol after stimulation with corticotrophin releasing hormone (CRH) (Gold *et al.*, 1986a; Gold *et al.*, 1986b). This hormonal release pattern occurs in approximately 50% of patients with a major depressive disorder (Maes *et al.*, 1990; Holsboer, 2000). Enlargements of the pituitary and adrenal glands have been shown in depressed patients, also indicating a hyperactive HPA-axis (Owens & Nemeroff, 1993; Gold *et al.*, 1986a). It has also been shown that depressed patients display a blunted ACTH response to CRH administration (Nemeroff, 1996; Gold *et al.*, 1986a). Finally, postmortem studies of suicide victims have shown increased CRH values in cerebrospinal fluid (CSF) and a decreased CRH receptor density in the brain (Arato *et al.*, 1989; Nemeroff *et al.*, 1984), as well as reduced mineralocorticoid receptor (MR) (Lopez *et al.*, 1998).

1.1.2.3 The role of corticoid receptors

Occupation of MR and GR receptors by cortisol is an important factor in the regulation of HPA axis activity (Reul & De Kloet, 1985; McEwen *et al.*, 1986). In particular the correct balance of MR and GR mediated effects may be vital to restrain HPA activity (De Kloet *et al.*, 1998). An imbalance in MR/GR actions may lead to alterations in the behavioral adaptation necessary to normalize the stress response. When the MR/GR imbalance persists and surpasses a predisposed individual threshold, cognition is compromised and vulnerability for developing affective disorders may also increase (De Kloet *et al.*, 1998; De Kloet *et al.*, 1999). Importantly, the time course of antidepressant-induced corticoid receptor up-regulation coincides with normalization of HPA-axis activity and closely follows

clinical improvement of depression (Reul *et al.*, 1993; Reul *et al.*, 1994; Yu *et al.*, 2008).

1.1.2.4 The role of CRH

It has been proposed that increased CRH plays a role in the pathogenesis of depression (Raadsheer *et al.*, 1994; Nemeroff, 1988). Accordingly, it has been postulated that any intervention restoring HPA dysfunction may have an antidepressant effect (Nestler, 1998). Additional support for the stress/HPA-axis hypothesis is derived from the observation that depressive episodes follow traumatic life events. Such stressful life events are characterized by chronic activation of the HPA-axis. This might lead to changes in GR and MR density and also an impaired GR/MR balance, eventually resulting in HPA-axis dysfunction.

Using the dexamethasone/CRH suppression test, both in depressed patients and in their non-depressed relatives showed a blunted cortisol response in both groups. The study suggested that genes play a role in the vulnerability for stress (Holsboer *et al.*, 1995). On the other hand, the study also showed that a dysfunctional HPA-axis does not lead to an affective disorder per se. Yet, it is clear that the HPA-axis plays a role in the etiology and treatment of affective disorders (Ising *et al.*, 2005).

1.1.2.5 HPA-axis and 5-HT

There is extensive pharmacological and neuro-anatomical evidence that 5-HT containing neurons regulate the HPA-axis in rats (Fuller & Snoddy, 1990). Serotonergic neurotransmission has an excitatory influence on HPA-axis activity. For instance, local application of serotonin dose-dependently increases CRH in the hypothalamus (Holmes *et al.*, 1982). Serotonin directly stimulates the release of ACTH from the pituitary (Spinedi & Negro-Vilar, 1983). Moreover, serotonin increases corticosteroid receptor densities in hippocampus through 5-HT_{1A} receptors (Budziszewska *et al.*, 1995). Thus the serotonergic system influences HPA-axis feedback mechanisms at different levels.

Vice versa, the HPA-axis influences serotonergic neurotransmission. For instance corticosteroids influence 5-HT synthesis through the enzyme tryptophan hydroxylase, thereby affecting 5-HT turnover but not 5-HT levels (Azmitia, Jr. & McEwen, 1969; Azmitia, Jr. *et al.*, 1970). It has also been reported that local application of GR receptor agonists into the dorsal raphe nucleus compromises the functioning of the 5-HT1A autoreceptor (Laaris *et al.*, 1995). Furthermore, normalization of HPA-axis after antidepressant treatment may be due to antidepressant-induced changes in brain corticoid receptor capacity (Barden *et al.*, 1995). This normalization of HPA-axis activity is likely to be related to a restoration of the negative feedback between corticosteroids and the HPA-axis, possibly via increased corticosteroid receptor gene expression. Thus, non-suppression of dexamethasone in patients with depression disappears after recovery, and

normalisation of the HPA-axis is considered essential for permanent improvement (Ribeiro *et al.*, 1993; Firk & Markus, 2007).

1.1.3 Neuroplasticity hypothesis

Antidepressants immediately increase extracellular monoamine levels in the brain, yet their therapeutic effect is delayed for several weeks. The hypothesis that a simple shortage of monoamines would explain the etiology of major depression is therefore rather unlikely. Consequently, it has been proposed that adaptations have to take place to facilitate the therapeutic effect of antidepressants. Advances in both clinical and preclinical research have led to the proposition that neuroplasticity plays an important role in both the etiology and treatment of affective disorders.

The neuroplasticity hypothesis states that major depression results from an inability of the organism to make appropriate adaptive responses to stress or other aversive stimuli (Duman *et al.*, 1999). These inabilities could indicate an impaired capacity of the brain to correctly process such stimuli. In other words, the brain may have become rigid and according to this hypothesis, depressed.

