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**ON ANTIDEPRESSANT EFFECTS OF RUNNING AND SSRI:
FOCUS ON HIPPOCAMPUS AND STRIATAL DOPAMINE PATHWAYS**

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To my family

ABSTRACT

Considering the heterogeneous character of depression, it seems likely that many brain regions are involved mediating the diverse symptoms of depression. For example, the mesolimbic DA system has a central function for motivational behaviors and pleasure seeking, and it is possible that malfunctioning of brain reward systems could be an underlying mechanism of the anhedonia experienced in depressive illness. The hippocampal formation is essential for learning and memory and depressed patients often suffer of cognitive disturbances. Image studies have shown that there is shrinkage of the hippocampal formation in depressed patients. In this thesis we have used an animal model of depression, the Flinders Sensitive Line strain (FSL), to analyze the effects of physical activity, which has antidepressant effect in humans, and the SSRI-type drug escitalopram. Special focus has been on the brain reward pathways and the hippocampus.

To monitor depressive-like behavior we have used Porsolt's swim test, which can discriminate between a norepinephrine-like and serotonin-like mediated antidepressant responses. We also examined how environmental factors can modulate the response to antidepressant treatments. When comparing the antidepressant response of wheel running to the response of escitalopram we found the following: Wheel running had an antidepressant norepinephrine-like effect in the Porsolt swim test in FSL rats. Escitalopram had a serotonergic-like antidepressant response in the Porsolt swim test in single housed FSL rats with a running-wheel as cage enrichment (barely used for running). In contrast, escitalopram did not have an antidepressant-like effect in single housed FSL rats without a running-wheel as cage enrichment. Thus environmental factors can facilitate the antidepressant action of escitalopram.

Major depression in humans is associated with shrinkage of hippocampus. In this thesis we noted that the "depressed" FSL strain had lower adult hippocampal cell proliferation than the "non-depressed" FRL strain. Interestingly, wheel running normalized this mismatch. Moreover, wheel running, and escitalopram with or without access to running wheels increased the survival of newly proliferated cells. Thus decreased hippocampal cell proliferation could be one factor that contributes hippocampal shrinkage in depressed patients and antidepressant treatment could have the capacity to normalize this. However, the newly proliferated cells require a diverse cocktail of regulatory molecules to differentiate and migrate into deeper layers and send out axons that integrate into functional neuronal networks. In this thesis we have analyzed the levels of mRNAs encoding the neuropeptide tyrosine (NPY) and one of its receptor Y1 and the Brain Derived Neurotrophic Factor (BDNF), which are factors with a putative role in these processes. We found that escitalopram, running and a combination of these two increased the number of newly proliferated hippocampal BrdU-immunoreactive cells. NPY mRNA was elevated by running and the combined treatment and correlated positively to the number of newly proliferated cells and climbing behavior in the Porsolt swim test (noradrenergic response). Running elevated BDNF mRNA and correlated positively to climbing. Y1 receptor mRNA was elevated by running and the combined treatment and correlated to swimming in the Porsolt swim test (serotonergic response). Escitalopram alone did not regulate any of the molecules analyzed. Thus, different patterns of hippocampal NPY, Y1, BDNF mRNAs correlated to the norepinephrinergic and serotonergic PST response suggesting different mechanisms to achieve an antidepressant-like response by the treatments. Finally we analyzed the effect of the mild stress of social isolation in female FSL rats and SD controls with focus on hippocampus and on the dopamine D2 receptor in brain reward pathways. We demonstrated that socially isolated but not group housed FSL rats had lower dopamine D2 receptor mRNA levels compared to "non-depressed" Sprague Dawley rats. Our

findings of decreased dopamine D2 receptor levels in socially isolated FSL rats suggest that low D2 receptor expression may play a role in the pathophysiology of depression. Analysis of the hippocampus in the same model revealed that social isolation increased newly proliferated BrdU-immunoreactive cells in the FSL rats whereas it had no impact on the number of cells in the Sprague Dawley strain. Group housed “depressed” Flinders rats had a lower expression of mRNAs encoding BDNF, NPY and the serotonin 5HT2A receptor than Sprague Dawley, however social isolation down regulated these molecules in Sprague Dawley rats and washed-out the differences between the two strains. To summarize, our finding support that physical activity has a comparable antidepressant effect as a SSRI-type drug and that the drug is dependent on the environmental context. Moreover, we have found a complex regulation of the patterns of hippocampal cell proliferation and regulation of different receptor and neuropeptide mRNAs in hippocampus and brain reward pathways that could have a major impact for depression and antidepressant treatments.

Keywords: Depression, exercise, neuropeptides, cell proliferation, neurogenesis, NPY, BDNF, Flinders Sensitive Line.

LIST OF PUBLICATIONS

- I. **ASTRID BJØRNEBEKK, ALEKSANDER A. MATHÉ AND STEFAN BRENÉ.**
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- II. **ASTRID BJØRNEBEKK, ALEKSANDER A. MATHÉ AND STEFAN BRENÉ.**
(2006) Running has differential effects on NPY, opiates and cell proliferation in an animal model of depression and controls. *Neurophysopharmacology* 31(2):256-64
- III. **ASTRID BJØRNEBEKK , ALEKSANDER A MATHÉ, SUSANNE HM GRUBER AND STEFAN BRENÉ.** (2007) Housing conditions modulate escitalopram effects on antidepressive-like behavior and brain neurochemistry. *Submitted manuscript*
- IV. **ASTRID BJØRNEBEKK , ALEKSANDER A MATHÉ AND STEFAN BRENÉ**
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- V. **ASTRID BJØRNEBEKK , ALEKSANDER A MATHÉ AND STEFAN BRENÉ**
Isolated Flinders Sensitive Line rats have decreased dopamine D2 receptor mRNA *NeuroReport (in press)*
- VI. **ASTRID BJØRNEBEKK , ALEKSANDER A MATHÉ, SUSANNE HM GRUBER AND STEFAN BRENÉ** Social isolation increases number of newly proliferated cells in hippocampus in female Flinders Sensitive Line rats *Submitted manuscript*

TABLE OF CONTENTS

INTRODUCTION	9
MAJOR DEPRESSIVE DISORDER	9
CAUSES OF CLINICAL DEPRESSION	9
HEREDITY	9
PHYSIOLOGY	9
TREATMENTS	10
PHARMACOLOGICAL TREATMENTS	11
PSYCHOTHERAPY	11
ELECTROCONVULSIVE THERAPY	11
RUNNING/PHYSICAL EXERCISE	11
NEURONAL CIRCUITRY OF DEPRESSION	12
THE HIPPOCAMPAL FORMATION	12
MONOAMINERGIC PROJECTIONS TO DENTATE GYRUS	13
HIPPOCAMPUS IN DEPRESSIVE ILLNESS	13
GENERATION OF NEW CELLS IN THE ADULT HIPPOCAMPUS	14
THE NEUROGENIC SUBGRANULAR CELL LAYER	14
THE STRIATAL DOPAMINE SYSTEM	14
PLASTICITY MARKERS	16
BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)	16
NEUROPEPTIDES	16
SUBSTANCE P	17
NEUROPEPTIDE Y	17
THE ENDOGENOUS OPIOID PEPTIDES	18
DOPAMINE AND DOPAMINE RECEPTORS	19
SEROTONIN RECEPTORS	19
GENETIC ANIMAL MODEL OF DEPRESSION	20
FLINDERS SENSITIVE LINE	20
GENDER	20
AIMS	22
MATERIAL AND METHODS	23
ANIMALS	23
BEHAVIORAL EXPERIMENTS	23
WHEEL RUNNING	23
SOCIAL ISOLATION	23
PORSOLT SWIM TEST	23
ADMINISTRATION OF BRDU	25
ADMINISTRATION OF ESCITALOPRAM	25
IN SITU HYBRIDIZATION	25
IMMUNOHISTOCHEMISTRY	26
STEREOLOGY	26
RESULTS AND DISCUSSION	27

