Identification and characterisation of novel antidepressant-responsive genes in mouse brain

Dissertation
an der Fakultät für Biologie
der Ludwig-Maximilians-Universität München

vorgelegt von
Dipl.-Biol. Karin Ganea
geboren am 26.09.1978
in München

München, Januar 2008

1. Gutachter: Prof. Dr. R. Landgraf

2. Gutachter: Prof. Dr. G. Grupe

Tag der mündlichen Prüfung: 14. Januar 2009

Contents

I Introduction

"Depression" by Szuzsanna Szegedi

"A conceptually novel antidepressant that acted rapidly and safely in a high proportion of patients would almost certainly become the world's bestselling drug."
(Wong and Licinio, 2004)

1.1 Preface

Depression is ranked as the fourth leading cause of disease burden worldwide and is expected to become the second most disabling disorder by 2010 (Üstün et al., 2004; Simon, 2003). Currently available antidepressant drugs are safe and effective, but shortcomings range from a delayed onset of action to a significant rate of non-responders. As mood disorders are associated with a significant risk for suicide, the latency until the onset of antidepressant efficacy makes a more rapid action a desirable attribute for novel antidepressants (Nemeroff and Owens, 2002). Therefore, intense attention is currently being given to the identification of potential novel drug targets. Both, genetic determinants and environmental factors act together to predispose to, or on the other hand protect against psychiatric diseases like depression, and they are influencing an individual's response to pharmacological treatment (for review see: Lesch, 2004).

A previous hypothesis-free approach to identify novel antidepressant-responsive genes was performed: To analyse the interaction between genes, behaviour and response to psychoactive substances, Sillaber et al. used antidepressant-responsive DBA/2OlaHsd mice to characterise their behaviour under basal conditions and after chronic antidepressant treatment (Sillaber et al, 2007, submitted). In this study the antidepressant paroxetine, a selective serotonin reuptake inhibitor (SSRI) commonly and effectively used to treat clinical depression and anxiety disorders, was administered. Genechip microarray analysis was applied for large-scale gene expression profiling in the hippocampus in a separate cohort of mice to investigate antidepressant-induced changes in gene expression (Sillaber et al, 2007, submitted). The hippocampus, receiving serotonergic input, was investigated because of its key role in the coordination of behavioural and neuroendocrine responses to stress and its implication in stress-related disorders including anxiety disorders and depression (Djavadian, 2004).

Work of the thesis:

Selected genes of the microarray analysis, which are potentially involved in pathways of affective disorders, could be validated by *in situ* hybridisation. In a second step the *in vivo* analysis of the distinct gene function, especially of previously unknown genes, was performed. Finally, we aimed at transferring our animal data to the clinical situation and analysed single nucleotide polymorphisms (SNP) in our genes of interest in cohorts of depressive patients. The principal object of the thesis was the identification and detailed characterisation (*in vitro* and *in vivo*) of novel genes involved in the mechanisms of action of antidepressant treatment, thus opening new avenues for discovering new drug targets of depressive disorders.

1.2 Depressive disorders

Depression is a multifactorial psychiatric disease, which affects up to 20% of the population across the world, and has profound social and economic consequences. It is a recurring, chronic and potentially life-threatening disorder with a variable combination of symptoms (Berton and Nestler, 2006). Patients suffering from major depression exhibit not only low or depressed mood, but also sleep or psychomotor disturbances, low self-esteem, anhedonia, reduced food intake and body-weight dysregulation, withdrawal from activities, impaired concentration, and finally suicidal tendency (for review see Wong and Licinio, 2001; Nestler et al., 2002). In the general population, suicide is one of the leading causes of death among adults and it has been estimated that 50-80% of completed suicides are associated with mood disorders. About 15% of patients suffering from depressive disorder commit suicide (Kasper et al., 1996).

Unfortunately, we still do not understand the underlying pathophysiology of depression; as though there have been various hypotheses related to environmental, genetic and biological factors (Rosenzweig-Lipson et al., 2006). Since the serendipitous discovery of the first effective antidepressants, monoamine oxidase inhibitors and tricyclic antidepressants respectively, the discussion about the possible pathophysiology of depression has been dominated by the so-called monoamine hypothesis, which postulates a deficit in noradrenaline and/or serotonin in distinct regions in the brain of depressed patients (Hindmarch, 2002).

Another prominent biological model for depression comprises the dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis (Holsboer, 2000). The body's physiological reaction to stressful situations comprises the fast activation of the adrenomedullary system (catecholamine release) and the activation of the HPA axis. This secondary stress response is slower and characterised by the final release of corticosteroids from the adrenal cortex (see figure 1)(de Kloet et al., 2005; Korte, 2001).

Moreover, there is a large body of evidence implicating that depression is substantially influenced by genetic factors and that the genetic component is highly complex and polygenic (Fava and Kendler, 2000; Ising and Holsboer, 2006). This makes depression a highly heritable disorder (up to 70%), but the search for specific genes that confer this risk has not been very successful (Nestler et al., 2002; Lesch, 2004). Due to the complexity of the disease it is likely that many genes are possibly involved in the development and there are only very few examples of psychiatric illnesses known to be inherited in a strictly Mendelian fashion so far (Burmeister, 1999). In addition, individual vulnerability to depression is only partly genetic,

with environmental factors like stress or trauma also being important contributors (Nestler et al., 2002).

Despite this lack of knowledge regarding the underlying pathophysiology of depression there are already effective treatment strategies available for this disorder.

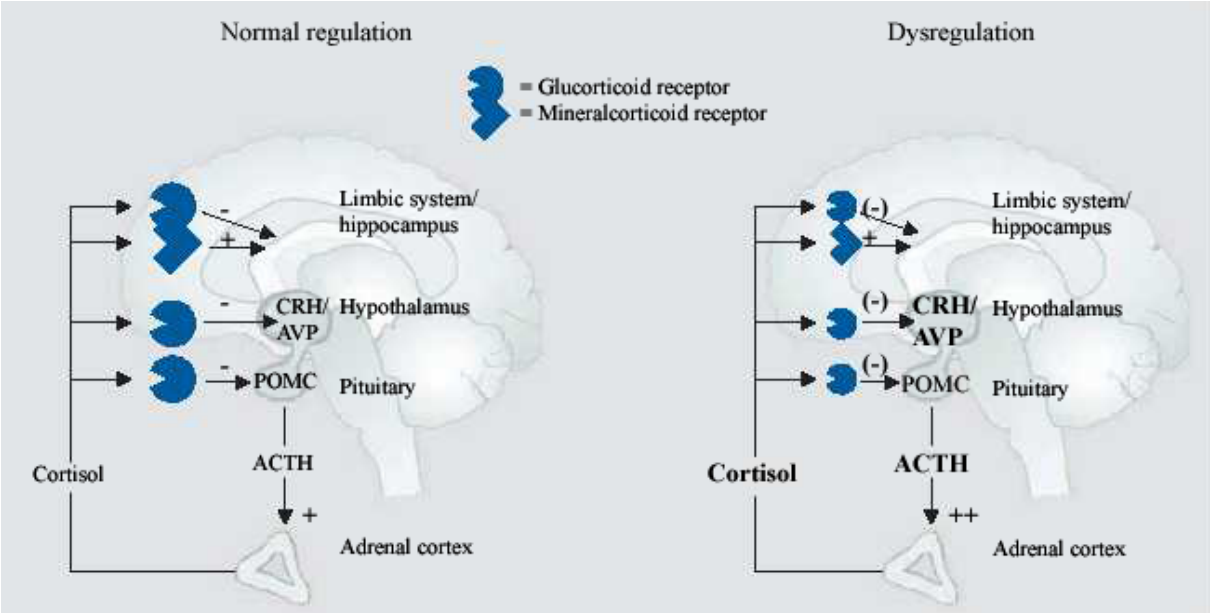


Figure 1 (adapted from Ising and Holsboer, 2006)**:** Model for normal and impaired regulation of the hypothalamic-pituitary-adrenocortical axis. CRH = corticotrophin-releasing hormone; AVP = arginin-vasopressin; POMC = pro-opiomelanocortin; ACTH = adrenocorticotropic hormone.
In stressful situations CRH and AVP are secreted from neurons of the hypothalamus. They are transported to the anterior pituitary through the portal blood vessel system and stimulate the secretion of stored ACTH from corticotrope cells. ACTH in turn stimulates the adrenal cortex to release glucocorticoids, in particular cortisol (human) or corticosterone (rodents). Cortisol/corticosterone is a major stress hormone and has effects on many tissues in the body, including the brain. It acts at two types of receptors - mineralocorticoid receptors and glucocorticoid receptors. When the organism is persistently exposed to stress, these hormones can be pathogenic.
Impaired corticosteroid signalling results in an attenuation of the negative feedback inhibition of CRH, AVP and ACTH, which can lead to chronically elevated levels of cortisol/corticosterone.

1.3 Antidepressant drugs

Despite the fact that we still do not understand the aetiology and precise pathophysiology of depression, existing antidepressant treatments are safe and effective, but far from ideal. The therapeutic effects take several weeks to manifest and these effects are often accompanied by unwanted side-effects. Astonishingly enough, fewer than 50% of all patients show full remission after treatment with a single antidepressant drug (for review see: Fava and Kendler, 2000; Nemeroff and Owens, 2002). The history of the treatment of depression started about 50 years ago, when two classes of substances were discovered by serendipity: the monoamine oxidase inhibitors and the tricyclic antidepressants. Most of today's psychopharmacological drugs are based on the mechanism of action of tricyclic antidepressants (TCA), which are believed to act by modulating the serotonin and/or noradrenaline system (Wong and Licinio,

2001). Currently available antidepressants are mediating their effects by increasing the monoamine neurotransmission: (1) by blocking the reuptake or inhibiting the metabolisation of the neurotransmitter(s) or (2) by blocking receptors that secondarily increase the signal transduction through other receptors, stimulate the release of neurotransmitters or increase the neuronal firing rate (Delgado, 2004). This generation of antidepressant drugs can be classified in selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NRIs), serotonin and noradrenaline reuptake inhibitors (SNRI) and noradrenaline and dopamine reuptake inhibitors (NDRIs) (Yadid et al., 2000). Unlike TCA, most of these drugs lack affinity for other neurotransmitter receptors, like muscarinic receptors, and their application improved the side effect profile and safety of antidepressants (Lucki and O'Leary, 2004). However, these substances effect the targeted neurotransmitter systems within hours, whereas the effects on mood need weeks to manifest (Wong and Licinio, 2001). These findings imply that it is not the monoaminergic signalling and the reuptake inhibition *per se* but rather yet unknown long-term neuroadaptive changes that may underlie these therapeutic effects (Boehm et al., 2006).

Besides the monoamine hypothesis of depression, another well accepted hypothesis has been developed, which relates aberrant stress hormone dysregulation to the causality of depression (Holsboer, 2000). A major focus of the investigation of the relationship between stress and neurobiological changes seen in mental disorders has been the hypothalamic-pituitary-adrenal (HPA) axis, both as a marker of the stress response and as a mediator of additional downstream pathophysiologic changes (Mello et al., 2003). According to Holsboer and Barden, there is considerable evidence that HPA dysregulation is causally implicated in the onset of depression and that mechanisms of action of antidepressant drugs include actions on the HPA system (Holsboer and Barden, 1996). Therefore great interest is given on the development of antagonists for the CRH receptors type 1 and 2, two key players in the HPA-axis pathway. Other possible antidepressant drug targets within the HPA system are vasopressin and glucocorticoid receptors (e.g. glucocorticoid receptor antagonists) (Berton and Nestler, 2006; Clark et al., 2007).

Furthermore, hopeful candidates in the focus of the search for novel antidepressant drugs are for example the endocannabinoid system, the neurokinin system, opioid receptors, cytokines, histone deacetylase inhibitors and neurotrophic factors (for a detailed review see: Berton and Nestler, 2006).

Regardless these innovative approaches, SSRIs are generally acknowledged to be the first-line pharmacological treatment for depression at the moment (Nemeroff, 2007).

For the treatment of severe and treatment-resistant depression, electroconvulsive seizure therapy (ECT), in which a generalized epileptic seizure is provoked by electrical stimulation of the brain, has a proven therapeutic application (Frey et al., 2001). Despite the efficacy of electroconvulsive seizure as a non-chemical antidepressant treatment, the mechanism of action remains still unclear (Newton et al., 2006). But there is growing evidence that the effects of ECT could be mediated by increasing neurogenesis (the birth of new neurons) (Madsen et al., 2000; Altar et al., 2004; Duman and Monteggia, 2006; Newton et al., 2006; Ploski et al., 2006). Adult neurogenesis, especially in the dentate gyrus of the hippocampus, might be implicated in the aetiology and treatment of depression. It has already been shown that stress can be associated with morphometric brain changes, neuronal atrophy, and reduced neurogenesis in the dentate gyrus. On the other hand, all antidepressant drugs studied to date, including substances of various classes, ECT and behavioural treatments increase neurogenesis in the hippocampal dentate gyrus (Duman et al., 2001; Drew and Hen, 2007; Paizanis et al., 2007).

1.4 The hippocampus, depression and neurogenesis

One region of high interest in mood disorder research is the hippocampus as receiving input about the "external world" and the homeostatic and emotional "internal world" (Buzsaki, 1996). The hippocampal dentate gyrus receives the principal afferent input about the external world from the entorhinal cortex via the perforant pathway (see figure 2). CA3 neurons in turn receive their main input from the dentate gyrus via the mossy fibres. The CA1 represents the last stage in the intrahippocampal trisynaptic loop and is the major target of CA3 pyramidal cell axons, the Schaffer collaterals. The pathway from CA1 to the subiculum and on to the entorhinal cortex forms the principal hippocampal output (Freund and Buzsaki, 1996). Moreover, the hippocampus receives input from the amygdala and the claustrum, the septal complex and the supramammillary area, the hypothalamus, thalamus and the brainstem. The hippocampus, in turn projects to the septal nuclei, the thalamus, the mamillary and amygdaloid complexes and the striatum (Rosene and Van Hoesen, 1987). The hippocampal formation is part of the limbic system, which is a major centre for emotion formation and processing, for memory, and for learning. Therefore, limbic structures might serve as a link between the stress system and neuropsychiatric disorders (Smith and Vale, 2006). Additionally, the hippocampus plays a role in modulating body physiology, including the activity of the HPA axis, the immune system, blood pressure, and reproductive function. It

has been proposed that the hippocampus acts, in parallel with the amygdala, to modulate body physiology in response to cognitive stimuli (for review see: Lathe, 2001).

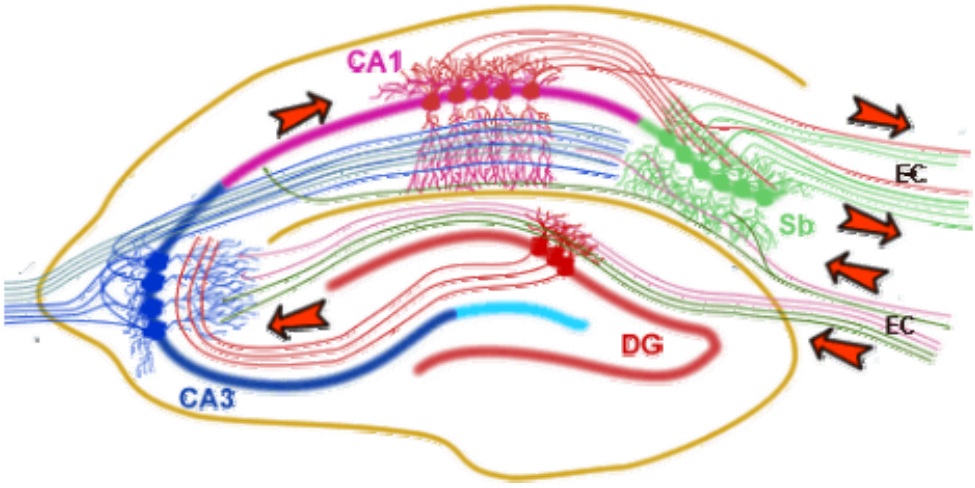


Figure 2 (adapted from http://www.bristol.ac.uk/Depts/Synaptic/info/pathway/figs/hippocampus.gif)**:** The hippocampal dentate gyrus (DG) receives the major afferent input from the entorhinal cortex (EC) via the perforant path. The granule cells of the dentate gyrus in turn send their axons (the mossy fibers) to innervate the CA3 region. The pyramidal neurons of the CA3 region project via the Schaffer collaterals to the CA1 pyramidal neurons. The principal output of the hippocampus finally forms the connection of the CA1 to the subiculum (Sb) and on to the entorhinal cortex (Morris and Johnston, 1995).

The hippocampus is one of the most sensitive and plastic regions in the brain. Both physical and psychosocial stress cause adaptive plasticity in the brain, like suppression of neurogenesis in the dentate gyrus (McEwen, 2000b; Malberg, 2004). The hippocampal dentate gyrus is one of the only two brain structures known to retain the capability to produce new neurons in adulthood (Christie and Cameron, 2006; Drew and Hen, 2007). The function of adult neurogenesis is not yet well understood, but it has been hypothesised that the decrease and increase of neurogenesis in the hippocampal formation are important features associated with depressive episodes (Jacobs et al., 2000; Paizanis et al., 2007). Already existing studies observed dysfunction or significant reduction of the volume of the hippocampus in patients with depression or post-traumatic stress disorder (Sheline et al., 1996; Bremner et al., 2000; Sapolsky, 2000; MacQueen et al., 2003; Malberg, 2004). Moreover, brain regions of depressed patients, such as the hippocampus, show altered activity patterns in functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) (McEwen, 2006).

Regarding the time delay for the onset of action of antidepressant treatment, it is very likely that adaptations of gene and protein expression could contribute to the therapeutic effect of antidepressants. In respect of the opposing actions of stress and antidepressant treatment on hippocampal neurogenesis, multiple studies investigate the regulation and function of

neurotrophic factors like brain-derived neurotrophic factor (BDNF) in depression and treatment (Duman and Monteggia, 2006). BDNF synthesis in the hippocampus is suppressed by stress, at least partially through a sustained modification of chromatin structure. Essentially all antidepressant drugs increase BDNF synthesis and signalling in the prefrontal cortex and hippocampus. Neurotrophic factors are seen as tools in the activity-dependent modulation of neuronal plasticity and could therefore contribute to the therapeutic benefit of antidepressants (for review see : Castren et al., 2007).

One technology capable of identifying underlying pathways in diseases or genes affected by antidepressant treatment is gene expression profiling (Boehm et al., 2006). Unbiased approaches like microarray analysis are able to uncover novel pathways and changes in gene expression levels, which are yet unknown to be involved in disorders like depression.

1.5 Animal models of depression

Studying the neurobiological mechanisms underlying depression and the therapeutic effects of antidepressant drugs on the human brain is quite difficult for various reasons, including ethical issues. Moreover, factors like the genetic and environmental prehistory of the patients can hardly be controlled for and are difficult to quantify. Therefore, subjects cannot be randomised to treatment groups (Shively, 1998). For those reasons, animal models are essential tools to study parameters that have been implicated in the neurobiology of depression in humans (Cryan et al., 2002). Animal models of human diseases should fulfil three criteria: *construct validity*, *predictive validity* and *face validity*. High *construct validity* is achieved when both, the features of depression that are modelled and the behaviour in the model are homologous and can be unambiguously interpreted. Further, the modelled feature should have an empirical and theoretical relation to depression. *Predictive validity* is attained when a model correctly identifies diverse antidepressant substances and when the treatment effect in the model correlates to the effects in the clinical situation. *Face validity* is given when the behaviour and the specific symptoms exhibited by the animal model are similar to the human condition (Willner, 1984; McArthur and Borsini, 2006). A forth criterion was established by Geyer and Markou in 1995: the a*etiological validity*. This concept is related to the causes of human disease. As causes of mood disorders are seldom known, the validity is limited to the hypothesis regarding the possible underlying aetiology (Geyer and Markou, 1995). Some symptoms of major depression are impossible to model in rodents, like thoughts of death or suicide and feelings of worthlessness or guilt, while other symptoms like changes

in appetite or weight gain and psychomotor agitation or slowness of movements can be observed in animals (Cryan and Holmes, 2005).

There are various existing models and approaches to model affective disorders and to assess the activity of potential therapeutics in mice. Many models are based on genetically modified mice, like serotonin or noradrenaline receptor or transporter knockout mice. Many of these modified mouse strains have been generated, largely because of known associations between the targeted gene and antidepressant action or the pathology of depression (Cryan and Mombereau, 2004). The limiting factors in knockout mice are the time consuming generation, which allows no high throughput approaches, and compensatory gene regulation after the knockout, a factor which can not be properly controlled for and which may influence the observed phenotype. Another approach is the use of specific inbred mouse strains like the DBA/2 strain. They display a high level of innate anxiety-like behaviour (Ohl et al., 2003; Yilmazer-Hanke et al., 2003) and show changes in stress-induced hormone levels (Thoeringer et al., 2007) and, therefore, might be suitable to investigate the effects of antidepressant-like substances.

Behavioural paradigms that aim at modelling depression- and anxiety-related phenotypes and investigate the potential antidepressant-like effects of novel substances often involve exposure to stressful situations. Exposure to persisting stress and trauma has been shown to be one of the main predisposing factors to depression. Among those paradigms are the forced swim test, the tail suspension test, the learned helplessness paradigm, maternal deprivation or chronic mild stress (for review see: Cryan and Slattery, 2007). The probably most frequently used test is the Porsolt's forced swim test (FST) (Porsolt et al., 1977). In this test, rodents are placed in a cylinder filled with water, which they cannot escape from. Passive or immobile behaviour (floating) is thought to reflect depressive-like behaviour (Porsolt et al., 1977). Antidepressant drugs increase the active (swimming, struggling) and decrease the passive (floating) coping strategies of mice or rats in this paradigm (Cryan and Slattery, 2007).

Taken together, despite the difficulties to create an ideal model of depressive disorder, a number of different animal models show substantial *construct validity* and have been widely used (Cryan and Slattery, 2007).

1.6 Gene expression studies in mood disorders

Microarray analysis offers the possibility to screen nearly all genes expressed in a distinct tissue or to investigate the changes in gene expression levels associated with antidepressant treatments. Therefore, in the last years this technology has been increasingly used to study

polygenic and multifactorial diseases, like psychiatric disorders (Sequeira and Turecki, 2006). An increasing number of pharmacogenomic studies have been conducted to reveal the molecular pathways underlying the effects of antidepressant drugs: Drigues et al. examined the gene expression profile in the hippocampus in rats after chronic treatment with three different antidepressants and exposure to the forced swim test (Drigues et al., 2003). Wong et al. studied the hypothalamic gene expression in rats after treatment with the tricyclic antidepressant imipramine and the herbal product St. John's worth (Wong et al., 2004). Two other groups investigated the effects of electroconvulsive seizure on gene expression levels in the rat frontal cortex and hippocampus (Altar et al., 2004; Ploski et al., 2006). Conti et al. performed microarray analysis in seven different rat brain regions following three antidepressant treatments: electroconvulsive seizure, sleep deprivation and fluoxetine (Conti et al., 2007). Until now there is only a small number of microarray studies conducted in mice. Boehm et al. for example analysed the subchronic (4 days) and chronic (28 days) effects of a tricyclic antidepressant on gene expression in the nucleus accumbens of mice (Boehm et al., 2006). Another study integrates brain gene expression data from mice (acutely treated with a stimulant, and/or the mood stabilizer valproate) with human data (genetic studies in post-mortem brains) to identify genes involved in bipolar disorders (Ogden et al., 2004). Microarray analyses are preferably performed in animals as this allows controlling for various conditions, like drug application or environment, and opens the possibility to use genetically more homogenous individuals (Sillaber et al., submitted).

1.7 A hypothesis-free approach to identify novel antidepressant-responsive genes in the mouse brain

By means of a reverse pharmacological approach Sillaber et al. aimed at determining alterations of hippocampal gene expression in DBA/2OlaHsd mice after chronic antidepressant treatment with paroxetine, a selective serotonin reuptake inhibitor (Sillaber et al., submitted). Paroxetine is a common and widely used antidepressant drug. The chronic treatment was chosen to mimic the clinical situation, in which the antidepressant effects of drugs need several weeks to manifest. Four parallel groups of mice were treated chronically with either water or paroxetine by oral gavaging (twice daily, 10 mg/kg, 28 days). In two groups potential behavioural changes induced by the treatment were assessed, in the other two groups paroxetine-induced gene expression alterations in the whole hippocampus were investigated by means of microarray analysis. Four hours after the last treatment three different behavioural tests were performed: the forced swim test (FST), the modified hole

board (mHB) and the dark/light box (DaLi). Antidepressant treatment significantly decreased passive stress coping behaviour in the FST. The same decrease in floating behaviour could be detected in a retest 18 h after the last administration of the substance. In the mHB and the DaLi anxiolytic effects of chronic paroxetine treatment could be shown, as the paroxetine-treated animals spend significantly more time on the exposed board in the middle of the mHB and in the lit compartment of the DaLi.

The groups taken for gene expression profiling were sacrificed 4 hours after the last treatment, then the hippocampus was dissected and antidepressant-induced gene expression changes were examined using three different microarray chips (CodeLink, Affymetrix and the house-intern MPI-Chip). The underlying hypothesis stated that genes, which showed altered mRNA expression levels, could be involved in the mechanism of action of paroxetine.

For the present thesis, eleven genes out of the created list of all regulated genes of the three microarrays were chosen (see table 1) to further validate the results by *in situ* hybridisation studies. Selection criteria were among others the known distribution of the gene in behaviour-relevant brain regions or the relation to depression-relevant genes. After validation, the most promising candidates were examined with respect to their role in mediating the antidepressant-like effects of paroxetine *in vivo*.

Symbol	Name	Fold-Regulation (on one or two platforms)	Confirmation by *in situ* hybridisation
Acvr1	**Activin receptor, IA**	**+ 1,31 + 1,43**	**yes**
Cckbr	**Cholecystokinin B receptor**	**- 1,46**	**yes**
Chrm1	**Cholinergic receptor, muscarinic 1, CNS**	**- 1,32**	**no**
Gabrd	**Gamma-aminobutyric acid (GABA-A) receptor, subunit delta**	**- 1,56 - 1,61**	**yes**
Gmeb1	**Glucocorticoid modulatory element binding protein 1**	**+ 1,34**	**no**
Grp	**Gastrin releasing peptide**	**+ 1,79**	**yes**
Inha	**Inhibin alpha**	**- 1,36**	**yes**
Inhba	**Activin βA/Inhibin βA**	**+ 2,87 + 2,7**	**yes**
Nr3c1	**Nuclear receptor subfamily 3, group C, member 1**	**- 1,36**	**yes**
Penk1	**Preproenkephalin 1**	**+ 3,04 + 2,75**	**yes**
Plat	**Plasminogen activator, tissue**	**+ 1,46**	**yes**

Table 1: List of selected genes

1.8 Scope of the thesis

As illustrated in the introduction, mood disorders have profoundly deleterious consequences on well-being and their toll on economic productivity and quality of life matches that of heart disease. Both, genetic determinants and environmental factors act together to predispose to, or protect against, psychiatric diseases, like depression, and they are influencing an individual's response to pharmacological treatment. Currently available antidepressant drugs are safe and effective, but they are not directly targeting at the underlying pathophysiology of depression. Therefore, intense attention is currently being given to the identification of potential novel drug targets. The studies presented in this thesis all together aim at identifying such potential novel drug targets by investigating the following objectives:

(1) Validation of distinct genes out of an existing microarray by *in situ* hybridisation to prove the induced gene regulation by chronic antidepressant treatment with paroxetine
(2) Investigation of the time course of gene expression regulation of selected genes
(3) *In vivo* analysis of the role of the selected genes in the mechanism of action of antidepressant treatment

In **project 1** the antidepressant-induced gene expression regulation of eleven different genes is validated by *in situ* hybridisation. Nine out of the eleven genes could be confirmed to be regulated in different regions of the mouse brain. These results represent the basis for the following experiments: in **project 2** the regional and temporal specifity of the gene regulation of three validated candidate genes is described. The mice were treated acutely, subchronically and chronically with paroxetine in order to investigate the correlation between the onset of action of the antidepressant drug and the onset of gene regulation. In **project 3** the antidepressant-like effects of one of the most promising genes out of the microarray (activin βA) were investigated by administration of the protein into two different brain regions of mice. To further clarify the neurobiological mechanisms underlying the outcoming antidepressant-like effects of the protein, we performed electrophysiological tests. Additionally, we wanted to know whether the candidate gene identified might also play a role in affective disorders, or in the clinical phenotype and treatment response. Therefore, corresponding genetic alterations in patients were compared to our data. **Project 4** focuses on the *in vivo* behavioural analysis of a second gene, the gastrin releasing peptide. The effects of a stereotactic infusion of the peptide in to the hippocampus were investigated in specific depression and anxiety-related paradigms.

In consideration of the heterogeneity of the questioning and research objectives each project will be introduced and discussed separately.

II Materials and methods

2.1 Animals and housing conditions

Experiments were carried out with male DBA/2OlaHsd mice (except project 3, experiment 2, 3 and 4) from Harlan Winkelmann (Borchen, Germany) or with male DBA/2Ico mice (project 3, experiment 2 and 3) or male Bl6 mice (project 3, experiment 4) from Charles River Laboratories (France or Sulzfeld, Germany). The animals were between 4-8 weeks old on the day of arrival. All animals were housed in groups of 4 until an age of 8 weeks, then they were singly housed in standard cages (45 cm×25 cm×20 cm) under a 12L:12D cycle (lights on at 7:00) and constant temperature (23±2°C) conditions. Food and water were provided *ad libitum*. The experiments were carried out at the animal facility of the Max Planck Institute of Psychiatry in Munich, Germany, and started when mice attained a minimum weight of 25 g and were between 10-14 weeks (except project 3, experiment 2 and 3) or 16 weeks (project 3, experiment 2 and 3). In project 3, experiment 4 mice were 6-8 weeks old when the experiments were performed.

The experiments were carried out in accordance with European Communities Council Directive 86/609/EEC. All efforts were made to minimize animal suffering during the experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany.

2.2 Drug application

Chronic antidepressant treatment

Animals were randomly distributed to the vehicle or paroxetine treatment group. Paroxetine (Seroxat) was obtained from GlaxoSmithKline (Munich, Germany) and was diluted in water to a final concentration of 1 mg/ml. The drug (10 mg/kg) or vehicle (water) was given orally by gavage twice per day over a period of 28 days. On the last day, the animals were treated in the morning at 8:00 and were sacrificed 4 h later. After decapitation, brains were removed, frozen in isopentane at –40°C and stored at –80°C for *in situ* hybridisation studies.

Acute and subchronic antidepressant treatment

Animals were randomly distributed to the vehicle or paroxetine treatment group. Paroxetine (10 mg/kg) or vehicle (water) was given orally by gavage. Animals were treated once (acute treatment schedule) or for 7 days (subchronic treatment schedule). In both cases the animals received their only/last treatment in the morning at 8:00 and were sacrificed 4 h later. After decapitation, brains were removed, frozen in isopentane at –40°C and stored at –80°C for *in situ* hybridisation studies.

2.3 Surgery: Intracerebral application of substances

Mice were anesthetized with pentobarbital sodium diluted 1:20 in 0,9% NaCl (80 mg/kg bodyweight, i.p., Narcoren, Rhone Merieux, Paupheim, Germany). The animals were placed in a stereotactic apparatus (Stoelting Co., Wood Dale, USA). Stainless steel guide cannulas (Hamilton, 23 gauges) were bilaterally implanted under local anaesthesia with lidocaine (Xylocain, Astra GmbH, Wedel, Germany). The coordinates for the amygdala and the hippocampal dentate gyrus relative to bregma were the following (project 3): -1,0 mm anterior-posterior, ±3,1 mm lateral and –1,8 mm dorsoventral and –1,4 mm anterior-posterior, ± 1,0 mm lateral and -1,1 mm dorsoventral (according to the Paxinos and Watson brain atlas). The cannulas in project 4 were placed at coordinates relative to the bregma –1,5 mm anterior-posterior, ±1,0 mm lateral and -1,5 mm dorsoventral. After surgery the animals recovered for 14 days before the experiment started.

Mice were randomly distributed into two groups to be bilaterally infused. In project 3 they received either recombinant human/mouse/rat activin A (1 μg/side; carrier free; R&D systems, Minneapolis, MN USA) in 0,1% BSA in PBS or vehicle (0,1% BSA in PBS) (as described previously Dow et al., 2005). In project 4 we administered either human gastrin releasing peptide (1 μg/side; Bachem) in 0,9% saline or vehicle (0,9% saline). A total volume of 1 μl was infused and injections were made over 1 min with injection cannulas (Hamilton, 31 gauges) that extended 1 mm (dentate gyrus and project 4) and 1,2 mm (amygdala) beyond the tip of the guide cannula. The injection cannulas were left in place for additional 1 min to allow for diffusion of injectate. During experimental manipulation the animals were not under anaesthesia and hand-held. Correct cannula placement was determined by post mortem histological verification. Mice with misplaced cannulas were excluded from the final analysis.