Several studies support the idea that the integrity of the brain is affected in major depressive disorders. Neuroimaging studies, for example, have demonstrated a reduced gray-matter volume in the prefrontal cortex (PFC) and striatum of depressed patients. Furthermore, enlargements of the third ventricle have been reported in patients, that were compared to healthy volunteers (Drevets, 2000). Neuropathological studies in post-mortem tissue from depressed patients also showed a reduction of cortical volume. In addition, glia cell count was reduced as well as neuron size in the PFC and amygdala (Rajkowska *et al.*, 1999; Rajkowska, 2000). Reduced glial density and glia to neuron ratios in the amygdala have also been reported (Hamidi *et al.*, 2004). Notably, glial cells play a critical role in regulating synaptic glutamate concentration, central nervous system (CNS) homeostasis and in the release of trophic factors (Araque *et al.*, 2000;Ullian *et al.*, 2004)

1.1.3.1 Neurogenesis

There is a growing body of preclinical evidence in support of the neuroplasticity hypothesis. Gould and colleagues made a real advance, showing that against all expectations neurogenesis occurs in the adult brain. First, this was shown in rodents and non-human primates, but more recently it was also shown in humans (Gould *et al.*, 1997; Eriksson *et al.*, 1998). Next, it was demonstrated that stress reduced neurogenesis in *scadentia*, a species phylogenetically in between insectivores and primates, and in primates (Gould *et al.*, 1997; Gould *et al.*, 1998). Conversely, neurogenesis is stimulated in rodents placed in a rich environment (Kempermann *et al.*, 1997; van Praag *et al.*, 1999). When animals were

subsequently trained to perform an associative learning task, the survival rate of the newly formed cells increased (Gould *et al.*, 1999). During stress, apoptosis or cell death appeared to be increased. The effects were region specific, but not restricted to the granular cell layer in the dentate gyrus of the hippocampus (Lucassen *et al.*, 2001).

These observations imply that:

- *The adult brain is capable of creating new neurons and other cells.*
- *The process of neurogenesis and cell survival can be influenced by various environmental stimuli.*
- *Neurogenesis combined with apoptosis may represent a flexible system, which has evolved to enable adaptation of the organism to changing environmental conditions.*
- *This system of flexibility is apparently compromised by chronic stress.*

Different types of antidepressant treatment also appear to influence neurogenesis. For example, electroconvulsive shock treatment of animals was shown to have a positive effect on neuropeptide system plasticity (Altar *et al.*, 2004). It has also been shown that electric convulsive therapy (ECT) induces neurogenesis and that antidepressants stimulate cytogenesis (Madsen *et al.*, 2000; Malberg *et al.*, 2000).

The general consensus is now that the stressed/depressed brain is maladaptive, implying that the organism is less capable to adapt and react to external stimuli. It is probably this state of the brain, which causes depression. There is compelling evidence that the reduced neurogenis observed in several animals models can be reversed by antidepressants (Dranovsky & Hen, 2006). And it could be that stimulation of the hippocampal neurogenesis is part of the therapeutic effect of antidepressant drugs (Sahay & Hen, 2007). The lack of neuroplasticity might also offer a viable explanation for the co-occurrence of affective disorders with such neurodegenerative disorders as Parkinson's disease.

1.1.3.2 Synaptic plasticity

As mentioned previously, glial function is compromised in depressed patients. Glia cells are mostly thought of for having a supportive role for neurons and as playing a role in synaptic activity. In the adult mammalian brain glial cells outnumber neurons by a factor 10-15. In the brain 3 types of glial cells can be found;

microglial cells, oligodendrocytes and astrocytes. Microglial cells are small cells of distinct shape. When activated they play a major role in immunological protection of the brain. Oligodendrites are involved in axon isolation and signal transduction, a function similar to the Schwann cells in the peripheral nervous system. Astrocytes are the most abundant glial cell type in the brain. They are found in close association with axons, blood capillaries and cell bodies. Several essential functions have been attributed to these cells such as, the supply of energy and nutrients to neurons, neurotransmitter metabolism, maintenance of ion homeostasis in the extracellular space, regulation of neuronal migration, secretion of growth factors and participation in immune and inflammatory responses. Because of their close association with the blood capillaries, they are a vital part of the blood-brain-barrier (BBB). Astrocytes also possess receptors for neurotransmitters and steroid hormones, that can trigger electrical and biochemical events inside the cell, comparable to neurons. Astrocytes can release glutamate and d-serine, enabling them to modulate neuronal activity.

Moreover, astrocytes influence both synaptogenesis and synaptic transmission. A recent study in chronically stressed tree shrews has demonstrated a reduction in the number astrocytes expressing glial fibrillary acidic protein (GFAP, a cytoskelatal protein) as well as a decreased cell body volume. Glial cells also play an important role in synaptic function (Barres, 2008). In the CA1 hippocampal area a single astrocyte can contact with up to 140,000 synapses (Bushong *et al.*, 2002). In this specific region about 60% of mainly glutamatergic synapses have intimate contact with surrounding astrocytes (Ventura & Harris, 1999). Astrocytes influence neuronal morphology in two ways: first, by releasing compounds that enhance synaptic transmission, such as d-serine, which acts as co-agonist of the NMDA receptor. Second, by releasing factors, that influence axonal and dendritic outgrowth, and thus the number of synaptic connections (Ullian *et al.*, 2004;Pittenger *et al.*, 2007).

Chronic stress has a major impact on the shape of dendrites in the hippocampal formation. It has been reported that the shape of the dendrites influences the firing rate of neurons (van Ooyen *et al.*, 2002). Magarinos (1996) has shown that chronic stress leads to dendritic retraction of the pyramidal cell in the CA3 area of the hippocampus (Magarinos *et al.*, 1996). This retraction of dendritic outgrowth was found with various stress paradigms, and may lead to a reduction of effective synapses. When animals are housed in a stimulatory environment the opposite occurs and increased synapse formation and an increased length of the dendrites are observed (van Praag *et al.*, 2000).

The volume loss observed in the brains of depressed patients could be related to stress. In animal studies it has been shown that hippocampal volume is reduced after chronic stress. It has been suggested that a hyperactive HPA-axis is responsible for this volume loss. However, this volume loss could not be induced by hypercortisolemia (Sousa *et al.*, 2000; Leverenz *et al.*, 1999). Moreover, the number of neurons is not significantly decreased as shown by post-mortem studies

in severely depressed patients or patients chronically treated with steroid hormones such as prednisolone (Muller *et al.*, 2001). An alternative explanation for the loss of brain volume could be a reduction of extracellular space (ECS) (Sykova & Chvatal, 2000). The ECS of the central nervous system is the microenvironment of neurons and glial cells and its composition and size changes during neuronal activity. Suggestions have been made that the volume of the ECS might be compromised during pathological states of the brain. One must realize that this is relatively unknown territory, which deserves further investigation.