ANTIDEPRESSANT-LIKE EFFECT OF WHEEL-RUNNING IN “DEPRESSED RATS” (PAPERS I & III)	27
ANTIDEPRESSANT-LIKE EFFECT OF WHEEL-RUNNING DIFFERENT FROM THAT OF ESCITALOPRAM (PAPERS I & III)	27
MECHANISMS ASSOCIATED WITH ANTIDEPRESSANT-LIKE EFFECT OF RUNNING AND ESCITALOPRAM (PAPERS I-IV)	28
HIPPOCAMPUS	28
STRIATAL DOPAMINE PATHWAYS	30
ESCITALOPRAM ATTENUATES RUNNING BEHAVIOR (PAPER III)	31
ENVIRONMENTAL INFLUENCE ON ANTIDEPRESSANT-LIKE EFFECT OF ESCITALOPRAM (PAPER III)	32
ENVIRONMENT AND GENDER INFLUENCES (PAPER III, V & VI)	32
<u>CONCLUSIONS</u>	35
<u>ACKNOWLEDGEMENTS</u>	36
<u>REFERENCES</u>	42

INTRODUCTION

MAJOR DEPRESSIVE DISORDER

Major depressive disorder (MDD) is a state of intense sadness, despair or melancholia that is disruptive to the individual's wellbeing and social functioning in every day life. It is noteworthy that major depressive disorder is a clinical diagnosis and may be different from the everyday meaning of "being depressed".

Major depressive disorder affects 7% to 18% of the population on at least one occasion (1), and is currently the leading cause of disability world wide, and the 4th leading contributor to the global burden of disease (WHO). Women are overrepresented in the depressed population. About twice as many women receive a diagnosis of depression than men (2, 3). The reason for this gender difference is not clear, but most likely involves a combination of biological and sociocultural factors (4).

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) criteria for a diagnosis of MDD, the patient must display symptoms of either depressed mood or anhedonia over a two-week period, in conjunction with at least five other symptoms. These symptoms include: feelings of overwhelming sadness or emptiness, decreased interest or pleasure in daily activities, loss of weight (or weight gain), sleep disturbances, feelings of worthlessness, loneliness, and or anxiety, psychomotor agitation or retardation, fatigue or loss of energy, trouble with concentration and focus, as well as a generalized slowing and reduction of cognition and memory functioning. MDD may occur as discrete events described as "a single" episode, but more commonly reoccurs over the lifespan and is then specified as "recurrent" MDD. Moreover, episodes of MDD can further be divided into mild, major or severe. MDD is considered to be a life threatening disease in the sense that about 15% of depressed individuals commit suicide.

CAUSES OF CLINICAL DEPRESSION

Despite of intensive investigations, the underlying biological causes of MDD or other forms of depressive illness have not been elucidated.

HEREDITY

Some types of depression run in families and suggest that a biological vulnerability can be inherited (5, 6). MDD is from 1.5-3 times more frequent among first-degree biological relatives of persons with the disorder than among the general population (7). However, MDD also occurs in people without a family history of depression.

PHYSIOLOGY

A common held view is that clinical depression is caused by an imbalance of neurotransmitters in the brain. This theory is based on observations in the 1950s that antidepressants alter monoamine neurotransmitter levels as well as altering depressive symptoms. The focus was primarily on two neurotransmitters of the monoamine class, serotonin and norepinephrine, because the antidepressant medications had the effect of increasing their availability at synaptic junctions. These observations led to the *monoamine hypothesis* that thought depression was

due to a depletion of one or both of these neurotransmitters (8-10). During the last decades the view of the pathophysiology of mood disorders has been strongly influenced by the monoamine hypothesis of depression (11, 12). However, when presented to the public the relationship between monoamine levels, such as serotonin and norepinephrine, and depression is mostly oversimplified and not supported by research evidence. It is recognized that most antidepressants have a delayed onset before there is a significant difference in the efficacy compared to placebo treatment. Usually several weeks of administration is required to improve depressive symptoms (13, 14), suggesting that other mechanisms rather than increased levels of serotonin or norepinephrine per se are mediating the antidepressant effects.

In the wake of the monoamine hypothesis of depression a new research focus was formed. It was based on the discovery that antidepressants after long-term treatment produce adaptive changes in monoamine receptors and their coupling to intracellular signal transduction (15). This new direction is sometimes called a *chemical or molecular hypothesis of depression*, and presumes that mood disorders are produced by long-term changes in production or activity of molecules in the brain such as growth factors or neuropeptides, and that these molecular changes may be counteracted by antidepressant treatments (12, 16, 17).

In the last few years an alternative hypothesis to the molecular hypothesis of depression has developed (11, 18). The *network hypothesis* suggests that depression is caused by problems in activity-dependent neuronal communication, and that antidepressants induce plastic changes in neuronal connectivity that gradually leads to improved neuronal information processing in the affected neuronal network and recovery of mood (11). A key aspect of the network hypothesis is the recognition that information in the brain is not stored in a chemical form. Instead information is being processed by complex interactions of neuronal networks that develop by interactions with the environment, and are constantly refined through synaptic plasticity to assure optimal processing and storage of information (11). The network hypothesis does not exclude the chemical or molecular hypothesis of depression, rather they complement each other. Whereas synthesis and release of signaling molecules are regulated by neuronal activity, changes in the activity of neuronal networks influence the concentration of these molecules. Thus, whereas the initial effects of antidepressive treatments are chemical, directed towards the metabolism of monoamines, they result in adaptive changes in concentration of signaling molecules linked to the structure of neuronal networks. Evidence for a network hypothesis of depression is mostly indirect. Reduced hippocampal gray matter, and decreased hippocampus and frontal cortex volumes are frequently observed in depressed patients (19, 20), as well as memory impairments (21) which possibly imply reduced neuronal complexity and connectivity. One of the most compelling evidence for the network hypothesis is the capability of various antidepressant treatments to increase hippocampal neurogenesis. Moreover, the delayed onset of most antidepressants (13) is similar to the time frame it takes newly formed neurons to migrate into the granule cell layer and get integrated into functional neuronal networks, which supports the network hypothesis of depression.

TREATMENTS

Despite our limited understanding of depressive illness there are several efficient treatments. The majority of patients show improvements with any of several antidepressants or with electroconvulsive seizures (ECT) (18). In addition there exist several different forms of psychotherapy that are efficient for many patients with mild to moderate cases of depression. A

combination of medication and psychotherapy is often recommended and is considered to have the best outcome (22).

PHARMACOLOGICAL TREATMENTS

In the 1950s two classes of chemical antidepressants, the tricyclic antidepressants and the monoamine oxidase inhibitors were discovered, in part by chance, and led to a revolution in the treatment of depression (14). The mechanisms of action of these antidepressants were inhibition of serotonin or norepinephrine reuptake transporters by tricyclic antidepressants, and inhibition of monoamine oxidase, which is a major catabolic enzyme for monoamines, by monoamine oxidase inhibitors (23). Despite the numerous antidepressant compounds available today, most agents have the same basic mechanism of action as the tricyclics discovered 50 years ago, and their efficacy and the range of patients they can treat are no better than with the early tricyclics. The serotonin-selective reuptake inhibitors (SSRIs) are the current first choice in standard drug treatment. They have a better side effect profile than the older agents, and are thus easier to tolerate. A complete remission is obtained in about 50% of all patients on SSRIs, whereas partial improvements are found in up to 80% of patients.