2.4 In situ hybridisation analysis

Frozen brains were sectioned coronally at -20°C in a cryostat microtome at 16 μm. The sections were thaw-mounted on superfrost slides, dried at 32°C and kept at -80°C. *In situ* hybridisation using a ^{35}S-UTP labelled ribonucleotide probe was performed as described previously (Schmidt et al., 2002). Briefly, for riboprobe *in situ* hybridisation sections were fixed in 4% paraformaldehyde and acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine/HCl. Subsequently, brain sections were dehydrated in increasing concentrations of ethanol. Tissue sections (4 brain sections per slide) were saturated with 100 μl of hybridisation buffer containing approximately $1x10^6$ cpm ^{35}S-UTP labelled riboprobe. Brain sections were coverslipped and incubated overnight at 55°C. The following day the

sections were rinsed in 2xSSC (standard saline citrate), treated with RNAse A (20 mg/l) and washed in increasingly stringent SSC solutions at room temperature. Finally sections were washed in 0.1xSSC for 1 h at 65°C and dehydrated through increasing concentrations of alcohol.

The tissue plasminogen activator (Plat) cDNA, the delta subunit of the GABA-A receptor (Gabrd) cDNA, the gastrin releasing peptide (Grp) cDNA and the activin βA (Inhba) cDNA were generated by PCR amplification from mouse hippocampal tissue and subsequent cloned into a pCR®II-TOPO® vector (Invitrogen). The used primers are listed in table 2. The cDNA templates for the genes including T7 and SP6 promotors for sense and antisense riboprobe *in vitro* transcription were generated by PCR amplification from the vector using a T7 primer (5'-GAA TTG TAA TAC GAC TCA CTA TAG GGC GAA TTG-3') and a SP6 primer (5'-CCA AGC TAT TTA GGT GAC ACT ATA GAA TAC T-3'). The pGEM vector for activin receptor IA (Acvr1, 470 bp) *in situ* hybridisation was generously provided by the lab of D. Huylebroeck (Verschueren et al., 1995). The following vectors came from the microarray clone bibliography of the Max Planck Institute of Psychiatry, Munich: For preproenkephalin 1 (Penk1), cholecystokinin B receptor (Cckbr) and muscarinic cholinergic receptor 1 (Chrm1) *in situ* hybridisation the templates were generated of a linearised pT7T3D vector, whereas the template of glucocorticoid modulatory element binding protein 1 (Gmeb1) was generated out of a pT7T3D-PacI vector. The template for inhibin alpha (Inha) was derived from a pCMV-SPORT 2 vector. Finally, the glucocorticoid receptor probe (GR, 1250 bp) in a pCR®II-TOPO® vector was kindly provided by the research group of Dr. Deussing of the Max Planck Institute of Psychiatry. Both antisense and sense ^{35}S-UTP labelled ribonucleotide probes were tested on mouse brain tissue. Absence of label after hybridisation with radiolabelled sense probe confirmed the specifity of the antisense probe signal.

The slides were apposed to Kodak Biomax MR films (Eastman Kodak Co., Rochester, NY) and developed. Autoradiographs were digitized, and relative expression was determined by computer-assisted optical densitometry (Scion Image, Scion Corporation, Frederic, USA). The mean of the measurements of 4 different brain slices was calculated from each animal. The data were analysed blindly, always subtracting the background signal of a nearby structure not expressing the gene of interest from the measurements.

Gene	Primer sequence	Length	GC-amount (%)	Product lenght (bp)
Plat upstream	5‘-ATG AGG CAT CGT CTC CAT TC-3‘	20-mer	50	410 bp
Plat downstream	5‘-CCT TTT AGG CGC ATC TTC TG-3‘	20-mer	50	410 bp
Gabrd upstream	5‘-TGG CTT AAT GGA GGG CTA TG-3‘	20-mer	50	379 bp
Gabrd downstream	5‘-GTA CTT GGC GAG GTC CAT GT-3‘	20-mer	55	379 bp
Grp upstream	5‘-CAC GGT CCT GGC TAA GAT GT-3‘	20-mer	55	387 bp
Grp downstream	5‘-GGG TTT TGT TTT GCT CCT TG-3‘	20-mer	45	387 bp
Inhba upstream	5‘-TGG ATG GAG ATG GGA AGA AG-3‘	20-mer	50	508 bp
Inhba downstream	5‘-TCC ATT TTC TCT GGG ACC TG-3‘	20-mer	50	508 bp

Table 2: List of used primers

2.5 Behavioural test paradigms

Porsolt forced swim test (FST)

The forced swim test was applied in this thesis as it is the most commonly used experimental paradigm to assess antidepressant-like properties of compounds, due to its ability to detect activity in a broad spectrum of clinically effective antidepressants (Cryan and Mombereau, 2004). Three behavioural patterns of the animals are classified in this test: floating (interpreted as despair), swimming (seen as neutral behaviour) or struggling (interpreted as escape behaviour) (Ohl, 2005). Mice received 15 min (project 3) or 30 min (project 4) before the first trial of the forced swim test a single bilateral infusion of either vehicle or activin A/gastrin releasing peptide. Each animal was placed into a beaker (diameter: 13 cm, height: 24 cm) filled with water (temperature 25±1°C) to a height of 15 cm for a test period of 5 min. The parameters swimming, struggling (vigorous escape-oriented activity), and floating (immobile posture with only small movements to keep balance) were scored by a trained observer blind to the treatment. After the performance mice were removed from the water and dried with a towel. The animals in project 4 (Grp) were immediately sacrificed after the first testing and brains were processed for *in situ* hybridisation analysis (see 2.4).

In order to investigate the effects of a first FST exposure under vehicle or activin A treatment on the stress-coping strategy, in project 3 all animals were retested 24 h after the infusion. Animal were immediately sacrificed after the second trial of the FST, brains were removed, frozen in isopentane at –40°C and stored at –80°C for histological localization or *in situ* hybridisation studies.

Modified hole board (mHB)

As we were interested to get a comprehensive overview on behavioural changes under mild-stressful conditions and to detect changes in locomotor activity we performed the modified hole board test (see for details Ohl et al., 2001) in project 3. The apparatus consisted of a gray PVC box (100x50x50 cm) in the middle of which a gray PVC board (60x20 cm) with 23 holes (1,5x0,5 cm) covered by lids is placed, thus representing the central area of an open field. Mice received a single infusion of vehicle or activin A and were tested in the mHB 20 h after the treatment. The animals were placed always in the same corner of the outer area of the test apparatus for a test period of 5 min each. The following test parameters were scored during the test period: board visits, rearing, grooming behaviour, stretched attends and total covered distance. Animals were immediately sacrificed after the test, brains were removed, frozen in isopentane at –40°C and stored at –80°C for histological localization or *in situ* hybridisation studies.

Elevated plus maze (EPM)

To assess changes in anxiety-related behaviour in project 4, we performed the elevated plus maze test, which is used as a rodent test of anxiety, in a separate cohort of animals. The elevated plus maze consisted of two opposing open arms (30x5x0,5 cm) and two opposing enclosed arms (30x5x15 cm) of gray PVC which were connected by a central platform (5x5 cm) shaping a plus sign. Animals were placed 30 min after a single bilateral infusion of either vehicle or Grp in the center of the plus maze and were allowed to explore it for 5 minutes. Time spent in the open arms, stretching and rearing behaviour as well as number of head dips were recorded (Rodgers and Dalvi, 1997).

Open field

In order to exclude behavioural changes due to alterations in the locomotor activity of mice in project 4 we performed the open field test 15 min after the elevated plus maze. Open field arenas (50x50x50 cm) were made of gray PVC and evenly illuminated during testing (20 lux). General locomotor activity in vehicle and Grp injected mice was recorded for 5 minutes (distance traveled) using a video-tracking system (Anymaze 4.20, Stoelting, Illinois, USA).

2.6 Electrophysiology

Brain slice preparation

Transverse hippocampal slices (350 μm) were obtained from the brains of adult, 6-8 weeks

old mice that were anaesthetized with isoflurane and then decapitated. The brain was rapidly removed, and slices were prepared in icy Ringer solution using a vibroslicer. All slices were placed in a holding chamber for at least 60 min and were then transferred to a superfusing chamber for extracellular or whole-cell recordings. The flow rate of the solution through the chamber was 1.5 ml/min. The composition of the solution was 124 mM NaCl, 3 mM KCl, 26 mM NaHCO3, 2 mM CaCl2, 1 mM MgSO4, 10 mM D-glucose, and 1.25 mM NaH2PO4, bubbled with a 95% O2-5% CO2 mixture, and had a final pH of 7.3. All experiments were performed at room temperature.

Electrophysiologic recording

Extracellular recordings of field excitatory postsynaptic potentials (fEPSPs) were obtained from the dendritic region of the CA1 region of the hippocampus using glass micropipettes (1-2 MΩ) filled with superfusion solution. For LTP induction, high-frequency stimulation conditioning pulses (100 Hz/1 s) were applied to the Schaffer collateral-commissural pathway. Measurements of the slope of the fEPSP were taken between 20 and 80% of the peak amplitude. Slopes of fEPSPs were normalized with respect to the 20-min control period before tetanic stimulation.

Isolated NMDA-receptor-mediated EPSCs (NMDA-EPSCs) were measured in the presence of 5 µM 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quino–xaline-7-sulphon–amide (NBQX) 50 µM picrotoxin and 200 µM 3-aminopropyl(diethoxymethyl)phosphonic acid (CGP 35348). Drugs were applied via the superfusion system. Pipettes had a series resistance of 4-6 MW, when filled with a solution containing (in mM) K-D-gluconat 130, KCl 5, EGTA 0.5, MgCl2 2, HEPES 10, D-glucose 5, Na2-phosphocreatine 20 (all from RBI/Sigma, Deisenhofen, Germany). Currents were recorded with a switched voltage-clamp amplifier (SEC 10L, NPI electronic, Tamm, Germany) with switching frequencies of 60-80 kHz (25% duty cycle). Series resistance was monitored continuously and compensated in bridge mode. fEPSPs and EPSC were evoked by stimuli (0.033Hz, 4-5 V, 20 µs), delivered via bipolar tungsten electrodes insulated to the tip (5-µm tip diameter) and positioned in the Schaffer collateral-commissural pathway. The recordings were amplified, filtered (3 kHz), and digitized (9 kHz). The digitized responses were stored to disk on a Macintosh computer using a data acquisition program.

Substance application

Either activin A (40 nM) or vehicle (0,1% BSA in PBS) were applied to the perfusion system

for 1 h, then LTP was induced.

2.7 Human genetic association study

Patient sample

224 patients (46% male, 54% female; mean age = 48.17; SD = 13.89) were admitted to the hospital of the Max Planck Institute of Psychiatry (MPI), Munich/Germany, for treatment of a depressive disorder presenting with major depression (88%) or bipolar disorder (12%). Part of the patients had participated in previous studies (Künzel et al., 2003). Patients were included in the study within 1-3 days of admission, and the diagnosis was ascertained by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Patients with depressive disorders due to a medical or neurological condition were excluded. Trained raters using the 21-item Hamilton Depression Rating Scale (HAM-D) assessed the severity of psychopathology at admission. Patients fulfilling the criteria for at least a moderate depressive episode (HAM-D>=14) entered the analysis. All patients were Caucasian; ethnicity was recorded using a self-report questionnaire about nationality, first language and ethnicity of the subject and of all 4 grandparents. The study was approved by the local ethics committee and written informed consent obtained from all subjects.

Combined dex/CRH-test

Patients underwent combined dexamethasone (dex) / corticotropin releasing hormone (CRH) tests at admission (1 to 10 days after admission) and at discharge (10 days or less prior to discharge). The procedure of the combined dex/CRH tests was described in detail elsewhere (Künzel et al., 2003; Heuser et al., 1994; Holsboer et al., 1987; Zobel et al., 2001). Briefly, 1.5 mg dexamethasone is orally administered at 11 p.m. the day before stimulation with 100 µg human CRH. Blood samples are drawn at 15:00, 15:30, 15:45, 16:00, and 16:15 pm. CRH is injected just after the first sample is collected. Plasma ACTH concentrations were analysed using an immunometric assay without extraction (Nichols Institute, San Juan Capistrano, CA; detection limit 4.0 pg/ml). Plasma cortisol concentrations were determined by radioimmunoassay (ICN Biomedicals, Carson, CA; detection limit 0.3 ng/ml).

DNA preparation, SNP selection and genotyping

At the time of enrolment in the study, 40 ml of EDTA blood were drawn from each patient. DNA was extracted from fresh blood using the Puregene® whole blood DNA-extraction kit (Gentra Systems Inc; MN).

We analysed 40 SNPs in the genes encoding activin A and its receptors. Activin A is a homodimer consisting of activin ßA subunits encoded by the gene Inhba. Genes Acvr1, Acvr2a and Acvr2b code for Activin receptor IA, Activin receptors IIA and IIB, respectively. SNPs located within as well as 10 kb up- and downstream of genes Inhba, Acvr1, Acvr2a and Acvr2b as included in the Sentrix Human-1 Genotyping BeadChip and HumanHap300 were genotyped using Illumina BeadChip technology (Illumina Inc., San Diego, USA) according to the manufacturer's standard protocols. Information on selected SNPs, their position, quality control, and minor allele frequencies are available in table 3.

Gene	Chr	SNP	Position	Location	HWE	MAF	CR	Alleles
INHBA	7	rs2237435	41697577	Intron	0,108	0,283	1,000	A/C
INHBA	7	rs2237432	41701558	Intron	0,268	0,238	1,000	G/A
INHBA	7	rs3801158	41705665	Intron	0,723	0,169	1,000	G/C
INHBA	7	rs2877098	41709818	Promoter	0,078	0,309	1,000	T/C
INHBA	7	rs2007475	41713081	Promoter	0,684	0,449	0,992	A/G
ACVR1	2	rs12997	158301602	3' UTR	0,370	0,268	0,995	G/A
ACVR1	2	rs1220133	158311561	Intron	1,000	0,243	1,000	A/G
ACVR1	2	rs10497189	158333049	Intron	1,000	0,105	1,000	C/T
ACVR1	2	rs1146037	158342534	Intron	0,211	0,237	0,997	C/T
ACVR1	2	rs3820742	158344586	Intron	0,210	0,237	1,000	T/C
ACVR1	2	rs10497190	158347485	Intron	0,264	0,196	1,000	T/C
ACVR1	2	rs7565550	158362536	Intron	0,363	0,208	1,000	A/C
ACVR1	2	rs10497191	158375462	Intron	0,249	0,126	1,000	T/C
ACVR1	2	rs4380178	158376690	Intron	0,307	0,180	1,000	A/G
ACVR1	2	rs10497192	158379945	Intron	0,808	0,292	1,000	C/T
ACVR1	2	rs4233672	158400171	Intron	0,458	0,215	1,000	A/G
ACVR1	2	rs13398650	158402625	Intron	0,173	0,105	1,000	A/G
ACVR1	2	rs2883605	158404774	Promoter	0,322	0,114	0,992	T/G
ACVR2A	2	rs1364658	148324277	Intron	0,905	0,299	0,997	G/C
ACVR2A	2	rs6747792	148327997	Intron	0,298	0,215	1,000	G/T
ACVR2A	2	rs1895694	148336215	Intron	0,606	0,413	1,000	G/A
ACVR2A	2	rs929939	148343997	Intron	0,815	0,311	0,997	A/C
ACVR2A	2	rs12987286	148380037	Intron	0,812	0,301	1,000	T/G
ACVR2A	2	rs1469211	148383304	Intron	0,298	0,215	1,000	A/G
ACVR2A	2	rs3768689	148386243	Intron	0,905	0,304	0,995	C/G
ACVR2A	2	rs3820716	148396729	Intron	1,000	0,474	1,000	C/T
ACVR2A	2	rs2303392	148396896	Intron	1,000	0,310	0,990	G/C
ACVR2B	3	rs3792527	38468215	Promoter	0,603	0,408	1,000	A/G

ACVR2B	3	rs2268753	38475192	Intron	0,677	0,411	0,992	G/A
ACVR2B	3	rs7431353	38494428	Intron	0,506	0,346	1,000	A/G
ACVR2B	3	rs4407366	38496428	Intron	0,439	0,348	1,000	C/T
ACVR2B	3	rs1046048	38499745	Exon/syn	0,916	0,401	0,990	G/A
ACVR2B	3	rs7374458	38506214	Promoter	0,439	0,348	1,000	T/G
ACVR2B	3	rs9838614	38512674	Promoter	0,438	0,348	0,997	C/A

Table 3: Information and quality control of genotyped SNPs. Chr=chromosome; position according to genome build hg18; HWE=p-values for deviation from Hardy-Weinberg equilibrium; MAF=minor allele frequency; CR=call rate; Alleles=minor/major allele.

Statistical analysis

For all SNPs exact tests for Hardy-Weinberg Equilibrium were performed (Wigginton et al., 2005). No SNPs were excluded due to significant deviation from Hardy-Weinberg Equilibrium, 3 SNPs were excluded due to a minor allele frequency below 2.5%, and 3 SNPs due to a call rate less than 98%, and this resulted in 34 SNPs entering the analysis (table 3). The genotype-related effects on the ACTH and cortisol response in dex/CRH tests at admission and discharge were assessed by calculation of areas under the curve (AUC) using the trapezoid rule. To correct for the effects of gender and age, standardised regression residuals corrected for these potential confounder effects were used for the data analysis. We tested both allelic and genotypic modes of inheritance. P values were adjusted for multiple comparisons by a re-sampling method (100,000 permutations) according to Westfall and Young (1993). The level for significance was set at 5%. We also analysed gene-wide associations applying the multivariate Fisher-Product Method (FPM) for all SNPs within one gene and for all phenotypes tested (ACTH and cortisol response to dex/CRH tests at admission and at discharge, respectively). For in-depth analysis of significantly associated SNPs we performed repeated measure ANCOVAs with repeated plasma ACTH and cortisol values during the dex/CRH test as within subjects factor and genotypes as between subjects factors; age and sex were included as covariates.

To test for genetic interactions we performed an ANCOVA with genotypes of the 2 highest associated SNPs as independent and ACTH and cortisol AUC at admission and discharge as dependent outcome variables as well as age and gender as covariates. Due to small groups for the rare alleles we excluded patients with genotype ‘GG’ (rs2237432) and ‘AA’ (rs1469211) from the analyses.

Permutation analyses were performed with the program "Permer" (available at http://www.wg-permer.org), all other analyses were performed with SPSS (Release 12.01, SPSS Inc., Chicago, USA).

2.8 Statistical analysis

Data analysis

The commercially available program SPSS 12 was used for statistical analysis. Comparisons of two groups were made by unpaired t-test. The level of significance was set at $p<0.05$. Data are presented as mean + SEM.

III Results

3.1 Validation of potential antidepressant target genes

3.2 Transcriptional changes following paroxetine administration are dependent on the duration of treatment and the neuroanatomical region

3.3 Activin βA is induced by chronic paroxetine treatment and exerts antidepressant-like effects in vivo

3.4 Characterisation of behavioural effects of central gastrin releasing peptide administration

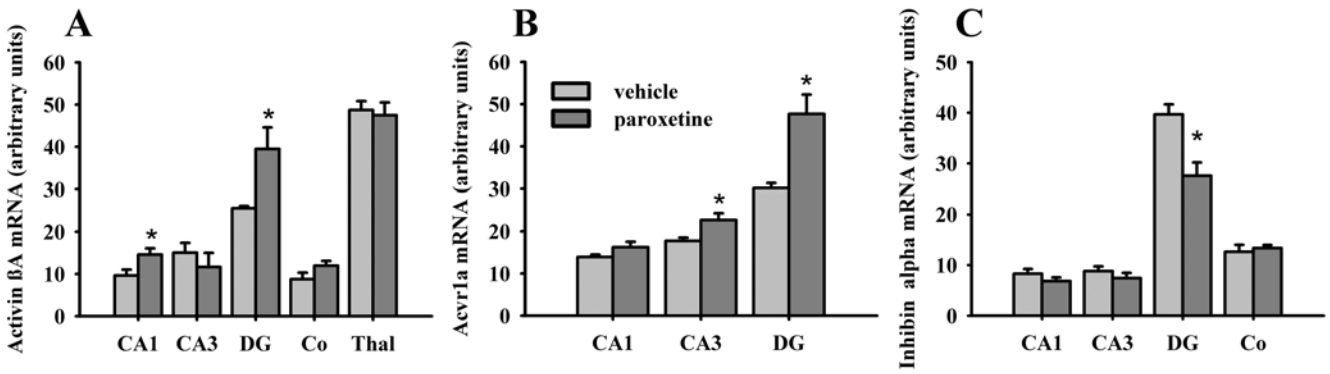
1. Validation of potential antidepressant target genes

Besides the modulation of distinct neurotransmitter systems like the monoamines, a major component of antidepressant drug action is their impact on the regulation of gene expression (Tardito et al., 2006). Therefore an increasing number of hypothesis-free microarray experiments is conducted to investigate the association between antidepressant treatment and the resulting changes in expression levels of genes, which might be unknown by now to be implicated in mood disorders. Sillaber et al. aimed to determine changes in hippocampal gene expression in DBA/2OlaHsd mice after chronic antidepressant treatment with paroxetine, a selective serotonin reuptake inhibitor (Sillaber et al., submitted). Depending on the cut-off-level of fold regulation, they found a different number of genes to be significantly regulated in the performed microarray: taking a cut-off-level of ±1,2 255 genes were found to be regulated, at a cut-off-level of ±1,35 the expression of 69 genes was found to be altered in the total hippocampus. Following these results we performed *in situ* hybridisation studies in order to validate distinct genes. Using the *in situ* hybridisation technique instead of real-time-PCR for validation provides the advantage of revealing brain-region specific differences in gene expression levels. Among the regulated genes we chose eleven genes (see table 1) which seemed promising with respect to the involvement in the pathophysiology and treatment of depressive disorders. The selected genes showed different levels and direction of regulation after chronic paroxetine treatment. Functionally they belong to different groups, e.g. to growth and differentiation or transcription factors or to the opioid system. The following genes were elected from the microarray: three genes of the activin family (*activin βA*, *inhibin alpha* and *activin receptor IA*). Activin is of growing interest in respect to its neuroprotective and potential antidepressant-like effects (Kupershmidt et al., 2007; Dow et al., 2005). The widespread distribution in the CNS and the various proposed neuronal functions of the *cholecystokinin B receptor* suggested it as promising candidate for our studies (for review see: Crawley and Corwin, 1994). The *muscarinic acetylcholine receptor 1 (CNS)* was selected due to its known implications in various psychiatric diseases like Alzheimer's disease, anxiety and depression (Levey, 1996; File et al., 2000; Chau et al., 2001). Furthermore, the *gamma-aminobutyric acid receptor, subunit delta* gene was chosen due to its expression in brain regions involved in depression and anxiety like the dentate gyrus of the hippocampus and the amygdala (Fujimura et al., 2005; Persohn et al., 1992; Peng et al., 2002). Additionally, we were interested in the *glucocorticoid modulatory element binding protein 1* and its ability to modulate the glucocortocoid receptor regulated gene induction (Oshima and Simons, Jr.,

1992; Kaul et al., 2000). The *gastrin releasing peptide* was of high interest in respect to its neuropeptidergic function and its possible involvement in neurochemical alterations associated with psychiatric disorders (Yamada et al., 2002; Roesler et al., 2006). In addition, our interest was laid on the *glucocorticoid receptor*, which is already well known for its central role in the feedback regulation of the HPA-axis (DeRijk et al., 2002). The *preproenkephalin 1* gene as precursor of several endogenous opioid peptides was analysed due to the well-known fact that many stressors interact with the endogenous opiate systems (for review see: Vaccarino and Kastin, 2001). Moreover, inhibition of enkephalin degradation exerts antidepressant-like effects in different behavioural paradigms (Baamonde et al., 1992; Smadja et al., 1995; Jutkiewicz et al., 2006). Finally, we were interested in the *tissue plasminogen activator* gene. Tissue plasminogen activator deficient mice were shown to display an impaired response to stress (Pawlak et al., 2003).

Results

Activins, inhibins and receptors: We analysed alterations in gene expression levels of activin βA, inhibin α and activin receptor IA after chronic oral paroxetine treatment compared to the vehicle treated control group. For mRNA levels of activin βA we could detect a significant increase in the CA1 and the dentate gyrus region of the hippocampus in the paroxetine-treated group (t-test, $p<0.05$) (figure 3A), whereas we could not observe differences in the CA3 of the hippocampus, the cortex and the thalamus (figure 3A). For activin receptor IA mRNA we found a significant increase in the CA3 and dentate gyrus of the hippocampus after chronic antidepressant treatment (t-test, $p<0.05$) (figure 3B) and no changes in mRNA levels in the CA1 of the hippocampus (figure 3B). The levels of inhibin α mRNA were significantly decreased in the dentate gyrus in paroxetine-treated animals (t-test, $p<0.05$) (figure 3C). Figures 3D, E and F show the respective, representative autoradiographs.



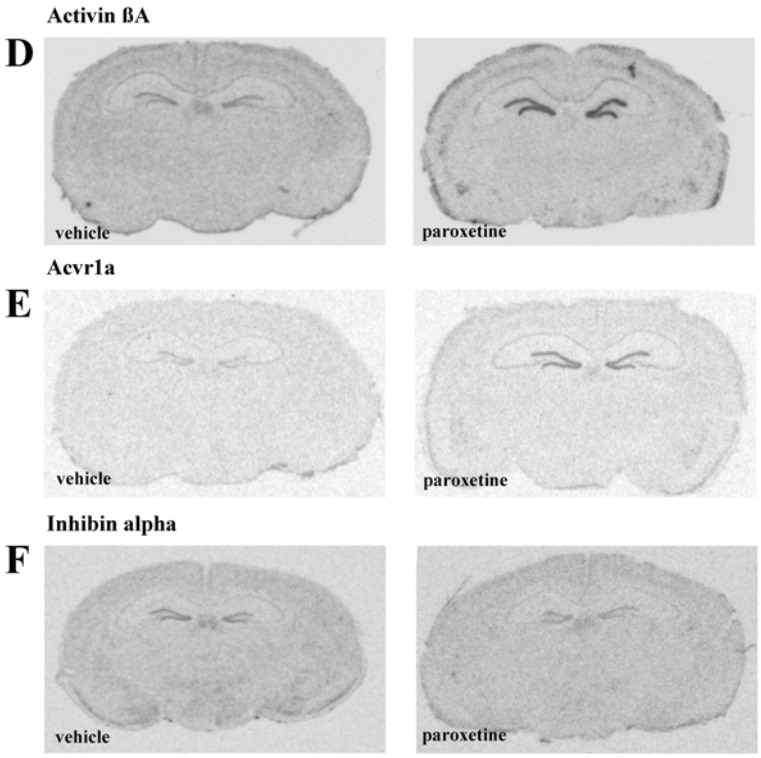


Figure 3:
Expression levels of activin βA, activin receptor IA (Acvr1a) and inhibin α mRNA in various brain regions after chronic paroxetine treatment compared to the control group. Activin βA mRNA in the CA1, CA3, dentate gyrus (DG), the cortex (Co) and the thalamus (Thal) (A). Activin receptor IA mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus (B). Inhibin α mRNA in the CA1, CA3, dentate gyrus (DG) and the cortex (Co) (C). Representative activin βA mRNA (D), activin receptor IA mRNA (E) and inhibin α mRNA expression autoradiographs (F). n=9 per group.
* significant from control group, $p<0.05$.
Data are presented as mean + SEM.

Cholecystokinin B receptor: The analysis of cholecystokinin B receptor mRNA expression in distinct brain regions after chronic paroxetine administration revealed a significant decrease in the CA1 and CA3 region of the hippocampus and the cortex compared to the control group (t-test, $p<0.05$) (figure 4A). In the dentate gyrus of the hippocampus and the hypothalamus no differences were found between both treatment groups (figure 4A). Figure 4B shows the respective, representative autoradiograph.

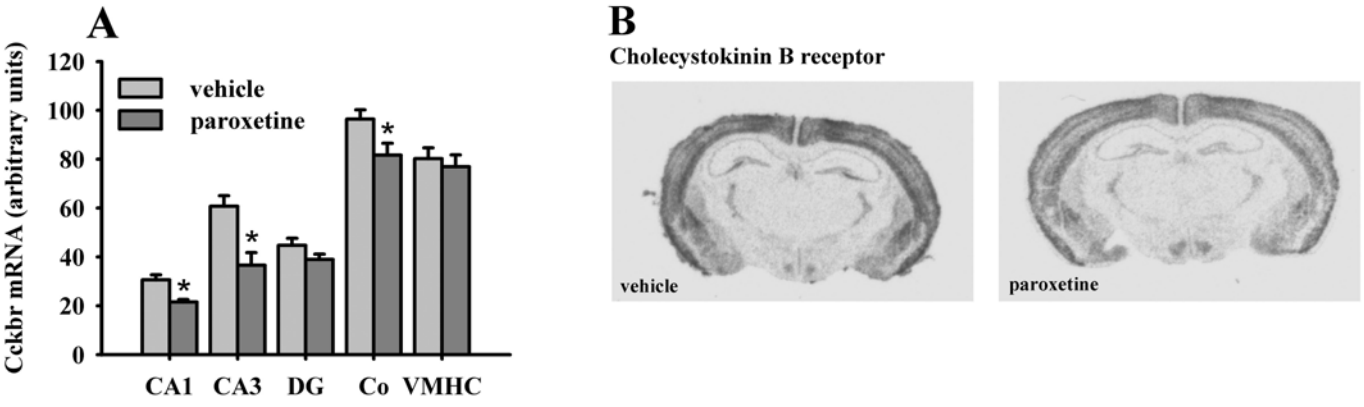


Figure 4:
Expression levels of cholecystokinin B receptor mRNA in the CA1, CA3, dentate gyrus (DG) of the hippocampus, the cortex and the hypothalamus (VMHC = ventromedial hypothalamic nucleus, central part) (A). Representative cholecystokinin B receptor mRNA expression autoradiographs (B). Vehicle group n=10; paroxetine group n=9. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Muscarinic acetylcholine receptor 1: For muscarinic acetylcholine receptor 1 mRNA levels we could not detect significant differences in the CA1, CA3 and the dentate gyrus of the hippocampus between paroxetine and vehicle treated mice (figure 5A). Figure 5B shows the respective, representative autoradiograph.

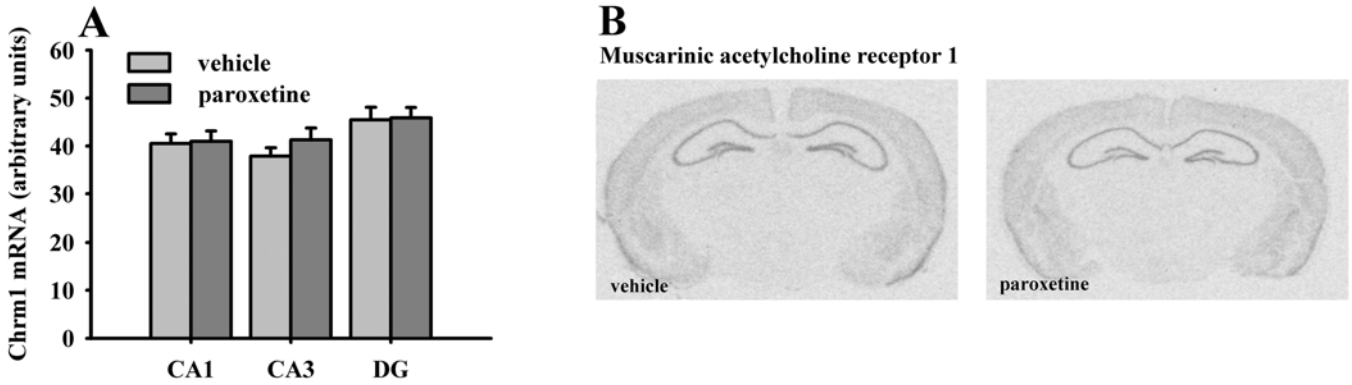


Figure 5:
Expression levels of muscarinic acetylcholine receptor 1 mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus (A). Representative muscarinic acetylcholine receptor 1 mRNA expression autoradiographs (B). Vehicle group n=10; paroxetine group n=9. Data are presented as mean + SEM.

$GABA_A$ receptor, subunit delta: After analysis of alterations in expression levels of $GABA_A$ receptor, subunit delta mRNA we could detect a significant decrease in the dentate gyrus of the hippocampus as well as in the cortex (t-test, $p<0.05$) (figure 6A), whereas no differences could be found in mRNA levels in the CA1 region and the thalamus (figure 6A). Figure 6B shows the respective, representative autoradiograph.