1.1.3.3 Intracellular Plasticity

The alleged compromised neuroplasticity in affective disorders may involve changes at different cellular levels. For example changes of the cAMP cascades have been reported by several groups (Perez *et al.*, 2000;Perez *et al.*, 1989;Ozawa & Rasenick, 1991). Also cAMP response element binding protein (CREB), a transcription factor that mediates many of the actions of cAMP, is up-regulated by chronic, but not acute antidepressant treatment (Nibuya *et al.*, 1996). In addition, a post-mortem study has shown that expression of CREB is decreased in the temporal cortex of patients with depression, whereas antidepressant treatment was shown to reverse this effect (Dowlatshahi *et al.*, 1998). Chronic antidepressant treatment not only increased percentage of phosphorylated state of CREB proteins, but it also increased the expression levels of brain derived neurotrophic factor (BDNF), a major gene target regulated by CREB (Duman, 2002). BDNF belongs to the family of neurotrophic factors, which also includes the nerve growth factors neurotrophin 3 and 4 (Thoenen, 1995). Initially neurotrophic factors were being characterized for regulating neuronal growth and differentiation during development, but now they are also recognized as potent regulators of synaptic plasticity and survival of adult neurons and glia (Patterson *et al.*, 1996;Korte *et al.*, 1996).

1.2 Affective disorders and the immune system

Previously, we have focused primarily on the central nervous system, but immune function is playing an important role in affective disorders too.

The involvement of the immune system in affective disorders is well known. For instance, patients treated with interleukin-1 develop depressive-like symptoms, like disturbances in circadian rhythm as witnessed by an altered sleep/wake cycle. Depressed patients are also more susceptible to infections, allergies and autoimmune diseases (Whitlock & Sisking, 1979). This so-called sickness behavior suggests impairments of immune system function. Antidepressant treatment does not only alleviate symptoms of depression, but it also improves immune system function (Castanon *et al.*, 2002).

Several lines of evidence indicate that central nervous system (CNS) and immune system interact. For instance, sympathetic nerve endings were shown to be present in lymphoid tissue. Also, cytokinine receptors have been found on CNS neurons, amongst others in brain structures that are associated with depression, such as hypothalamus, hippocampus and locus coeruleus (Turnbull & Rivier, 1999). Moreover, activated T cells and monocytes can pass the blood brain barrier and bind to brain cells through cell-membrane specific molecules (Maes et al., 1995).

The presence of monoamine receptors on lymphocytes and the production of HPA-axis mediators such as ACTH by lymphocytes suggest a direct communication between CNS and immune system during stressful events. It has been reported that (large) increases of cortisol levels are capable of inducing thymocyte apoptosis (van den Brandt et al., 2007). Social defeat in rats results in more persistent alterations of thymus function (Engler & Stefanski, 2003). Moreover, both surgical and physical stress suppresses the activation of natural killer (NK) cells in rats (Ben Eliyahu et al., 1999).

Several studies have been performed in vitro to investigate the effect of antidepressants on the immune system, for instance, by testing the proliferation rate of isolated lymphocytes under different antidepressant treatment regimes. It appeared that all tested antidepressants dose-dependently reduced the capacity of lymphocytes to proliferate in response to lectin or antigen activation (Castanon et al., 2002).

1.3 Animal models of affective disorders

Studying the mechanisms underlying affective disorders in humans has many restrictions. Invasive techniques for the brain are not allowed and ethical considerations limit the use of psychological trauma models that are indispensable for inducing pathological effects reminiscent of those in major depression.

For this reason animal models have been developed to study the biological processes implicated in major depression (Shively, 1998). Such animal models must fulfil three important criteria viz. high face-, construct- and predictive-validity. High face-validity is achieved when a model replicates several of the symptoms seen in depressed patients, such as anhedonia and low locomotor activity. A model has high construct-validity when it is homologous in etiology and has an empirical and theoretical relationship to the disorder. Finally, having a high predictive-validity, means that the animal model is capable of correctly identifying efficacious pharmacotherapy. For major depression this implies that the behavioral correlates of the animal model must respond to chronic antidepressant treatment. As yet, no animal model has been able to unambiguously meet all these three criteria.

Animal models for depression can be divided in two groups. The first group is more or less based on clinical observations. In these models, the focus is on observable

features, which can be specifically influenced by antidepressants. The second group is based on etiological considerations, such as the notion that stressful life events are among the most potent triggers of a depressive episode (Kendler *et al.*, 1999). In the next sections examples of both types of animal models will be given, although more animal models have been developed over the years (McArthur & Borsini, 2006).

1.3.1 Animal models based on observations

In the learned helplessness paradigm animals are exposed to aversive stimuli, which they cannot predict, control or avoid (Seligman & Beagley, 1975). In this model the animal shows deficits in escape performance, but also decreased locomotor activity and a loss of appetite and weight. Treatment with antidepressants is capable of reversing these symptoms (Willner *et al.*, 1992;Yadid *et al.*, 2000)

In the Porsolt swim test (Porsolt *et al.*, 1977), also known as the forced swim test, a rat or a mouse is placed in a container filled with water from which it cannot escape. After some time the animal knows that it is useless to struggle, and it assumes an immobile posture. When placed for a second time in the container the period of struggling is shorter and the animal takes on its immobile posture more rapidly. The onset of this immobility is delayed by pre-treatment with antidepressants (Kawashima *et al.*, 1986).

In the chronic unpredictable stress paradigm (Katz, 1981) the animal is subsequently exposed to various kinds of stressors. This results in disrupted behavior in the open field test, which measures explorative behavior of the animal. Treatment with antidepressants during the chronic stress period was shown to prevent the aberrant behavior in the open field. The paradigm exists in several variations and is also known as the chronic mild stress model of depression (Papp *et al.*, 2002)

The animal models in this section are often used by the pharmaceutical companies in the screening process of new potential antidepressants (for a more elaborate overview see (Bourin *et al.*, 2001).