All commonly used antidepressant drugs today affect serotonin and/or norepinephrine and can be classified as: tricyclic antidepressants, the norepinephrine-selective reuptake inhibitors (NRIs), the serotonin-selective reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOs), and more receptor specific agents. As previously mentioned the delayed onset of antidepressants suggests that adaptive mechanisms rather than serotonin and norepinephrine neurotransmission per se is responsible for the alleviation of depressive symptoms.

PSYCHOTHERAPY

With psychotherapy the depressed patient receives assistance in understanding and resolving habits or problems that may have contributed to the cause of depression. Medication and psychotherapy are generally complementary. However, efficient psychotherapy may result in differential habitual thinking and actions and lead to better recovery and lower relapse rate than medications alone. There are many different psychotherapeutic approaches. *Cognitive Therapy*, often combined with *behavioral therapy*, is demonstrated to be more efficient in reducing depressive symptoms and reducing relapse rate than traditional “talk” therapies (22).

ELECTROCONVULSIVE THERAPY

In severe cases of depression, or when other treatments fail electroconvulsive therapy (ECT) may be used. In this treatment short bursts of a controlled electrical current of electricity, induce a brief seizure while the patient is under anesthesia. The benefit with ECT treatment is a very fast response, and it is therefore the treatment of choice in emergencies. Common side effects are short-term memory loss and disorientation. However long term memory functions are preserved (24). Like antidepressants little is known about the actual mechanisms of action of ECT. However, there is evidence that electroconvulsive seizures induce; massive release of monoamines, hormones and neuropeptides, neurotrophic factors and increases neurogenesis (25-28).

RUNNING/PHYSICAL EXERCISE

Exercise promotes physical health and has an antidepressant effect in patients, similar to conventional antidepressant treatments and psychotherapy. Exercise is also thought to help antidepressants and psychotherapy to work better and faster. Follow-up studies of patients have

shown that continued exercise is more efficient in preventing depressive relapses than antidepressant medication (29-32).

Since anhedonia is a dominant feature of depression, it can be difficult to find the motivation to exercise in severe depression. Physical activity such as running is a natural rewarding behavior that activates the brain reward system (33, 34). A well-known phenomenon that illustrates the activation of the brain reward system is the “runner’s high”, that can be regarded as a state of euphoria experienced immediately after a session of running (35). Running increases brain dopaminergic and noradrenergic activity, levels of endogenous opioid peptides, and the expression of neurotrophic factors and hippocampal neurogenesis (36-44). It is possible that exercise, which increases these factors, thereby also potentiates the functional and structural reorganization of brain that could be necessary for the antidepressant effect. In this thesis we analyze regulation of hippocampal cell proliferation/neurogenesis as well as candidate molecules thought to be involved in neuronal plasticity in the brain reward system and hippocampus that may be involved in the antidepressant properties of running. Moreover, the antidepressant properties of voluntary wheel running are compared to those of escitalopram, a commonly used SSRI.

NEURONAL CIRCUITRY OF DEPRESSION

Our understanding of the neuronal circuitry involved in the pathophysiology of depressive disorders is still very rudimentary. There is no consensus regarding the site of pathology. The wide variety of symptoms of depression suggests that many brain regions might be involved mediating the different symptoms. In this thesis we have focused on the pathophysiology of depression, and adaptive changes after antidepressant treatments in the hippocampal formation and the brain reward pathway, regions implicated in the cognitive disturbances and the decreased motivation observed in depressed patients. However, several cortical and subcortical structures such as the prefrontal cortex, the amygdala, the hypothalamus and the brainstem among others (18), which are implicated in for example attention, motivation and fear, are also involved.

THE HIPPOCAMPAL FORMATION

The hippocampal formation consists of six regions; the dentate gyrus (DG), hippocampus that is subdivided into three fields (CA1, CA2 and CA3), subiculum, presubiculum, parasubiculum and entorhinal cortex (Fig. 1) (45). The major input into the hippocampal formation is from the entorhinal cortex via the perforant pathway. The perforant pathway terminates on dendrites of granule cells in the molecular layer of the dentate gyrus. The granule cells then project via the mossy fibers to the CA3 field of the hippocampus. Pyramidal cells of the CA3 field project via the Schaffer collaterals to the CA1 pyramidal neurons, which projects from the hippocampus to cortical areas via the subiculum. This circuitry is termed the trisynaptic circuit and glutamate acts as the major excitatory neurotransmitter in each of these pathways (45, 46).

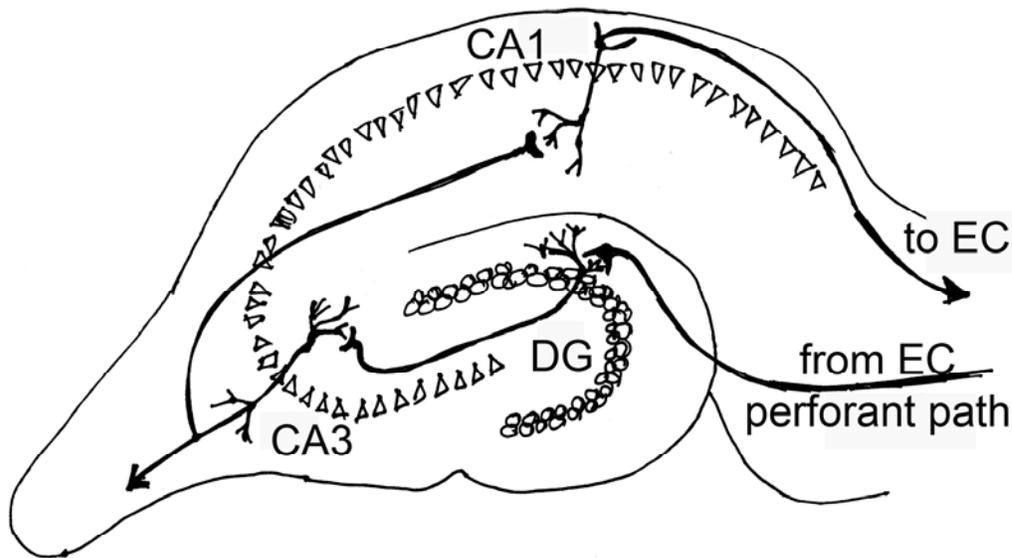


FIGURE 1. A schematic drawing of the trisynaptic circuit in the hippocampal formation.

There is also a direct projection from the entorhinal cortex to the CA1 and CA3 pyramidal cells. In addition, a variety of interneurons, mainly GABAergic, that are located in the vicinity to the granule cell layer provide a mean of inhibitory control of granule cell activity (47).

MONOAMINERGIC PROJECTIONS TO DENTATE GYRUS

Whereas entorhinal cortex provides the major input to the dentate gyrus the subcortical input to the dentate gyrus mainly originates from the septal nuclei, the supramammillary region of the posterior hypothalamus, and from several monoaminergic nuclei in the brain stem, especially the locus coeruleus and the raphe nuclei (45). Of the monoaminergic projections to the hippocampal formation, noradrenergic innervations are the most prominent. The dentate gyrus noradrenergic input is primarily from the pontine nucleus locus coeruleus, and the noradrenergic fibers terminate mainly in the polymorphic layer of the dentate gyrus (45, 48-50). The serotonergic projections from the raphe nucleus also terminate in the dentate polymorphic layer. The dentate gyrus also receives a minor and diffusely distributed dopaminergic innervations from cells in the ventral tegmental area (51).