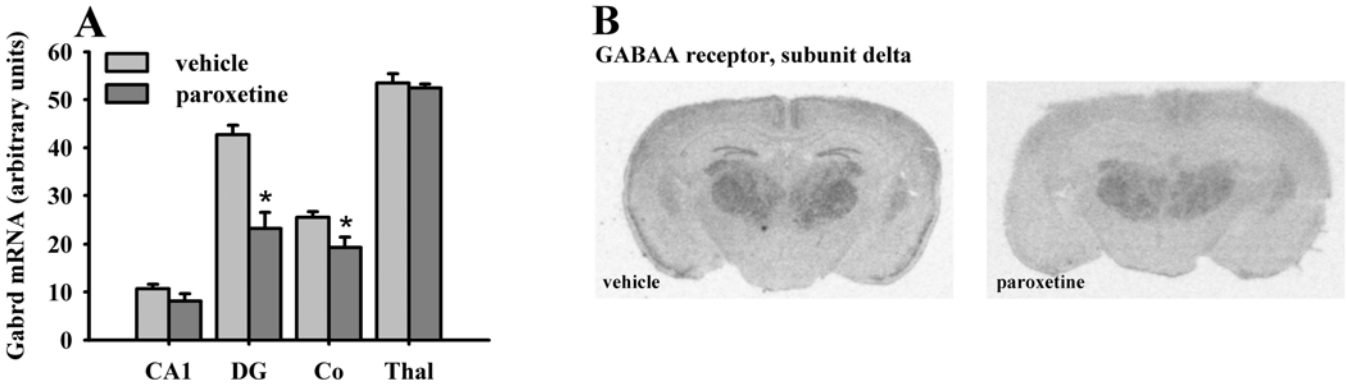


Figure 6:
Expression levels of $GABA_A$ receptor, subunit delta mRNA in the CA1, the dentate gyrus (DG) of the hippocampus, the cortex (Co) and the thalamus (Thal) (A). Representative $GABA_A$ receptor, subunit delta mRNA expression autoradiographs (B). n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Glucocorticoid modulatory element binding protein 1: For Gmeb1 mRNA expression no significant differences between treatment groups were revealed in the CA1, CA3 and the dentate gyrus of the hippocampus (figure 7A). Figure 7B shows the respective, representative autoradiograph.

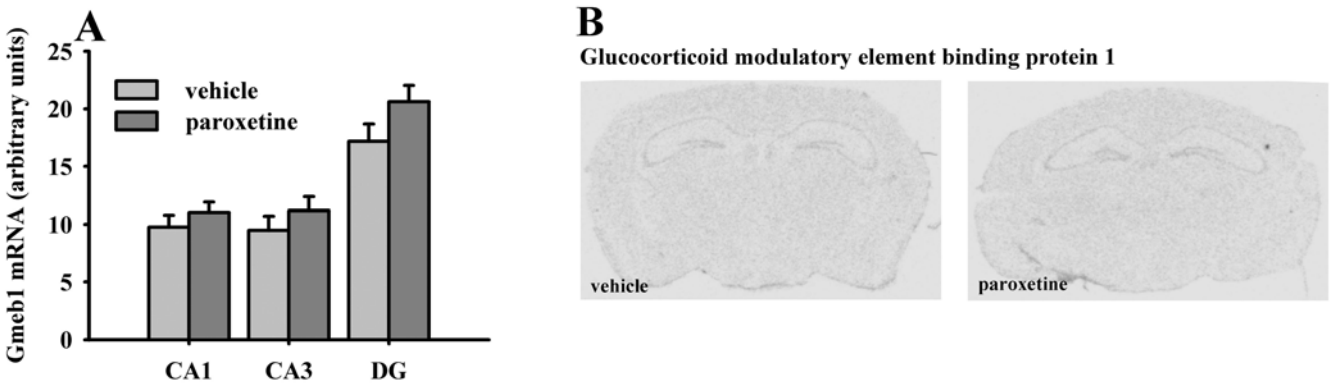


Figure 7:
Expression levels of the glucocorticoid modulatory element binding protein 1 mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus (A). Representative Gmeb1 mRNA expression autoradiographs (B). Vehicle group n=10; paroxetine group n=9. Data are presented as mean + SEM.

Gastrin-releasing peptide: In chronically paroxetine-treated mice we could only observe a significant upregulation of gastrin-releasing peptide expression levels in the dentate gyrus of the hippocampus (t-test, $p<0.05$) (figure 8A). No differences in mRNA levels could be detected in the CA1 and CA3 of the hippocampus, the accessory basal and the lateral nucleus of the amygdala (figure 8A). Figure 8B shows the respective, representative autoradiograph.

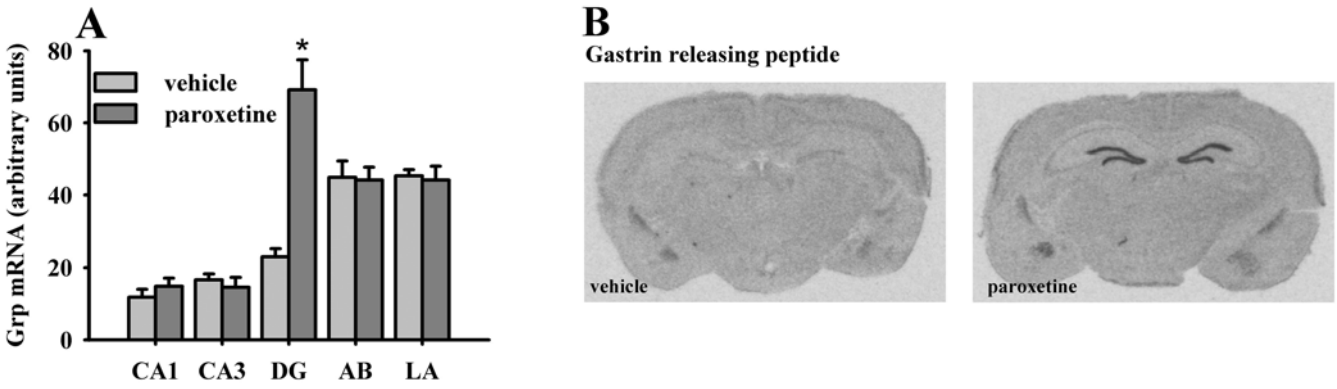


Figure 8:
Expression levels of gastrin releasing peptide mRNA in the CA1, CA3, dentate gyrus (DG) of the hippocampus and the accessory basal nucleus (AB) and lateral nucleus (LA) of the amygdala (A). Representative gastrin-releasing peptide mRNA expression autoradiographs (B). n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Glucocorticoid receptor: The expression of the glucocorticoid receptor gene was significantly decreased in the dentate gyrus region (t-test, $p<0.05$) (figure 9A). However, we could not identify differences between treatment groups in the CA1 and CA3 region of the hippocampus (figure 9A). Figure 9B shows the respective, representative autoradiograph.

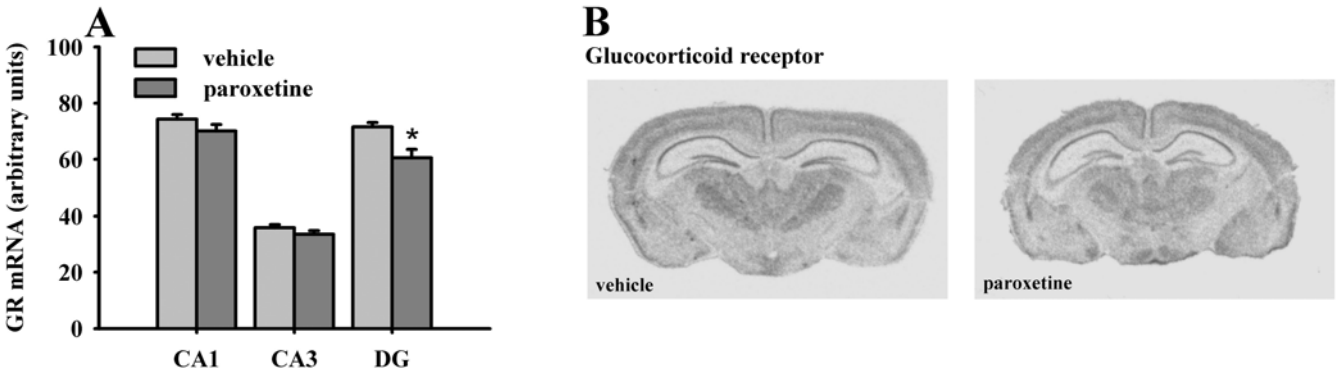


Figure 9:
Expression levels of the glucocorticoid receptor mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus (A). Representative GR mRNA expression autoradiographs (B). n=9 per group. * significant from control group, $p<0.05$ Data are presented as mean + SEM.

Preproenkephalin 1: Analysis of preproenkephalin 1 mRNA in paroxetine- and vehicle-treated mice brains revealed a significant increase after antidepressant treatment in the dentate gyrus of the hippocampus and the amygdala (t-test, $p<0.05$) (figure 10A): In contrast there was no difference in gene expression in the CA1 of the hippocampus (figure 10A). Figure 10B shows the respective, representative autoradiograph.

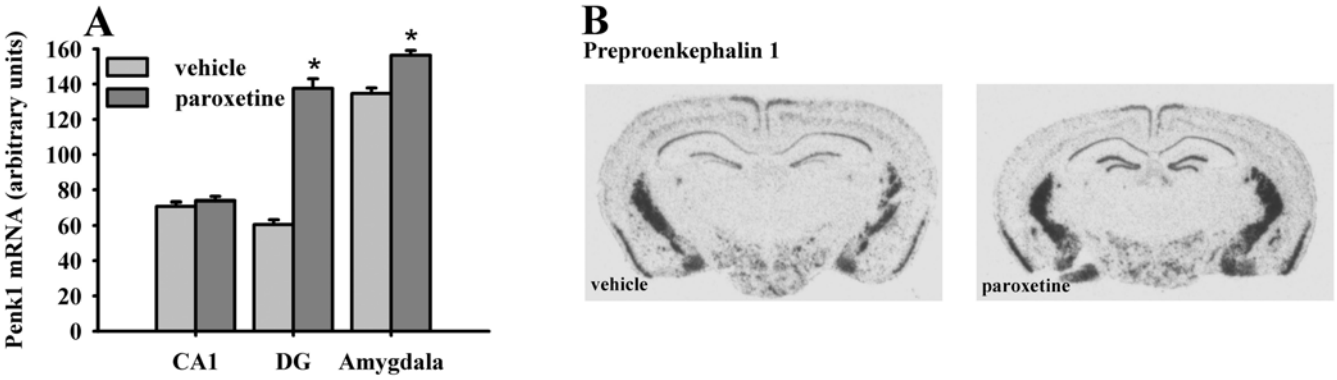


Figure 10:
Expression levels of the preproenkephalin 1 mRNA in the CA1, the dentate gyrus (DG) of the hippocampus and the amygdala (A). Representative preproenkephalin 1 mRNA expression autoradiographs (B). Vehicle group n=10; paroxetine group n=9. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Tissue plasminogen activator: For tissue plasminogen activator mRNA we could detect a significant upregulation in the CA1 and the dentate gyrus of the hippocampus after paroxetine administration (t-test, $p<0.05$) (figure 11A). Both treatment groups did not differ in tissue plasminogen activator mRNA levels in the CA3 of the hippocampus and the cortex (figure 11A). Figure 11B shows the respective, representative autoradiograph.

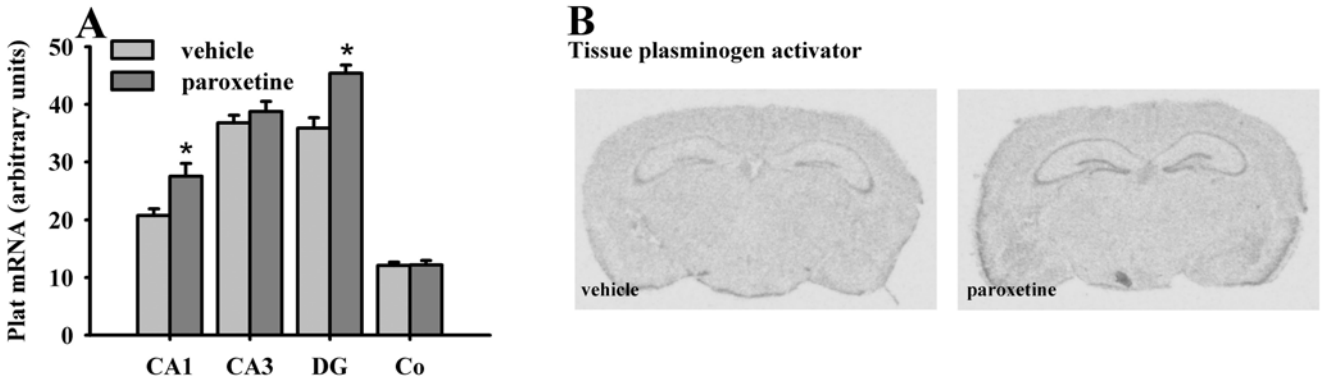


Figure 11:
Expression levels of the tissue plasminogen activator mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus and the cortex (A). Representative plasminogen activator expression mRNA autoradiographs (B). n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Discussion

In this study we were interested in the investigation of novel genes, which are potentially implicated in the mechanism of action of the antidepressant paroxetine and accordingly might be involved in the pathophysiology of depression. 11 genes were selected from a microarray study performed by Sillaber et al. (Sillaber et al., submitted). We were able to validate nine of the eleven genes by *in situ* hybridisation technique. These findings suggest an influence of chronic antidepressant treatment on the expression of various genes. Furthermore, by means of *in situ* hybridisation we could not only show alterations in gene expression levels in the hippocampus, where tissue for microarray analysis was taken from, but also in other regions, which are relevant for anxiety and depression like the amygdala and the cortex. In the following, a short review is given on the influence of chronic paroxetine administration on the regulation of selected genes and their functions:

Activins, inhibins and receptors: Corresponding to the paroxetine-induced upregulation of activin βA and activin receptor IA mRNA in the microarray analysis we could detect an upregulation of both genes in distinct regions of the hippocampus. For activin βA gene expression we could show a significant increase in the CA1 and the dentate gyrus of the hippocampus, for activin receptor IA expression an upregulation in the CA3 and the dentate gyrus after chronic antidepressant treatment. For the inhibin α subunit we could confirm a significant mRNA decrease in the dentate gyrus region of the hippocampus.

Activins and inhibins belong to the transforming growth factor β (TGF-β) family, which are known as multifunctional factors (Shi and Massague, 2003; Flanders et al., 1998). Both proteins are composed of two subunits. Until now, five β subunits (βA, βB, βC, βD, βE) and one single α subunit were identified. Activins are homodimers (βA-βA , βB-βB) or heterodimers (βA-βB) of the activin β-subunits, whereas inhibins are heterodimeric proteins composed of one β-subunit which is disulfide-linked to a structurally related α-subunit (Gray et al., 2005) Regarding the strong regulation of the activin βA subunit, in this thesis we focused on activin A consisting of two βA subunits. Activin A exerts its biological activity by two different types of receptors, the type I (Acvr1 and Acvr1b) and the type II receptors (Acvr2a and Acvr2b) (Florio et al., 2007). Since the discovery of activin in 1986 (Ling et al., 1986; Vale et al., 1986) it has been demonstrated to exert a broad range of biological effects. Originally, activin has been identified as cytokine, neuroendocrine hormone, and growth and differentiation factor (Peng and Mukai, 2000; Danila et al., 2002). Activin βA mRNAs were detected in the ovary, testis, placenta, adrenal tissue, bone marrow, spleen, spinal cord and the brain (Meunier et al., 1988). Several roles of activin in the CNS have been characterised like

neuronal development and protection (Tretter et al., 1996; Iwahori et al., 1997; Trudeau et al., 1997). Regarding the activity of activin A in the hippocampus, it has already been shown to exert neurotrophic effects on cultured hippocampal neurons and furthermore to be involved in neural plasticity (Iwahori et al., 1997; Inokuchi et al., 1996; Shoji-Kasai et al., 2007). Activin A is not yet mentioned frequently in the context of anxiety and depression, only recently Dow et al. could show antidepressant-like effects of activin A infusion into the dentate gyrus, whereas infusion into the CA1 region showed no effects (Dow et al., 2005).

Cholecystokinin B receptor (Cckbr): We were able to validate the results of the microarray analysis providing evidence for paroxetine-induced regulation of the Cckb receptor. We detected a significant decrease in the CA1 and CA3 region of the hippocampus and the cortex in paroxetine-treated animals.

Cholecystokinin (Cck) is a regulatory peptide hormone originally discovered in the gastrointestinal tract, but also present as a neuropeptide throughout the nervous system. The biological effects of Cck are mediated by two specific G-protein coupled receptor subtypes, designated Cckar and Cckbr (Herranz, 2003). Together with Cck, the structurally related gastrin, a peptide hormone of the gastrointestinal system, is also physiological ligand for the Cckbr/gastrin receptors (Rehfeld et al., 2007). The major population of central Cck receptors are of Cckbr subtype, but it can also be found in areas of the gastrointestinal tract and on pancreatic acinar and parietal cells (Hill et al., 1987; Noble and Roques, 1999). The widespread distribution of Cckb receptors in the central nervous system is consistent with the various proposed functions of neural Cck like a role in the regulation of satiety, in learning and memory, analgesia and neuropsychiatric disorders such as anxiety and panic attacks (for review see: Crawley and Corwin, 1994). Existing studies could show that activation of the brain Cckb receptor induces anxiety and that on the other hand selective receptor antagonists produce anxiolytic effects in rats (Singh et al., 1991; Rezayat et al., 2005). Bilateral injection of a selective Cckb receptor antagonist into the CA1 region of the dorsal hippocampus produced significant anxiolytic behaviour in the elevated plus maze test (Rezayat et al., 2005). As paroxetine has also anxiolytic effects, our observed downregulation of the Cckb receptor in the CA1 may contribute to this part of its therapeutic action. Moreover, there is evidence that the receptor might be implicated in depressive disorders, as antagonism of Cckb receptors mediates antidepressant-like effects in the forced swim test in mice (Derrien et al., 1994; Hernando et al., 1994). Additionally, data of Cckb receptor knockout mice suggest a role for the receptor in the negative feedback control of the opioid

system, as deletion of the receptor resulted in an activation of the endogenous opioid system (see preproenkephalin 1, page 48) (Pommier et al., 2002).

Cholinergic receptor, muscarinic 1, CNS (Chrm1): In case of the Chrm1 we could not confirm the analysed alterations in mRNA levels in the microarray after paroxetine treatment. The neurotransmitter acetylcholine binds to two different types of receptors, one is the family of nicotinic receptors and the other one the family of muscarinic receptors. The muscarinic acetylcholine receptors (mAChRs) participate in a number of various functions in the periphery and brain, like arousal, sensory processing, cognition and motor control (Felder et al., 2000). They are belonging to the super family of plasma membrane-bound G-protein coupled receptors and five subtypes have been cloned so far (M1-M5) (Kubo et al., 1986; Bonner et al., 1987; Bonner et al., 1988). The mAChRs exhibit a remarkable similarity in sequence homology and pharmacology across the mammalian species, especially in rat, mouse and humans (Bymaster et al., 2003). The muscarinic acetylcholine receptor 1 is not widely expressed in the body periphery, but a few studies reported its presence in the sympathetic nervous system and the salivary glands (Caulfield and Birdsall, 1998; Levey, 1993). However, the expression of Chrm1 in the brain is widespread, including the cortex, striatum, and the hippocampus (Levey, 1993). There is already evidence that the receptor might be implicated in psychiatric diseases: several studies have reported the potential effects of selective M1 agonists for the treatment of Alzheimer's disease (Levey, 1996; Fisher et al., 2003; Caccamo et al., 2006) and File et al. suggest a role for Chrm1 agonists in the dorsal hippocampus in mediating anxiolytic-effects (File et al., 2000). Moreover, application of a M1 antagonist in the nucleus accumbens of rats elicited antidepressant-like effects in the Porsolt forced swim test (Chau et al., 2001).

Gamma-aminobutyric acid (GABA-A) receptor, subunit delta (Gabrd): In situ hybridisation analysis revealed corresponding to the microarray data a significant decrease in $GABA_A$ receptor, subunit delta mRNA in the dentate gyrus and the cortex after chronic antidepressant treatment.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. The transmitter exerts its physiological actions via two different receptors, $GABA_A$ and $GABA_B$ (Shiah and Yatham, 1998). The $GABA_A$ receptor is a ligand-gated chloride-ion channel and is composed of five subunits. So far 16 different subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, θ) have been cloned from the mammalian nervous system (Möhler, 2006). However, the unique functional and pharmacological profile conferred by each of the various subunits is not well understood. $GABA_A$ receptors containing the δ subunit are restricted to

extrasynaptic somatic and dendritic membranes (Nusser et al., 1998). An already known feature of the subunit delta of $GABA_A$ receptors is the sensitivity to neuroactive steroids, which have been implicated in the genesis of depression and anxiety disorders (Spigelman et al., 2003; Stell et al., 2003; Eser et al., 2006). In the hippocampus, neuroactive steroids seem to specifically modulate an inhibitory conductance via δ subunit containing $GABA_A$ receptors (Stell et al., 2003). GABA itself is already known to be involved in depression (for review see: Shiah and Yatham, 1998) and Merali et al. could show a dysregulation of $GABA_A$ receptors subunits, including the δ subunit, in the frontal cortex of depressed suicide victims (Merali et al., 2004). Moreover, the subunit is expressed in brain regions involved in anxiety and depression, like the amygdala and the dentate gyrus of the hippocampus (Fujimura et al., 2005; Persohn et al., 1992; Peng et al., 2002).

Glucocorticoid modulatory element binding protein 1 (Gmeb1): For the Gmeb1 gene we could not validate the microarray data in the analysed regions.

The glucocorticoid modulatory element binding protein 1 is a highly conserved structure of high interest in respect of its multiple activities (Zeng et al., 2000; Chen et al., 2002). It has already been identified as part of a 550-kDa heterooligomeric protein complex that binds to the glucocorticoid modulatory element (GME), a tyrosine aminotransferase gene sequence (Oshima et al., 1995). The binding of Gmeb1 to GME is highly associated with its ability to modulate glucocorticoid receptor (GR)-regulated gene induction (Oshima and Simons, 1992; Kaul et al., 2000). Additionally, Gmeb1 interacts with CREB-binding protein, which coordinates the function of multiple transcription factors (Kaul et al., 2000; Leahy et al., 1999). Moreover, it has been characterised as a member of a family of transcription factors called KDWK proteins or SAND domain proteins (Kaul et al., 2000; Bottomley et al., 2001). The SAND domain is found in a number of nuclear proteins, which are known to be involved in chromatin-dependant transcriptional control (Bottomley et al., 2001). In other contexts than transcriptional activity, Gmeb1 has been found to bind the heat shock protein 27, which is an antiapoptotic protein (Thériault et al., 1999; for review see: Garrido et al., 2001). Further, it has been identified as being involved in *Parvovirus* replication (Christensen et al., 1997). The highest abundance of Gmeb1 is present in fetal and developing tissues, which suggest a role in developmental processes (Zeng et al., 2000).

Gastrin releasing peptide (Grp): In paroxetine-treated animals we could observe a significant upregulation of gastrin releasing peptide mRNA in the dentate gyrus. These results are in line with the microarray data.

Bombesin is a 14 amino acid peptide isolated in 1971 from the skin of a frog (Anastasi et al., 1971). Some years later the mammalian 27 amino acid homologous counterpart was identified, the gastrin releasing peptide (McDonald et al., 1979; Spindel et al., 1984). The expression of Grp mRNA is widespread throughout the brain, with high levels being found in the isocortex, the hippocampus, the amygdala, the thalamus and the hypothalamus (Wada et al., 1990). The distribution of the gastrin releasing peptide receptor, a member of the G-protein coupled receptor superfamily, is similar to its ligand (Moody and Merali, 2004). The peptide is known as a gastrointestinal hormone, growth factor and neuropeptide (Lebacq-Verheyden et al., 1988). Moreover, gastrin releasing peptide is supposed to be involved in the physiological control of feeding behaviour (Stein and Woods, 1982; Ladenheim et al., 1996; Merali et al., 1999; Fekete et al., 2002), in the modulation of emotionally-motivated memory (Shumyatsky et al., 2002; Martins et al., 2005), in the mediation and/or modulation of the stress response (Kent et al., 1998; Merali et al., 2002), and in the control of hypothalamic-pituitary-adrenal hormone secretion (Garrido et al., 1998; Garrido et al., 1999; Garrido et al., 2002). Additionally, there is evidence that Grp and its receptor might also be involved in the neurochemical alterations associated with psychiatric disorders like depression and anxiety (Yamada et al., 2002; Roesler et al., 2006). Underlining this assumption it has been found that Grp infusion into the ventral hippocampus of rats increased extracellular GABA levels in the hippocampus (Andrews et al., 2000). The observed Grp increase in the dentate gyrus of chronic paroxetine-treated mice might therefore have an influence on hippocampal GABA neurotransmission. As antidepressant treatments are supposed to have the ability to increase GABA neurotransmission, the regulation of the gastrin releasing peptide might contribute to the therapeutical effect of paroxetine (Leung and Xue, 2003).

Nuclear receptor subfamily 3, group C, member 1 (Nr3c1) / glucocorticoid receptor (GR): For the GR gene *in situ* hybridisation analysis revealed a significant decrease in the dentate gyrus of the hippocampus after chronic paroxetine treatment, thus confirming the microarray data.

Under the control of the hypothalamic-pituitary-adrenal axis (HPA axis) cortisol (human)/corticosterone (rodent) is secreted from the adrenal cortex following stress exposure. Central in the feedback regulation of the HPA-axis are two receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). While the MR shows high, the GR shows a 10 times lower affinity for cortisol/corticosterone (DeRijk et al., 2002). Once bound by the ligand, both receptors dimerize and translocate to the nucleus, where they recruit co-activators to stimulate gene transcription (de Kloet, 2003). MR is supposed to respond to low,

basal circadian levels of circulating glucocorticoids, whereas GR mediates the feedback action of high-stress levels of glucocorticoids to restore disturbances induced by stress. Both receptors are highly abundant in the hippocampus, but the GR receptor is additionally more ubiquitously expressed in the brain, for instance in the cortex, the brain stem, the amygdala and the thalamus (de Kloet et al., 1990). Due to the essential function of GR in the feedback loop of the HPA-axis, a malfunction of the receptor has serious consequences. It is already well known that one characteristic of depressive disorders is a dysfunctional GR system (Barden, 2004). Existing data suggest that the function of the GR is reduced in major depression in the absence of clear evidence of a decreased GR expression. Further, antidepressant treatment seems to have direct effects on GR, leading to enhanced GR expression in animal studies (for review see: Pariante and Miller, 2001). Contrary to this data are findings of Brady et al., who investigated the effects of three different antidepressant drug classes (SSRIs, monoamine oxidase inhibitors (MAO) and α2-adrenergic receptor antagonists) on the expression levels of various genes in the rat brain. The SSRI and the MAO failed to alter GR levels in the hippocampus after 2 and 8 weeks of treatment (Brady et al., 1992). In contrast to these findings is the observed downregulation of GR in our experiment. This effect could be due to species or strain differences of the chosen animals, application differences or differences in the chemical structure and pharmacology of the administered antidepressant. Bjartmar and colleagues demonstrated that chronic treatment with different subclasses of antidepressants had different and region-specific effects on the direction of GR regulation. They found a significant decrease in GR expression in the dentate gyrus of rats after fluoxetine treatment, whereas a 5-HT1A-receptor agonist significantly increased GR mRNA levels (Bjartmar et al., 2000). Further elucidation of the molecular and biochemical mechanisms involved in GR changes in major depression might lead to new insights into the pathophysiology and treatment strategies of affective disorders (Pariante and Miller, 2001).

Preproenkephalin 1 (Penk1): After analysis of gene expression levels of Penk1 by *in situ* hybridisation we could confirm the microarray results. Penk1 mRNA was upregulated in the dentate gyrus and the amygdala of chronically antidepressant-treated mice.

Endogenous opioid peptides and their receptors are distributed throughout the central nervous system and various tissues of the mammalian organism (Janecka et al., 2004). The functions of opioid peptides reach from a modulatory role in gastrointestinal, autonomic and endocrine functions to the modulation of learning and memory (Akil et al., 1998). The known endogenous opioid peptides derive from three distinct protein precursors encoded by the proopiomelanocortin (POMC), prodynorphin (Pdyn) and preproenkephalin 1 (Penk1) genes

(Chaturvedi, 2003). Seven mature pentapeptides are in turn derived by proteolytic processing of Penk1: one copy of leu-enkephalin and six copies of met-enkephalin (Legon et al., 1982). The existence of three major opiate receptors has been proven: δ, μ and κ receptors (Kieffer and Gaveriaux-Ruff, 2002). Leu- and met-enkephalin have high affinities for δ receptors, ten-fold lower affinities for μ receptors and marginal affinity for κ receptors (Janecka et al., 2004). Neurons containing high levels of Penk1 mRNA are predominantly interneurons and are distributed in the limbic system like the septum, the hippocampus, the bed nucleus of stria terminalis and the amygdala (Chaturvedi, 2003; Wiedenmayer et al., 2002). In the hippocampus it has already been shown that high frequency stimulation of the dentate gyrus is associated with an increase of preproenkephalin mRNA in the granule cells, suggesting a role for hippocampal preproenkephalin in mediating functional plasticity (Roberts et al., 1997). Corresponding to these data we found a strong increase in the Penk1 mRNA in the dentate gyrus after chronic paroxetine treatment, which is supposed to enhance neurogenesis and plasticity in the hippocampus. The observed upregulation of Penk1 in the amygdala might contribute to the anxiolytic effects of paroxetine, as previous findings could show a potentiation of the anxiolytic effects of benzodiazepine by overexpression of preproenkephalin in the amygdala (Kang et al., 2000). Moreover, inhibition of enkephalin degrading enzymes was proven to exert antidepressant-like effects in various behavioural paradigms (Baamonde et al., 1992; Smadja et al., 1995; Jutkiewicz et al., 2006). The antidepressant-like and anxiolytic effects are thought to be mediated through the δ opioid receptor (Jutkiewicz, 2007). In contrast, mice deficient in the preproenkephalin gene are more anxious compared to wildtype mice, but show no depression-related phenotype (König et al., 1996; Bilkei-Gorzo et al., 2007). Interestingly, there is a neurobiological link between the opioid system and the Cckb receptor (see page 44), as the antidepressant-like effect evoked by inhibiting enkephalin degrading enzymes is enhanced by a Cckb receptor antagonists (Roques, 2000).

Plasminogen activator, tissue (Plat): Finally, we were able to validate the microarray data regarding the tissue plasminogen activator gene. *In situ* hybridisation analysis could reveal an upregulation in the dentate gyrus after paroxetine administration as well.

Plasminogen activators are serine proteases that cleave the inactive zymogen plasminogen into the active protease plasmin, which is capable of degrading a broad spectrum of substrates (Melchor and Strickland, 2005). The primary function of plasmin is degrading fibrin, which is involved in blood clotting and activating matrix metalloproteinases that, in turn, degrade the extracellular matrix (Collen, 1999). Besides these functions, there is growing

evidence that Plat also plays a role in the CNS (Benchenane et al., 2004). In the adult mouse brain tissue plasminogen activator mRNA is highly expressed in regions involved in autonomic and endocrine functions (hypothalamus), anxiety (amygdala), and learning and memory (hippocampus) (Melchor and Strickland, 2005). In the mouse hippocampus it has been reported that Plat converts the precursor proBDNF to the mature BDNF (brain-derived neurotrophic factor) by activating plasmin (Pang et al., 2004). Regarding the assumed role of the neurotrophic factor BDNF in the pathophysiology of depression and antidepressant treatment effects, the paroxetine-induced observed increase of Plat in the hippocampus might contribute to the neuroplasticity-enhancing effects of antidepressant drugs (Duman and Monteggia, 2006). Investigations about the role of Plat in the amygdala showed a significant upregulation of total Plat activity in the amygdala after acute restraint stress (Pawlak et al., 2003). In a following study it could be revealed that Plat activity in the amygdala is upregulated by corticotropin-releasing factor, a major stress neuromodulator (Matys et al., 2004). In contrast, Plat deficient mice display an impaired response to stress and a maladaptive hormonal stress response (Pawlak et al., 2003). These results indicate that Plat might play a role in emotional learning and might contribute to the control of hormonal stress response.

Taken together we were able to validate nine of eleven genes from the microarray performed by Sillaber et al. and could therefore confirm a high reliability of the microarray results. With respect to the problems of microarray analysis in general in producing reliable data due to many aspects of the analysis, which are error-prone, our validation rate is quite high (Asyali et al., 2004). Our data may help to achieve a better understanding of the pharmacological action of the selective serotonin reuptake inhibitor paroxetine on various gene expression levels and signalling cascades. The knowledge of so far unknown genes, which are associated with the mechanism of action of antidepressant drugs might help to improve their efficacy or identify novel targets for a better therapy of depressive disorder in the future.

2. Transcriptional changes following paroxetine administration are dependent on the duration of treatment and the neuroanatomical region

Depressive disorders are among the most prevalent and costly diseases of the central nervous system worldwide with a serious impact on the affected peoples quality of life (Üstün et al., 2004). Despite decades of research in that field, progress in understanding the underlying neurobiology is slow, and still relatively little is known about both the disease-relevant pathophysiological processes and the mechanisms of action of current treatment strategies. Although there already are a number of safe and effective antidepressant drugs available, co-morbidities, treatment resistance and the latency until the first clinical signs of improvement often significantly complicate successful treatment and clinical management of these severe and life-threatening disorders. The development of novel antidepressant substances which acted rapidly and safely in a high proportion of patients therefore is an unmet need in current drug discovery research (Nemeroff and Owens, 2002; Taylor et al., 2005).