1.3.2 Animal models based on etiology

Animal models based on etiology mostly refer to the notion that a stressful life event is likely to increase susceptibility for affective disorders. As the etiology for depression is still not clear, several models have been developed based on the assumption that stress plays a major factor in developing depression. Although the following list is not complete, it illustrates the diversity of the animal models based on etiology.

Prenatal stress exists of inflicting stressful stimuli to pregnant rats, such as repeated restraint stress for 30 minutes. Fride (1986) demonstrated that the offspring of these rats showed reduced stress coping abilities and an increased emotionality, as witnessed by an increased anxious reaction in fear related tests (Fride *et al.*, 1986). Furthermore HPA-axis response to stressful stimuli in the offspring was more profound (Bhatnagar *et al.*, 2005; Coe *et al.*, 2003).

Maternal separation is based on removing the pups for a variable period of time from their mother during the weaning period. These pups show a long-lasting HPA-axis response to stress. Deficits in attention and emotions were also reported, but not in all studies. Sometimes the opposite effect was noted which according to Staton *et al.* (1988) might relate to the time point of maternal deprivation (Stanton & Levine, 1988; Millstein & Holmes, 2007).

Social defeat models refer to the increased risk for developing an affective disorder when humans lose their social status (Brown *et al.*, 1990). Many species have a strong hierarchical social structure, primarily based on confrontations between males. Loss of rank within such hierarchical structure may model the loss of social status in humans. It has been proposed that the loss of rank in animals in an established social group might serve as model for loss of self-esteem in humans, which could lead to depression (Willner, 1995; Rygula *et al.*, 2008)

Primates live in hierarchal structures, and social status is of eminent importance for reproduction, food intake and other privileges. It appears that basal cortisol levels are correlated with the social status of an individual within the group. For example dominant squirrel monkeys in captivity have significantly lower levels of cortisol (Manogue *et al.*, 1975). High-ranking male olive baboons living in the wild also showed lower plasma cortisol levels, and a stronger cortisol response during stressful encounters (Sapolsky, 1982). However, markedly increased basal cortisol levels were measured in higher ranked males when their authority was compromised during social instability. This rise in basal cortisol levels was not only seen in the higher ranked animals, but occasionally also in lower ranked animals involved in the social instability. Apparently individual coping styles determine HPA-axis reaction during social instability and hence the physiological consequences of this hypercortisolism (Sapolsky, 1994; Shively *et al.*, 2006).

Several animal models are based on social defeat. For example the resident intruder paradigm, which has been developed for several species but is most investigated in rats. Social defeat elicits long lasting behavioral and physiological changes in rats, however, these effects can be prevented by chronic antidepressant treatment (Koolhaas *et al.*, 1990; Rygula *et al.*, 2008).

Variations of the resident intruder paradigm are also used in mice and tree shrews (Fuchs & Flugge, 2002). The tree shrew variant will be discussed in more thoroughly in chapters 2 and 3 this thesis

1.4 Neuropeptides and affective disorders

Despite major advances in biological research in the past decades, no revolutionary new therapies to treat affective disorders have emerged. New generations of antidepressants still have to be administered over an extended period of time before any therapeutic effect can be observed. Also antidepressant treatment is still associated with undesirable side effects, often leading to discontinuation of treatment.

Research may not have led to major breakthroughs in pharmacotherapy, but it has sharpened the insights in the processes and the brain areas, that play a role in affective disorders. Here we will discuss neuropeptides and their (neuro-) modulatory role in brain areas allegedly involved in affective disorders such as hypothalamus, amygdala, peri-aquaductal gray (PAG) and hippocampus.

1.4.1 Neuropeptides

The neuropeptides form a steadily growing group of more than 50 endogenous compounds with a broad spectrum of biological effects. Neuropeptides are conserved between species and can be subdivided into families. Some neuropeptides are associated with rather distinct behaviors. For example, oxytocin and vasopressin have striking and specific effects on social behaviour, including maternal behavior and pair bonding. Other well-known peptides are cholecystokinin, dynorphin, substance P and neuropeptide Y.

1.4.1.1 Function

Neuropeptides interact with G-protein-coupled receptors (also called: metabotrobic receptors), which are expressed by selective populations of neurons. Their chemical structure consists of 4 to 131 amino acid residues and distribution throughout the body is widespread. They function as molecular messengers between neurons, thereby influencing the neuron excitability through a process of depolarisation or hyperpolarisation, a property they have in common with classical neutransmitters, such as dopamine, noradrenaline and serotonine. However, neuropeptides can have divergent effects, such as modulation of gene expression, local blood flow, synaptogenesis, and glial cell morphology.

1.4.1.2 Neuropeptides versus 'classical' neurotransmitters

Many neurons are capable of making both a 'classical' neurotransmitter (such as glutamate, GABA or dopamine) and one or more neuropeptides. However, storage and release of these latter chemical messengers differs from 'classical'