HIPPOCAMPUS IN DEPRESSIVE ILLNESS

One of the most consistent findings of pathology in depressive illness is the finding of reduced hippocampal volume in patients diagnosed with MDD (MDD)(19, 20, 52, 53). Moreover, the degree of hippocampal volume reduction is correlated with duration of the disease (20, 21). The hippocampal formation has an important role in learning and memory (54) and it is also one of the two major regions in the adult brain where new neurons are formed (55). One hypothesis of depressive illness is that it is caused by a reduced neuronal plasticity with a disturbed hippocampal neurogenesis (56).

GENERATION OF NEW CELLS IN THE ADULT HIPPOCAMPUS

Until recently a central dogma of neuroscience was that neurons were not produced in the adult brain, despite early evidence of the contrary (55, 57, 58). Currently it is widely accepted that neurogenesis occurs in the subgranular zone of the dentate gyrus and in the subventricular zone. The physiological role of neurogenesis is still controversial. Two popular perspectives of the role of the new neurons in hippocampus is that they are implicated in learning and memory formation (59, 60) and that reduced hippocampal plasticity and reduced neurogenesis is associated with depressed mood and that the antidepressant effects are achieved by increased plasticity with newly formed neurons added in the dentate gyrus (61, 62). The neurogenesis hypothesis of depression is based on a number of findings that stress, a key factor implicated in the cellular pathology of depression, decreases neurogenesis (61, 63), whereas treatments that have an antidepressant effect in patients, e.g. antidepressants drugs (62), electroconvulsive treatment (64) and physical exercise increase hippocampal neurogenesis (36).

THE NEUROGENIC SUBGRANULAR CELL LAYER

In hippocampus, the majority of the progenitor cells are found in the “subgranular zone”. After mitosis the daughter cells migrate into the granule cell layer where they differentiate into glial cells and mature neurons, which send projection axons to the CA3 region of the pyramidal cell layer (Fig. 2)(65-67). It has been suggested that production of neurons in the dentate gyrus may be stimulated by activation of certain receptors located on granule precursor cells.

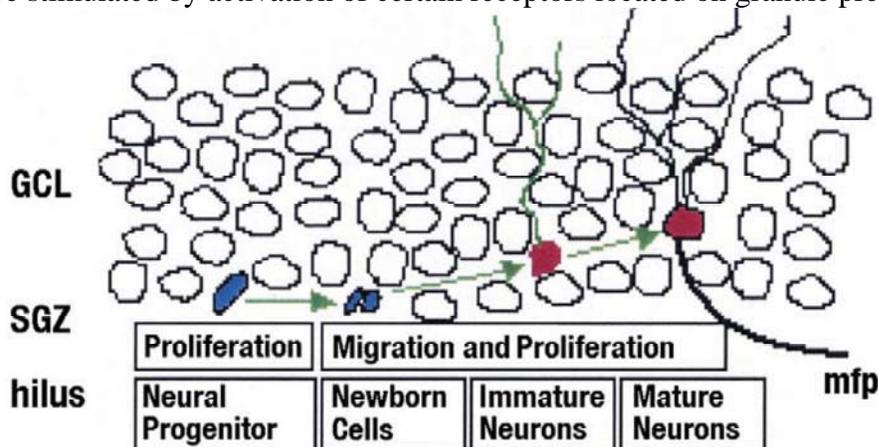


FIGURE 2. Neurogenesis in hippocampus. Progenitor cells are located in the subgranular zone (SGZ) of the hippocampus, at the border between the hilus and the granule cell layer (GCL). Approximately 80% of the newborn cells become neurons, and the remaining glial cells. Newly proliferated cells migrate into the granule cell layer, mature and become mature adult granule cells, which send axons into the pyramidal cell layer via the mossy fiber pathway (mfp). Modified from Duman et al. (The Journal of Pharmacology and Experimental Therapeutics 2001; 299(2):401-407, with permission from Elsevier)

THE STRIATAL DOPAMINE SYSTEM

The striatum in rat is a relatively large gray mass in the deepest part of the cerebral hemisphere. It is a rather homogenous structure formed by two major subdivisions, the *ventral* and *dorsal striatum*, which share numerous cytoarchitectural and neurochemical features and afferent connectivity, but to some extent differ in the type of information that they process (68).

The neurons in the terminal field of dorsal and ventral striatum can be divided into three groups; medium size spiny neurons, medium size aspiny neurons and large aspiny neurons (69). The most abundant neuronal type is the GABAergic medium size spiny neurons representing approximately 95% of the striatal neuronal population. Medium size spiny neurons are projection neurons (70), whereas the medium size aspiny neurons are interneurons (68). Medium size spiny neurons can further be divided into two populations according to their projection area and their peptide and receptor expression. One population projects to the substantia nigra and expresses preferentially the dopamine D1 receptor subtype and the neuropeptides dynorphin and substance P. The other population of medium size spiny neurons projects to the globus pallidus, and expresses preferentially dopamine D2 receptors and the neuropeptide enkephalin (Fig. 3) (68).

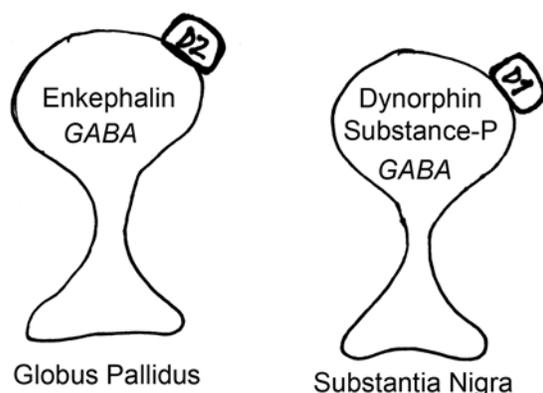


FIGURE 3. GABAergic medium size spiny neurons are divided into two populations, one that projects to the globus pallidus and express the dopamine D2 receptor and the enkephalin. The other population of expresses the dopamine D1 receptor and the neuropeptides dynorphin and substance-P.

The dorsal striatum consist of the caudate putamen. In primates the internal capsule separates the caudate nucleus and the putamen from each other. In rodents however the internal capsule is poorly developed, and therefore the entity is referred to as the caudate-putamen complex. The dorsal striatum receives afferents from sensorimotor cortex, thalamus and dopaminergic projections from the substantia nigra pars compacta (68). There are two main pathways that project from striatum to the cortex via basal ganglia and thalamus. The “direct” striatonigral pathway projects directly to the substantia nigra pars compacta (the so-called direct pathway), and the “indirect pathway” that projects via the globus pallidus and the subthalamic nuclei to the substantia nigra. From the substantia nigra the information is sent back to the thalamus and to cortex. These pathways are critically involved in control of voluntary movements. The striatum integrates sensorimotor associative and limbic information to produce motor behavior. The important role for striatal pathways in control of movements are obvious in diseases such as Parkinson’s and Huntington’s where death of dopaminergic neurons that form the nigrostriatal pathway (Parkinson’s) or degeneration of the medium spiny neurons in the striatum (Huntington’s) are causing symptoms of slowness of movements (bradykinesia), tremor and rigidity (Parkinson’s), or irregular movements and rigidity (Huntington’s).

The ventral striatum should be regarded as a ventral continuation of the dorsal striatum as there is no clear boundary between the regions. The accumbens is part of the ventral striatum and is often referred to as the link between the limbic system and the motor system (71). Accumbens receives dopaminergic input from the ventral tegmental area and limbic excitatory input from the hippocampal formation, entorhinal cortex and amygdala (72-75). It can further be divided into a medial and a lateral part, termed shell and core respectively (72, 76, 77). Functionally, the core division is considered to have a role in motor function whereas the shell is more limbic. Both core and shell project into the ventral pallidum and ventral mesencephalon (78, 79). In addition, the shell also projects into the bed nucleus of the stria terminalis, hypothalamus, mesencephalic reticular formation, and central grey (68).