The most widely used antidepressants, originally discovered by serendipity about 50 years ago, achieve their effect by targeting monoaminergic neurotransmitter systems and, in particular, the serotonergic system. The postulated mechanism of action of selective serotonin reuptake inhibitors (SSRIs) is to increase the extracellular levels of serotonin by inhibiting its reuptake into the presynaptic cell. Interestingly, antidepressant drugs affect their presumed target system already within hours of initial treatment which is in marked contrast to the fact that the clinical effects of antidepressants take several weeks to develop. This latency of antidepressant drug action strongly supports the hypothesis that antidepressant treatment induces complex adaptive changes in brain structures affected by depression, including regulation of neural gene expression (Celada et al., 2004). In support of this hypothesis, there is growing evidence that chronic, but not acute, treatment with different classes of antidepressant drugs increases cell proliferation as well as neurogenesis in the hippocampus (for review see: Duman et al., 2001; Malberg, 2004; Paizanis et al., 2007).

So far, the majority of approaches to investigate the underlying neurobiology of depression are hypothesis-driven and are largely based on the presumed effects of existing therapeutic agents. Over the last years these approaches were successful in developing a growing number of more and more selective drugs targeting brain monoaminergic systems. However, they do not allow for the identification of novel and innovative drug targets, which, at best, will provide the basis for the development of causal treatment strategies with a more rapid onset of antidepressant action in the future.

In order to identify those potential novel target genes unbiased, hypothesis-free approaches such as proteomics, large-scale genetic or microarray studies are currently undertaken (for review see: Sequeira and Turecki, 2006). For reasons of genetic homogeneity and minimisation of confounding variables, such investigations are preferably performed in animals. Several pharmacogenomic studies using cDNA microarrays have been conducted mostly in rats to uncover the molecular mechanisms underlying the actions of different antidepressant drugs, such as fluoxetine (Conti et al., 2007), imipramine (Wong et al., 2004), paroxetine and mirtazapine (Landgrebe et al., 2002). However, none of these studies investigated whether the applied antidepressant treatment schedule elicited antidepressant-like behavioural effects in the animals.

In an attempt to analyse the interaction between genes, behaviour and response to psychoactive substances, Sillaber et al., (submitted) investigated antidepressant-responsive genes in the mouse hippocampus by microarray analysis. Antidepressant-responsive DBA/2OlaHsd mice were treated for 4 weeks with paroxetine, an SSRI which is commonly and effectively used to treat clinical depression and anxiety disorders (Feighner et al., 1993; Nemeroff, 1994), thus mimicking the clinical situation. In a first step, Sillaber et al. could show that chronic paroxetine treatment exerted clear antidepressant-like effects in specific behavioural paradigms, providing the basis for a subsequent hypothesis-free discovery of novel genes relevant for the antidepressant efficacy of this drug. Therefore hippocampal brain tissue was analysed by means of genechip microarray analysis. Previous findings suggest that the behavioural effects of chronic administration of antidepressants may be mediated by the stimulation of neurogenesis in the hippocampus (Santarelli et al., 2003), indicating the hippocampus as a region of high interest for microarray analyses.

The present study focuses on the characterisation of three selected genes that have been shown to be significantly regulated by chronic paroxetine treatment (Sillaber et al.), namely the endogenous opioid precursor preproenkephalin 1 (Penk1), tissue plasminogen activator (Plat) and the delta subunit of the $GABA_A$ receptor (Gabrd). Penk1 is of high interest due to the already known implication of opioids in mood disorders, Plat has been shown to be involved in aspects of neuronal plasticity and activation of GABA receptors causes a broad range of physiological and behavioural processes. In contrast to the majority of pharmacogenomic studies published so far, we were not only interested in validating the results obtained by expression profiling (see project 1), but we went further to systematically analyse two major questions: 1) the time course and onset of gene expression regulation, i.e. whether the genes of interest are regulated acutely, following one week or four weeks of

paroxetine administration and 2) the regional specificity of gene expression changes induced by paroxetine. The latter aspect takes into account that one of the major needs in the field of depression research is a better understanding of the involved neural circuits in the brain. Particularly interesting are the circuits that control mood under normal circumstances, that mediate abnormalities in mood seen in depression and that are targeted by the molecular and cellular effects of antidepressant treatment. Based on the complexity of depressive symptoms, it is likely that the pathophysiology of this disorder and the mechanisms by which currently available treatments reverse its symptoms, involve numerous brain regions and remodelling of neuronal circuits (Yamada 2005).

Corresponding to the known delayed onset of antidepressant drug action, the most prominent effects on gene regulation were observed in the hippocampus following 28 days of paroxetine administration (see project 1). The mRNA of the $GABA_A$ receptor delta subunit was found to be significantly regulated already following 7 days of antidepressant treatment, whereas no acute or subchronic effects of paroxetine on the expression of the tissue plasminogen activator gene could be observed. The effects of paroxetine on the expression of preproenkephalin were shown to be dependent on the duration of treatment: both subchronic and chronic (project 1) treatment significantly increased preproenkephalin expression in the hippocampal dentate gyrus with the extent of mRNA increase being correlated to the duration of paroxetine administration. Additional acute effects of paroxetine on the expression of preproenkephalin in the amygdala could be observed; interestingly, the direction of gene regulation after acute treatment was in the opposite direction compared to the effects of subchronic and chronic treatment.

Results

Transcriptional changes of preproenkephalin 1 mRNA following paroxetine are dependent on duration of treatment and neuroanatomical region

Acute treatment with paroxetine significantly downregulated Penk1 mRNA in the amygdala (t-test, $p<0.05$) (figure 12A), whereas no difference could be observed in the CA1 region and the dentate gyrus of the hippocampus (figure 12A).

Both subchronic and chronic paroxetine treatment (see project 1) induced a significant increase in preproenkephalin 1 mRNA levels in the dentate gyrus of the hippocampus (t-test, $p<0.05$) (figure 12B). In chronically treated animals, a significant increase in Penk1 mRNA could also be observed in the amygdala (project 1, figure 10A).

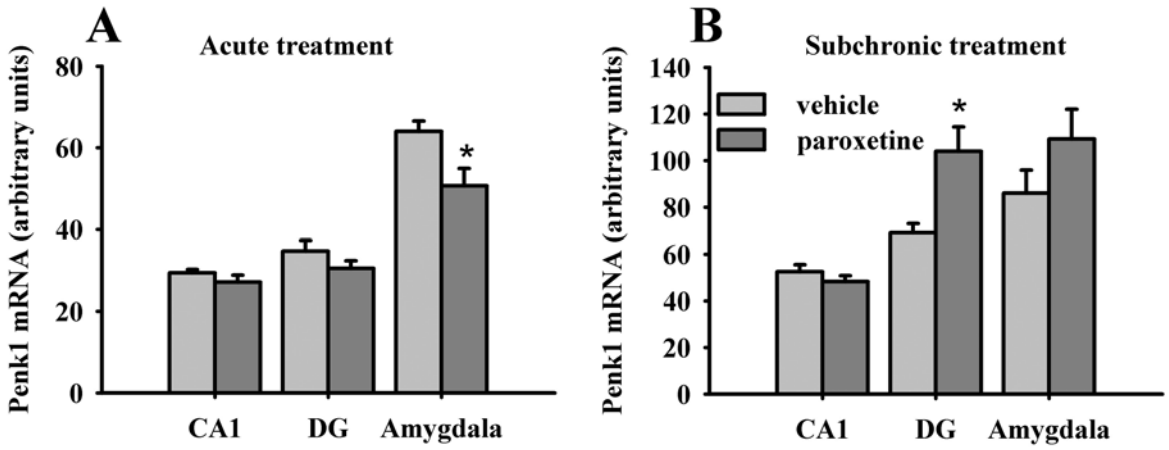


Figure 12:
Expression levels of Penk1 mRNA in the hippocampus and the amygdala after acute (A) and subchronic (B) paroxetine treatment compared to the control group. Penk1 mRNA in the CA1, dentate gyrus (DG) and the medial amygdaloid nucleus, posteroventral part (MePV) (A,B). n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Downregulation of $GABA_A$ receptor subunit delta mRNA after subchronic and chronic paroxetine treatment

Acute treatment with paroxetine did not result in an alteration in the expression level of the $GABA_A$ receptor subunit delta mRNA in any of the brain regions examined (CA1 and the dentate gyrus of the hippocampus, cortex and thalamus) (figure 13A). Subchronic paroxetine treatment significantly downregulated $GABA_A$ receptor subunit delta mRNA in the dentate gyrus of the hippocampus and the CA1 region (t-test, $p<0.05$) (figure 13B). However, no differences could be observed in the cortex and the thalamus (figure 13B). As already mentioned before (project 1) after chronic paroxetine administration Gabrd mRNA was

significantly decreased in the dentate gyrus of the hippocampus and the cortex when compared to the control group (figure 6A).

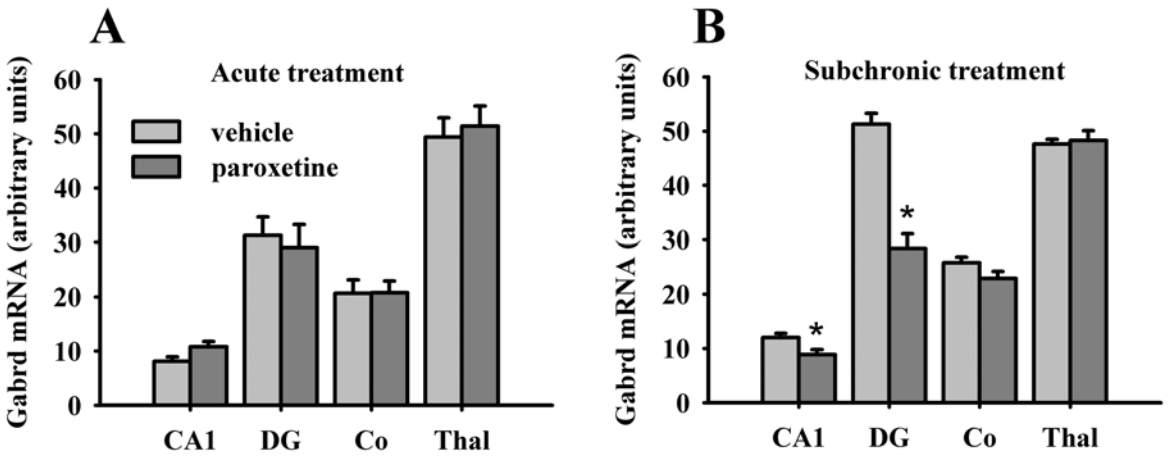


Figure 13:
Expression levels of Gabrd mRNA in the hippocampus, the cortex (Co), and the thalamus (Thal) after acute (A) and subchronic (B) paroxetine treatment compared to the control group. n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Hippocampal tissue plasminogen activator (Plat) mRNA expression is regulated following chronic paroxetine treatment

Neither acute nor subchronic treatment with paroxetine resulted in any change in the expression of tissue plasminogen activator mRNA levels in the CA1, CA3 and the dentate gyrus of the hippocampus or the cortex (figure 14A and B).

Chronic paroxetine treatment significantly induced tissue plasminogen activator mRNA levels in the CA1 and the dentate gyrus of the hippocampus compared to vehicle treated mice (see project 1, figure 11A), whereas no differences were found in the CA3 of the hippocampus and the cortex.

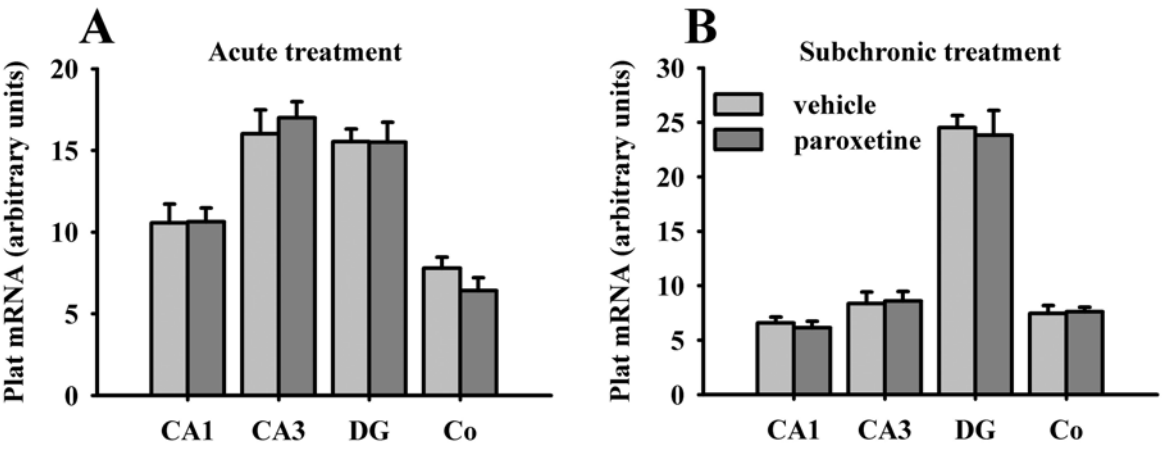


Figure 14:
Expression levels of Plat mRNA in the cortex and the CA1, CA3 and the dentate gyrus (DG) of the hippocampus after acute (A) and subchronic (B) paroxetine treatment compared to the control group. n=9 per group. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Discussion

The present study revealed the regional and temporal specificity of paroxetine-induced gene regulation in the adult mouse brain. Three potential novel antidepressant target genes, Gabrd, Penk1 and Plat that previously had been identified in a reverse pharmacological hypothesis-free approach were shown to be specifically and selectively regulated in different neuroanatomical subregions of the hippocampus, the amygdala, the cortex and the thalamus. Those brain areas have previously been demonstrated to be affected by changes in blood flow or related measures in depressed patients and have been implicated in a neural circuitry of mood disorders. As potential target sites of antidepressant action, those neuroanatomical regions are of particular interest when investigating the molecular and cellular effects of antidepressant treatment (Drevets, 2001).

Although antidepressants have been used for more than 50 years, knowledge about their molecular mechanisms of action is still limited. The same holds true for the neurobiological mechanisms and pathophysiological processes underlying the development and maintenance of human affective disorders. During the last years, more and more findings revealing neuroplastic and complex adaptive effects of antidepressant drugs have begun to challenge the "monoamine hypothesis of depression" (for review see: Millan, 2004; Berton and Nestler, 2006), and two major aspects might now be considered as widely accepted in psychopharmacology research: 1) that successful antidepressant treatment strategies result in molecular and cellular adaptation processes that are downstream of their presumed acute effects on monoaminergic neurotransmission (Vaidya and Duman, 2001) and 2) that given the complexity and diversity of depressive symptoms, it is likely that the pathophysiology of the disorder and the mechanisms by which successful treatments reverse its symptoms involve numerous brain regions interacting in neuronal circuits.

Region-specific regulation of novel antidepressant target genes is dependent on duration of paroxetine-treatment: the endogenous opioid system as an "early response" candidate

The SSRI paroxetine is one of the most widely used antidepressant drugs to treat depression and anxiety disorders. Paroxetine acutely enhances serotonergic neurotransmission by the potent and selective inhibition of the serotonin transporter (SERT) resulting in a decreased neuronal reuptake of serotonin. It has also been shown to be a weak inhibitor of norepinephrine reuptake (Nemeroff and Owens, 2003). After acute application peak plasma concentrations are reached in humans about five hours after oral administration, whereas steady-state plasma concentrations are reached after 7 to 14 days of treatment (Gunasekara et

al., 1998). While the preclinical *in vivo* pharmacology of paroxetine is consistent with its primary action as an antagonist of the SERT, the typical delay of 3-5 weeks following initiation of antidepressant treatment until a clinical response can be observed has stimulated the interest in elucidating the neurochemical and molecular changes that occur following chronic antidepressant administration.

At the level of serotonergic neurotransmission, acute application of SSRIs in rats results in an inhibition of neuronal firing and reduced release of serotonin (Hjorth and Auerbach, 1994; Hjorth and Auerbach, 1996; Hajós et al., 1995). It is assumed that this effect arises from a blockade of serotonin reuptake in the raphe nuclei, which is followed by an indirect activation of somatodentritic 5-HT1A autoreceptors. This receptor activation is thought to counteract the enhancing effect of paroxetine on serotonergic neurotransmission. Corresponding to the delayed onset of the therapeutic action of paroxetine in treatment of depression, the activation of the 5-HT1A autoreceptors become desensitised after approximately two weeks and the firing rate of serotonergic neurons returns to normal. Moreover, adaptive changes, like decreased responsiveness of synaptic serotonergic receptors, which in turn leads to enhanced serotonin release, occur after two to three weeks of treatment as well (Gunasekara et al., 1998). Those differences between acute and long-term adaptive changes in neurotransmission and receptor properties might be responsible for the observed differences in downstream gene regulation following increasing duration of treatment. In the present investigation, the three novel antidepressant target genes, Gabrd, Penk1 and Plat, were shown to be regulated in the mouse hippocampus following chronic (4 weeks) paroxetine treatment (project 1), paralleling the antidepressant-like behavioural effects in this unbiased study design (Sillaber et al.). However, genes that are already regulated following 1 week of paroxetine administration or even acutely could be early antidepressant-responsive genes, and, as early players in a complex cascade of antidepressant-regulated genes, could be of interest to further investigate. We therefore extended our analyses and examined whether Gabrd, Penk1 and Plat are regulated in the brains of mice that received the antidepressant for 1 week or acutely. Only Penk1 could be shown to be regulated in the mouse amygdala following a single administration of paroxetine. Interestingly, the direction of gene regulation after acute treatment was in the opposite direction compared to the effects of subchronic and chronic treatment: acute treatment resulted in a downregulation of Penk1 mRNA in the amygdala, whereas the extent of mRNA increase in the hippocampal dentate gyrus was correlated to the duration of paroxetine administration.

The endogenous opioid system and the neuropeptide Penk1 in particular, have already been recognized as potential drug target in mood disorders as inhibition of enkephalin degrading enzymes proved to exert antidepressant-like effects in various behavioural paradigms (Baamonde et al., 1992; Smadja et al., 1995; Jutkiewicz et al., 2006).

Endogenous opioid peptides are the naturally occurring ligands for opioid receptors and are synthesised in the central nervous system and in various glands throughout the body, such as the adrenal glands and the pituitary. Opioid peptides function both as neuromodulators and as hormones and, therefore generate a variety of physiological effects (Janecka et al., 2004). The known endogenous opioid peptides derive from three distinct protein precursors encoded by proopiomelanocortin (POMC), prodynorphin (Pdyn) and preproenkephalin 1 (Penk1) genes (Akil et al., 1984). The high-affinity δ and μ opioid receptors for the mature pentapeptides leu-and met-enkephalin are ubiquitously expressed throughout the brain, including the limbic system (Gulya et al., 1986; Moskowitz and Goodman, 1984). Neuropeptides commonly occur with, and are complementary to, classic neurotransmitters. (Hökfelt et al., 2003). Penk1-derived peptides have been shown to inhibit GABA release from inhibitory interneurons in the hippocampal formation which, in turn, results in increased excitability of dentate gyrus granule cells and hippocampal pyramidal cells (Simmons and Chavkin, 1996). Moreover, it has been reported that stimulation of the dentate gyrus or NMDA release in the region of granule cell dendrites causes a dramatic increase in preproenkephalin mRNA (Morris and Johnston, 1995). Accordingly, electroconvulsive seizures (ECS) have been shown to increase the expression of endogenous opioid peptides in various brain regions, including the hypothalamus (Yoshikawa et al., 1985) and the limbic system (Kanamatsu et al., 1986). Even if the clinical antidepressant effects of ECS and antidepressant drugs are very similar, their presumed mode of action might not necessarily be the same. To the best of our knowledge, there is no data on antidepressant-induced modulation of Penk1 expression in the limbic system so far. Our finding that paroxetine treatment, either acute, subchronic or chronic (project 1), significantly and specifically modulates the expression of Penk1 in the amygdala and the hippocampal formation adds further evidence to the potential involvement of the endogenous opioid system in mood disorders. Opioids might act as mediators of hippocampal plasticity and might be involved in neuroplastic phenomena that are most likely to underlie successful antidepressant therapy.

Another important aspect to discuss is the regional specificity of paroxetine-induced gene regulation. Our findings underline the complexity of antidepressant-induced neurobiological effects, which are dependent on the neuroanatomical region or duration of treatment. There is

only a very limited number of studies investigating region-dependent neurobiological effects of antidepressant treatment so far (D'Sa et al., 2005; Serres et al., 2006; Dagestad et al., 2006; Conti et al., 2007). Most studies focus on one particular region of interest. However, to advance our understanding of the therapeutic actions of antidepressants, we must now extend our efforts beyond theories based on dysfunction of selected neuroanatomical regions. While many brain regions have been implicated in regulating emotions, we still have a very rudimentary understanding of the neural circuitry underlying normal mood and the abnormalities in mood that are the hallmark of depression. It is likely that many brain regions together mediate the diverse symptoms of depression (Drevets, 2001). Of course, these various brain regions operate in a series of highly interacting parallel neuronal circuits.

The delta subunit of the $GABA_A$ receptor as antidepressant target gene: potential involvement of neurosteroids

We identified the $GABA_A$ receptor subunit delta mRNA to be significantly downregulated in the hippocampal dentate gyrus following chronic paroxetine treatment (project 1). Validation of these data by means of *in situ* hybridisation provided evidence that the $GABA_A$ receptor subunit delta mRNA was also downregulated in the cortex of paroxetine-treated mice. We could further show that downregulation of Gabrd mRNA in the dentate gyrus was already significant following 7 days of treatment. In contrast, acute antidepressant treatment had no effects on the expression level of this gene.

$GABA_A$ receptors and GABAergic mechanisms are already known to be directly implicated in behavioural and physiological processes (Shiah and Yatham, 1998; Korpi et al., 2002), and have been suggested to play a role in human affective disorders (Brambilla et al., 2003). It has been reported that chronic administration of antidepressants (i.e. phenelzine and imipramine) or benzodiazepines may differentially modulate the gene expression of GABA receptor subunits in rat brain (Heninger et al., 1990; Kang and Miller, 1991; Tanay et al., 1996). These studies showed that the modulation of GABA receptor subunits may vary in different brains regions, suggesting a regional heterogeneity that may be implicated in the mechanisms of action of antidepressants in mood disorder patients. However, there was no evidence so far supporting the $GABA_A$ receptor subunit delta mRNA as antidepressant target gene. $GABA_A$ receptors mediate the majority of fast inhibitory neurotransmission in the brain. They are composed of a combination of five different subunits exhibiting specific individual expression patterns in distinct brain regions and even in their subcellular localisation. Furthermore, each receptor subtype shows a unique pharmacological profile and therefore many drug binding

sites exist (McKernan and Whiting, 1996; Korpi et al., 2002). Interestingly, GABA receptors containing the δ subunit are insensitive to diazepam and are located exclusively at extrasynaptic sites as shown in the hippocampal dentate gyrus and cerebellum, the thalamus, cortical areas and the striatum (Wisden et al., 1992; Fritschy and Möhler, 1995; Pirker et al., 2000; Sperk et al., 1997). They are tailor made for tonic inhibition, due to their high affinity for GABA and slow desensitization kinetics (Möhler, 2006). GABA receptors containing the δ subunit are the target of neuroactive steroids such as allopregnanolone, dehydroepiandrosterone (DHEA) or 3α,5α-Tetrahydrodeoxycorticosterone (3α,5α-TH DOC), which are synthesised in the central nervous system (Stell et al., 2003). Neuroactive steroids are potent modulators of $GABA_A$ receptors and specifically enhance a tonic inhibitory conductance in hippocampal neurons (for review see: Belelli and Lambert, 2005; Stell et al., 2003). Additionally, it has been reported that the effects of neuroactive steroids are reduced in δ subunit knockout mice (Mihalek et al., 1999; Spigelman et al., 2003). Changes in neurosteroid levels are associated with various physiological and pathophysiological conditions including stress, pregnancy and postpartum depression as well as ageing. Moreover, neurosteroids have been shown to exert potent anxiolytic/antidepressant-like effects in different behavioural paradigms: allopregnalonone, for example, exerts antidepressant-like effects in the mouse forced swim test (Khisti and Chopde, 2000; Khisti et al., 2000). Dysregulation in the neurosteroid response may, therefore, predispose for the development of mood disorders (Stell et al., 2003; Girdler and Klatzkin, 2007). Concerning a potential role of neurosteroids in mood disorders, the dominant idea emerging from clinical and preclinical studies is that neurosteroids could be involved in the pathophysiology of affective disorders as well as in the mechanism of action of SSRI (for review see: Brambilla et al., 2003; Van Broekhoven and Verkes, 2003; Pinna et al., 2006). Indeed, it was previously shown that antidepressant treatment, especially with SSRIs, increases the concentrations of neurosteroids in the cerebrospinal fluid or plasma of patients suffering from major depression (Uzunova et al., 1998; Ströhle et al., 2002). Moreover, fluoxetine potentiates the antidepressive effect of allopregnalonone by enhancing the GABAergic tone but, remarkably, not by enhancing serotonergic neurotransmission (Khisti and Chopde, 2000). Our finding that paroxetine treatment regulates the expression of the $GABA_A$ receptor δ subunit mRNA in the mouse hippocampus might therefore add further evidence to the involvement of neurosteroids and the Gabrd in the mechanism of action of antidepressant drugs, the more as the δ subunit was the only GABA receptor subunit that was found to be significantly regulated by paroxetine treatment in the microarray analyses (data not shown, Sillaber et al., submitted).

The observed changes in the expression level of the GABA receptor δ subunit might be discussed in the context of altered circulating neurosteroid levels following chronic antidepressant treatment. Previous studies revealed a rapid and specific regulation of the δ subunit during the estrous cycle (Maguire and Mody, 2007) and following stress (Maguire and Mody, 2007). Those two conditions are known to be associated with changes in brain neurosteroid content. However, those experimental conditions are different from our study design regarding the variations in circulating neurosteroid concentrations occur regularly (during the estrous cycle) or very rapidly (already 30 minutes following acute stress).

Tissue plasminogen activator mediates synaptic plasticity and BDNF action

The third potential antidepressant target gene in our focus is the tissue plasminogen activator (Plat or tPA). Plasminogen activators are serine proteases of tryptic specifity that catalyse the conversion of the inactive proenzyme plasminogen into the active serine protease plasmin. Plasmin has a very broad spectrum of substrates and is capable of degrading indirectly or directly most of the extracellular proteins (Vassalli et al., 1991). The primary substrate of active plasmin in the vasculature is fibrin. Plasmin breaks down fibrin clots, aiding in vascular patency and haemostasis (Melchor and Strickland, 2005). Beside its fibrinolytic function, there is growing evidence that Plat also plays an intriguing role in the CNS (Benchenane et al., 2004). It has been shown that Plat is secreted by neurons during neurite outgrowth and tissue remodelling. Moreover, several lines of evidence suggest that Plat activity might be important for learning-related synaptic plasticity. Baranes et al. could show that Plat contributes to the late phase of LTP and induces both axonal elongation and the production of new synaptic structures in the hippocampal mossy fibre pathway (Baranes et al., 1998). One potential mechanism by which Plat exerts its effects on synaptic growth is the conversion of the BDNF precursor to the mature BDNF by activation of plasmin. Such conversion is critical for late-LTP expression in mouse hippocampus (Pang et al., 2004; Melchor and Strickland, 2005). Taking into account that the precursor and the mature BDNF can have opposite biological effects (pro-apoptotic *versus* neurotrophic effects), proteolytic processing of the precursor by the Plat/plasminogen system represents an interesting mechanism to control the direction of BDNF action (Teng et al., 2005). The potential implications of the striking degree of overlap between the molecular and cellular changes induced by antidepressant treatment and the molecular mechanisms of neuroplasticity, especially in synaptic plasticity have been reviewed in detail most recently (Pittenger and Duman, 2008). The involvement of Plat in synaptic plasticity and the BDNF pathway in particular has encouraged us to study the

antidepressant-induced regulation of Plat expression in more detail. Chronic (see project 1), but neither acute nor subchronic treatment with paroxetine resulted in a significant upregulation of Plat mRNA in the dentate gyrus and the CA1 area of the hippocampal pyramidal cell layer. This paroxetine-induced upregulation of Plat could lead to an increase in mature BDNF protein, which, in turn, has been supposed to normalise depression-associated dysfunction of neuronal circuits by supporting synaptic and neuronal remodelling and increasing hippocampal neurogenesis (Duman and Monteggia, 2006). Interestingly, the well described antidepressant-induced upregulation of BDNF has recently been shown to occur following chronic (three weeks of treatment), but not acute antidepressant treatment, thus paralleling the time course of paroxetine-induced regulation of Plat in the present analysis (Khundakar and Zetterstrom, 2006).

In summary, our present study was designed for identifying molecular events downstream of direct antidepressant actions on monoamines (i.e. downstream of paroxetine's direct action on serotonine reuptake), thus generating new theories about the pathophysiology of depression and the potential action of antidepressant medication. We could confirm and further investigate three potential novel antidepressant target genes which previously had been identified by means of a hypothesis-free approach (see project 1). The three candidates Penk1, Plat and Gabrd show distinct patterns with respect to temporal specificity of gene expression regulation, suggesting that there are "early" and "late" responder genes to antidepressant treatment: as Penk1 is already regulated following acute and subchronic paroxetine treatment, it may represent a novel target for faster onset antidepressant drugs. The Gabrd is crucially important for the action of neurosteroids which have been shown to be involved in emotional regulation. Plat is a highly interesting antidepressant target gene with respect to its involvement in the BDNF pathway and mechanisms of neuroplasticity.
Furthermore, all three genes show region-specific patterns of antidepressant-induced expression changes. This regional specificity underlines the importance to extend our efforts beyond theories based on dysfunction of selected neuroanatomical regions and to study neuronal circuits when investigating the complex mechanisms by which successful treatments might reverse depressive symptoms. Ongoing studies will continue to further elucidate the role of the three novel candidate genes in antidepressant action, finally aiming at the development of more specific and effective therapeutic interventions with a more rapid onset of action in the future.

3. Activin βA is induced by chronic paroxetine treatment and exerts antidepressant-like effects in vivo

Affective disorders are among the leading causes of morbidity and mortality world-wide (Kessler et al., 2003; Andlin-Sobocki and Wittchen, 2005). Since the serendipitous discovery of tricyclic antidepressants about half a century ago, the lack of a clear understanding of the pathophysiological mechanisms underlying depression has prevented the development of drugs targeting specific proteins. Theories about the mechanisms of action of currently available antidepressants have focused primarily on their effects on synaptic neurotransmission such as inhibition of neurotransmitter uptake (e.g. selective serotonin reuptake inhibitors). Those currently available antidepressant drugs are safe and effective, but only less than half of the patients attain complete remission after therapy with a single antidepressant drug after 6-8 weeks (Nemeroff and Owens, 2002). Moreover, the vast majority of patients does not respond until 3-5 weeks after initiation of the treatment. It has been hypothesised that this delay in the onset of action of existing antidepressant drugs is due to the fact that they initially act on substrates (e.g. neurotransmitter uptake) considerably upstream of yet unknown targets, which are ultimatively responsible for the antidepressant effects. In this context one of the major systems postulated to mediate the delayed adaptive effects of antidepressants are neurotrophic signalling cascades (Nestler et al., 2002).

In an unbiased approach to identify potential novel genes and signalling pathways mediating antidepressant-like effects, Sillaber et al. (submitted 2007) performed a brain-region specific microarray analysis for large-scale gene expression profiling in antidepressant-responsive DBA/2OlaHsd mice treated 4 weeks with the SSRI paroxetine. In a first step they could show that chronic paroxetine treatment exerted clear antidepressant-like effects in specific behavioural tests providing the basis for a subsequent hypothesis-free discovery of novel genes relevant for the antidepressant efficacy of paroxetine. Among other interesting potential targets, Sillaber and co-workers could show that a group of genes that are involved in neurogenesis as well as in neuronal plasticity (e.g. BDNF, Egr1, Egr1 and Stat3) and neuronal cell differentiation (e.g. Vimentin) are significantly upregulated by chronic paroxetine treatment, supporting the intriguing hypothesis that neurotrophic factors and adult neurogenesis play critical roles in mediating the behavioural responses to antidepressants (for review see: Duman and Monteggia, 2006).

One of the genes that was found to be significantly upregulated by chronic paroxetine treatment was activin βA (Sillaber et al., 2007). Activins and inhibins are members of the transforming growth factor β superfamily and are dimeric proteins composed of α- and/or β-

subunits. Activins are homo- or heterodimers of activin β-subunits, whereas inhibins are heterodimeric proteins composed of one β-subunit which is linked to the structurally related α-subunit by disulfide bounds (for review see: Luisi et al., 2001; Bernard et al., 2001). Activin has originally been shown to control many physiological processes such as cell proliferation, cell differentiation, immune responses, wound repair and various endocrine activities (for review see: Luisi et al., 2001; Munz et al., 1999). Inhibin α, activin βA and activin βB mRNAs were detected in a variety of organs: in the bone marrow, kidney, spinal cord and in the brain (Meunier et al., 1988). Activin signalling is mediated by two different transmembrane receptor serine/threonine kinases (type 1 and type 2 receptors) whose expression is relatively widespread, with prominent expression sites in the central nervous system (e. g. the hippocampal dentate gyrus and other areas of the limbic system in the case of activin type 2 receptors, Acvr2a) (Cameron et al., 1994). Binding of activin to type 2 receptors (Acvr2a or Acvr2b) is followed by phosphorylation and activation of type 1 receptors (Acvr1 and Acvr1b), which in turn triggers a transient association and phosphorylation of SMAD2 or 3. This leads to a translocation to the nucleus, enabling the SMADs to target a variety of DNA binding proteins regulating transcriptional response (Pangas and Woodruff, 2000).