neurotransmitters which are reflected at their functional level. For instance, the large dense vesicles (LDVs), in which neuropeptides are stored, are more sensitive to local intracellular calcium variations, leading to a more subtle release mechanism in comparison with the classical neurotransmitters (De Camilli, 1991). Release of the latter is according to a kind of 'go or no-go' principle. When a certain threshold is reached, exocytosis is a fact, while with neuropeptides release is generally more gradual. Neuropeptides and 'classical' neurotransmitters also display major differences in synthesis. Neuropeptides are synthesized at the endoplasmatic reticulum as pro-peptides, which must be spliced first by enzymes in order to become the bioactive neuropeptide. This splicing takes place during axonal transport and the neuropeptides are subsequently stored in LDVs (Hokfelt *et al.*, 2000; Hokfelt *et al.*, 2001). In contrast, classical neurotransmitters are synthesized by enzymes from amino acids readily available in the body and in case of serotonin from dietary tryptophan. Biosynthesis can take place at the release site, thereby avoiding the time consuming process of axonal transport (Sulzer *et al.*, 2005). After synthesis, 'classical' neurotransmitters are stored in small synaptic vesicles (SSVs). Because LDVs are 10 times more sensitive to elevations of the intracellular calcium concentration than SSVs, neuropeptides may serve to fine-tune the action of classical neurotransmitters (De Camilli, 1991). This increased sensitivity of the LDVs for calcium is likely to be related to the activity of a voltage dependant L-type calcium channels (tue-Ferrer *et al.*, 1993). When these channels are specifically blocked, both the electrical and potassium evoked release of substance P is inhibited (Holz *et al.*, 1988). Blockade of L-type calcium channels does, however, not inhibit potassium-evoked release of noradrenaline, indicating that the release of neuropeptides is regulated differently compared to 'classical' neurotransmitter release (Perney *et al.*, 1986). LDVs are distributed throughout the neuron, including soma, dendrites, axonal swellings and nerve endings, whereas the SSVs are mainly clustered at synaptic junctions. Another major difference between classical neurotransmitters and neuropeptides is in the duration of neurotransmission, which are milliseconds versus minutes, respectively. This difference may be related to the time needed to remove the transmitter from the target area. Monoaminergic neurons possess a highly efficient reuptake mechanism and the enzymes responsible for the degradation of monoamines viz. catechol-O-methyl transferase (COMT) and mono amine oxidase (MAO) are present in abundance (Bortolato *et al.*, 2008). Except for cholecystokinin (CCK) a reuptake mechanism for neuropeptides has not been demonstrated yet. Moreover, neuropeptides are inactivated through several steps of enzymatic degradation. For instance, substance P is inactivated by endopeptidases, ACE and aminopeptidases, but sometimes a second enzymatic degradation step is required to fully inactivate substance P (Michael-Titus *et al.*, 2002). De novo-synthesis of the neuropeptides is also a limiting factor, because they have to be synthesized at the endoplasmatic reticulum as pro-peptide, transported from the endoplasmatic reticulum to the release site and spliced by enzymes before they can be released.

1.4.1.3 Extrasynaptic transmission

The prolonged action of neuropeptides might imply a more modulating role reminiscent of hormonal function. Accordingly, neuropeptides may play a role in paracrine transmission, also referred to as volume or extrasynaptic transmission (Fuxe *et al.*, 1996; Vizi *et al.*, 2004). The predominant extrasynaptic location of neuropeptides and the notion that release sites of neuropeptides do not always face the target receptors are equally in support of a paracrine-like function.

1.4.1.4 Interactions between neuropeptides and monoamine neurotransmitters

Interactions between neuropeptides and 'classical' neurotransmitters can take different forms. First, as main transmitters of peptidergic neurons they can activate specific receptors located on axon terminals of glutamatergic or GABA-ergic neurons. In this way neuropeptides may fine-tune the effects of these major excitatory and inhibitory neurotransmitters on monoaminergic systems, the prime targets of antidepressants. Examples are the regulation of 5-HT release in dorsal raphe nucleus by CRH through GABA and substance P via glutamate. Second, neuropeptides may be co-localized with monoamines. An interesting example is 5-HT moduline, a peptide strongly associated with the function of 5-HT_{1B} receptors. Deactivation of 5-HT moduline by selective antibodies has anxiolytic-like activity in mice as assessed in the open field and elevated plus maze tests (Fillion *et al.*, 1996; Moret & Briley, 2000). It is clear from these examples that neuropeptides modulate monoaminergic transmission. Therefore, interfering with ex- and intrinsic neuropeptidergic processes may have antidepressant/anxiolytic potential (Holmes *et al.*, 2003). Prime examples are cortorticotrophin releasing hormone (CRH), oxytocin (OXT) and arginine-vasopressin (AVP) and substance P.

1.4.1.5 Corticotrophin releasing hormone

The neuropeptide CRH has been implicated in affective disorders in two ways. Firstly, CRH plays a crucial role in the regulation of HPA-axis activity. It is released from the paraventricular nucleus (PVN) into the hypothalamo-hypophyseal portal vessels, where it stimulates ACTH release from the anterior pituitary, which eventually leads to the release of cortisol from the adrenals. Release of CRH is also subject to feedback regulation controlling HPA-axis activity. Infusion of CRH fragments into the brain of rodents leads to anxious behavior and HPA-axis dysfunction. Evidence has been presented that unrestrained secretion of CRH in the central nervous system induces depressive symptoms by activating CRH1 receptors continuously. All currently known antidepressants restore the negative feedback between corticosteroids and the HPA-axis, possibly by increasing corticosteroid receptor gene expression. Notably, in an open-label safety study the CRH1 receptor antagonist R121919 significantly reduced depression and anxiety

scores, however not with a faster onset of action than regular antidepressants (Zobel *et al.*, 2000).

The second way is implied in affective disorders, which involves the CRH neuronal circuitry in the brain. The CRH neuronal pathway originates in the amygdale, projecting to the bed nucleus of the stria terminalis (BNST), the PAG, dorsal raphe nucleus (DRN) and locus coerulus (LC). The latter two brain areas contain the majority of serotonergic and noradrenergic cell bodies, respectively. Arguably, regulation of monoaminergic activity by CRH embeds the peptide in the monoamine hypothesis of depression. In particular interactions between CRH and 5-HT in dorsal raphe nucleus may be of interest in the pathophysiology of depression, as witnessed by the increased CRH immunoreactivity in dorsal raphe nucleus of depressed suicide victims. Valentino and Commons suggested that 5-HT neuronal activity in dorsal raphe nucleus is controlled by CRH_1 and CRH_2 receptors via opposite actions on GABA (Valentino & Commons, 2005). According to these authors, the abundant expression in dorsal raphe nucleus of NK_1 receptors in the vicinity of neurons that co-localize 5-HT and CRH suggests that the dorsal raphe nucleus is an important locus for 5-HT-CRH-NK interactions. These interactions could play a role in the anxiolytic-like activity of NK_1 receptor antagonists through inhibition of CRH/5-HT neurons that project from dorsal raphe nucleus to the central nucleus of the amygdala.

Based on their observations Valentino and Commons suggest that combining classic antidepressants with CRH_1 and NK_1 receptor antagonists might help to individualize and optimize efficacy and minimize side effects of treatment (Valentino & Commons, 2005).