The dopaminergic projection from the ventral tegmental area of the midbrain to the nucleus accumbens (the mesolimbic dopamine system) plays a critical role in reward. Virtually all drugs that are abused by humans mediate a dopamine release in the nucleus accumbens in animal models (80), and this increase is thought to contribute to the rewarding effect of the drugs (81, 82). However, the reward mediating properties of the mesolimbic dopamine system has obviously not evolved to make us prone to abuse drugs. Natural rewards as food, sex and running also activate the brain reward system although with much lower potency. The symptoms of anhedonia, reduced motivation and lack of energy seen in most individuals with depression suggest that the striatal dopamine pathways are critically involved in the pathophysiology of depression.

PLASTICITY MARKERS

BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family-also comprising nerve growth factor, neurotrophin-3 and neurotrophin-4, and has an important role in neuronal growth, survival and differentiation. Moreover, trophic factors are suggested to have an important role for adaptive changes and BDNF is important for learning, memory and LTP in hippocampus (83, 84). Recently a role for neurotrophic factors, and in particular BDNF, in mood disorders was suggested by Duman and coworkers (85). This “neurotrophic” hypothesis of depression is partly based on observations that chronic administration of different classes of antidepressant drugs, electroconvulsive seizures (ECS) and physical exercise increase the expression of BDNF and its receptor, trkB mRNA, in hippocampal regions (86-88), and BDNF is implied to have a role in the control of plastic changes induced by antidepressant treatments (85). BDNF and its receptor trkB are distributed throughout the brain. In hippocampus BDNF expressing neurons are found in the granule cell layer, the pyramidal cell layer as well as in hilus (89, 90). BDNF is also known to increase the expression of neuropeptides such as NPY and CCK, whereas it decreases the expression of dynorphin. It is possible that BDNF may play a role in modulating neuropeptide expression in the normal brain as well as in various pathophysiological states (91).

NEUROPEPTIDES

Neuropeptides act as neurotransmitters, neuromodulators or as trophic factors. Today about 65 different mammalian neuropeptides are known (92, 93).

The mechanisms for synthesis and packaging of neuropeptides into synaptic vesicles are different from those used for small molecule neurotransmitters. Classic neurotransmitters such

as glutamate, catecholamines (dopamine, norepinephrine, adrenaline, serotonin) and GABA are stored in small clear synaptic vesicles close to the presynaptic membrane. In contrast, neuropeptides are synthesized in the cell body as pre-propeptides, which are much larger than the “mature” peptide. Pre-propeptides are synthesized in the rough endoplasmic reticulum where the signal sequence of amino acids is removed and the propeptides remain. The propeptides are processed in the Golgi network and packed into vesicles in the cell body, that are transported by fast axonal transport to the synaptic terminal of the neuron. Neuropeptides are released by exocytosis when vesicles fuse with the presynaptic membrane as a response of Ca^{2+} influx (94). Whereas small molecule neurotransmitters are released upon low-frequency stimulation, neuropeptide release is mainly observed upon high frequency stimulation (95).

Propeptide precursors are typically larger than their active peptide product, and can give rise to more than one type of neuropeptide. That means that multiple neuropeptides can be released from a single vesicle. Moreover, neuropeptides are often co-released with small molecule neurotransmitters, thus giving rise to complex post-synaptic responses (96).

Neuropeptides initiate their effects by binding to G-protein coupled receptors at low concentrations compared to the concentration required to activate receptors for small molecule neurotransmitters. These properties make it possible for neuropeptides to activate post-synaptic targets quite far from the presynaptic terminal, and can directly or indirectly regulate several physiological responses of nearby neurons (92, 97).

SUBSTANCE P

The study of neuropeptides began nearly eighty years ago with the discovery of substance P (SP) by Von Euler and Gaddum in 1931. Substance P is an 11-aminoacid peptide belonging to the tachykinin family of peptides, and is present both in the PNS and CNS with high concentrations in human hippocampus, neocortex, striatum, and in the gastrointestinal tract. The gene encoding for SP also encodes for other neuropeptides including, neurokinin A, neuropeptide K and neuropeptide γ . SP is thought to be involved in memory, reward and in suppression of pain (94, 98). Moreover, elevated levels of SP have been found in the cerebrospinal fluid of depressed patients (99) and attempts have been made to treat depression by blocking the tachykinin NK1 receptor that has the highest selectivity for SP (100), suggesting a potential role in the pathophysiology of depression.

NEUROPEPTIDE Y

Neuropeptide tyrosine (Y) (NPY) is a 36-amino-acid peptide named by its many tyrosine residues. NPY is widely distributed within the nervous system, with high concentration in several limbic and cortical regions. NPY is involved in regulation of a multiple functions in the CNS; including feeding behavior (101) learning and memory (102), locomotion (103), sexual behavior and seizure control(104). Clinical and experimental data suggest a role for NPY in the pathophysiology of depression (27, 105-107). Enhancement of the NPYergic transmission is a mechanism that is shared by antidepressants and electroconvulsive therapy (27, 108, 109). Moreover, a role for NPY in promoting proliferation of neuronal precursor cells, possibly mediated by the Y1 receptor has been suggested (110, 111).

The Y1 receptor

The effects of NPY are mediated by activation of G-protein coupled receptors. Six receptors (Y1-Y6) with high affinity for NPY are cloned (112). In this thesis we have focused solely on the Y1 receptor, due to its possible involvement in antidepressant action and promoting neuronal proliferation (106, 110, 111). High levels of the Y1 receptor are detected in the neocortex, the medial preoptic and the arcuate nucleus of the hypothalamus, several thalamic nuclei, and the hippocampal dentate gyrus and in the olfactory tubercle (113). The distribution of the Y1 receptor is species specific and in humans Y1 receptors are only found in the dentate gyrus (114).

THE ENDOGENOUS OPIOID PEPTIDES

Enkephalin, dynorphin and β -endorphin are endogenous opioid peptides important for brain reward and interacting with the dopamine system (115, 116). They are called *opioids* because they bind to the same receptors that are activated by opium. Morphine is the predominantly active ingredients in opium, and has a high analgesic and addictive potential. The opioid peptides were discovered in the 1970s when searching for endorphins, *endogenous* compounds that mimic the actions of morphine (94). By now over 20 endogenous ligands of the opioid receptors have been identified. They are divided into three classes; the endorphins, the enkephalins and the dynorphins. Endogenous opioid peptides are widely distributed throughout the brain and are often co-localized with other small molecule neurotransmitters. Endogenous opioid peptides modulate the activity of dopaminergic neurons, and vice versa (115-117).

There are three types of G-protein coupled opioid receptors, mu (μ), kappa (κ) and delta (δ) (118), to which the opioid peptides bind with different affinity. Enkephalins have high affinity for the delta receptor, but also bind to mu receptors. β -endorphins bind to both delta and mu receptors. Dynorphins are the only opioid peptides with high affinity for kappa receptors (119). The rewarding effects of opioids are mediated via activation of mu and delta receptors, whereas kappa receptor agonists produce dysphoria (120, 121).