Despite the interesting expression pattern of activin and its receptors in the brain and particularly in the limbic system, there is only relatively sparse data on its putative effects on central nervous system function focussing on the role of activin A in neuronal development and neuroprotection. Activin supports neuron survival in primary cell cultures of rat hippocampus (Iwahori et al., 1997) and facilitates neuronal development (Trudeau et al., 1997). Furthermore, noxious stimuli such as kainic acid lesions of the hippocampus increase the expression of activin βA mRNA (Tretter et al., 1996) and infusion of activin A, in turn, was shown to reduce neuronal damage after excitotoxic brain injury (Tretter et al., 2000). Those neuroprotective properties of activin A are further supported by data from transgenic mice (dnActRIB) in which activin signalling is selectively disrupted in limbic brain areas. DnActRIB transgenic mice are significantly more vulnerable to the excitotoxin kainic acid than their control littermates (Müller et al., 2006). Most recently, Dow et al. described an increase of activin βA mRNA in the rat dentate gyrus following chronic electroconvulsive seizures. However, the investigators failed to detect an upregulation of activin mRNA following chronic antidepressant treatment with the SSRI fluoxetine (Dow et al., 2005).

Given the described neuroprotective properties, activin A is an interesting novel candidate gene as antidepressant target. Therefore, we selected – among a variety of other genes

regulated in the microarray experiment (Sillaber et al.) – activin A to be investigated in more detail and to examine its role in mediating the antidepressant-like effects of paroxetine. In a first step, we validated the antidepressant-induced upregulation of activin βA by means of *in situ* hybridisation (see project 1). In this study we additionally intended to examine the time-course of paroxetine-mediated gene regulation. We then investigated the behavioural effects of stereotactic infusion of activin A into the dentate gyrus or the amygdala to determine whether activin A exerts region-dependent antidepressant-like or anxiolytic effects in specific behavioural paradigms *in vivo*.

To further clarify the neurobiological mechanisms underlying the antidepressant-like effects of activin A, we studied whether activin A is able to modulate the electrophysiological properties of hippocampal neurons. We could show that even nanomolar concentrations of activin A significantly block hippocampal LTP, and that this blockade of LTP is not mediated via NMDA receptors. As we were finally able to identify genetic variants in the human activin signalling pathway that are associated with specific endophenotypes of depression, there is strong evidence that the activin system is an interesting candidate system for the development of novel antidepressant drugs in the future.

Results

Experiment 1:

Effects of acute and subchronic treatment with paroxetine on activin βA mRNA levels

Acute paroxetine treatment (10 mg/kg) had no significant effect on activin βA mRNA expression levels in the CA1, CA3 or the dentate gyrus of the hippocampus, nor in the cortex (figure 15A). In contrary, subchronic treatment with paroxetine resulted in an increase of activin βA mRNA levels in the CA1 and the dentate gyrus of the hippocampus as observed following chronic treatment (t-test, $p<0.05$), whereas additionally the activin βA gene was significantly downregulated in the CA3 (t-test, $p<0.05$) (figure 15B).

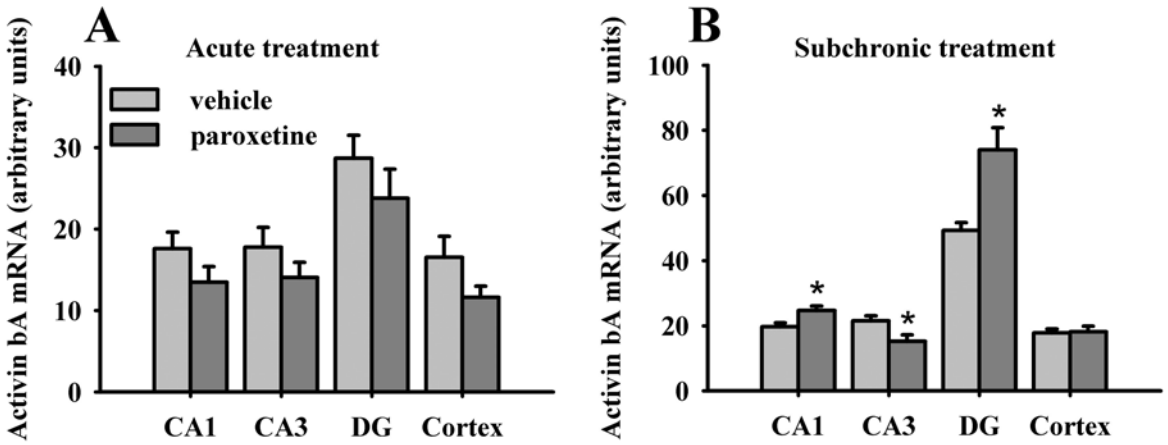


Figure 15:
Expression levels of activin βA mRNA in the CA1, CA3 and the dentate gyrus (DG) of the hippocampus and the cortex after acute (A) and subchronic (B) paroxetine treatment compared to the control group. Acute treatment n=6, subchronic treatment n=9. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Experiment 2:

Activin A injection into the dentate gyrus resulted in an antidepressant-like effect in the Porsolt forced swim test (FST) (Porsolt et al., 1977)

The forced swim test was applied in this study as it is the most commonly used experimental paradigm to assess antidepressant-like properties of compounds (Cryan and Mombereau, 2004). In rats, generally two trials (one pretreatment-trial before drug application) are required to generate a stable immobility readout, whereas for yet unexplained reasons in mice one exposure is sufficient after drug application (Cryan and Mombereau, 2004). In our case we were interested in investigating the effects of a single FST exposure following vehicle or activin A treatment on the stress-coping strategy, so all animals were retested 24 h after the infusion.

Due to a strong upregulation of activin βA in the dentate gyrus of DBA mice after chronic

paroxetine treatment, we wanted to examine the behavioural effects of bilateral microinjections of activin A into the dentate gyrus. Therefore, we infused activin A (1 µg/side) or 0,1 % BSA in the dentate gyrus and performed behavioural testing 15 min after the infusion. We could observe a strong antidepressant-like effect of activin A as animals that received activin A showed a significant increase in struggling (t-test, $p<0.05$) and a significant decrease in floating behaviour (t-test, $p<0.01$) with no change in swimming 15 min after treatment (figure 16A). The differences between treatment groups in struggling behaviour could still be observed 24 h after the activin A infusion (t-test, $p<0.05$) (figure 16B).

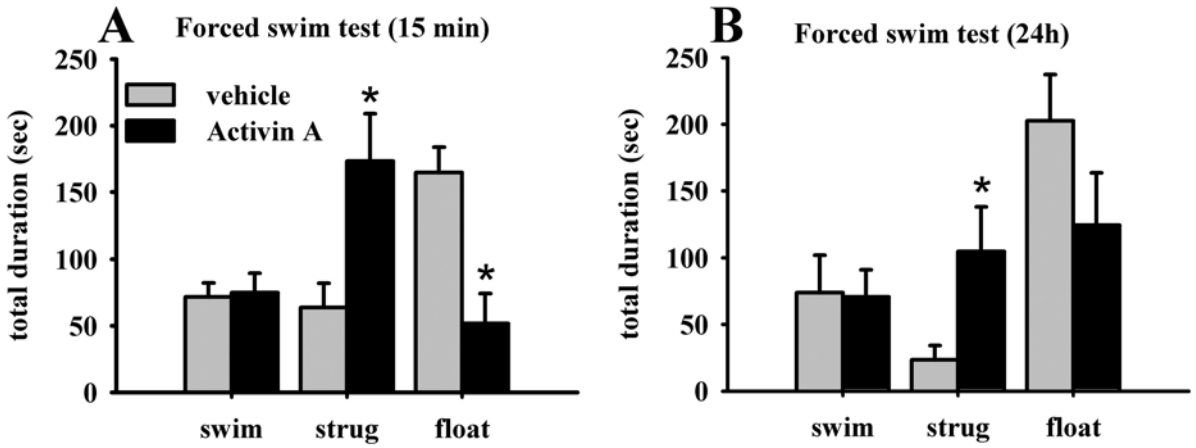


Figure 16:
Acute infusion of activin A into the dentate gyrus of the hippocampus produces antidepressant-like effects in the forced swim test 15 min after infusion (A), which lasted over 24 h (B). Vehicle group n=5, activin A group n=4. Swim=swimming, strug=struggling and float=floating. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

As we were interested in region-specific effects, we decided to investigate the effects of bilateral activin A infusion into the amygdala as well. Animals that received activin A showed no changes in struggling, floating or swimming behaviour 15 min after treatment (figure 17A). Only 24 h after treatment we found a tendency that activin A infusion caused an increase in swimming (t-test, $p=0.056$) (figure 17B).

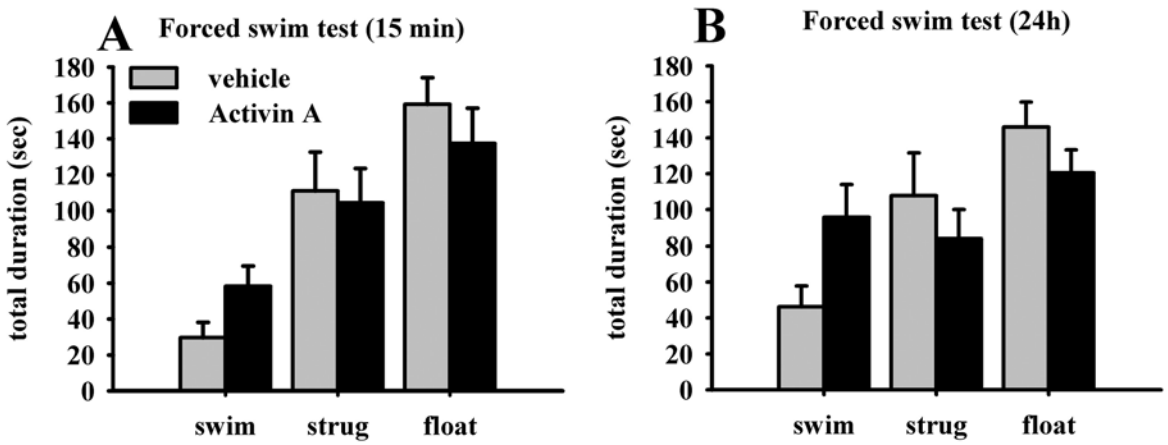


Figure 17:
Animals that received acute bilateral activin A infusion into the amygdala showed no changes in struggling, floating or swimming behaviour 15 min after treatment (A). Only 24 h after treatment we found a tendency that activin A infusion caused an increase in swimming (B; p=0.056). Vehicle group n=7, activin A group n=10. Swim=swimming, strug=struggling and float=floating. Data are presented as mean + SEM.

Experiment 3:

Reduction of anxiety-like behaviour in the modified hole board after activin A injection into the amygdala

The modified hole board was used to get a comprehensive overview on behavioural changes under mild stressful conditions and to assess alterations in locomotor activity due to acute activin A treatment.

After activin A infusion into the dentate gyrus, no differences between activin A- and vehicle treated animals were observed for the measured time in the exposed area, i.e. on the board, the latency until first entry on board or the entries on board (data not shown). Both groups did not differ in risk assessment behaviour reflected by the number of stretch attends (data not shown). Furthermore, parameters indicating activity and exploration were not significantly different between groups: the general locomotor activity during 5 minutes of hole board exposure was not altered after activin A treatment (figure 18A) and the number of rearings was not significantly changed. Grooming behaviour did not differ between the groups (data not shown).

Activin A infusion into the amygdala revealed a slight but not significant difference between activin A- and vehicle treated animals for the measured time in the exposed area, i.e. on the board (t-test, p=0.087) (figure 19A). No behavioural changes were observed for the latency until the first entry on board or the entries on board (data not shown). Parameters reflecting risk assessment or exploration were not significantly different between groups. Activin A

treatment had no influence on general locomotor activity during 5 minutes of hole board exposure (figure 18B). Finally, there was a remarkable but not significant different effect in grooming behaviour, activin A treated animals tend to groom less than control animals (t-test, p=0.057) (figure 19B).

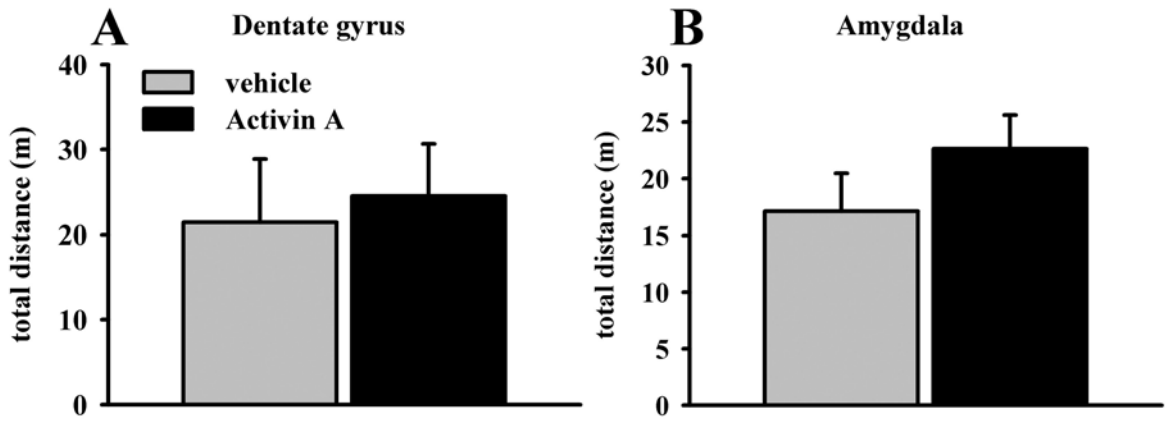


Figure 18:
Influence of activin A infusion into the dentate gyrus of the hippocampus (A) and the amygdala (B) on locomotor activity was measured 20 h after the treatment in the modified hole board. There was no significant treatment effect on the locomotion of the mice. Dentate gyrus n=4; amygdala n=8. Data are presented as mean + SEM.

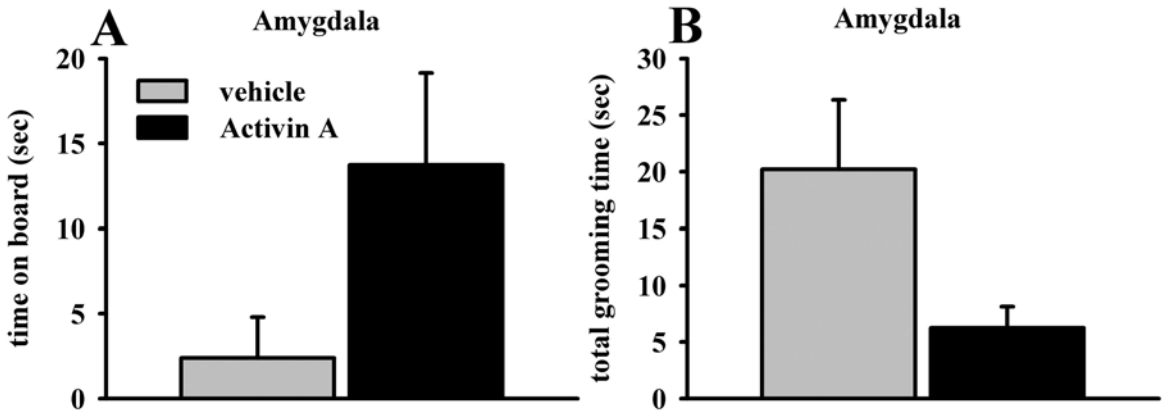


Figure 19:
20 h after activin A infusion into either the dentate gyrus or the amygdala mice performed the modified hole board test. After infusion into the dentate gyrus no differences between activin A- and vehicle treated animals could be observed (data not shown).
Activin A infusion into the amygdala revealed a slight but not significant difference between activin A- and vehicle treated animals for the measured time in the exposed area, i.e. on the board (A; p=0.087). Grooming behaviour was reduced in activin A treated animals (B; p=0.057). n=8 per group. Data are presented as mean + SEM.

Experiment 4 (in cooperation with Gerhard Rammes,Max Planck Institut of Psychiatry):

Activin A blocks hippocampal long-term potentiation

To assess whether activin A causes alterations in synaptic plasticity in the CA1 region of the dorsal hippocampus, we studied long-term potentiation (LTP) in the Schaffer collateral-commissural pathway in acute brain slices. When slices were superfused with activin A (40nM), LTP was significantly weaker than under control conditions, at least 60 minutes after high-frequency stimulation (t-test, $p<0.05$) (figure 20).

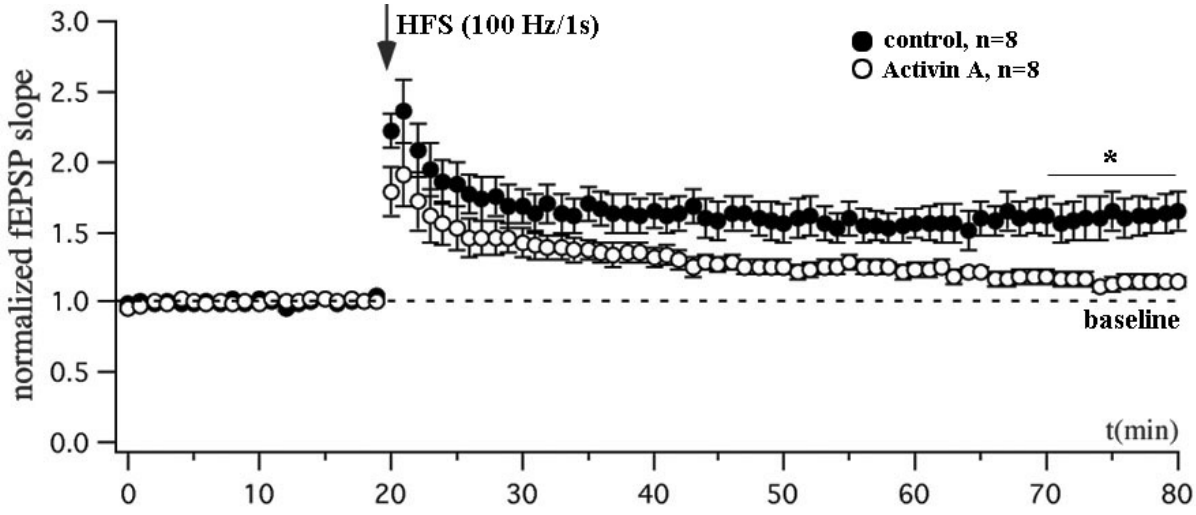


Figure 20:
Effects of activin A on long-term potentiation in the hippocampal CA1 area. HFS=high-frequency stimulation. n=8 per group. * significant from control group, $p<0.05$. Data are presented as mean ± SEM.

Experiment 5 (in cooperation with Susanne Lucae, Max Planck Institut of Psychiatry):

Human genetic association study

Dex/CRH test

Three intronic SNPs located in the gene coding for activin A (Inhba) were significantly associated with the ACTH response in the dex/CRH test at discharge in a genotypic mode of inheritance ($p_{corrected}$ for rs2237432, rs2237435 and rs2877089 = 0.00305, 0.0105 and 0.0452, respectively). ACTH and cortisol responses for SNP rs2237432 are presented in figure 21. In the gene coding for Activin receptor IIA (Acvr2A) 2 SNPs were associated with cortisol response at discharge in an allelic mode of inheritance ($p_{corrected}$ for rs1469211 and rs6747792 = 0.0489 for both SNPs). Furthermore SNPs of both genes and in the genes Acvr1 and Acvr2b were nominally associated with ACTH and cortisol responses at admission and discharge, but these associations did not withstand correction for multiple testing.

When testing all SNPs within one gene against a combination of all four phenotypes in an FPM-analysis we found the Activin A gene to be highly associated ($p_{genotypic} = 0.00481$).

We tested for genetic interactions of SNPs rs2237432 (Inhba) and rs1469211 (Acvr2a) for all 4 phenotypes and found nominally significant interaction effects for the ACTH response at admission ($p = 3.942 \times 10^{-4}$, $F_{df3} = 6.333$) and discharge ($p = 2.122 \times 10^{-5}$; $F_{df3} = 8.581$).

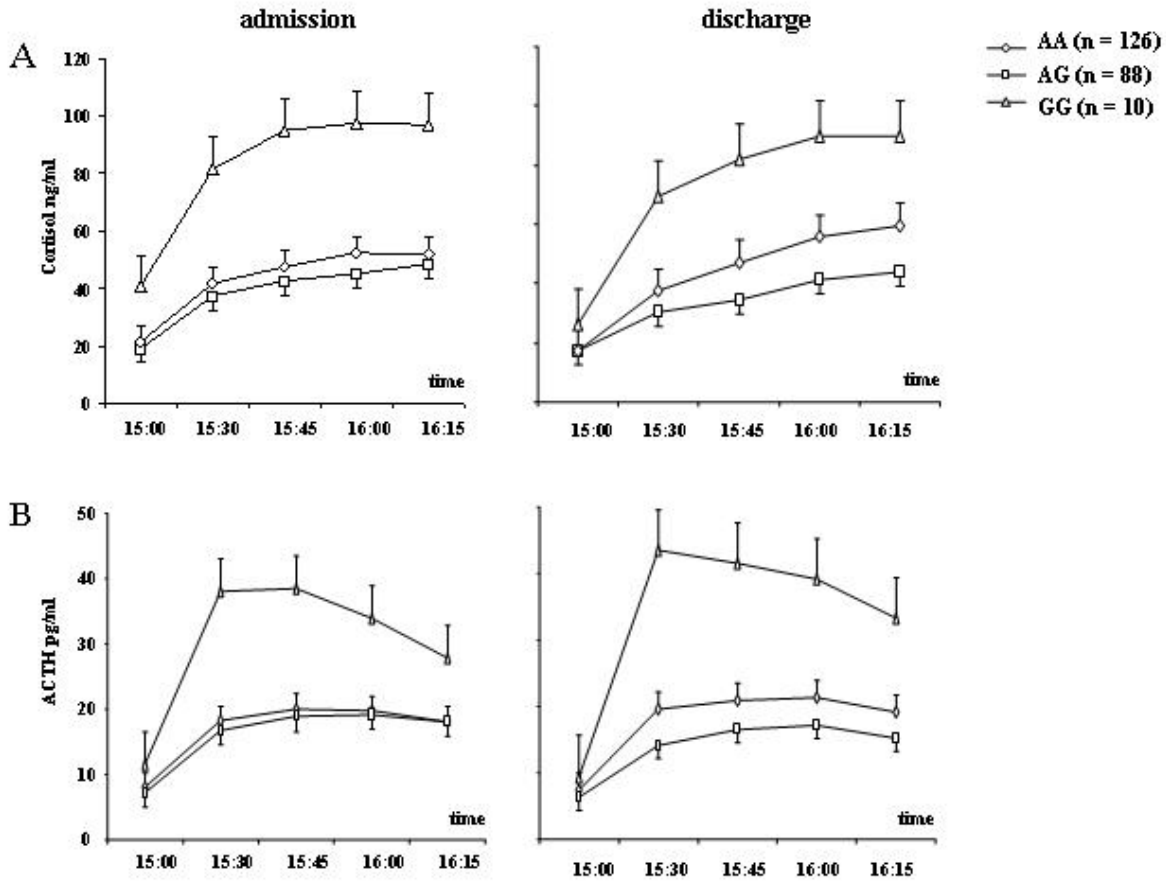


Figure 21:
Cortisol (A) and ACTH (B) response in the dex/CRH test of depressed in-patients at admission (left) and discharge (right) according to rs2237432 genotypes.

Discussion

Induction of activin βA mRNA after paroxetine treatment

Based on the data from an unbiased approach to identify novel antidepressant target genes, we could show that chronic antidepressant-treatment with paroxetine resulted in a significant upregulation of activin βA in the CA1 and the dentate gyrus of the hippocampus (project 1). These findings are in line with previous reports demonstrating an upregulation of activin βA mRNA in the rat hippocampus after acute and chronic electroconvulsive seizure (ECS), an effective treatment for depression (Andreasson and Worley, 1995; Dow et al., 2005; Conti et al., 2007). In contrast to our study, Dow et al. could not find an upregulation of activin βA mRNA levels in the hippocampus after chronic treatment with desipramine (a norepinephrine reuptake inhibitor) or fluoxetine (a serotonin reuptake inhibitor), whereas Conti et al. observed a slight increase of activin βA mRNA levels in the hippocampus after two weeks of fluoxetine treatment.

So far there is evidence that activin A, consisting of two activin βA subunits, might play a role in neuronal development and neuroprotection. As neuroplastic alterations in the hippocampus have been discussed to be involved in the pathophysiology of depression (Paizanis et al., 2007), our findings could indicate that activin βA participates in the neurotrophic or neuroprotective action of antidepressant treatment. Activin has been shown to support survival in primary cell cultures of rat hippocampal neurons (Iwahori et al., 1997) and to facilitate neuronal development in amygdala neurons of the rat (Trudeau et al., 1997). Daadi et al demonstrated that activin and fibroblast growth factor 2 play a role in regulating the fate of the embryonic basal forebrain ventricular zone progenitors (Daadi et al., 1998). In addition, activin stimulates somatostatin expression and differentiation of developing ciliary ganglion neurons (Coulombe et al., 1993). Furthermore there is evidence that long-term potentiation increases activin βA mRNA *in vitro* in granule cell neurons of the hippocampus and *in vivo* in rat hippocampus (Andreasson and Worley, 1995; Inokuchi et al., 1996). *In vivo* experiments underline these results: Tretter et al could show an increase of expression of activin βA mRNA after kainic acid lesions of the hippocampal CA3 region (Tretter et al., 1996) and in following experiments infusions of recombinant activin A reduced neuronal damage after excitotoxic brain injury (Tretter et al., 2000). Inokuchi et al. could show a significant induction of the expression of activin βA mRNA as a result of excitotoxic injury as well (Inokuchi et al., 1996). In transgenic mice expressing a dominant-negative activin receptor 1b blocking the activin signalling, hippocampal neurons were significantly more vulnerable to intracerebroventricular injection of the excitotoxin kainic acid than those from

control mice (Müller et al., 2006). These findings suggest a role of activin A in the neuroprotective action of antidepressant drugs. To exclude the possibility that the upregulation of activin βA might lead to formation of inhibin A, which consists of an inhibin α and an activin βA subunit, we performed also an *in situ* hybridisation analysis for inhibin α (project 1). The analysis revealed a significant downregulation of inhibin α mRNA in the dentate gyrus region of the hippocampus, indicating that the increase in activin βA mRNA expression might rather lead to formation of activin A than inhibin A.

According to the delayed onset of action of antidepressant treatment we could not show any differences in the activin βA mRNA levels in the mouse brain after acute paroxetine treatment, whereas for subchronic treatment a significant increase of activin βA gene expression could be observed in the CA1 and the dentate gyrus region of the hippocampus. This is corresponding to the results after chronic administration of paroxetine (project 1). In contrast to the effects of chronic antidepressant drug application, we additionally found a significant decrease of activin βA mRNA levels in the CA3 region of the hippocampus.

Infusion of activin A into the dentate gyrus, but not the amygdala, elicits antidepressant-like behaviour

In this study we could show that infusion of the dimeric protein activin A into the dentate gyrus of mice resulted in antidepressant-like effects in the forced swim test lasting up to 24 h, whereas we could show no such behavioural effects after infusion into the amygdala. The provoked behavioural alterations in the forced swim test after hippocampal activin A application are in line with a previous study, in which activin A infusion into the dentate gyrus resulted in an antidepressant-like phenotype in rats as well (Dow et al., 2005). Moreover, they could show that coinfusion of activin A and inhibin A blocked the activin A elicited behavioural changes. Inhibin A alone did not alter the phenotype in the FST compared to the control group, which clearly supports the hypothesis that activin A and not inhibin A might contribute to the therapeutic actions of antidepressant treatment.

Preclinical and clinical studies have shown that neuronal atrophy and loss might result from stress conditions and are possibly involved in the development of depression (for review see: Duman et al., 1999). On the other hand, neurotrophic factors like BDNF and neurotrophin-3 are known to be critical for the survival of neurons (for review see: McAllister et al., 1999) and are thought to be implicated in the mechanism of action of antidepressant treatment (Nibuya et al., 1995; Shirayama et al., 2002). Interestingly, a single infusion of BDNF into the hippocampal dentate gyrus exerted antidepressant-like effects in the FST 3 to 10 days after

substance application (Shirayama et al., 2002). Activin βA has also been shown to hold neurotrophic or neuroprotective abilities (Iwahori et al., 1997; Tretter et al., 2000; Müller et al., 2006). Moreover, it has been hypothesised that disturbed hippocampal activity could underlie some of the depressive symptoms, at least in part, because of loss of synaptic contacts, a process that is likely to be reversed by antidepressant drugs (Hajszan et al., 2005). Shoji-Kasai and colleagues demonstrated that activin is upregulated during hippocampal long-term potentiation and increases the number of synaptic contacts as well as the length of dendritic spine necks (Shoji-Kasai et al., 2007). Regarding the fast onset of antidepressant-like effects of activin A after 15 min in the forced swim test, it is not very likely that neurotrophic actions or synaptic remodelling are mediating the acute behavioural changes in mice. As there are various studies reporting on acute modulation of synaptic transmission by neurotrophic factors this could be a possible mechanism underlying the actions of activin as well (Levine et al., 1995; Poo, 2001; Müller et al., 2006). Future studies will be needed to identify the molecular adaptations underlying the actions of activin A and their translation into antidepressant-like effects.

In order to compare brain area-specific functions of activin A we were additionally interested in the effects of activin A infusion into the amygdala, a region which is richly endowed with activin receptors (Jakeman et al., 1992; Cameron et al., 1994; Funaba et al., 1997) and highly involved in mediation of physiological and behavioural responses associated with fear and strong emotions (for review see: McEwen, 2005). We could not show antidepressant-like effects of activin A application into the amygdala in the forced swim test, but we were able to detect slight anxiolytic effects of the protein in the modified hole board test.

Infusion of activin A into the amygdala tend to produce anxiolytic effects

In the modified hole board a broad spectrum of behaviour patterns can be tested (Ohl et al., 2001). We were mostly interested in anxiety-like behaviour and locomotor activity of the treatment groups. The influence of activin A on the locomotion was determined to investigate whether the decrease of immobility and the increase of struggling in the FST are due to a general effect on locomotor activity. As we could not observe any changes in locomotor activity between activin A treated and control animals, we can conclude that the observed antidepressant-like effects arise from application of activin A. These findings are in line with the results of Dow and colleagues (Dow et al., 2005).

Interestingly, in contrast to the results of the FST, in the modified hole board infusion of activin A into the amygdala turned out to have slight, but not significant, anxiolytic effects,

whereas there was no difference in behaviour after activin A injections into the hippocampal dentate gyrus compared to vehicle. These data propose a region-specific mechanism of action of activin A. The amygdala is considered to be a key brain-region in assigning emotional significance to a specific sensory input, and conditions like anxiety are supposed to be linked to its abnormal function (Wand, 2005). Animals that received activin A infusion tend to spend more time on the exposed inner board of the modified hole board, indicating that the mice were less anxious than the vehicle group. Furthermore, the time spent with grooming, which is considered as displacement activity in uncertain situations, was slightly reduced (Cohen and Price, 1979). Like in the hippocampus, for the amygdala a possible neurotrophic role of activin A is suggested, which might support anxiolytic effects (Trudeau et al., 1997). It has been already suggested that the stimulation of neurotrophic factors, like BDNF, may also prove to arise anxiolytic effects (Gorman, 2003).

Taken together we could show that activin A exerts distinct behavioural effects, depending on the brain region, in which it is injected. Of course, there is need of further studies to elucidate the role of activin A in modulating emotional behaviour in more detail.

Activin A blocks hippocampal long-term potentiation

Electrophysiological experiments revealed that nanomolar concentrations of activin A significantly block hippocampal long-term potentiation (LTP) in the CA1 region of the mouse hippocampus. Additionally, we could exclude that this inhibition is mediated by N-methyl-D-aspartate (NMDA) receptors, which are normally required for LTP induction in the CA1 region of the hippocampus (Malenka and Nicoll, 1993). The sparse data on the influence of activin and its receptors on synaptic activity and plasticity are quite contrary. Inhibition of LTP in the hippocampus by activin A has already been reported. Ikegaya and collegues could show that activin selectively blocks the induction of LTP evoked by threshold tetanic stimulation but not robust LTP elicited by strong tetanic stimulation of the perforant path. They proposed that activin plays a role in the decision-making process whether a LTP is essential or indefinite (Ikegaya et al., 1997). In contrast to these findings it has been found that expression of activin βA subunit mRNA is induced in the dentate gyrus of rats by high frequency stimulation of the perforant pathway. In this experiment it could be shown that the increase of gene expression was NMDA receptor-dependent (Inokuchi et al., 1996). Furthermore, it has been shown that activin increases the length of dendritic spine necks and number of synaptic contacts in cultured neurons (Shoji-Kasai et al., 2007). In a recent study of Alzheimer and colleagues in transgenic mice expressing a dominant-negative activin IB

receptor in forebrain neurons, a reduced NMDA current response and impaired LTP in the CA1 region could be observed, which in turn resulted in impaired synaptic plasticity (Müller et al., 2006). Taken together, the studies suggest that activin A plays in interesting role in neuronal activity and plasticity in the rodent brain, but the specific function still remains unclear.