1.4.1.6 Oxytocin and arginine-vasopressin

The neuropeptides oxytocin and arginine vasopressin (AVP) are produced in the supraoptic and paraventricular nucleus (PVN) of the hypothalamus. Peripheral release is via pituitary projections to the neuro-hypophysis. However, oxytocin and AVP are also directed centrally into the brain (Landgraf & Neumann, 2004). Both oxytocin and AVP are long acting neuron-modulators, and in addition to their peripheral functions they may be involved in stress responses, learning and memory. Furthermore, AVP is co-localized with CRH. In animal models oxytocin has anxiolytic-like effects while AVP has anxiogenic effects (Landgraf, 2006). A recent study has shown that the CRH_1 receptor antagonist SSR125543A, the V_{1b} AVP receptor antagonist SSR149415 and the clinically effective antidepressant fluoxetine all reverse chronic mild stress induced suppression of neurogenesis in mice. It is likely that reversal in neurogenisis is cased by increased expression of the cAMP response element-binding protein (CREB) in dentate gyrus (Alonso *et al.*, 2004; Stemmelin *et al.*, 2005). Both oxytocin and AVP have been implicated in obsessive behaviors and depression. Postmortem analysis of brain tissue from patients with mood disorder has shown increased numbers of AVP and oxytocin

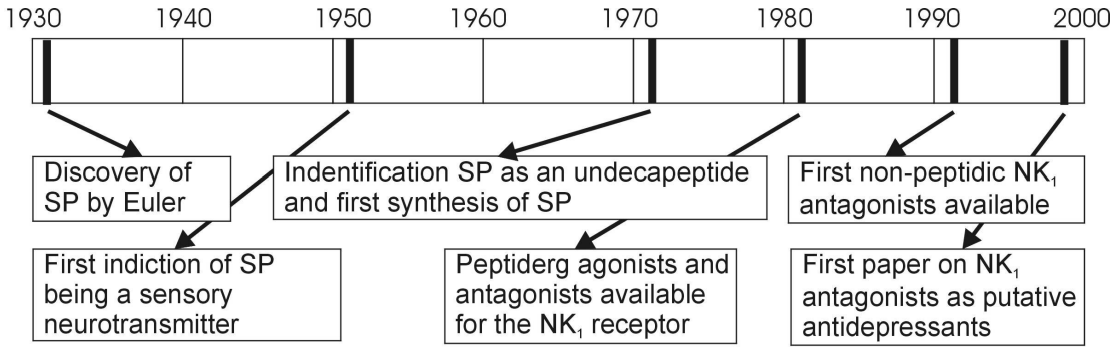
immunoreactive neurons in the PVN (Swaab *et al.*, 2005). Plasma levels of AVP in depression (especially in patients with melancholic type) were higher than normal, while no differences were observed for oxytocin levels, although there was a tendency to increased levels. Oxytocin and its precursor molecule have been associated with clinical response to electro-convulsive shock therapy (ECT), which could however not be replicated by others (Smith *et al.*, 1994; Devanand *et al.*, 1998). Yet, both clinical and preclinical studies suggest a role for oxytocin as an endogenous antidepressant (Rosat Consiglio, 2006). Acute as well as chronic administration of SSRIs increases blood oxytocin levels in rats. These observations suggest that antidepressant treatment could be capable of normalizing the decreased oxytocin plasma levels in depressed patients (Uvnas-Moberg *et al.*, 1999).

1.5 Substance P

The neurokinin substance P, first known for its involvement in nociception, has been the subject of renewed interest since it was discovered that neurokinin 1 antagonists possess antidepressant properties. It is also the main subject of this thesis. And will therefore be discussed in more detail

1.5.1 History of substance P

In 1931 von Euler described, an atropine resistant substance, from the gastro-intestinal tract of rabbits in the Journal of Physiology. He named this unidentified compound substance P and suggested that it could be found in the brain as well. Pernow, a student of Von Euler, continued his work on substance P. He purified the substance and was able to elucidate the distribution of substance P in the spinal cord, the brain and the regional distribution within these areas. He also demonstrated the neuronal localization of substance P. At the same time a group in Austria under supervision of Lembeck presented convincing evidence that substance P is a sensory neurotransmitter. It was not until the early seventies that Leeman and colleagues identified substance P as an undecapeptide. Her group was also the first to synthesise substance P and to set-up a radioimmunoassay for the peptide. This enabled functional and immuno-histochemical studies. Finally, it was also possible to measure the release of substance P, which turned out to fulfill several important criteria of neurotransmitter release. It was now possible to describe the role of the peptide in spinal cord pain transmission in more detail, during this period substance P received its label as being a pain transmitter.



1.5.2 Biochemistry of substance P

Substance P is synthesized from preprotachykinin-A. This pro-peptide can be cleaved in 4 ways resulting in substance P alone; a combination of substance P, NK-A and NPK; or substance P, NK-A and NP-Y. Thus maturation of the pro-peptide can result in mono-transmission or co-transmission, which is solely determined by the enzyme involved in the cleaving process. Substance P preferably binds to neurokinin 1 (NK_1) receptors, but it also displays affinity for NK_2 and NK_3 receptors. In the activated state, peptide-receptor-complexes internalize into the target cell in endosomes. This internalization occurs within one minute after activation of the receptor by substance P. Studies in spinal cord and striatum indicate that after 30 minutes the cell membrane will return to its original state (Mantyh *et al.*, 1995a; Mantyh *et al.*, 1995b; Wang & Marvizon, 2002),

1.5.3 Clinical targets of substance P

In the early eighties, the first agonists and antagonists fort the neurokinin receptors became available. They were all based on peptide structures and were found to be adequate tools for investigation of the physiological role of substance P. Clinically these compounds were less relevant, because they did not pass the gastro-intestinal tract due to metabolic instability.