Dynorphin

Prodynorphin is the precursor protein of the dynorphins, which gives rise to several active peptide fragments. Dynorphin is distributed in the hippocampus, the striatum and in the hypothalamic pituitary axis. In hippocampus, dynorphin is densely expressed in granule cell layer of the dentate gyrus (122, 123), and in the nucleus accumbens and patch compartments of the caudate and putamen in the human striatum (124). Dynorphin acting on kappa receptors in the nucleus accumbens reduces dopamine overflow, whereas a kappa receptor antagonist increases dopamine overflow (116). The reduced overflow following kappa receptor stimulation is associated with both reduced dopamine release and increased uptake (125).

Enkephalin

Proenkephalin is the precursor of the enkephalins. Proenkephalin is distributed throughout the brain, with a strong expression in striatal subregions where about 50% of the GABAergic medium spiny neurons express enkephalin and the D2 receptor (126), and a moderate expression in the hippocampus. Whereas agonist binding to the kappa receptors decreases dopamine levels in the nucleus accumbens, agonist binding to mu and delta receptors increases dopamine levels in accumbens (116, 127). There is evidence that the rewarding effects of running partly are mediated via endogenous opioids (128, 129). Enkephalin has been reported

to be involved in several important functions in the CNS including analgesia, generation of euphoria, respiration and learning (130). In the ancient times opium was also a common treatment for melancholia as well as other disorders, suggesting a potential role in antidepressant action.

DOPAMINE AND DOPAMINE RECEPTORS

Dopamine is the most abundant catecholamine in the brain (131), and is synthesized from tyrosine in midbrain neurons of the retrorubral field, substantia nigra, hypothalamus, olfactory bulb and the ventral tegmental area (132). The dopamine neurons of the midbrain give rise to several ascending pathways; the mesolimbic (from VTA to nucleus accumbens), the mesocortical (from VTA to cortical regions) (133) and the nigrostriatal (from substantia nigra to dorsal striatum) (134). Dopaminergic neurotransmission has been extensively studied and dopamine has an essential role in motor control, but also affects mood and motivation. Dysfunctions of the dopamine system have been implicated in several diseases such as Parkinson's, Huntington's, ADHD, schizophrenia and addictive behavior.

Dopamine receptors

Dopamine acts by binding to G-protein coupled receptors. There are five different dopamine receptors (D1-D5) identified and classified into D1 and D2-like receptors. D1 like receptors comprise the D1 and the D5 receptor, whereas D2, D3 and D4 comprise the D2-type. Receptors of the D1-like family are positive regulators of cyclic adenosine monophosphate (AMP) levels, while inhibition of cyclic AMP synthesis is a common property of the D2-like subfamily (135). In my thesis we have investigated the D1 and D2 receptors that are highly expressed in the striatum (Fig.) (136).

Dopamine D1 receptors are mainly expressed on the medium-sized spiny striatonigral neurons. The dopamine D2 receptors are mainly expressed on medium-sized spiny striatopallidal neurons and on large sized aspiny cholinergic interneurons (126, 137, 138). However, D2 receptors are also located presynaptically on dopamine neurons in the substantia nigra and the ventral tegmental area, where they act as autoreceptors (139). The D2 receptor seems to be involved in the pathophysiology of a variety of psychiatric disorders including addictive behavior and possibly also affective disorders (140-147). In this thesis we investigate post-synaptic D2 receptors of the striatum.

SEROTONIN RECEPTORS

The brain serotonergic system was mapped by Dahlström and Fuxe (1964). Nine groups of serotonergic neurons located mainly within the raphe nuclei were identified and named B1-B9 (132). Serotonergic cells of the raphe nuclei complex extensively innervate the rat forebrain, and regulate sleep and wakefulness (94). A large number of drugs used in the treatment of anxiety and depression act upon 5-HT neurotransmission. Many antidepressants act by inhibiting uptake of serotonin (SSRIs), and the immediate effect is an increased concentration of 5-HT in the synaptic cleft. However, as previously described, antidepressant effects are usually observed several weeks after the onset of treatments (13). This has led to suggestions that the therapeutic effects of SSRIs are mediated via adaptive changes in various 5-HT receptor systems (148, 149). A large number of 5-HT receptors have been identified, most of them are metabotropic, G-protein coupled receptors.

5HT1A

The 5-HT_{1A} receptor is widely distributed throughout the brain, with high densities in the dorsal hippocampus and dorsal raphe (150). The 5-HT_{1A} is a G-protein coupled receptor, negatively coupled to adenylyl cyclase. In the dorsal raphe nucleus it acts as presynaptic autoreceptor, whereas it is located postsynaptically in limbic structures such as the hippocampus (151). Activation of postsynaptic 5-HT_{1A} receptors located on dentate granule precursor cells has been suggested to stimulate the production of neurons in the dentate gyrus (59).

5HT2A

The 5HT_{2A} receptor subtype has been suggested to play an important role in psychopathological conditions such as anxiety, depression, suicide, schizophrenia and alcoholism (152-154). The 5HT_{2A} receptor is highly expressed in neurons of the olfactory bulb and olfactory regions, nucleus accumbens, ventral pallidum, Islands of Calleja and neocortex. A moderate expression of the 5HT_{2A} receptor is found in pyramidal cells in the CA fields of hippocampus. 5HT_{2A} receptor expression has been identified on subpopulations of GABAergic interneurons and cholinergic neurons but also on astrocytes (155-157).

GENETIC ANIMAL MODEL OF DEPRESSION

It is recognized that a combination of genetic vulnerability and environmental factors contributes to the pathophysiology of MDD. Moreover, antidepressant drugs do not elevate mood in non-depressed subjects. Therefore to investigate mechanisms of antidepressant action of running and SSRI, and to explore the involvement of candidate molecules in depressive illness, we used a genetic rat model of depression in our studies.

FLINDERS SENSITIVE LINE

The Flinders Sensitive Line (FSL) rats have been proposed to be a genetic animal model of depression. FSL and its control Flinders Resistant Line (FRL) were originally bred to be resistant to the effects of the organophosphate anticholinesterase agent, diisopropyl fluorophosphates (DFP). The breeding led to one strain that was genetically more sensitive to DFP, the FSL strain, but not a line that was more resistant, thus Flinders Resistant Line rats are only more resistant in comparison to the FSL strain (158). The FSL were proposed as an animal model of depression following evidence that depressed patients were sensitive to cholinergic agonists (159, 160). The FSL rats exhibit several characteristics that are similar to those observed in depressed patients such as psychomotor retardation, increased anhedonia in response to mild stress, lower body weight and elevated REM sleep (158). Moreover, in the Porsolt swim test, FSL have a depressive-like phenotype that is normalized by chronic but not acute antidepressant treatments such as tricyclics and SSRIs (161, 162). Thus, the FSL represents an appropriate model for studying neurochemical mechanisms involved in depressive illness.

GENDER

The vast majority of animal studies of depression are performed in male animals, despite the recognition that women are overrepresented in the population of depressed subjects (2, 3). There is also clinical and preclinical evidence that males and female have differential responses to environmental challenges, such as stress (163, 164). Therefore, female rats are used in some of our studies.

AIMS

GENERAL AIM

The overall aim of this thesis was to add knowledge to the neurobiology underlying depressive illness and its treatments. A more defined focus was to reveal and compare antidepressant mechanisms of running to that of the SSRI-type drug escitalopram. In particular we studied the involvement of hippocampal plasticity mechanisms and key receptors and neuropeptides in the brain reward system with a putative role in the anhedonia symptoms of depression.