Over the last years data accumulated indicating that different classes of antidepressant drugs and electroconvulsive therapy exert their effects at least partially via inhibition of LTP, mostly mediated via inhibition of NMDA receptors (Anwyl et al., 1987; Stewart and Reid, 1993; Watanabe et al., 1993; Massicotte et al., 1993; Li et al., 2006; Szasz et al., 2007). Moreover, NMDA receptor antagonists have proven to elicit antidepressant-like effects in rodents and humans (Rogóz et al., 2002; Zarate, Jr. et al., 2006). In our experiment we could not show that the blockade of LTP by activin A is mediated by NMDA receptors. This could be due to several reasons, like the minor concentrations we used or a NMDA receptor independent mechanism. Whether the LTP blockade of activin A is responsible for the acute antidepressant-like effects of activin in the forced swim test remains to be investigated.

Endophenotypes of major depression are associated with genetic variants in the activin signalling pathway

Using a translational approach, we investigated whether genetic variants (single nucleotide polymorphisms, SNPs) in genes involved in the activin signalling pathway are associated with specific endophenotypes of major depression in patients. A host of data implicates central and peripheral disturbances of stress hormone regulation (hypothalamic-pituitary-adrenocortical axis, HPA axis) in the pathogenesis of depression (Holsboer, 2000) and normalization of these defects as a prerequisite of clinical response to medication depression (Nemeroff and Owens, 2002).

There is evidence in the literature suggesting an interaction between the activin signalling pathway and stress hormone regulation. It was shown that activin A inhibits proopiomelanocortin (the precursor of ACTH) mRNA accumulation and ACTH secretion from the anterior pituitary, suggesting that locally secreted activins may exert a tonic inhibitory influence on pituitary corticotropes (Bilezikjian et al., 1991). Central administration of activin A, in contrast, evoked significant elevations of circulating ACTH levels in rats (Plotsky et al., 1991). The effects of activin A on HPA system regulation, therefore, seem to be more complex and dependent on whether activin A is administered centrally or peripherally.

In depressed patients, the combined dexamethasone/CRH test is a sensitive test for detecting altered HPA system regulation. The outcome in the DEX/CRH test has recently been shown to predict antidepressant treatment response in major depression, thus being considered a potential biomarker of antidepressant response (Ising et al., 2007). It has been suggested that differences in response to antidepressant treatment could be related to innate differences in HPA axis regulation (Binder et al., 2004).

In the present study, we genotyped SNPs in the genes encoding for activin A (Inhba), the activin I, IIA and IIB receptors in depressed individuals and found significant associations with results in the combined dexamethasone-CRH test: three intronic SNPs located in the gene coding for activin A were significantly associated with the ACTH response in the dex/CRH test at discharge: individuals carrying the associated genotype GG had more HPA hyperactivity. Moreover, in the gene coding for activin receptor IIA (Acvr2a) two SNPs were associated with cortisol response at discharge.

Resolution of depressive symptomatology has been associated with a normalization of HPA axis hyperactivity (Holsboer, 2000). The association between genetic variants in the activin signalling pathway and altered stress hormone regulation in depressed patients strengthens our preclinical data and emphasizes the potential role of activin signalling in antidepressants' mechanism of action.

4. Characterisation of behavioural effects of central gastrin releasing peptide administration

Depression is a disease with an enormous social and economic burden. It is not simply a condition of excessive unhappiness, but core symptoms include insomnia, decreased interest in pleasurable stimuli and restlessness (Nestler et al., 2002; Simon, 2003). The pathophysiology of the disease is multifactorial and implicates genetic as well environmental factors (McEwen, 2000a). Available antidepressants are mediating their effects mainly in targeting monoamine neurotransmission (Wong and Licinio, 2001). Current pharmacotherapies are limited and suboptimal with regard to their tolerability and efficacy (Holmes et al., 2003; Nemeroff and Owens, 2002). Regarding the additional delayed onset of action of antidepressant treatment, it is not very likely that they directly target causal pathways or structures (Taylor et al., 2005). Thus, it is necessary to identify alternative drugs that act faster and are more specific.

To address this question, research over the last years has focused on novel drug targets for antidepressant treatment. A main class of identified therapeutic targets was neuropeptides like Galanin, Neurokinin 1 or Neuropeptide Y (for review see: Ögren et al., 2006)(Swanson et al., 2005; Kuteeva et al., 2007; Kramer et al., 1998). Usually neuropeptides are 3-100 amino-acid residues long and therefore up to 50 times larger than classic neurotransmitters. They exert a broad spectrum of functions in the brain, ranging from pain transmission and control of food intake to functions as neurotransmitter, hormones or growth factor (Hökfelt et al., 2003; Landgraf and Neumann, 2004). Neuropeptides coexist in most of the cases with at least one of the classical messengers, including serotonin and noradrenalin, which are thought to be major transmitters implicated in depression and therapeutic effects of antidepressant drugs (Chronwall, 1985; Hökfelt, 1991). This aspect proposes them as ideal candidates for mediating the pathophysiology of psychiatric disorders. In addition, neuropeptides possess more recognition sites for the receptors compared to the smaller classic neurotransmitters and contain more chemical information. Consequently, their binding affinity and selectivity is enhanced, which means in regard to their pharmacological use for instance less side effects, as a smaller amount of antagonists or agonists is needed (Hökfelt et al., 2003).

Here we used an unbiased approach to identify potential novel drug targets or associations between genes and disease. In a previous study Sillaber et al. (submitted, 2007) aimed at identifying novel antidepressant-responsive genes in the mouse brain by microarray analysis. Mice were chronically treated with the selective serotonin reuptake inhibitor paroxetine and subsequent large-scale gene expression profiling in the mouse hippocampus was performed.

Gastrin releasing peptide (Grp) was identified as a potential target gene, as it was significantly upregulated after chronic paroxetine treatment. By using *in situ* hybridisation techniques we could confirm a significant increase of Grp mRNA levels in the dentate gyrus region of the hippocampus (see project 1). Grp is already known as a gastrointestinal hormone, growth factor and neuropeptide (Lebacq-Verheyden et al., 1988). Like several other neuropeptides the gastrin releasing peptide is localised in brain areas that mediate affective behaviours and responses to stress, like the hippocampus, the amygdala, the thalamus and the hypothalamus (Wada et al., 1990). The distribution of the gastrin releasing peptide receptor, a member of the G-protein coupled receptor superfamily, is similar to its ligand (Moody and Merali, 2004). Furthermore, double-labelling immunohistochemistry revealed already that subpopulations of the Grp receptor are present in GABAergic neurons of the amygdala (Kamichi et al., 2005). Regarding the functions of gastrin releasing peptide, it exerts a wide spectrum of biological effects on metabolism, digestion, memory and behaviour (Wada et al., 1997; Roesler et al., 2006). To date, Grp and its receptor are not well described in correlation with anxiety and depression. We hypothesised that the increase in Grp gene expression may at least partially underlie the efficacy of chronic antidepressant treatment. To test this assumption, we investigated the behavioural effects of a stereotactic infusion of Grp into the hippocampus in specific depression and anxiety-related paradigms *in vivo* (experimental design figure 22).

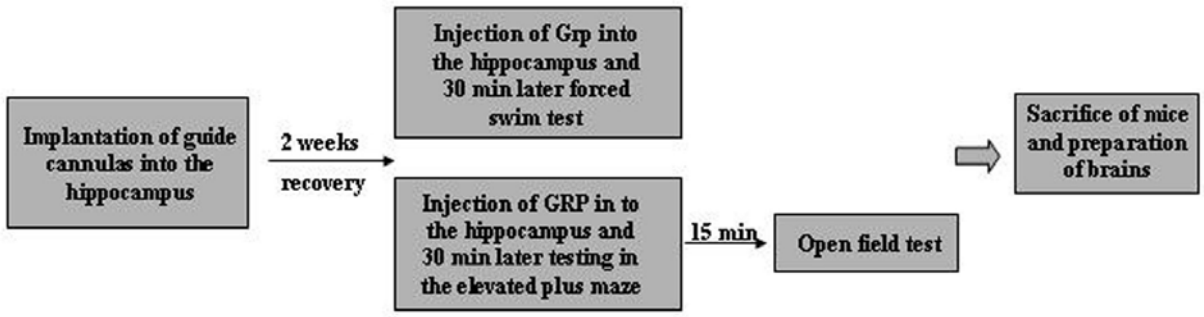


Figure 22: Experimental design

Results

We wanted to examine the behavioural effects of bilateral microinjections of Grp into the dentate, because we had a strong upregulation of Grp in the dentate gyrus of DBA mice after chronic paroxetine treatment (see project 1). After investigation of the cannula placement we noticed that most of the cannulas terminated in the CA1 region. This deviation might be a result of a technical defect of the steretactic apparatus or potentially aberrant head sizes of the test animals, which were used to assign the coordinates for the dentate gyrus, compared to the animals used in the experiment. Therefore all of our results display the effects of a Grp infusion into the CA1 region of the hippocampus. Animals with misplaced cannulas were excluded.

Grp injection into the CA1 region had no effects on antidepressant-like behaviour

We infused Grp (1 μg/side) or 0,9% saline in the CA1 area and tested 30 min after the infusion. We could observe no antidepressant-like effects of Grp in the CA1 region in the forced swim test, as swimming, struggling and floating behaviour was not significantly altered in Grp injected animals compared to the control group (figure 23).

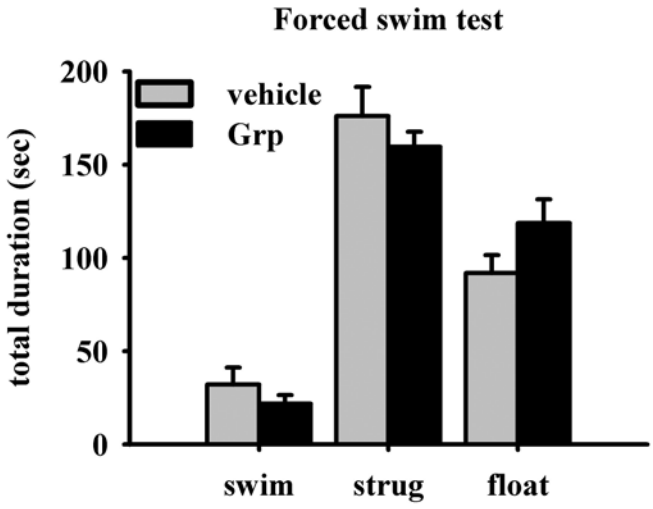


Figure 23:
Animals that received acute bilateral Grp infusion into the CA1 region showed no significant changes in struggling (strug), floating (float) and swimming (swim) behaviour 30 min after treatment. Vehicle group n=4; Grp group n=3. Data are presented as mean + SEM.

Effects of intrahippocampal Grp injection on anxiety-related behaviour

We could detect no significant differences regarding anxiety-like behaviour as Grp injected animals did not differ in the time spent on the open arm of the elevated plus maze compared to the control group (figure 24A). Furthermore, the parameter head dips, which is an indicator for anxiety and exploration (Fernández, 1997; Rodgers and Dalvi, 1997), was not significantly changed after Grp infusion (figure 24B). However, Grp treated animals performed significantly less stretch attends (t-test, $p<0.05$) (figure 24C), which are associated with risk assessment and exploration, and total rears, which display vertical activity (t-test, $p<0.05$) (figure 24D) (Rodgers and Dalvi, 1997). The total distance travelled in the elevated plus maze was significantly reduced in mice, that received a Grp infusion (t-test, $p<0.05$).

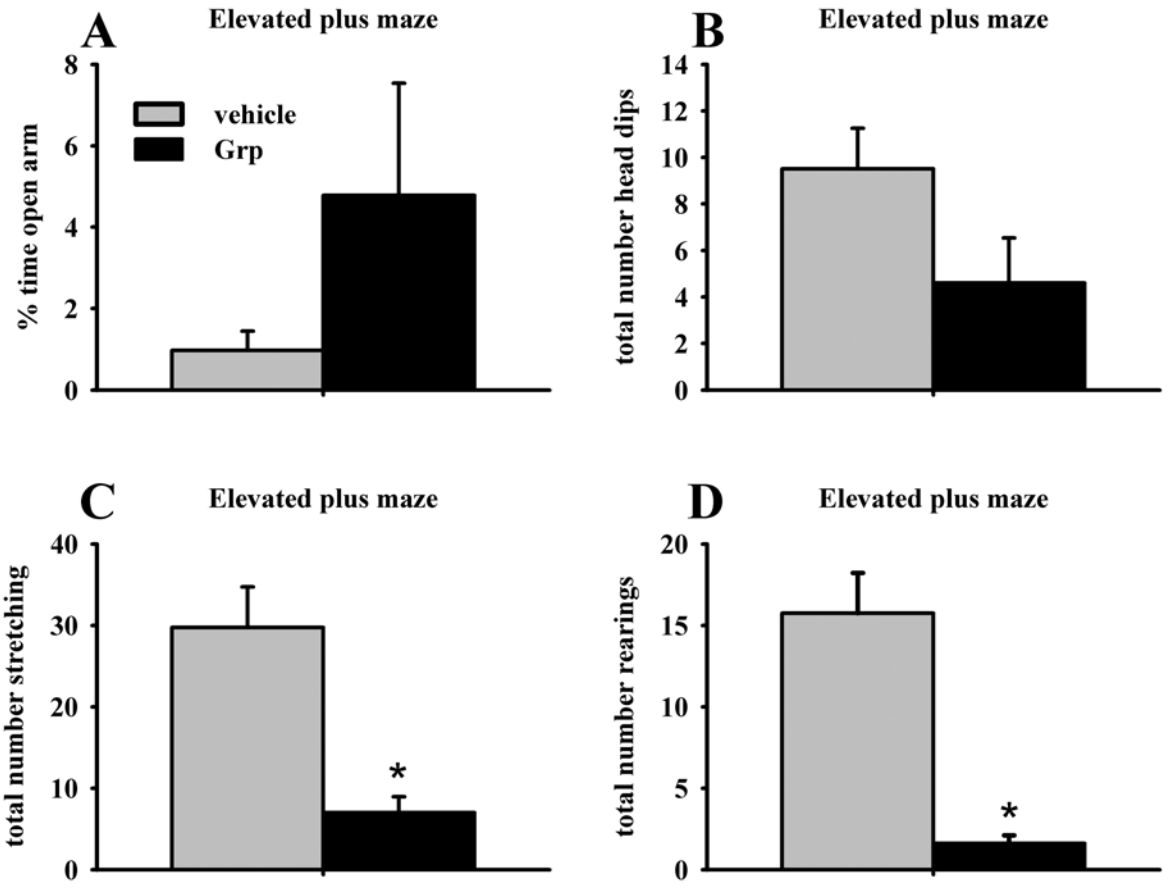


Figure 24:
30 min after Grp or saline infusion we could detect no significant differences in the percent time spent on the open arm (A) and the total number of head dips (B) on the elevated plus maze, whereas the number of stretch attends (C) and rearings (D) was significantly reduced in gastrin releasing peptide infused animals. Vehicle group n=4; Grp group n=5. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Effects of intrahippocampal Grp injection on locomotor activity

In the open field test we reveal a significant difference in general locomotor activity between groups. Grp treated animals displayed a significant reduction of locomotor activity compared to the control group (t-test, $p<0.05$) (figure 25A). Consequently, entries and time spent in the inner zone of the open field were decreased after Grp infusion (t-test, $p<0.05$) (figure 25B and C). The latency until the first entry into the inner zone was not significantly altered between groups, but saline treated animals tend to enter the unprotected area faster than the Grp treated mice (t-test, $p=0.065$) (figure 25D).

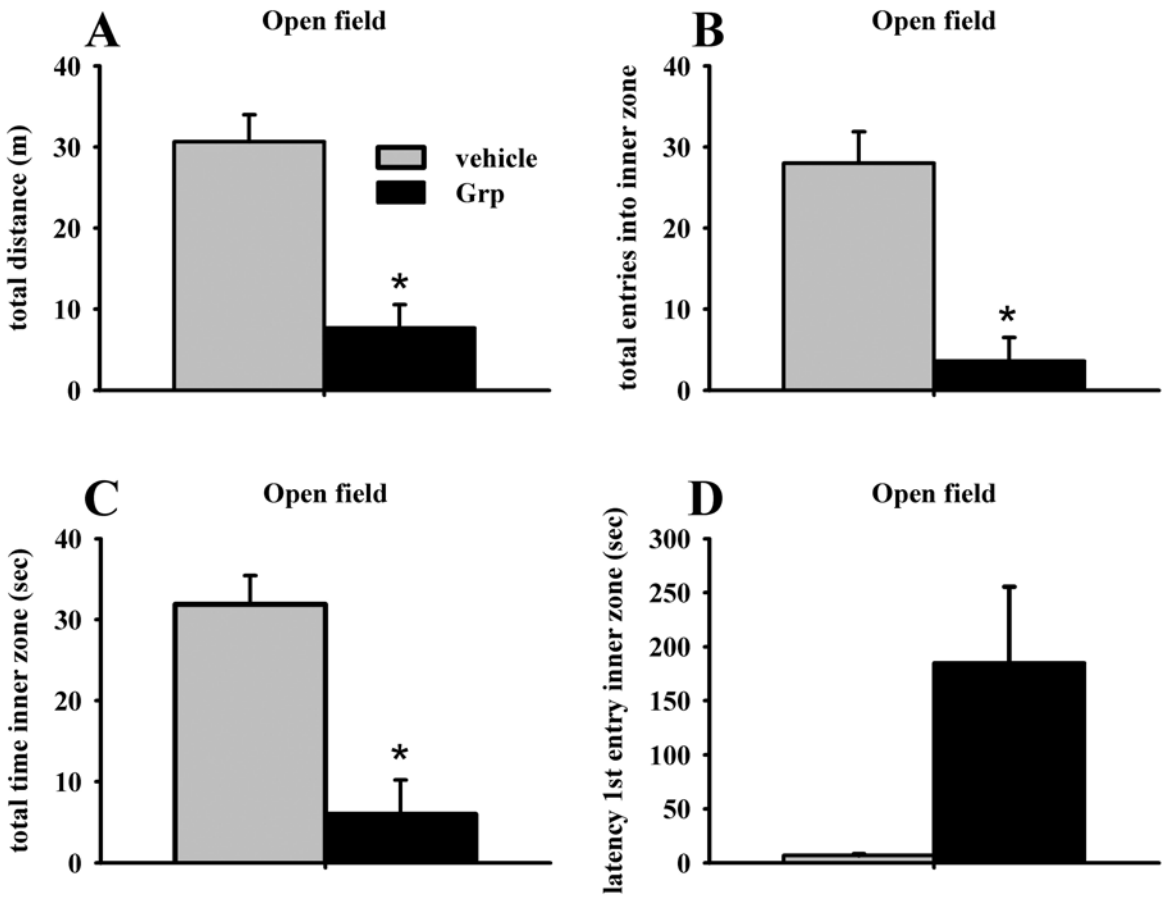


Figure 25:
Influence of Grp infusion into the CA1 region of the hippocampus on locomotor activity was measured 45 min after the treatment in the open field test. There was a significant treatment effect on the locomotion regarding the total distance travelled of animals that received Grp infusion into the CA1 region (A). The total number of entries (B) and time spent (C) in the inner zone of the open field was significantly reduced in Grp treated mice. There was no significant difference regarding the latency until the first entry into the inner zone between groups (D). Vehicle group n=4; Grp group n=5. * significant from control group, $p<0.05$. Data are presented as mean + SEM.

Discussion

In the current study we tested the hypothesis that Grp is directly involved in mediating the antidepressant and anxiolytic effects of the selective serotonin reuptake inhibitor paroxetine. We tested mice, which received Grp infusion into the CA1 region of the hippocampus in two behavioural test paradigms, the forced swim test and the elevated plus maze. In the FST antidepressant-like effects of drugs are measured, whereas in the EPM test the anxiety-related effects of pharmacological agents are assessed.

Effects of Grp injection into the CA1 region in the forced swim test

In the forced swim test we could not detect an antidepressant-like effect of Grp treatment compared to the control group, as swimming, struggling and floating behaviour was not significantly different between both groups. It has already been shown that gastrin releasing peptide has an influence on the hippocampal GABAergic system. Grp has been reported to depolarise GABAergic interneurons in the stratum oriens layer of the hippocampus and local infusion of Grp by reverse dialysis significantly raises levels of GABA in the ventral hippocampus (Lee et al., 1999; Andrews et al., 2000). These data suggest a potential implication of Grp in the mechanism of action of antidepressant drugs, as deficits in GABAergic neurotransmission are supposed to play a role in the aetiology of pathological anxiety and mood disorders (for review see: Brambilla et al., 2003)(Earnheart et al., 2007). The distinct patterns of gene expression regulation after paroxetine treatment we found in our previous studies suggest a highly region-specific function of the investigated genes. As we found a strong increase of Grp mRNA in the dentate gyrus and not the CA1 region of the hippocampus after chronic antidepressant treatment (project 1), it remains to be investigated in a further study whether the infusion of Grp into the dentate gyrus would reveal antidepressant-like effects.

Effects of Grp injection into the CA1 region in the elevated plus maze and the open field test

Testing of Grp treated animals in the EPM revealed no changes in anxiety-related behaviour compared to the vehicle treated animals, as the percentage of time spent on the open arm was not different between treatment groups. In contrast, parameters, which reflect risk assessment and exploration, were decreased in animals that received a Grp infusion. Moreover, the locomotor activity of the Grp group was significantly decreased. The latter result could be confirmed in the open field test. Additionally, Grp treated animals spent significantly less time in the inner zone of the open field. This seemingly anxiogenic effects of Grp treatment in

the open field must so far be interpreted as a result of the strongly reduced locomotor and explorative activity of Grp treated mice, because we could not reveal any anxiogenic effects of Grp in the elevated plus maze despite altered locomotion.

There are inconsistent findings regarding the effects of Grp and its receptor on locomotor activity. Wada and colleagues reported increased locomotor activity in Grp receptor deficient mice during the dark period, the active phase of mice (Wada et al., 1997). These results would be in line with our findings, indicating that functional Grp signalling decreases locomotion, whereas a disconnection of Grp signalling via Grp receptor knockout leads to enhanced locomotor activity. It has been reported that the altered locomotion might be associated with dopaminergic mechanisms, as dopamine receptor antagonists attenuate the generated effects on locomotion (Piggins and Merali, 1989). In contrast, other studies could not find effects on locomotion in the open field and the elevated plus maze after systemic injection of a Grp receptor antagonist, but they could show impaired habituation in the open field (Martins et al., 2005). Regarding these findings, more research needs to be done to clarify the role of central and peripheral Grp signalling. The data support the hypothesis that central and systemic administration of Grp might elicit different effects due to different target sites. Moreover, the investigation of central Grp should take in account that behavioural effects of Grp infusion could be highly dependent on the brain region. Furthermore, there could be a dose-dependant effect of Grp, as Mountney and colleagues revealed contrasting results on the expression of learned fear after infusion of different doses of Grp into the amygdala (Mountney et al., 2006). Despite the potential influence Grp on locomotion, several studies have reported that central administration of Grp and highly related peptides activates the HPA axis and therefore might have downstream effects on antidepressant- and anxiety-like behaviour (Garrido et al., 1998; Kent et al., 1998).

The data from this experiment have to be interpreted with caution for several reasons. In the first place our initial intention would have been the investigation of the behaviour of mice after Grp infusion into the dentate gyrus of the hippocampus, due to our precedent result of a prominent increase of Grp in the dentate gyrus after chronic paroxetine treatment. The dentate gyrus coordinates we predefined in test surgeries did not work for the experimental animals due to potentially aberrant head sizes or maybe a technical defect of the stereotactic apparatus. However, most of the cannulas were placed into the CA1 region of the hippocampus and we therefore investigated the effects of Grp infusion into the CA1 region in the behavioural paradigms. After exclusion of animals with misplaced cannulas only a small number of mice

remained per treatment group, which is critical in regard to the achievement of representative data. Further, it would be interesting to clarify whether the potential anxiogenic effects of Grp in the open field test are really due to locomotor alterations, because in the elevated plus maze the altered locomotor activity did not result in altered anxiety-related behaviour. Another reason for this discrepancy could be the difference between time point of injection and the particular test, as the EPM test was performed 30 min and the open field 45 min after injection. It has already been suggested that certain effects of drugs can be dependant on the time difference between administration and assessment of data (Khundakar and Zetterstrom, 2006). Finally, in future studies we should also investigate dose-dependent effects of Grp. Flood and colleagues could demonstrate a dose-dependent modulation of memory processing after intraperitoneal Grp injection (Flood and Morley, 1988). For our experiment we chose a relatively high dose, which might not reflect the normally occurring endogenous Grp concentrations and therefore have divergent effects.

Taken together we could show no antidepressant-like effects in the forced swim test after Grp microinfusion into the hippocampal CA1 region. However, we observed a decreased locomotor activity in Grp treated mice in the open field and elevated plus maze test. Additionally, we found a reduced explorative behaviour after Grp infusion in the elevated plus maze. To gain a more detailed insight in the hippocampal functions of Grp a comparison of the behavioural effects of Grp microinfusion into the dentate gyrus and the CA1 region would be helpful, regarding the fact, that we only had a significant increase of Grp mRNA in the dentate gyrus and not the CA1 region after chronic paroxetine treatment.

IV Discussion

Discussion

Major depressive disorder is a psychiatric disease that is becoming an increasingly important problem in modern society. The World Health Organization estimated that nearly 340 million people are affected by depression worldwide and categorized major depression as among the most disabling clinical diagnosis in the world (Greden, 2001). Moreover, depression has a serious impact on the quality of life of affected people (Üstün et al., 2004). Patients who suffer from this multifactorial disease present a combination of psychological, behavioural and physical symptoms like depressed mood, low self esteem, anxiety and insomnia or hypersomnia (Nestler et al., 2002). Additionally, affected people have an increased risk of mortality as depression aggravates many medical conditions like cardiovascular disease or diabetes and causes suicide (Angst et al., 1999; Angst et al., 2002). Many patients have recurring episodes of depression and require therefore long-term treatment of their illness (Kupfer, 1991). Despite our limited understanding of the underlying pathophysiology of depression, there are many effective treatments, as tricyclic antidepressants or selective serotonin reuptake inhibitors (Nestler et al., 2002). Still, further knowledge and improved treatment strategies are necessary, as 20% of the patients do not respond at all to pharmacological or psychotherapeutic treatment and 30% remain symptomatic and disabled (Sackeim, 2001). Shortcomings of antidepressant drugs range from intolerable side-effects like nausea, somnolence, dizziness and weight gain, to a slow onset of effects (4-8 weeks) (Keith, 2006; Gelenberg and Chesen, 2000). Regarding these drawbacks, especially the suicidality of patients, there is a desperate need of the development of innovative drugs that act faster and more specific.

Antidepressant drugs primarily modulate monoaminergic neurotransmission. However, the latency period of several weeks before the clinical effects of antidepressant therapy occur, suggests adaptive neuronal changes following manifold alterations in gene expression, which are not necessarily linked to the monoaminergic system (Duman, 1998; Bjartmar et al., 2000). Over the last years research is undertaken to identify conceptually novel drug targets or pathways. One possible approach is the investigation of genes that respond to antidepressant treatment, which may lead to the design of novel substances beyond the “monoamine hypothesis” and allow therefore a more successful treatment (Yamada and Higuchi, 2002).

Although the identification of novel target structures for the treatment of major depressive disorder through preclinical research should be considered critically, in the past decades, the use of mice in neuropsychiatric research has become more and more important. Besides the practical and economic advantages of using mice as they are proficient breeders and can be

housed in large numbers, the genetic and environmental background and experimental settings are more controllable than in humans (Cryan and Holmes, 2005). Moreover, approximately 99% of the mouse genes hold a homologue in the human genome (Waterston et al., 2002).

In this thesis we used an inbred mouse strain (DBA/2), with a high innate anxiety-like behaviour (Ohl et al., 2003; Yilmazer-Hanke et al., 2003) to identify and characterise novel genes that might be implicated in the action of antidepressant treatment. In a first step we were successful in validating the detected regulation of 82% of the selected genes of the microarray performed by Sillaber and colleagues (submitted 2007). Considering the multifactorial problems of microarray studies in producing reliable data, the validation rate of 82% is quite high (Asyali et al., 2004). This experiment was mainly conducted to reveal potential novel targets for a more direct antidepressant therapy. Subsequently, we were interested in the time course and onset of gene expression regulation following different periods of paroxetine treatment and the regional specifity of the gene expression changes. Therefore, three promising candidate genes were chosen: preproenkephalin 1, tissue plasminogen activator and the delta subunit of the $GABA_A$ receptor. The potential novel antidepressant targets were shown to be selectively and specifically regulated in different depression-relevant brain-regions like the hippocampus, the cortex, the amygdala and the thalamus in a time-dependent manner. The well-known delay of the onset of action of antidepressant drugs (Nemeroff and Owens, 2002) suggests that the primary mechanism of action is not the antagonism of serotonin reuptake (Vaswani et al., 2003), but supports the hypothesis that downstream adaptations mediate the therapeutical effects (Nickel et al., 2003; Thakker-Varia et al., 2007; Abumaria et al., 2007). As we could show that distinct genes (Gabrd and Penk1) were already regulated following one week of paroxetine treatment or even acutely, these genes could be putative early-responsive targets of antidepressant substances. This finding is very interesting in respect to the aim of developing antidepressant drugs that act more rapidly than the existing substances. Especially the faster onset of action is a desirable attribute for novel drugs regarding the high suicidal tendency of affected patients. Only recently Lucas and colleagues could demonstrate that novel $serotonin_4$ ($5\text{-}HT_4$) receptor agonists exert antidepressant properties at the behavioural and molecular level as early as after three days of treatment. The observed alterations were comparable to the effects of classical antidepressants after two to three weeks of application (Lucas et al., 2007). Hopefully, the identification of genes that respond already after a few days of paroxetine treatment may lead to the detection of novel pathways, which are causally involved in the

pathophysiology of major depressive disorder and treatment response. To prove the direct antidepressant effects of one of our targets we conducted a further experiment *in vivo*. Therefore we chose the activin βA gene and the respective activin A protein, which consists of two activin βA subunits. For this gene, a significant regulation could already been shown after one week of treatment. The protein is assumed to play a role in neuroprotection and neuronal development (Trudeau et al., 1997; Müller et al., 2006). Most of the currently available antidepressants are known to increase neurogenesis in the dentate gyrus, what has been suggested to be one potential mechanism crucial for antidepressant efficacy (Lesch, 2001; Duman et al., 2001; Shirayama et al., 2002; Santarelli et al., 2003; Duman and Monteggia, 2006). Furthermore, there is evidence to suggest that major depression might be induced or accompanied by the activation of inflammatory processes and that antidepressants exert their effects through their negative immunoregulatory capacities (Malaguarnera et al., 1998; Maes, 2001). Inflammation is capable of inducing apoptosis and depression has been shown to be accompanied by atrophy of the hippocampus, neurotrophic or neuroprotective factors are promising candidates for the treatment and improvement of depressive symptoms (Leonard and Myint, 2006; Schmidt and Duman, 2007). For activin A we could clearly show an antidepressant-like phenotype in the forced swim test directly after injection into the dentate gyrus of the hippocampus of DBA mice. Additionally, the infusion of the protein in the amygdala tended to exert anxiolytic effects in the modified hole board test. We performed electrophysiological studies on hippocampal mouse brain slices in order to further clarify potential underlying neurobiological mechanisms being responsible for the anxiolytic and antidepressant-like effects of activin A. We detected that even nanomolar concentrations of activin A were able to significantly block hippocampal long-term potentiation (LTP) in the CA1 of the hippocampus. Furthermore, we could show that this inhibition is not mediated by N-methyl-D-aspartate (NMDA) receptors. In a previous study, Ikegaya and colleagues revealed that activin selectively blocks the induction of LTP evoked by threshold tetanic stimulation, but not robust LTP elicited by strong tetanic stimulation. This data could indicate that activin participates in 'deciding' which LTP is essential and selectively excludes indefinite LTP (Ikegaya et al., 1997). It has already been shown that, for instance, acute and chronic administration of tricyclic antidepressants cause a reduction in the magnitude of LTP in hippocampal slices (Watanabe et al., 1993; Massicotte et al., 1993). Moreover, repeated electroconvulsive therapy, which is the most effective form of treatment for patients with treatment-resistant depression, reduces long-term potentiation in rat hippocampal slices (Anwyl et al., 1987; Stewart and Reid, 1993). However, the potential role of hippocampal

LTP inhibition in the therapeutic effect of antidepressant drugs remains to be investigated. Ultimately, besides the promising data of our preclinical experimental design, we were able to identify human genetic variants of the activin family that are associated with distinct endophenotypes of depression. Taken together, in this study we were able to bridge the gap between basic science (bench) and clinical data (bed), using a translational approach. We identified the activin pathway as a potential antidepressant target in mice and could further show that genetic alterations in members of the activin family are associated with a distinct clinical phenotype.