A major breakthrough in substance P research took place in the beginning of the nineteen-nineties, when the first selective non-peptidergic NK_1 receptor antagonists were synthesized (Snider *et al.*, 1991). These new compounds made it possible to clinically target the substance P system, because they were metabolically more stable and thus suitable for oral administration. The initial therapeutic target for the NK_1 antagonists was analgesia, because of substance P's alleged role in nociception. However, the first clinical results were disappointing, but new therapeutic targets soon emerged. Primarily based on the anatomical distribution of substance P within the brain (e.g. nucleus tractus solitarius, area

postrema, striatum, amygdala and PAG) emesis, depression and schizophrenia were among the candidate therapeutic targets.

1.5.4 Substance P and pain

Initially substance P research was focused on the role of substance P in pain transmission. In the spinal cord substance P is abundantly present. When the sensory nerve endings are stimulated, substance P is massively excreted in the dorsal horn and a pain signal is perceived. For example capsaicin (the active compound in Spanish peppers) induces, when injected subcutaneously, a severe pain sensation. This pain sensation is mediated by substance P interneurons. These interneurons are part of glutamatergic pain transmission cascades in the spinal cord. Unfortunately, blocking the effect of substance P by selective NK_1 receptor antagonists did not alleviate the pain. It appeared, that pain is perceived through two different pathways and substance P plays a role in only one of these pathways. Thus pain signals were still able to enter the brain through the second pathway, making substance P a less interesting target to alleviate pain.

1.5.5 Substance P and affective disorders

In the 80's of the last century it became clear, that substance P might be involved in affective disorders. It appeared that intra cranial injection of substance P or particular fragments leads to anxious behavior. More extensive research was, however, impossible because the right pharmacological tools were not available at the time. This changed when a non-peptidergic and selective substance P antagonist became available in 1991. After the disappointing results in analgesia experiments, which are partly explained by the poor penetration of NK_1 antagonists into the brain, a new therapeutic target was found in emesis (Urban & Fox, 2000). The ability of NK_1 antagonists to suppress emeses was a major success. Aprepitant is a selective NK_1 receptor antagonist that effectively prevents cisplatin-induced emeses. Its mode of action is in the brain stem solitary tract, the emeses centre, where it blocks substance P induced emeses.

In 1998 Kramer and colleagues from Merck research laboratories published a paper in Science, wherein preclinical and clinical evidence was presented for antidepressant activity of NK_1 antagonists (Kramer *et al.*, 1998). Clearly, this stimulated preclinical research into the role of substance P in affective disorders.

For instance, substance P and NK_1 receptors were found in brain areas involved in emotion, stress and arousal, such as the amygdala and the limbic system (Harbuz & Jessop, 2001). It also appeared that chronic antidepressant treatment in rats reduces substance P concentrations in these brain areas (Shirayama *et al.*, 1996). A confounding factor was the species variety of NK_1 receptors, which has hindered research into the role of substance P in affective disorders considerably. Because the alleged antidepressant effect of NK_1 antagonists could not be tested in rat and

mice models of depression, research had to be redirected to other species such as gerbils, guinea pigs and tree shrews. For instance, in the social investigation test in gerbils, NK_1 receptor antagonists (dose-dependently) increased the time spent on social investigation to a similar extent as the anxiolytic drug chlordiazepoxide (Gentsch *et al.*, 2002). Neonatal vocalizations after maternal separation in guinea pigs were reduced after treatment with antidepressants, but not with anxiolytics. It was discovered that the NK_1 receptor antagonist L-733,060 was also able to reduce these vocalizations (Kramer *et al.*, 1998), suggesting again that NK_1 receptor antagonists may have antidepressant properties as well. Such combined anxiolytic and antidepressant activity would be a positive feature of NK_1 receptor antagonists, because comorbidity of anxiety disorders and depression is high.

1.5.6 Biological substrate for the antidepressant activity of NK_1 antagonists

Several attempts have been made to define the biological substrate for the antidepressant activity of NK_1 antagonists, and to give substance P a place in current hypothesis of major depression.

1.5.6.1 Substance P and the monoamine hypothesis

Co-transmission of substance P and serotonin has been shown for descending projections from raphe nuclei to the spinal cord. Moreover, in humans it has been shown that 50 % of the dorsal raphe neurons co-express serotonin and substance P (Sergeyev *et al.*, 1999). However, co-localisation could not be demonstrated in rats. An electrophysiological study in brain slices has shown that substance P does not directly interact with serotonergic neurones. Excitation of serotonergic neurones by substance P appears to be indirectly through glutamatergic interneurones, and can be blocked by the glutamate AMPA receptor antagonist CNQX (Liu *et al.*, 2002). Apparently, species differences do not only relate to the NK_1 receptor subtype, but also to the anatomical organization of substance P neurones. This complicates research into the functionality of substance P considerably. Because depression research is mostly done in mice and rats, any extrapolation, in the case of substance P and NK_1, to humans must be treated with caution. Yet the obtained data from research may give some indication, as illustrated by a study in guinea pigs, wherein an increase in firing rate of dorsal raphe serotonergic neurons was observed after administration of NK_1 receptor antagonists, which had also been observed in mice (Conley *et al.*, 2002; Santarelli *et al.*, 2001). Interestingly, the firing rate was increased after blockade of the NK_1 receptor, without a subsequent increase in basal serotonin efflux in the projection area. A lot of functional studies have been performed in NK_1 receptor knock-out mice. In these animals the 5-HT_{1A} receptor appeared to be desensitized. Desensitization of this receptor is also seen after chronic treatment with SSRIs and

according to some groups this a crucial aspect of the antidepressant effect of SSRIs (Santarelli *et al.*, 2001; Froger *et al.*, 2001).

Substance P interacts not only with the serotonergic system, but also with the noradrenergic system. Substance P containing fibers have also been identified in the locus coeruleus and NK_1 receptors are found on the noradrenergic cell bodies (Hahn & Bannon, 1998). Like serotonergic neurons, the firing rate of noradrenergic neurons increases after application of NK_1 receptor antagonists, but in contrast to serotonin, an increased efflux of norepinephrine was observed in the dorsal hippocampus and frontal cortex of the guinea pig (Millan *et al.*, 2001; Maubach *et al.*, 2002).