SPECIFIC AIMS

- At a behavioral level analyze whether wheel-running has an antidepressant-like effect in the Porsolt swim test in FSL rats, and to compare the putative antidepressant-like effect of running with that of the SSRI compound, escitalopram.
- Evaluate if disturbed hippocampal cell proliferation can be an underlying cause of depression in FSL rats and also whether this disturbance can be normalized by running and/or treatment with the SSRI-drug escitalopram.
- Correlate the noradrenergic and serotonergic-like response in the Porsolt swim test in FSL rats after running and/or escitalopram to hippocampal cell proliferation/neurogenesis and mRNA levels of NPY, Y1 receptor and BDNF.
- Analyze mRNA levels of receptors and neuropeptides in the brain reward pathways with a putative role in anhedonia after running and/or escitalopram in FSL rats.
- Investigate if social isolation can alter levels of key regulators of transmission and plasticity in hippocampus and brain reward pathways.
- Compare the response to social isolation in “depressed” and “non-depressed” rats on mRNAs encoding key receptors and neuropeptides in brain reward pathways.

MATERIAL AND METHODS

ANIMALS

FSL rats bred at the Karolinska Institute were used in all experiments included in the thesis. Male rats were used in manuscript I and II, whereas female rats were used in manuscript III-VI. In manuscript I and II Flinders Resistant Line were used as controls, whereas Sprague Dawley rats were used as a control strain in manuscript V and VI. For information about weight and age, see the respective papers.

BEHAVIORAL EXPERIMENTS

WHEEL RUNNING

In paper I-IV antidepressant mechanisms of wheel-running were investigated. The animals were placed in individual cages with free access to running wheels (diameter 34 cm, one revolution corresponding to 1.07 m), and non-running controls were placed in individually cages in the same room. The running behavior of each animal was recorded by a computer based data collection system.

SOCIAL ISOLATION

It is recognized that a combination of genetic and environmental factors plays a crucial role in the pathogenesis of depressive disorders. Social isolation is considered to be stressful for rodents, and increases anxiety-like behavior (165). Moreover, group housing can buffer the influence of certain types of stress on the activity of the hypothalamic pituitary adrenal axis (HPA) (166). We investigated the effect of social isolation on mRNA regulation in the brain reward system and hippocampus as well as investigating the impact of the stress of social isolation on newly proliferated cells in the dentate gyrus (paper V & VI). Social isolation experiments were performed in adult animal that were 29-30 weeks old at the start of the experiment. Prior to the experiment all animals were housed with standard housing conditions (4/cage) and with access to food and water ad libitum. The animals were randomly assigned into two groups, one with standard group housing conditions (4/cage) and one housed in individual cages. These housing conditions were maintained for 7 weeks. The animals were only handled when weighed once a week. After 7 experimental weeks the animals were sacrificed. The brains were rapidly removed and stored in -80°C for analyzes of mRNAs encoding dopamine receptors, endogenous opioids and neuropeptides.

PORSOLT SWIM TEST

The forced swim test or Porsolt swim test (PST) was originally described by Porsolt (167, 168) and is today the most widely used behavioral model, to assess antidepressant activity. The test is based upon the assumption that rodents will try to escape from aversive stimulus, but eventually give up (develop immobility) when placed in a cylinder of water. Immobility behavior is sometimes explained as “learned helplessness” behavior, and the duration of immobility in the PST defines the magnitude of depressive-like behavior (169, 170). Treatments with antidepressant effect reduce the amount of immobility and increase the amount of active escape behaviors displayed during the test.

The PST has also been shown to be sensitive to factors that influence the development of depressive illness in humans, such as genetic predisposition (animal models of depression) and previous exposure to stress (maternal separation) (Cryan, 2005).

The PST is sensitive to all types of antidepressant treatments, including TCA, MAOI, SSRIs, NRIs and electroconvulsive treatment (See Borsini and Meli, 1988). Escape behavior in the swim test that results in decreased immobility time can be swimming or climbing behavior. Moreover, it has been demonstrated that diverse antidepressant drugs produce different patterns of these behaviors in the swim test. Antidepressant drugs that enhance serotonin levels at synaptic junctions (SSRIs) decrease immobility behavior and increase swimming in the swim test without affecting climbing behavior. In contrast, selective norepinephrine reuptake inhibitors reduce immobility without altering swimming behavior (171). Lastly, drugs that affect both serotonin and norepinephrine neurotransmission increase both swimming and climbing behavior (172) (Fig. 4).

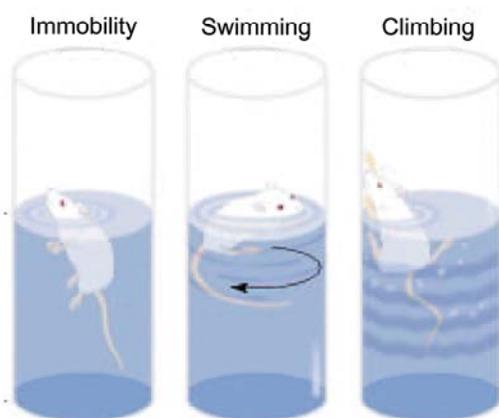


FIGURE 4. The Porsolt swim test (PST). The PST was conducted in transparent cylinders filled with water to the level, that prevents the animals from reaching the bottom of the cylinder. The rats can engage in immobility behavior, swimming and climbing. The swim test was videorecorded and an observer blinded to the animal's treatment condition quantified the time engaged in different behavioral parameters. Modified from Cryan et al. (Trends in Pharmacological Sciences 2002; 23(5):240, with permission from Elsevier)

A modified swim test was performed after chronic antidepressant treatments, such as running (Paper I, II, III, IV), the SSRI escitalopram, or both (paper III, IV). Transparent cylinders (24 cm diameter) containing 25 cm deep water (25°C) were used. After 15 min, the animals were removed from the water, dried with towels and placed into a warmed enclosure before being returned to the home cage. The test was video-recorded and later analyzed by an observer blind to the treatment condition. Immobility was defined as a stationary posture when the only movements of the rat are those necessary to keep the head above the water. At least three of the rat's paws had to be immobile. Climbing was defined as time spent with upward-directed movements of the forepaws along the side of the swim chamber and swimming was defined as time with horizontal movement in the cylinder.

ADMINISTRATION OF BRDU

Bromodeoxyuridine (BrdU) is a thymidin analogue that is incorporated into the DNA, of dividing cells, and is a marker of proliferating cells. BrdU labeling can be visualized with immunohistochemical techniques, and the number of newly divided cells can be estimated by stereological counting procedures. In paper I BrdU was administered by 4 injections i.p. (75mg/kg) with 2-hour interval, and the animals were sacrificed approximately 20 hours after the last injection. With this administration regime we quantify cell proliferation of 1-day-old cells. The majority of newly proliferated cells in the dentate gyrus have neuronal phenotype. Whereas the great majority of the newly proliferated cells die within the first two weeks (59, 173), there is evidence that those survived migrate and form functional connections (66).

In paper IV and VI BrdU was administered in the drinking water (1 mg BrdU/ml water, Sigma) during a ten or nine-day period, respectively (see papers). The purpose of this administration regime was to avoid the stress of needle injections, as well as allowing labeling of cells during the night hours, which is the period where the animals are in an active phase; e.g. run in running wheels, eat, drink, etc. To investigate how different antidepressant treatments or housing condition affected the pool of proliferated cells that had survived the critical first two weeks after migration, the animals in these studies were sacrificed 14 or 17 days after the last when animals were drinking water containing BrdU. This way of administering BrdU allows quantification of BrdU immunoreactive cells that are between 14-26 days old.

ADMINISTRATION OF ESCITALOPRAM

Escitalopram was administered in food pellets (Paper III & IV) according to a method developed by H Lundbeck A/S (Copenhagen, Denmark) and tested in collaboration with A. Mørk (174). Escitalopram doses of 0.35g, 0.45 and 0.57g/kg pellet have been used, resembling the clinical situation where a range of escitalopram (or other antidepressants) is used to treat depressed patients. We have measured daily intake of escitalopram containing pellets and calculated the average consumption of escitalopram/kg/day. After decapitation, trunk-blood samples were collected and serum levels of escitalopram were measured in collaboration with A. Mørk at H Lundbeck A/S.