As second candidate gene for our *in vivo* experiments we chose the gastrin releasing peptide. There is already evidence that Grp and its receptor might be involved in the neurochemical alterations associated with psychiatric disorders (Yamada et al., 2002; Roesler et al., 2006) Therefore, we investigated the behavioural effects of Grp infusion into the CA1 region of the hippocampus and could not observe an antidepressant-like effect in the forced swim test. However, we detected in two other behavioural paradigms (open field and the elevated plus maze) a decrease in locomotor activity in mice that had been injected with Grp. This decrease in explorative behaviour and risk assessment could indicate a potential anxiogenic effect of Grp. Due to several reasons the data from this experiment have to be interpreted with caution. A relatively small group size and the Grp-induced alteration of basal locomotor activity complicate the interpretation of the behavioural results. Interestingly, the search for a corresponding genetic alteration in the Grp gene in patients revealed an association of a distinct Grp SNP and an anxious phenotype (data not shown). Regarding the high rate of comorbidity between anxiety and depressive disorders, this findings suggest Grp as a promising candidate which should be further investigated in detail (Angst, 1996; Kessler et al., 1996; Lewinsohn et al., 1997; Mineka et al., 1998; Brown et al., 2001).

Taken together, in this thesis we could show that a hypothesis-free approach for investigating antidepressant-responsive genes in mice provides a promising tool to search for novel candidate genes and drug targets. The most fascinating aspect of the study was the possibility to bridge the gap between basic research and the human clinical situation. The identification of additional and novel genes, which were previously unknown to be implicated in antidepressant drug action, will hopefully help to improve the treatment of depression in the future.

V Summary

Summary

Depressive disorders are a highly prevalent public health problem and are a major cause of morbidity worldwide. They impinge on the quality of a patients' life in terms of suffering, social and economic consequences, and increased mortality. Present in all cultural circles they affect the complete patients' environment, including the functioning of the concerned families and the work environment of the affected person (Sartorius, 2003). Existing treatments, like pharmacotherapy, psychotherapy and electroconvulsive therapy, show antidepressant efficacy and can significantly improve symptoms of the disease. Unfortunately, the underlying pathophysiology of most mood and anxiety disorders has remained unknown so far (Nemeroff and Vale, 2005). Consequently, the current available antidepressant substances continue to have limitations of both, efficacy and tolerability. The therapy is still hampered by a delayed onset of clinical improvement and a series of side effects and relapse is common (Gumnick and Nemeroff, 2000; Binder and Holsboer, 2006; Holtzheimer and Nemeroff, 2006). Moreover, a substantial group of patients show limited or no response to even the most aggressive interventions (Keller et al., 1992; Fink, 2001; Sackeim, 2001). Further, almost half of the affected people experience to have residual depressive symptoms despite adequate treatment (Fava, 2003). In consideration of these shortcomings, intensive effort is given to identify potential novel antidepressant drug targets. High throughput techniques like microarray analysis offer the promise to identify novel neurobiological components of depressive disorders and antidepressant drug response in a hypothesis-free approach (Nemeroff and Vale, 2005).

In a previous study Sillaber and colleagues analysed the interaction between behaviour, gene expression and response to psychoactive substances (Sillaber et al, 2007, submitted). To identify novel antidepressant-responsive genes they used DBA/2OlaHsd mice to characterise their behaviour under basal conditions and after chronic treatment with the commonly used antidepressant paroxetine. In a separate cohort of mice, genechip microarray analysis was applied for large-scale gene expression profiling in the hippocampus to investigate antidepressant-induced changes in gene expression.

In **project 1** of the present thesis we chose among the regulated genes of the microarray analysis eleven genes to further validate and characterise them. After chronic paroxetine treatment of mice we performed *in situ* hybridisation studies on hippocampal brain slices to confirm the results of the microarray. 82% of the selected genes could be validated by *in situ* hybridisation: three genes of the activin family, the cholecystokinin B receptor, the delta subunit of the $GABA_A$ receptor, the gastrin releasing peptide, the glucocorticoid receptor,

preproenkephalin 1 and the tissue plasminogen activator. Based on these findings further experiments were conducted to characterise those potential novel target genes in more detail *in vitro* and *in vivo*.

Project 2 mainly focuses on the detailed time-dependent and regional expression patterns of three of the validated candidate genes, namely preproenkephalin (Penk1), tissue plasminogen activator (Plat) and the delta subunit of the $GABA_A$ receptor (Gabrd). Penk1 is the precursor gene of certain endogenous opioid neuropeptides that generate a variety of physiological effects like inducing pain relief or modulation of learning and memory (Legon et al., 1982; Janecka et al., 2004; Akil et al., 1998). The plasminogen activator protein is a serine protease with plasminogen as its primary substrate (Vassalli et al., 1991). It has already been reported that the Plat/plasminogen system might play a role in synaptic plasticity (Pang et al., 2004; Melchor and Strickland, 2005) and neurotrophic factors in turn are supposed to be implicated in the pathophysiology of depression and antidepressant treatment effects (Duman and Monteggia, 2006). Finally, we were interested in Gabrd. Most of the fast inhibitory neurotransmission in the brain is mediated by $GABA_A$ receptors. They consist of a combination of five different subunits, which exhibit specific individual expression patterns in distinct brain regions and have a unique pharmacological profile (McKernan and Whiting, 1996; Korpi et al., 2002). The analysis of the time course and onset of gene expression regulation in the hippocampus following antidepressant treatment revealed the most prominent effects after chronic antidepressant administration. All three genes turned out to be significantly regulated after chronic drug application of four weeks in distinct brain regions (data are shown in **project 1**). Additionally, gene expression of Gabrd and Penk1 was found to be significantly altered already after a subchronic treatment period of seven days, whereas Plat mRNA levels were not changed. The only gene that was significantly regulated after acute drug application was Penk1; interestingly, the direction of gene regulation after acute treatment was in the opposite direction compared to the effects of subchronic and chronic treatment. Taken together, we could show that the different genes are regulated in a unique time-dependent and region-specific manner following antidepressant treatment.

In **project 3** we examined another candidate gene in more detail *in vitro* and *in vivo*. Activin βA mRNA could also shown to be significantly regulated after chronic (see **project 1**) and subchronic drug application. The respective proteins, the activins and inhibins, are members of the transforming growth factor β superfamily and are dimeric proteins composed of α- and/or β- subunits (for review see: Luisi et al., 2001; Bernard et al., 2001). In this study we investigated the *in vivo* effects of activin A, which is composed of two activin βA subunits.

There are only a few studies that report on the function of activin A in the central nervous system, but it is suggested that it plays a role in neuronal development and neuroprotection (Iwahori et al., 1997; Trudeau et al., 1997). To get a more comprehensive overview of behavioural effects of activin A, we stereotactically injected the protein into the dentate gyrus or the amygdala of mice and performed specific depression- and anxiety-related behavioural tests. We could show that application of activin into the dentate gyrus exerted antidepressant-like effects, whereas infusion into the amygdala tended to elicit an anxiolytic effect. In a following step we were interested in characterising potential underlying neurobiological pathways of activin action by performing *in vitro* electrophysiological analyses. We could show that activin is able to significantly block hippocampal LTP. Furthermore, in a large cohort of depressive patients, we were able to identify human genetic variants in the activin signalling cascade that are associated with a specific endophenotype of depressive disorders.

The gene we analysed in **project 4**, the gastrin releasing peptide, was also significantly upregulated in hippocampal regions after chronic antidepressant treatment (**project 1**). As its name implies, it can function as gastrointestinal hormone, but also as a neuropeptide or growth factor (Lebacq-Verheyden et al., 1988). To examine whether the increase in Grp mRNA levels contributes to the efficacy of chronic antidepressant treatment, we investigated the behavioural effects of a stereotactic application of Grp into the hippocampus *in vivo*. In the forced swim test no antidepressant-like behavioural phenotype could be observed. Further testing in two additional behavioural paradigms that assess anxiety and locomotion, revealed a decrease in locomotor activity and risk assessment of Grp treated animals.

In conclusion, we could show that hypothesis-free approaches, like microarray analysis, are valuable for detecting promising candidate genes as potential novel antidepressant drug targets. In addition to the data obtained in animal experiments, one candidate gene even turned out to be associated with specific endophenotypes of depression and has therefore ultimately high clinical relevance. Approaches transferring knowledge from animal studies to the clinical situation and back may contribute to uncover the complex and heterogeneous genetic background of psychiatric diseases and therefore promote future research and development of antidepressant drugs.

VI Reference list

Reference List

Abumaria N, Rygula R, Hiemke C, Fuchs E, Havemann-Reinecke U, Ruther E, Flugge G (2007) Effect of chronic citalopram on serotonin-related and stress-regulated genes in the dorsal raphe nucleus of the rat. Eur Neuropsychopharmacol 17:417-429.

Akil H, Owens C, Gutstein H, Taylor L, Curran E, Watson S (1998) Endogenous opioids: overview and current issues. Drug Alcohol Depend 51:127-140.

Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM (1984) Endogenous opioids: biology and function. Annu Rev Neurosci 7:223-55.:223-255.

Altar CA, Laeng P, Jurata LW, Brockman JA, Lemire A, Bullard J, Bukhman YV, Young TA, Charles V, Palfreyman MG (2004) Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. J Neurosci 24:2667-2677.

Anastasi A, Erspamer V, Bucci M (1971) Isolation and structure of bombesin and alytesin, 2 analogous active peptides from the skin of the European amphibians Bombina and Alytes. Experientia 27:166-167.

Andlin-Sobocki P, Wittchen HU (2005) Cost of affective disorders in Europe. Eur J Neurol 12 Suppl 1:34-8.:34-38.

Andreasson K, Worley PF (1995) Induction of beta-A activin expression by synaptic activity and during neocortical development. Neuroscience 69:781-796.

Andrews N, Davis B, Gonzalez MI, Oles R, Singh L, McKnight AT (2000) Effect of gastrin-releasing peptide on rat hippocampal extracellular GABA levels and seizures in the audiogenic seizure-prone DBA/2 mouse. Brain Res 859:386-389.

Angst F, Stassen HH, Clayton PJ, Angst J (2002) Mortality of patients with mood disorders: follow-up over 34-38 years. J Affect Disord 68:167-181.

Angst J (1996) Comorbidity of mood disorders: a longitudinal prospective study. Br J Psychiatry Suppl 31-37.

Angst J, Angst F, Stassen HH (1999) Suicide risk in patients with major depressive disorder. J Clin Psychiatry 60 Suppl 2:57-62; discussion 75-6, 113-6.:57-62.

Anwyl R, Walshe J, Rowan M (1987) Electroconvulsive treatment reduces long-term potentiation in rat hippocampus. Brain Res 435:377-379.

Asyali MH, Shoukri MM, Demirkaya O, Khabar KS (2004) Assessment of reliability of microarray data and estimation of signal thresholds using mixture modeling. Nucleic Acids Res 32:2323-2335.

Baamonde A, Dauge V, Ruiz-Gayo M, Fulga IG, Turcaud S, Fournie-Zaluski MC, Roques BP (1992) Antidepressant-type effects of endogenous enkephalins protected by systemic RB 101 are mediated by opioid delta and dopamine D1 receptor stimulation. Eur J Pharmacol 216:157-166.

Baranes D, Lederfein D, Huang YY, Chen M, Bailey CH, Kandel ER (1998) Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron 21:813-825.

Barden N (2004) Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression. J Psychiatry Neurosci 29:185-193.

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565-575.

Benchenane K, Lopez-Atalaya JP, Fernandez-Monreal M, Touzani O, Vivien D (2004) Equivocal roles of tissue-type plasminogen activator in stroke-induced injury. Trends Neurosci 27:155-160.

Bernard DJ, Chapman SC, Woodruff TK (2001) Mechanisms of inhibin signal transduction. Recent Prog Horm Res 56:417-50.:417-450.

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137-151.

Bilezikjian LM, Blount AL, Campen CA, Gonzalez-Manchon C, Vale W (1991) Activin-A inhibits proopiomelanocortin messenger RNA accumulation and adrenocorticotropin secretion of AtT20 cells. Mol Endocrinol 5:1389-1395.

Bilkei-Gorzo A, Michel K, Noble F, Roques BP, Zimmer A (2007) Preproenkephalin Knockout Mice Show No Depression-Related Phenotype. Neuropsychopharmacology 32:2330-2337.

Binder EB, Holsboer F (2006) Pharmacogenomics and antidepressant drugs. Ann Med 38:82-94.

Binder EB, et al. (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nat Genet 36:1319-1325.

Bjartmar L, Johansson IM, Marcusson J, Ross SB, Seckl JR, Olsson T (2000) Selective effects on NGFI-A, MR, GR and NGFI-B hippocampal mRNA expression after chronic treatment with different subclasses of antidepressants in the rat. Psychopharmacology (Berl) 151:7-12.

Boehm C, Newrzella D, Herberger S, Schramm N, Eisenhardt G, Schenk V, Sonntag-Buck V, Sorgenfrei O (2006) Effects of antidepressant treatment on gene expression profile in mouse brain: cell type-specific transcription profiling using laser microdissection and microarray analysis. J Neurochem [Epub ahead of print].

Bonner TI, Buckley NJ, Young AC, Brann MR (1987) Identification of a family of muscarinic acetylcholine receptor genes. Science 237:527-532.

Bonner TI, Young AC, Brann MR, Buckley NJ (1988) Cloning and expression of the human and rat m5 muscarinic acetylcholine receptor genes. Neuron 1:403-410.

Bottomley MJ, Collard MW, Huggenvik JI, Liu Z, Gibson TJ, Sattler M (2001) The SAND domain structure defines a novel DNA-binding fold in transcriptional regulation. Nat Struct Biol 8:626-633.

Brady LS, Gold PW, Herkenham M, Lynn AB, Whitfield HJ, Jr. (1992) The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-releasing hormone and tyrosine hydroxylase mRNA levels in rat brain: therapeutic implications. Brain Res 572:117-125.

Brambilla P, Perez J, Barale F, Schettini G, Soares JC (2003) GABAergic dysfunction in mood disorders. Mol Psychiatry 8:721-37, 715.

Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS (2000) Hippocampal volume reduction in major depression. Am J Psychiatry 157:115-118.

Brown TA, Campbell LA, Lehman CL, Grisham JR, Mancill RB (2001) Current and lifetime comorbidity of the DSM-IV anxiety and mood disorders in a large clinical sample. J Abnorm Psychol 110:585-599.

Burmeister M (1999) Basic concepts in the study of diseases with complex genetics. Biol Psychiatry 45:522-532.

Buzsaki G (1996) The hippocampo-neocortical dialogue. Cereb Cortex 6:81-92.

Bymaster FP, McKinzie DL, Felder CC, Wess J (2003) Use of M1-M5 muscarinic receptor knockout mice as novel tools to delineate the physiological roles of the muscarinic cholinergic system. Neurochem Res 28:437-442.

Caccamo A, Oddo S, Billings LM, Green KN, Martinez-Coria H, Fisher A, LaFerla FM (2006) M1 receptors play a central role in modulating AD-like pathology in transgenic mice. Neuron 49:671-682.

Cameron VA, Nishimura E, Mathews LS, Lewis KA, Sawchenko PE, Vale WW (1994) Hybridization histochemical localization of activin receptor subtypes in rat brain, pituitary, ovary, and testis. Endocrinology 134:799-808.

Castren E, Voikar V, Rantamaki T (2007) Role of neurotrophic factors in depression. Curr Opin Pharmacol 7:18-21.

Caulfield MP, Birdsall NJ (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol Rev 50:279-290.

Celada P, Puig M, margos-Bosch M, Adell A, Artigas F (2004) The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. J Psychiatry Neurosci 29:252-265.

Chaturvedi K (2003) Opioid peptides, opioid receptors and mechanism of down regulation. Indian J Exp Biol 41:5-13.

Chau DT, Rada P, Kosloff RA, Taylor JL, Hoebel BG (2001) Nucleus accumbens muscarinic receptors in the control of behavioral depression: antidepressant-like effects of local M1 antagonist in the Porsolt swim test. Neuroscience 104:791-798.

Chen J, Kaul S, Simons SS, Jr. (2002) Structure/activity elements of the multifunctional protein, GMEB-1. Characterization of domains relevant for the modulation of glucocorticoid receptor transactivation properties. J Biol Chem 277:22053-22062.

Christensen J, Cotmore SF, Tattersall P (1997) Parvovirus initiation factor PIF: a novel human DNA-binding factor which coordinately recognizes two ACGT motifs. J Virol 71:5733-5741.

Christie BR, Cameron HA (2006) Neurogenesis in the adult hippocampus. Hippocampus 16:199-207.

Chronwall BM (1985) Anatomy and physiology of the neuroendocrine arcuate nucleus. Peptides 6 Suppl 2:1-11.:1-11.

Clark RD, Ray NC, Blaney P, Crackett PH, Hurley C, Williams K, Dyke HJ, Clark DE, Lockey PM, Devos R, Wong M, White A, Belanoff JK (2007) 2-Benzenesulfonyl-8a-benzyl-hexahydro-2H-isoquinolin-6-ones as selective glucocorticoid receptor antagonists. Bioorg Med Chem Lett 17:5704-5708.

Cohen JA, Price EO (1979) Grooming in the Norway rat: displacement activity or "boundary-shift"? Behav Neural Biol 26:177-188.

Collen D (1999) The plasminogen (fibrinolytic) system. Thromb Haemost 82:259-270.

Conti B, Maier R, Barr AM, Morale MC, Lu X, Sanna PP, Bilbe G, Hoyer D, Bartfai T (2007) Region-specific transcriptional changes following the three antidepressant treatments electro convulsive therapy, sleep deprivation and fluoxetine. Mol Psychiatry 12:167-189.

Coulombe JN, Schwall R, Parent AS, Eckenstein FP, Nishi R (1993) Induction of somatostatin immunoreactivity in cultured ciliary ganglion neurons by activin in choroid cell-conditioned medium. Neuron 10:899-906.

Crawley JN, Corwin RL (1994) Biological actions of cholecystokinin. Peptides 15:731-755.

Cryan JF, Holmes A (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775-790.

Cryan JF, Markou A, Lucki I (2002) Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23:238-245.

Cryan JF, Mombereau C (2004) In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9:326-357.

Cryan JF, Slattery DA (2007) Animal models of mood disorders: Recent developments. Curr Opin Psychiatry 20:1-7.

D'Sa C, Eisch AJ, Bolger GB, Duman RS (2005) Differential expression and regulation of the cAMP-selective phosphodiesterase type 4A splice variants in rat brain by chronic antidepressant administration. Eur J Neurosci 22:1463-1475.

Daadi M, Arcellana-Panlilio MY, Weiss S (1998) Activin co-operates with fibroblast growth factor 2 to regulate tyrosine hydroxylase expression in the basal forebrain ventricular zone progenitors. Neuroscience 86:867-880.

Dagestad G, Kuipers SD, Messaoudi E, Bramham CR (2006) Chronic fluoxetine induces region-specific changes in translation factor eIF4E and eEF2 activity in the rat brain. Eur J Neurosci 23:2814-2818.

Danila DC, Zhang X, Zhou Y, Haidar JN, Klibanski A (2002) Overexpression of wild-type activin receptor alk4-1 restores activin antiproliferative effects in human pituitary tumor cells. J Clin Endocrinol Metab 87:4741-4746.

de Kloet ER (2003) Hormones, brain and stress. Endocr Regul 37:51-68.

de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463-475.

de Kloet ER, Reul JM, Sutanto W (1990) Corticosteroids and the brain. J Steroid Biochem Mol Biol 37:387-394.

Delgado PL (2004) How antidepressants help depression: mechanisms of action and clinical response. J Clin Psychiatry 65 Suppl 4:25-30.:25-30.

DeRijk RH, Schaaf M, de Kloet ER (2002) Glucocorticoid receptor variants: clinical implications. J Steroid Biochem Mol Biol 81:103-122.

Derrien M, Durieux C, Roques BP (1994) Antidepressant-like effects of CCKB antagonists in mice: antagonism by naltrindole. Br J Pharmacol 111:956-960.

Djavadian RL (2004) Serotonin and neurogenesis in the hippocampal dentate gyrus of adult mammals. Acta Neurobiol Exp (Wars) 64:189-200.

Dow AL, Russell DS, Duman RS (2005) Regulation of activin mRNA and Smad2 phosphorylation by antidepressant treatment in the rat brain: effects in behavioral models. J Neurosci 25:4908-4916.

Drevets WC (2001) Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Opin Neurobiol 11:240-249.

Drew MR, Hen R (2007) Adult hippocampal neurogenesis as target for the treatment of depression. CNS Neurol Disord Drug Targets 6:205-218.

Drigues N, Poltyrev T, Bejar C, Weinstock M, Youdim MB (2003) cDNA gene expression profile of rat hippocampus after chronic treatment with antidepressant drugs. J Neural Transm 110:1413-1436.

Duman RS (1998) Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 44:324-335.

Duman RS, Malberg J, Thome J (1999) Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 46:1181-1191.

Duman RS, Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. Biol Psychiatry 59:1116-1127.

Duman RS, Nakagawa S, Malberg J (2001) Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 25:836-844.

Earnheart JC, Schweizer C, Crestani F, Iwasato T, Itohara S, Mohler H, Luscher B (2007) GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of trait anxiety and depression states. J Neurosci 27:3845-3854.

Eser D, Schule C, Baghai TC, Romeo E, Uzunov DP, Rupprecht R (2006) Neuroactive steroids and affective disorders. Pharmacol Biochem Behav 84:656-666.

Fava M (2003) Diagnosis and definition of treatment-resistant depression. Biol Psychiatry 53:649-659.

Fava M, Kendler KS (2000) Major depressive disorder. Neuron 28:335-341.

Feighner JP, Cohn JB, Fabre LF, Jr., Fieve RR, Mendels J, Shrivastava RK, Dunbar GC (1993) A study comparing paroxetine placebo and imipramine in depressed patients. J Affect Disord 28:71-79.

Fekete E, Vigh J, Bagi EE, Lenard L (2002) Gastrin-releasing peptide microinjected into the amygdala inhibits feeding. Brain Res 955:55-63.

Felder CC, Bymaster FP, Ward J, DeLapp N (2000) Therapeutic opportunities for muscarinic receptors in the central nervous system. J Med Chem 43:4333-4353.

Fernández EE (1997) Structure of the mouse behaviour on the elevated plus-maze test of anxiety. Behav Brain Res 86:105-112.

File SE, Kenny PJ, Cheeta S (2000) The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. Pharmacol Biochem Behav 66:65-72.

Fink M (2001) Convulsive therapy: a review of the first 55 years. J Affect Disord 63:1-15.

Fisher A, Pittel Z, Haring R, Bar-Ner N, Kliger-Spatz M, Natan N, Egozi I, Sonego H, Marcovitch I, Brandeis R (2003) M1 muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease: implications in future therapy. J Mol Neurosci 20:349-356.

Flanders KC, Ren RF, Lippa CF (1998) Transforming growth factor-betas in neurodegenerative disease. Prog Neurobiol 54:71-85.

Flood JF, Morley JE (1988) Effects of bombesin and gastrin-releasing peptide on memory processing. Brain Res 460:314-322.

Florio P, Gazzolo D, Luisi S, Petraglia F (2007) Activin A in brain injury. Adv Clin Chem 43:117-30.:117-130.

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6:347-470.

Frey R, Schreinzer D, Heiden A, Kasper S (2001) [Use of electroconvulsive therapy in psychiatry]. Nervenarzt 72:661-676.

Fritschy JM, Möhler H (1995) GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359:154-194.

Fujimura J, Nagano M, Suzuki H (2005) Differential expression of GABA(A) receptor subunits in the distinct nuclei of the rat amygdala. Brain Res Mol Brain Res 138:17-23.

Funaba M, Murata T, Fujimura H, Murata E, Abe M, Torii K (1997) Immunolocalization of type I or type II activin receptors in the rat brain. J Neuroendocrinol 9:105-111.

Garrido C, Gurbuxani S, Ravagnan L, Kroemer G (2001) Heat shock proteins: endogenous modulators of apoptotic cell death. Biochem Biophys Res Commun 286:433-442.

Garrido MM, Fuentes JA, Manzanares J (2002) Gastrin-releasing peptide mediated regulation of 5-HT neuronal activity in the hypothalamic paraventricular nucleus under basal and restraint stress conditions. Life Sci 70:2953-2966.

Garrido MM, Manzanares J, Fuentes JA (1999) Hypothalamus, anterior pituitary and adrenal gland involvement in the activation of adrenocorticotropin and corticosterone secretion by gastrin-releasing peptide. Brain Res 828:20-26.

Garrido MM, Martin S, Ambrosio E, Fuentes JA, Manzanares J (1998) Role of corticotropin-releasing hormone in gastrin-releasing peptide-mediated regulation of corticotropin and corticosterone secretion in male rats. Neuroendocrinology 68:116-122.

Gelenberg AJ, Chesen CL (2000) How fast are antidepressants? J Clin Psychiatry 61:712-721.

Geyer MA, Markou A (1995) Animal models of psychiatric disorders. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the forth generation of progress. New York: Raven Press 787-798.

Girdler SS, Klatzkin R (2007) Neurosteroids in the context of stress: Implications for depressive disorders. Pharmacol Ther 116:125-139.

Gorman JM (2003) New molecular targets for antianxiety interventions. J Clin Psychiatry 64 Suppl 3:28-35.:28-35.

Gray PC, Bilezikjian LM, Harrison CA, Wiater E, Vale W (2005) Activins and inhibins: Physiological roles, signaling mechanisms and regulation. Hormones and the brain, Springer Verlag 1-28.

Greden JF (2001) The burden of recurrent depression: causes, consequences, and future prospects. J Clin Psychiatry 62 Suppl 22:5-9.:5-9.

Gulya K, Gehlert DR, Wamsley JK, Mosberg H, Hruby VJ, Yamamura HI (1986) Light microscopic autoradiographic localization of delta opioid receptors in the rat brain using a highly selective bis-penicillamine cyclic enkephalin analog. J Pharmacol Exp Ther 238:720-726.

Gumnick JF, Nemeroff CB (2000) Problems with currently available antidepressants. J Clin Psychiatry 61 Suppl 10:5-15.:5-15.

Gunasekara NS, Noble S, Benfield P (1998) Paroxetine. An update of its pharmacology and therapeutic use in depression and a review of its use in other disorders. Drugs 55:85-120.

Hajós M, Gartside SE, Sharp T (1995) Inhibition of median and dorsal raphe neurones following administration of the selective serotonin reuptake inhibitor paroxetine. Naunyn Schmiedebergs Arch Pharmacol 351:624-629.

Hajszan T, MacLusky NJ, Leranth C (2005) Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. Eur J Neurosci 21:1299-1303.

Heninger C, Saito N, Tallman JF, Garrett KM, Vitek MP, Duman RS, Gallager DW (1990) Effects of continuous diazepam administration on GABAA subunit mRNA in rat brain. J Mol Neurosci 2:101-107.

Hernando F, Fuentes JA, Roques BP, Ruiz-Gayo M (1994) The CCKB receptor antagonist, L-365,260, elicits antidepressant-type effects in the forced-swim test in mice. Eur J Pharmacol 261:257-263.

Herranz R (2003) Cholecystokinin antagonists: pharmacological and therapeutic potential. Med Res Rev 23:559-605.

Heuser IJ, Yassouridis A, Holsboer F (1994) The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. Journal of Psychiatric Research 28:341-356.

Hill DR, Campbell NJ, Shaw TM, Woodruff GN (1987) Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J Neurosci 7:2967-2976.

Hindmarch I (2002) Beyond the monoamine hypothesis: mechanisms, molecules and methods. Eur Psychiatry 17 Suppl 3:294-9.:294-299.

Hjorth S, Auerbach SB (1994) Further evidence for the importance of 5-HT1A autoreceptors in the action of selective serotonin reuptake inhibitors. Eur J Pharmacol 260:251-255.

Hjorth S, Auerbach SB (1996) 5-HT1A autoreceptors and the mode of action of selective serotonin reuptake inhibitors (SSRI). Behav Brain Res 73:281-283.

Hökfelt T (1991) Neuropeptides in perspective: the last ten years. Neuron 7:867-879.

Hökfelt T, Bartfai T, Bloom F (2003) Neuropeptides: opportunities for drug discovery. Lancet Neurol 2:463-472.

Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G (2003) Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 24:580-588.

Holsboer F (2000) The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23:477-501.

Holsboer F, Barden N (1996) Antidepressants and hypothalamic-pituitary-adrenocortical regulation. Endocr Rev 17:187-205.

Holsboer F, von Bardeleben U, Wiedemann K, Müller OA, Stalla GK (1987) Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression. Implications for pathophysiology of DST nonsuppression. Biological Psychiatry 22:228-234.

Holtzheimer PE 3rd, Nemeroff CB (2006) Advances in the treatment of depression. NeuroRx 3:42-56.

Ikegaya Y, Saito H, Torii K, Nishiyama N (1997) Activin selectively abolishes hippocampal long-term potentiation induced by weak tetanic stimulation in vivo. Jpn J Pharmacol 75:87-89.

Inokuchi K, Kato A, Hiraia K, Hishinuma F, Inoue M, Ozawa F (1996) Increase in activin beta A mRNA in rat hippocampus during long-term potentiation. FEBS Lett 382:48-52.

Ising M, Holsboer F (2006) Genetics of stress response and stress-related disorders. Dialogues Clin Neurosci 8:433-444.

Ising M, Horstmann S, Kloiber S, Lucae S, Binder EB, Kern N, Kunzel HE, Pfennig A, Uhr M, Holsboer F (2007) Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? Biol Psychiatry 62:47-54.

Iwahori Y, Saito H, Torii K, Nishiyama N (1997) Activin exerts a neurotrophic effect on cultured hippocampal neurons. Brain Res 760:52-58.

Jacobs BL, Praag H, Gage FH (2000) Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry 5:262-269.

Jakeman L, Mather J, Woodruff T (1992) In vitro ligand binding of 125I-recombinant human activin A to the female rat brain. Endocrinology 131:3117-3119.

Janecka A, Fichna J, Janecki T (2004) Opioid receptors and their ligands. Curr Top Med Chem 4:1-17.

Jutkiewicz EM (2007) RB101-mediated protection of endogenous opioids: potential therapeutic utility? CNS Drug Rev 13:192-205.

Jutkiewicz EM, Torregrossa MM, Sobczyk-Kojiro K, Mosberg HI, Folk JE, Rice KC, Watson SJ, Woods JH (2006) Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. Eur J Pharmacol 531:151-159.

Kamichi S, Wada E, Aoki S, Sekiguchi M, Kimura I, Wada K (2005) Immunohistochemical localization of gastrin-releasing peptide receptor in the mouse brain. Brain Res 1032:162-170.

Kanamatsu T, McGinty JF, Mitchell CL, Hong JS (1986) Dynorphin- and enkephalin-like immunoreactivity is altered in limbic-basal ganglia regions of rat brain after repeated electroconvulsive shock. J Neurosci 6:644-649.

Kang I, Miller LG (1991) Decreased GABAA receptor subunit mRNA concentrations following chronic lorazepam administration. Br J Pharmacol 103:1285-1287.

Kang W, Wilson SP, Wilson MA (2000) Overexpression of proenkephalin in the amygdala potentiates the anxiolytic effects of benzodiazepines. Neuropsychopharmacology 22:77-88.

Kasper S, Schindler S, Neumeister A (1996) Risk of suicide in depression and its implication for psychopharmacological treatment. Int Clin Psychopharmacol 11:71-79.

Kaul S, Blackford JA, Jr., Chen J, Ogryzko VV, Simons SS, Jr. (2000) Properties of the glucocorticoid modulatory element binding proteins GMEB-1 and -2: potential new modifiers of glucocorticoid receptor transactivation and members of the family of KDWK proteins. Mol Endocrinol 14:1010-1027.

Keith S (2006) Advances in psychotropic formulations. Prog Neuropsychopharmacol Biol Psychiatry 30:996-1008.

Keller MB, Lavori PW, Mueller TI, Endicott J, Coryell W, Hirschfeld RM, Shea T (1992) Time to recovery, chronicity, and levels of psychopathology in major depression. A 5-year prospective follow-up of 431 subjects. Arch Gen Psychiatry 49:809-816.

Kent P, Anisman H, Merali Z (1998) Are bombesin-like peptides involved in the mediation of stress response? Life Sci 62:103-114.

Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS (2003) The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 289:3095-3105.

Kessler RC, Nelson CB, McGonagle KA, Liu J, Swartz M, Blazer DG (1996) Comorbidity of DSM-III-R major depressive disorder in the general population: results from the US National Comorbidity Survey. Br J Psychiatry Suppl 17-30.

Khisti RT, Chopde CT (2000) Serotonergic agents modulate antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice. Brain Res 865:291-300.

Khisti RT, Chopde CT, Jain SP (2000) Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. Pharmacol Biochem Behav 67:137-143.

Khundakar AA, Zetterstrom TS (2006) Biphasic change in BDNF gene expression following antidepressant drug treatment explained by differential transcript regulation. Brain Res 1106:12-20.