1.5.6.2 Substance P and the HPA-axis hypothesis

Chronic stress or a traumatic life event is thought to play a role in the etiology of major depression. Experimental acute and chronic stressors are known to alter the synthesis and secretion of various neuropeptides, including CRH, vasopressin, neurotensin and opioid peptides in various brain areas. Substance P neurons are equally responsive to aversive stimuli. Intermittent footschock in rats reduced substance P content in the ventral tegmental area, olifactory tubercle and several hypothalamic nuclei (Bannon *et al.*, 1986). At the same time, an increased substance P concentration was observed in the medial septum and dentate gyrus nuclei (Siegel *et al.*, 1987). NK_1 receptor internalization has been shown in the basolateral amygdala after maternal separation in guinea pigs and after immobilization stress in gerbils (Kramer *et al.*, 1998; Smith *et al.*, 1999). In gerbils the NK_1 receptor antagonist L-760,735 was able to prevent this internalization. Summarizing, substance P plays a role in stress- induced mechanisms in the brain, probably via activation of NK_1 receptors. Arguably, NK_1 receptor antagonists may be able to alleviate stress-induced symptoms of depression.

1.5.6.3 Substance P and the neuroplasticity hypothesis

The role of neuroplasticity in affective disorders has been discussed (see 1.1.3). Several studies support a role for substance P and its preferred NK_1 receptor in brain plasticity and neurogenisis. Persistent pain results in a stress-like effect on neurogenisis in the dentate gyrus of rat and decreases BDNF in the hippocampus (Duric & McCarson, 2006). Indeed NK_1 knockout mice appear to have a higher rate of neurogenisis in the dentate gyrus and an increased level of BDNF (Morcuende *et al.*, 2003)

1.5.6.4 Clinical support for a role of SP in depression

Attempts to support and explain the antidepressant properties of NK_1 antagonists have also been made in clinical settings. Infusion of substance P in healthy young men led to a worsening of mood, compared to placebo (Lieb *et al.*, 2002). A post

mortem study could demonstrate significant differences in NK_1 receptors densities in the anterior cingulate cortex between healthy volunteers and patients with unipolar depression, bipolar disorder and schizophrenia (Burnet & Harrison, 2000). In contrast, a *post-mortem* study in patients with major depression showed decreased binding of substance P in the rostral orbitofrontal cortex compared to control individuals (Stockmeier *et al.*, 2002). Furthermore, a preliminary study in patients with major depression has shown significantly increased serum levels of substance P, when compared to healthy volunteers (Bondy *et al.*, 2003).

A follow up study with the NK_1 antagonist MK-869 was performed, but could not demonstrate significant differences, when compared to placebo and the reference compound fluoxetine. Yet, the investigators claimed a significantly improved efficacy in severely depressed patients (Rupniak & Kramer, 1999). Other NK_1 receptor antagonists, such as NKP 608 of Novartis and TAK 637 of Takeda, have also been the subject of clinical trials. However, the development of NKP 608, TAK637 and MK 869 has been discontinued, because the results were not as good as expected. However, there may yet be a role for substance P and other neuropeptides in affective disorders. Major depression is a heterogeneous disease, and the role of neuropeptides may be too small to demonstrate significant differences in placebo controlled clinical studies. Clearly, more research is required to establish the exact role of substance P and NK_1 receptors in affective disorders.

1.6 Scope of the thesis

Depression is a multifactorial disease, with both genetic and environmental factors likely to play a role in its etiology. Traditionally, research has focused on the role of monoamines and their receptors in both the neurobiology and pharmacotherapy of depression. However, given the complexity of the CNS it is conceivable that other neuronal systems are involved. With the discovery that neurokinine NK_1 receptor antagonists display potential antidepressant activity, research into the substance P-NK_1 peptidergic system has intensified. This thesis investigates the effects of a NK_1 receptor antagonist relative to established antidepressants on several behavioral and biochemical markers in the psychosocial stress paradigm in tree-shrews. This animal model for depression has a high face-, predictive- and construct-validity. NK_1 receptor antagonists have been reported to augment the increase of extracellular 5-HT levels caused by an SSRI in rats, which makes them potential candidates for antidepressant augmentation strategies. In view of reported NK_1 receptor species differences, it was investigated whether this augmentation also takes place in guinea pigs. Knowledge of neuropeptide function in the CNS is severely limited due to the problems encountered when trying to measure these compounds *in vivo*. To circumvent this problem, sampling technique and chemical analysis of neuropeptides were improved, allowing a more reliable measurement of both basal levels and dynamics of neuropeptides *in vivo*.

The aims of the research presented in this thesis are:

- ***To assess behavioral, humeral, brain-metabolic and immunological parameters of the NK_1 receptor antagonist L-730735 as a putative antidepressant in the chronic psychosocial stress paradigm in tree-shrews and compare them to classical antidepressants.***
- ***To study the effects of combined administration of a NK_1 receptor antagonist and an SSRI on extracellular 5-HT levels in the guinea pig brain.***
- ***To improve the in vivo assessment of neuropeptides, in particular substance P, in the brains of laboratory animals.***

1.6.1 Outline of the thesis

In **chapter 2**, the behavioral and endocrine effects of a classical antidepressant and L-760735, a NK_1 receptor antagonist, are compared in the tree-shrew chronic psychosocial stress model.

In **chapter 3** the effects of chronic psychosocial stress in tree-shrews on brain metabolites, neurogenesis and hippocampal volume are examined. Furthermore, the effects of both clomipramine and L-760735 administration on these parameters are investigated.

In **chapter 4** the effects of chronic psychosocial stress alone and in combination with various antidepressants and L-760735 on stress responses and immune function are studied in the tree-shrew model.

In **Chapter 5** the effects of combined administration of fluoxetine, an SSRI, and GR 205171, a NK_1 receptor antagonist, on extracellular concentrations of serotonin, norepinephrine and dopamine are studied in guinea pigs.

In **chapter 6** several aspects of a microdialysis approach were improved, to enable a more reliable and sensitive in vivo assessment of small neuropeptides, including substance P, in the brains of laboratory animals.

In **chapter 7** a new LC-MS/MS method is developed and discussed, which can be used to measure substance P concentrations in brain microdialysates. The new method is compared to the traditional RIA method.

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