Kieffer BL, Gaveriaux-Ruff C (2002) Exploring the opioid system by gene knockout. Prog Neurobiol 66:285-306.

König M, Zimmer AM, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, Zimmer A (1996) Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383:535-538.

Korpi ER, Grunder G, Luddens H (2002) Drug interactions at GABA(A) receptors. Prog Neurobiol 67:113-159.

Korte SM (2001) Corticosteroids in relation to fear, anxiety and psychopathology. Neuroscience & Biobehavioral Reviews 25:117-142.

Kramer MS, et al. (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281:1640-1645.

Kubo T, Fukuda K, Mikami A, Maeda A, Takahashi H, Mishina M, Haga T, Haga K, Ichiyama A, Kangawa K, . (1986) Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323:411-416.

Künzel HE, Binder EB, Nickel T, Ising M, Fuchs B, Majer M, Pfennig A, Ernst G, Kern N, Schmid DA, Uhr M, Holsboer F, Modell S (2003) Pharmacological and nonpharmacological factors influencing hypothalamic-pituitary-adrenocortical axis reactivity in acutely depressed psychiatric in-patients, measured by the Dex-CRH test. Neuropsychopharmacology 28:2169-2178.

Kupershmidt L, Amit T, Bar-Am O, Youdim MB, Blumenfeld Z (2007) The neuroprotective effect of Activin A and B: implication for neurodegenerative diseases. J Neurochem 103:962-971.

Kupfer DJ (1991) Long-term treatment of depression. J Clin Psychiatry 52 Suppl:28-34.:28-34.

Kuteeva E, Wardi T, Hökfelt T, Ögren SO (2007) Galanin enhances and a galanin antagonist attenuates depression-like behaviour in the rat. Eur Neuropsychopharmacol 17:64-69.

Ladenheim EE, Wirth KE, Moran TH (1996) Receptor subtype mediation of feeding suppression by bombesin-like peptides. Pharmacol Biochem Behav 54:705-711.

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25:150-176.

Landgrebe J, Welzl G, Metz T, van Gaalen MM, Ropers H, Wurst W, Holsboer F (2002) Molecular characterisation of antidepressant effects in the mouse brain using gene expression profiling. J Psychiatr Res 36:119-129.

Lathe R (2001) Hormones and the hippocampus. J Endocrinol 169:205-231.

Leahy P, Crawford DR, Grossman G, Gronostajski RM, Hanson RW (1999) CREB binding protein coordinates the function of multiple transcription factors including nuclear factor I to regulate phosphoenolpyruvate carboxykinase (GTP) gene transcription. J Biol Chem 274:8813-8822.

Lebacq-Verheyden AM, Krystal G, Sartor O, Way J, Battey JF (1988) The rat prepro gastrin releasing peptide gene is transcribed from two initiation sites in the brain. Mol Endocrinol 2:556-563.

Lee K, Dixon AK, Gonzalez I, Stevens EB, McNulty S, Oles R, Richardson PJ, Pinnock RD, Singh L (1999) Bombesin-like peptides depolarize rat hippocampal interneurones through interaction with subtype 2 bombesin receptors. J Physiol 518:791-802.

Legon S, Glover DM, Hughes J, Lowry PJ, Rigby PW, Watson CJ (1982) The structure and expression of the preproenkephalin gene. Nucleic Acids Res 10:7905-7918.

Leonard BE, Myint A (2006) Inflammation and depression: is there a causal connection with dementia? Neurotox Res 10:149-160.

Lesch KP (2001) Serotonergic gene expression and depression: implications for developing novel antidepressants. J Affect Disord 62:57-76.

Lesch KP (2004) Gene-environment interaction and the genetics of depression. J Psychiatry Neurosci 29:174-184.

Leung JW, Xue H (2003) GABAergic functions and depression: from classical therapies to herbal medicine. Curr Drug Targets CNS Neurol Disord 2:363-374.

Levey AI (1993) Immunological localization of m1-m5 muscarinic acetylcholine receptors in peripheral tissues and brain. Life Sci 52:441-448.

Levey AI (1996) Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. Proc Natl Acad Sci U S A 93:13541-13546.

Levine ES, Dreyfus CF, Black IB, Plummer MR (1995) Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc Natl Acad Sci U S A 92:8074-8077.

Lewinsohn PM, Zinbarg R, Seeley JR, Lewinsohn M, Sack WH (1997) Lifetime comorbidity among anxiety disorders and between anxiety disorders and other mental disorders in adolescents. J Anxiety Disord 11:377-394.

Li YF, Zhang YZ, Liu YQ, Wang HL, Cao JB, Guan TT, Luo ZP (2006) Inhibition of N-methyl-D-aspartate receptor function appears to be one of the common actions for antidepressants. J Psychopharmacol 20:629-635.

Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R (1986) Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature 321:779-782.

Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Pineyro G, Sadikot AF, Debonnel G (2007) Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron 55:712-725.

Lucki I, O'Leary OF (2004) Distinguishing roles for norepinephrine and serotonin in the behavioral effects of antidepressant drugs. J Clin Psychiatry 65 Suppl 4:11-24.:11-24.

Luisi S, Florio P, Reis FM, Petraglia F (2001) Expression and secretion of activin A: possible physiological and clinical implications. Eur J Endocrinol 145:225-236.

MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, Nahmias C, Young LT (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. Proc Natl Acad Sci U S A 100:1387-1392.

Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A (2000) Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47:1043-1049.

Maes M (2001) The immunoregulatory effects of antidepressants. Hum Psychopharmacol 16:95-103.

Maguire J, Mody I (2007) Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. J Neurosci 27:2155-2162.

Malaguarnera M, Di F, I, Restuccia S, Pistone G, Ferlito L, Rampello L (1998) Interferon alpha-induced depression in chronic hepatitis C patients: comparison between different types of interferon alpha. Neuropsychobiology 37:93-97.

Malberg JE (2004) Implications of adult hippocampal neurogenesis in antidepressant action. J Psychiatry Neurosci 29:196-205.

Malenka RC, Nicoll RA (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. Trends Neurosci 16:521-527.

Martins MR, Reinke A, Valvassori SS, Machado RA, Quevedo J, Schwartsmann G, Roesler R (2005) Non-associative learning and anxiety in rats treated with a single systemic administration of the gastrin-releasing peptide receptor antagonist RC-3095. Peptides 26:2525-2529.

Massicotte G, Bernard J, Ohayon M (1993) Chronic effects of trimipramine, an antidepressant, on hippocampal synaptic plasticity. Behav Neural Biol 59:100-106.

Matys T, Pawlak R, Matys E, Pavlides C, McEwen BS, Strickland S (2004) Tissue plasminogen activator promotes the effects of corticotropin-releasing factor on the amygdala and anxiety-like behavior. Proc Natl Acad Sci U S A 101:16345-16350.

McAllister AK, Katz LC, Lo DC (1999) Neurotrophins and synaptic plasticity. Annu Rev Neurosci 22:295-318.:295-318.

McArthur R, Borsini F (2006) Animal models of depression in drug discovery: a historical perspective. Pharmacol Biochem Behav 84:436-452.

McDonald TJ, Jornvall H, Nilsson G, Vagne M, Ghatei M, Bloom SR, Mutt V (1979) Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. Biochem Biophys Res Commun 90:227-233.

McEwen BS (2000a) Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology 22:108-124.

McEwen BS (2000b) The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886:172-189.

McEwen BS (2005) Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism 54:20-23.

McEwen BS (2006) Protective and damaging effects of stress mediators: central role of the brain. Dialogues Clin Neurosci 8:367-381.

McKernan RM, Whiting PJ (1996) Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci 19:139-143.

Melchor JP, Strickland S (2005) Tissue plasminogen activator in central nervous system physiology and pathology. Thromb Haemost 93:655-660.

Mello AA, Mello MF, Carpenter LL, Price LH (2003) Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. Rev Bras Psiquiatr 25:231-238.

Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO, Anisman H (2004) Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. J Neurosci 24:1478-1485.

Merali Z, Kent P, Anisman H (2002) Role of bombesin-related peptides in the mediation or integration of the stress response. Cell Mol Life Sci 59:272-287.

Merali Z, McIntosh J, Anisman H (1999) Role of bombesin-related peptides in the control of food intake. Neuropeptides 33:376-386.

Meunier H, Rivier C, Evans RM, Vale W (1988) Gonadal and extragonadal expression of inhibin alpha, beta A, and beta B subunits in various tissues predicts diverse functions. Proc Natl Acad Sci U S A 85:247-251.

Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen RW, Homanics GE (1999) Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc Natl Acad Sci U S A 96:12905-12910.

Millan MJ (2004) The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur J Pharmacol 500:371-384.

Mineka S, Watson D, Clark LA (1998) Comorbidity of anxiety and unipolar mood disorders. Annu Rev Psychol 49:377-412.:377-412.

Möhler H (2006) GABA(A) receptor diversity and pharmacology. Cell Tissue Res 326:505-516.

Moody TW, Merali Z (2004) Bombesin-like peptides and associated receptors within the brain: distribution and behavioral implications. Peptides 25:511-520.

Morris BJ, Johnston HM (1995) A role for hippocampal opioids in long-term functional plasticity. Trends Neurosci 18:350-355.

Moskowitz AS, Goodman RR (1984) Light microscopic autoradiographic localization of mu and delta opioid binding sites in the mouse central nervous system. J Neurosci 4:1331-1342.

Mountney C, Sillberg V, Kent P, Anisman H, Merali Z (2006) The role of gastrin-releasing peptide on conditioned fear: differential cortical and amygdaloid responses in the rat. Psychopharmacology (Berl) 189:287-296.

Müller MR, Zheng F, Werner S, Alzheimer C (2006) Transgenic mice expressing dominant-negative activin receptor IB in forebrain neurons reveal novel functions of activin at glutamatergic synapses. J Biol Chem 281:29076-29084.

Munz B, Hubner G, Tretter Y, Alzheimer C, Werner S (1999) A novel role of activin in inflammation and repair. J Endocrinol 161:187-193.

Nemeroff CB (1994) The clinical pharmacology and use of paroxetine, a new selective serotonin reuptake inhibitor. Pharmacotherapy 14:127-138.

Nemeroff CB (2007) The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J Psychiatr Res 41:189-206.

Nemeroff CB, Owens MJ (2002) Treatment of mood disorders. Nat Neurosci 5 Suppl:1068-70.:1068-1070.

Nemeroff CB, Owens MJ (2003) Neuropharmacology of paroxetine. Psychopharmacol Bull 37 Suppl 1:8-18.

Nemeroff CB, Vale WW (2005) The neurobiology of depression: inroads to treatment and new drug discovery. J Clin Psychiatry 66 Suppl 7:5-13.:5-13.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002) Neurobiology of depression. Neuron 34:13-25.

Newton SS, Girgenti MJ, Collier EF, Duman RS (2006) Electroconvulsive seizure increases adult hippocampal angiogenesis in rats. Eur J Neurosci 24:819-828.

Nibuya M, Morinobu S, Duman RS (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 15:7539-7547.

Nickel T, Sonntag A, Schill J, Zobel AW, Ackl N, Brunnauer A, Murck H, Ising M, Yassouridis A, Steiger A, Zihl J, Holsboer F (2003) Clinical and neurobiological effects of tianeptine and paroxetine in major depression. J Clin Psychopharmacol 23:155-168.

Noble F, Roques BP (1999) CCK-B receptor: chemistry, molecular biology, biochemistry and pharmacology. Prog Neurobiol 58:349-379.

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693-1703.

Ogden CA, Rich ME, Schork NJ, Paulus MP, Geyer MA, Lohr JB, Kuczenski R, Niculescu AB (2004) Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. Mol Psychiatry 9:1007-1029.

Ögren SO, Kuteeva E, Hokfelt T, Kehr J (2006) Galanin receptor antagonists : a potential novel pharmacological treatment for mood disorders. CNS Drugs 20:633-654.

Ohl F (2005) Animal models of anxiety. Handb Exp Pharmacol 35-69.

Ohl F, Holsboer F, Landgraf R (2001) The modified hole board as a differential screen for behavior in rodents. Behav Res Methods Instrum Comput 33:392-397.

Ohl F, Roedel A, Binder E, Holsboer F (2003) Impact of high and low anxiety on cognitive performance in a modified hole board test in C57BL/6 and DBA/2 mice. Eur J Neurosci 17:128-136.

Oshima H, Simons SS, Jr. (1992) Modulation of transcription factor activity by a distant steroid modulatory element. Mol Endocrinol 6:416-428.

Oshima H, Szapary D, Simons SS, Jr. (1995) The factor binding to the glucocorticoid modulatory element of the tyrosine aminotransferase gene is a novel and ubiquitous heteromeric complex. J Biol Chem 270:21893-21901.

Paizanis E, Hamon M, Lanfumey L (2007) Hippocampal neurogenesis, depressive disorders, and antidepressant therapy. Neural Plast :73754.

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B (2004) Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306:487-491.

Pangas SA, Woodruff TK (2000) Activin signal transduction pathways. Trends Endocrinol Metab 11:309-314.

Pariante CM, Miller AH (2001) Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry 49:391-404.

Pawlak R, Magarinos AM, Melchor J, McEwen B, Strickland S (2003) Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat Neurosci 6:168-174.

Peng C, Mukai ST (2000) Activins and their receptors in female reproduction. Biochem Cell Biol 78:261-279.

Peng Z, Hauer B, Mihalek RM, Homanics GE, Sieghart W, Olsen RW, Houser CR (2002) GABA(A) receptor changes in delta subunit-deficient mice: altered expression of alpha4 and gamma2 subunits in the forebrain. J Comp Neurol 446:179-197.

Persohn E, Malherbe P, Richards JG (1992) Comparative molecular neuroanatomy of cloned GABAA receptor subunits in the rat CNS. J Comp Neurol 326:193-216.

Piggins H, Merali Z (1989) The effects of concurrent D-1 and D-2 dopamine receptor blockade with SCH 23390 and eticlopride, on bombesin-induced behaviours. Prog Neuropsychopharmacol Biol Psychiatry 13:583-594.

Pinna G, Costa E, Guidotti A (2006) Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. Psychopharmacology (Berl) 186:362-372.

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815-850.

Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33:88-109.

Ploski JE, Newton SS, Duman RS (2006) Electroconvulsive seizure-induced gene expression profile of the hippocampus dentate gyrus granule cell layer. J Neurochem 99:1122-1132.

Plotsky PM, Kjaer A, Sutton SW, Sawchenko PE, Vale W (1991) Central activin administration modulates corticotropin-releasing hormone and adrenocorticotropin secretion. Endocrinology 128:2520-2525.

Pommier B, Beslot F, Simon A, Pophillat M, Matsui T, Dauge V, Roques BP, Noble F (2002) Deletion of CCK2 receptor in mice results in an upregulation of the endogenous opioid system. J Neurosci 22:2005-2011.

Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24-32.

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327-336.

Rehfeld JF, Friis-Hansen L, Goetze JP, Hansen TV (2007) The biology of cholecystokinin and gastrin peptides. Curr Top Med Chem 7:1154-1165.

Rezayat M, Roohbakhsh A, Zarrindast MR, Massoudi R, Djahanguiri B (2005) Cholecystokinin and GABA interaction in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. Physiol Behav 84:775-782.

Roberts LA, Large CH, O'Shaughnessy CT, Morris BJ (1997) Long-term potentiation in perforant path/granule cell synapses is associated with a post-synaptic induction of proenkephalin gene expression. Neurosci Lett 227:205-208.

Rodgers RJ, Dalvi A (1997) Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev 21:801-810.

Roesler R, Henriques JA, Schwartsmann G (2006) Gastrin-releasing peptide receptor as a molecular target for psychiatric and neurological disorders. CNS Neurol Disord Drug Targets 5:197-204.

Rogóz Z, Skuza G, Maj J, Danysz W (2002) Synergistic effect of uncompetitive NMDA receptor antagonists and antidepressant drugs in the forced swimming test in rats. Neuropharmacology 42:1024-1030.

Roques BP (2000) Novel approaches to targeting neuropeptide systems. Trends Pharmacol Sci 21:475-483.

Rosene DL, Van Hoesen GW (1987) The hippocampal formation of the primate brain. Cerebral Cortex 6:448-450.

Rosenzweig-Lipson S, Beyer CE, Hughes ZA, Khawaja X, Rajarao SJ, Malberg JE, Rahman Z, Ring RH, Schechter LE (2006) Differentiating antidepressants of the future: Efficacy and safety. Pharmacol Ther 113:134-153.

Sackeim HA (2001) The definition and meaning of treatment-resistant depression. J Clin Psychiatry 62 Suppl 16:10-7.:10-17.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301:805-809.

Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57:925-935.

Sartorius N (2003) Physical symptoms of depression as a public health concern. J Clin Psychiatry 64 Suppl 7:3-4.:3-4.

Schmidt HD, Duman RS (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol 18:391-418.

Schmidt MV, Oitzl MS, Levine S, de Kloet ER (2002) The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. Brain Res Dev Brain Res 139:39-49.

Sequeira A, Turecki G (2006) Genome wide gene expression studies in mood disorders. OMICS 10:444-454.

Serres F, Sartori SB, Halton A, Pei Q, Rochat C, Singewald N, Sharp T, Millan M (2006) Stereoselective and region-specific induction of immediate early gene expression in rat parietal cortex by blockade of neurokinin 1 receptors. J Psychopharmacol 20:570-576.

Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A 93:3908-3913.

Shi Y, Massague J (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685-700.

Shiah IS, Yatham LN (1998) GABA function in mood disorders: an update and critical review. Life Sci 63:1289-1303.

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251-3261.

Shively CA (1998) Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. Biol Psychiatry 44:882-891.

Shoji-Kasai Y, Ageta H, Hasegawa Y, Tsuchida K, Sugino H, Inokuchi K (2007) Activin increases the number of synaptic contacts and the length of dendritic spine necks by modulating spinal actin dynamics. J Cell Sci 120:3830-3837.

Shumyatsky GP, Tsvetkov E, Malleret G, Vronskaya S, Hatton M, Hampton L, Battey JF, Dulac C, Kandel ER, Bolshakov VY (2002) Identification of a signaling network in lateral nucleus of amygdala important for inhibiting memory specifically related to learned fear. Cell 111:905-918.

Simmons ML, Chavkin C (1996) Endogenous opioid regulation of hippocampal function. Int Rev Neurobiol 39:145-196.

Simon GE (2003) Social and economic burden of mood disorders. Biol Psychiatry 54:208-215.

Singh L, Lewis AS, Field MJ, Hughes J, Woodruff GN (1991) Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. Proc Natl Acad Sci U S A 88:1130-1133.

Smadja C, Maldonado R, Turcaud S, Fournie-Zaluski MC, Roques BP (1995) Opposite role of CCKA and CCKB receptors in the modulation of endogenous enkephalin antidepressant-like effects. Psychopharmacology (Berl) 120:400-408.

Smith SM, Vale WW (2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin Neurosci 8:383-395.

Sperk G, Schwarzer C, Tsunashima K, Fuchs K, Sieghart W (1997) GABA(A) receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. Neuroscience 80:987-1000.

Spigelman I, Li Z, Liang J, Cagetti E, Samzadeh S, Mihalek RM, Homanics GE, Olsen RW (2003) Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the GABA(A) receptor delta subunit. J Neurophysiol 90:903-910.

Spindel ER, Chin WW, Price J, Rees LH, Besser GM, Habener JF (1984) Cloning and characterization of cDNAs encoding human gastrin-releasing peptide. Proc Natl Acad Sci U S A 81:5699-5703.

Stein LJ, Woods SC (1982) Gastrin releasing peptide reduces meal size in rats. Peptides 3:833-835.

Stell BM, Brickley SG, Tang CY, Farrant M, Mody I (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. Proc Natl Acad Sci U S A 100:14439-14444.

Stewart C, Reid I (1993) Electroconvulsive stimulation and synaptic plasticity in the rat. Brain Res 620:139-141.

Ströhle A, Romeo E, di MF, Pasini A, Yassouridis A, Holsboer F, Rupprecht R (2002) GABA(A) receptor-modulating neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment. Am J Psychiatry 159:145-147.

Swanson CJ, Blackburn TP, Zhang X, Zheng K, Xu ZQ, Hokfelt T, Wolinsky TD, Konkel MJ, Chen H, Zhong H, Walker MW, Craig DA, Gerald CP, Branchek TA (2005) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc Natl Acad Sci U S A 102:17489-17494.

Szasz BK, Mike A, Karoly R, Gerevich Z, Illes P, Vizi ES, Kiss JP (2007) Direct inhibitory effect of fluoxetine on N-methyl-D-aspartate receptors in the central nervous system. Biol Psychiatry 62:1303-1309.

Tanay VA, Glencorse TA, Greenshaw AJ, Baker GB, Bateson AN (1996) Chronic administration of antipanic drugs alters rat brainstem GABAA receptor subunit mRNA levels. Neuropharmacology 35:1475-1482.

Tardito D, Perez J, Tiraboschi E, Musazzi L, Racagni G, Popoli M (2006) Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. Pharmacol Rev 58:115-134.

Taylor C, Fricker AD, Devi LA, Gomes I (2005) Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways. Cell Signal 17:549-557.

Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A, Hempstead BL (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 25:5455-5463.

Thakker-Varia S, Krol JJ, Nettleton J, Bilimoria PM, Bangasser DA, Shors TJ, Black IB, Alder J (2007) The neuropeptide VGF produces antidepressant-like behavioral effects and enhances proliferation in the hippocampus. J Neurosci 27:12156-12167.

Thériault JR, Charette SJ, Lambert H, Landry J (1999) Cloning and characterization of hGMEB1, a novel glucocorticoid modulatory element binding protein. FEBS Lett 452:170-176.

Thoeringer CK, Sillaber I, Roedel A, Erhardt A, Mueller MB, Ohl F, Holsboer F, Keck ME (2007) The temporal dynamics of intrahippocampal corticosterone in response to stress-related stimuli with different emotional and

physical load: an in vivo microdialysis study in C57BL/6 and DBA/2 inbred mice. Psychoneuroendocrinology 32:746-757.

Tretter YP, Hertel M, Munz B, ten Bruggencate G, Werner S, Alzheimer C (2000) Induction of activin A is essential for the neuroprotective action of basic fibroblast growth factor in vivo. Nat Med 6:812-815.

Tretter YP, Munz B, Hubner G, ten Bruggencate G, Werner S, Alzheimer C (1996) Strong induction of activin expression after hippocampal lesion. Neuroreport 7:1819-1823.

Trudeau VL, Theodosis DT, Poulain DA (1997) Activin facilitates neuronal development in the rat amygdala. Neurosci Lett 237:33-36.

Üstün TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ (2004) Global burden of depressive disorders in the year 2000. Br J Psychiatry 184:386-392.

Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A (1998) Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A 95:3239-3244.

Vaccarino AL, Kastin AJ (2001) Endogenous opiates: 2000. Peptides 22:2257-2328.

Vaidya VA, Duman RS (2001) Depresssion--emerging insights from neurobiology. Br Med Bull 57:61-79.

Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J (1986) Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 321:776-779.

Van Broekhoven F, Verkes RJ (2003) Neurosteroids in depression: a review. Psychopharmacology (Berl) 165:97-110.

Vassalli JD, Sappino AP, Belin D (1991) The plasminogen activator/plasmin system. J Clin Invest 88:1067-1072.

Vaswani M, Linda FK, Ramesh S (2003) Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry 27:85-102.

Verschueren K, Dewulf N, Goumans MJ, Lonnoy O, Feijen A, Grimsby S, Vandi SK, ten Dijke P, Moren A, Vanscheeuwijck P, Heldin CH, Miyazono K, Mummery C, Van Den Eijnden-Van Raaij, Huylebroeck D (1995) Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. Mech Dev 52:109-123.

Wada E, Watase K, Yamada K, Ogura H, Yamano M, Inomata Y, Eguchi J, Yamamoto K, Sunday ME, Maeno H, Mikoshiba K, Ohki-Hamazaki H, Wada K (1997) Generation and characterization of mice lacking gastrin-releasing peptide receptor. Biochem Biophys Res Commun 239:28-33.

Wada E, Way J, Lebacq-Verheyden AM, Battey JF (1990) Neuromedin B and gastrin-releasing peptide mRNAs are differentially distributed in the rat nervous system. J Neurosci 10:2917-2930.

Wand G (2005) The anxious amygdala: CREB signaling and predisposition to anxiety and alcoholism. J Clin Invest 115:2697-2699.

Watanabe Y, Saito H, Abe K (1993) Tricyclic antidepressants block NMDA receptor-mediated synaptic responses and induction of long-term potentiation in rat hippocampal slices. Neuropharmacology 32:479-486.

Waterston RH, et al. (2002) Initial sequencing and comparative analysis of the mouse genome. Nature 420:520-562.

Westfall PH, Young SS (1993) Resampling-based multiple testing. John Wiley and Sons, New York.

Wiedenmayer CP, Noailles PA, Angulo JA, Barr GA (2002) Stress-induced preproenkephalin mRNA expression in the amygdala changes during early ontogeny in the rat. Neuroscience 114:7-11.

Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy-Weinberg equilibrium. American Journal Of Human Genetics 76:887-893.

Willner P (1984) The validity of animal models of depression. Psychopharmacology (Berl) 83:1-16.

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040-1062.

Wong ML, Licinio J (2001) Research and treatment approaches to depression. Nat Rev Neurosci 2:343-351.

Wong ML, Licinio J (2004) From monoamines to genomic targets: a paradigm shift for drug discovery in depression. Nat Rev Drug Discov 3:136-151.

Wong ML, O'Kirwan F, Hannestad JP, Irizarry KJ, Elashoff D, Licinio J (2004) St John's wort and imipramine-induced gene expression profiles identify cellular functions relevant to antidepressant action and novel pharmacogenetic candidates for the phenotype of antidepressant treatment response. Mol Psychiatry 9:237-251.

Yadid G, Nakash R, Deri I, Tamar G, Kinor N, Gispan I, Zangen A (2000) Elucidation of the neurobiology of depression: insights from a novel genetic animal model. Prog Neurobiol 62:353-378.

Yamada K, Santo-Yamada Y, Wada E, Wada K (2002) Role of bombesin (BN)-like peptides/receptors in emotional behavior by comparison of three strains of BN-like peptide receptor knockout mice. Mol Psychiatry 7:113-117.

Yamada M, Higuchi T (2002) Functional genomics and depression research. Beyond the monoamine hypothesis. Eur Neuropsychopharmacol 12:235-244.

Yilmazer-Hanke DM, Roskoden T, Zilles K, Schwegler H (2003) Anxiety-related behavior and densities of glutamate, GABAA, acetylcholine and serotonin receptors in the amygdala of seven inbred mouse strains. Behav Brain Res 145:145-159.

Yoshikawa K, Hong JS, Sabol SL (1985) Electroconvulsive shock increases preproenkephalin messenger RNA abundance in rat hypothalamus. Proc Natl Acad Sci U S A 82:589-593.

Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK (2006) A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63:856-864.

Zeng H, Kaul S, Simons SS, Jr. (2000) Genomic organization of human GMEB-1 and rat GMEB-2: structural conservation of two multifunctional proteins. Nucleic Acids Res 28:1819-1829.

Zobel AW, Nickel T, Sonntag A, Uhr M, Holsboer F, Ising M (2001) Cortisol response in the combined dexamethasone/CRH test as predictor of relapse in patients with remitted depression: a prospective study. Journal of Psychiatric Research 35:83-94.

List of abbreviations

ACTH	adrenocorticotropic hormone
Acvr1(a)	activin receptor IA
Acvr2a	activin receptor IIA
Acvr2b	activin receptor IIB
AVP	arginine vasopressin
BDNF	brain derived neurotrophic factor
BSA	bovine serum albumine
CA	cornu ammonis (part of the hippocampal formation)
Cck	cholecystokinin
Cckbr	cholecystokinin B receptor
Chrm1	cholinergic receptor, muscarinic 1, CNS
CNS	central nervous system
cpm	counts per minute
CRH	corticotropin releasing hormone
CRHR	corticotropin releasing hormone receptor
DaLi	dark/light box
Dex	dexamethasone
DG	dentate gyrus
ECS	electroconvulsive seizure
ECT	electroconvulsive seizure therapy
EPM	elevated plus maze
fEPSPs	field excitatory postsynaptic potentials
fMRI	functional magnetic resonance imaging
FST	forced swim test
GABA	gamma-aminobutyric acid
Gabrd	gamma-aminobutyric acid (GABA-A) receptor, subunit delta
Gmeb1	glucocorticoid modulatory element binding protein 1
GR (Nr3c1)	glucocorticoid receptor
Grp	gastrin releasing peptide
HAM-D	Hamilton Depression Rating Scale
HPA axis	hypothalamo pituitary adrenal axis
5-HT	serotonin
Inha	inhibin alpha

Inhba	activin βA/inhibin βA
i.p.	intraperitoneal
LTP	long-term potentiation
mAChR	muscarinic acetylcholine receptor
mHB	modified hole board
MPI	Max Planck Institute of Psychiatry
MR	mineralocorticoid receptor
NaCl	sodium chloride
NDRI	dopamine reuptake inhibitor
NMDA	N-Methyl-D-Aspartat
NRI	noradrenaline reuptake inhibitor
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Penk1	preproenkephalin 1
PET	positron emission tomography
Plat	tissue plasminogen activator
POMC	preopiomelanocortin
SEM	standard error of the mean
SERT	serotonin transporter
SNP	single nucleotide polymorphism
SNRI	noradrenaline reuptake inhibitor
SSC	sodium chloride-sodium citrate
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TGF-β	transforming growth factor β
UTP	uridine triphosphate

Assertion/Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig angefertigt habe. Es wurden nur die in der Arbeit ausdrücklich benannten Quellen und Hilfsmittel benutzt.

Des Weiteren erkläre ich, dass ich nicht anderweitig ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen. Die vorliegende Dissertation liegt weder ganz, noch in wesentlichen Teilen einer anderen Prüfungskommission vor.

Ort, Datum

Unterschrift Karin Ganea

List of publications

Schmidt MV, Levine S, Alam S, Harbich D, Sterlemann V, **Ganea K**, der Kloet ER, Holsboer F and Müller MB
Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse.
J Neuroendocrinol. 2006 Nov;18(11):865-74

Ganea K, Liebl C, Sterlemann V, Müller MB and Schmidt MV
Pharmacological validation of a novel home cage activity counter in mice.
J Neurosci Methods. 2007 May 15;162(1-2):180-6

Schmidt MV[a], Sterlemann V[a], **Ganea K**, Liebl C, Alam S, Harbich D, Greetfeld M, Uhr M, Holsboer F and Müller MB
a) both authors equally contributed to this work
Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence
Psychoneuroendocrinology. 2007 Jun;32(5):417-29

Sterlemann V, **Ganea K**, Liebl C, Harbich D, Alam S, Holsboer F, Müller MB and Schmidt MV
Long-term behavioral and neuroendocrine alterations following chronic social stress during adolescence: implications for stress-related disorders
Hormones and behavior, accepted November 2007

Curriculum vitae

Personal data

Name:	Karin Ganea
Date of birth:	26.09.1978
Place of birth:	Munich, Germany
Nationality:	German

School Education

1985-1989	Elementary school, Wolfratshausen
1989-1998	Gymnasium Geretsried
	Degree: Abitur

University Education

09/1999-12/2003	Ludwig-Maximilians-University, Munich
	Field: Biology
01/2004-10/2004	Diploma thesis on "Inactivation of Corticotropin-releasing hormone receptor 1 in the CNS by RNA-interference *in vivo*: effects on behaviour, neuroendocrine regulation and gene expression" at the Max-Planck-Institute of Psychiatry, Munich
10/2004	Master of science degree

PhD thesis

01/2005-12/2007	PhD thesis on "Identification and characterisation of novel antidepressant-responsive genes in mouse brain" at the Max-Planck-Institute of Psychiatry, Munich

Acknowledgements

First of all I would like to thank Dr. Marianne Müller for providing me the opportunity to realise my PhD in her research group. She has been my supervisor since the beginning of my study and supported me with scientific advice, helpful suggestions and constant encouragement during the course of this work.
Second, I owe my special thanks to Dr. Mathias Schmidt, who has always been available to provide me with valuable and constructive ideas and fruitful discussions that improved the quality of this study.
Further, I wish to thank all the people in our group for making the everyday work easier. I am deeply grateful to Vera and Claudia for their unselfish and gladly support in every respect and for their enriching friendship. Daniela and Stephanie are thanked for their assistance with all types of technical problems - at all times. Daniela – I will never forget the fun we had during the surgeries! I would like to thank Sebastian for the constructive proof-reading of my work. Especially, I thank Dr. Inge Sillaber and her team of the Affectis Pharmaceuticals AG for having laid of the cornerstone for this thesis and their continuous support. Many thanks to Inge for her patience answering the multitude of questions I had. I wish to thank Corinna for her unconfined help I could rely on whenever I needed it and her amicable and enjoyable company in my years at the institute. Thanks to Christine, Sabine and Yvonne for their help in performing surgery, immunohistochemistry and cell culture experiments.
I would like to express my gratitude to all those people of the institute who helped to make this thesis possible.
Finally, I want to thank Prof. Landgraf and Prof. Grupe for their unconditional readiness to read and evaluate my PhD thesis.