***IN SITU* HYBRIDIZATION**

The animals were decapitated, the brains rapidly removed and placed on ice before being stored at -80°C for subsequent histological analysis. In the striatum, 14- μ m coronal cryostat sections were collected, and in the hippocampus 30- μ m (paper IV and VI) or 40- μ m (paper I and II). Sections were thawed onto glass slides. *In situ* hybridization was carried out with radioactive oligonucleotide probes (175). The probes were 3'-end labeled with α -³³P-dATP (Perkin-Elmer) using terminal deoxynucleotidyl transferase (Gibco) to a specific activity of approximately 1×10^9 c.p.m./mg. For information about the 48-mer probes used see paper I-VI. Slides were incubated for 18h in a humidified chamber at 42°C. The hybridization cocktail contained 50% formamide, 4 x SSC (1xSSC is, in M, NaCl, 0.15; sodium citrate, 0.015, pH 7.0), 1 x Denhardt's solution, 1% Sarcosyl, 0.02M Na₃PO₄, pH 7.0, 10% dextransulphate, 0.06M dithiothreitol and 0.1 mg/mL sheared salmon sperm DNA. Following hybridization, the sections were rinsed 4 x 20 min each in 1 x SSC at 60°C. Finally, the sections were rinsed in autoclaved water for 10s, dehydrated in alcohol and air-dried. Thereafter, the slides were exposed to film (Kodak Biomax MR film, Kodak, Rochester, NY) and developed (see paper I-

VI for exposure time). Films were scanned and optical density values quantified using appropriate software (NIH image analysis program, version 1,62). A ^{14}C step standard (Amersham, Buckinghamshire, UK) was included to calibrate optical density readings and convert measured values into nCi/g. Optical densities were quantified by computerized image analysis software (NIH image and Image J, National institute of Health, USA).

IMMUNOHISTOCHEMISTRY

For the immunohistochemistry, coronal 30 μm sections were collected with a cryostat throughout the hippocampal formation. Antibodies and dilutions used were: mouse α -BrdU (1:100 DAKO A/S, Denmark), horse α -mouse-biotin (1:200 Vector, Burlingame, CA, USA). Immunohistochemistry for BrdU was performed as follows: sections were taken out of freezer (-20°C) and post fixed for 10 min in 4% formaldehyde, rinsed in PBS 4 x 5 min, incubated 30 min in 2M HCl at 37°C , rinsed 3 x 5 min in PBS, and incubated for 1 h in blocking solution (horse serum 10%, 0,1% tween in PBS) at room temperature. This 1 h incubation was followed by overnight incubation with mouse α -BrdU at 4°C . On day 2, the samples were rinsed 3 x 30 min in 0,1% tween PBS, incubated with horse α -mouse-biotin for 60 min at room temperature, rinsed again for 90 min in PBS 0,1% tween followed by 30 min in PBS only. The sections were then incubated for 40 min at room temperature with avidin-biotin-peroxidase complex (1:100 in PBS, Vectastain Elite, Vector, Burlingame, CA, USA), then rinsed in PBS for 1 h, followed by peroxidase detection (0.7 mg/ml, DAB dissolved in H₂O) (DAB Peroxidase Substrate, Sigma) for about 25 sec per section. The sections were rinsed in PBS and stained with a hematoxylin solution (Vector).

STEREOLOGY

For quantification of BrdU positive cells in the dentate gyrus the unbiased optical fractionator counting procedure was performed (176). Coronal 30- μm (paper III, IV) and 40- μm (paper I, II) sections were taken throughout the hippocampus and every fifteenth section (450- μm vs 650- μm apart) was selected for analysis of the right dentate gyrus. An unbiased counting frame with known area was superimposed on the field of view by appropriate software (StereologerTM, SPA inc.). The counting frames were systematically distributed with known x and y steps throughout the marked region from a random starting point. The area of the counting frame relative to the area associated with the x and y step gives the second fraction (area sampling fraction [asf]). The height of the optical disector relative to the thickness of the section results in the third fraction (height [h] / thickness [h]). The total number of neurons is given by $N_{total} = \sum Q \cdot \frac{1}{ssf} \cdot \frac{1}{asf} \cdot \frac{t}{h}$ where $\sum Q$ – is the number of neurons counted in the dissectors. The dentate gyrus was manually outlined using a 10x lens. Cell counts were performed with a 60x lens (numerical aperture = 1.4). Positive cells were counted if they were within the dissectors. Cells situated further than two cell body widths away from the base of the granular cell layer were defined as belonging to hilus, and thus not counted. Also, cells were excluded if they were situated in the lowermost focal plane. To estimate total number of BrdU cells per individual, a representative material of BrdU immunoreactive cells in the dentate gyrus of the left hemispheres was compared to that of the right hemispheres. T-tests showed that there were no differences in number of BrdU immunoreactive cells between the two hemispheres, and the total number of cells per individual was calculated.

RESULTS AND DISCUSSION

The results of papers I-VI are summarized and discussed below. For detailed descriptions, see appended publications and manuscripts.

ANTIDEPRESSANT-LIKE EFFECT OF WHEEL-RUNNING IN “DEPRESSED RATS” (PAPERS I & III)

Physical activity has a documented antidepressant-like effect in depressed patients similar to antidepressants and psychotherapy (29-32), as well as in a rodent model of learned helplessness (177). In paper I we found that wheel-running during 30 days induced an antidepressant-like effect selectively in the “depressed” FSL strain. The antidepressant-like effect was demonstrated by decreased immobility in the PST. In this experiment male rats were used. In paper III we confirmed the antidepressant-like effect of running, this time in female FSL rats, suggesting that running has an antidepressant property in “depressed” rats independent of gender.

ANTIDEPRESSANT-LIKE EFFECT OF WHEEL-RUNNING DIFFERENT FROM THAT OF ESCITALOPRAM (PAPERS I & III)

Different antidepressant compounds have differential effects on behavioral parameters in the PST. Whereas SSRI-type drugs prolong swimming time, drugs that act upon norepinephrine transmission increase climbing behavior (169, 171, 172). Prior to paper III there were no reports on whether the antidepressant-like effect of running is associated with a serotonergic or noradrenergic response in the PST. In paper III we compared the effect of running, escitalopram and the combination of the two treatments on antidepressant-like parameters in the PST. Running increased the time engaged in climbing behavior, a behavioral response in the PST that is similar to the responses induced by drugs that act upon norepinephrine neurotransmission. In contrast, the combined treatment with escitalopram and running-wheel access increased swimming time in the PST a serotonergic antidepressant-like response. Interestingly, as I will later describe escitalopram attenuated the running behavior of these rats, to such a degree that the majority of the animals in this group did not run at all. It is therefore plausible that the increased swimming observed is due to the effects of escitalopram and not running. Moreover, calculating daily escitalopram intake and serum levels revealed that this group did not differ in these aspects from the group receiving escitalopram without running-wheel access, suggesting that despite the physical appearance of a running-wheel in the combined treatment group these two groups should be identical. However, escitalopram without running wheel access had no antidepressant-like effect measured in the PST, and as we will see these two treatment groups have a different pattern of mRNA expression in brain reward pathways and in hippocampus. It is therefore plausible that the enriched housing condition in the combined treatment group facilitates the effects of escitalopram.

In conclusion, the differential behavioral responses in the PST suggest that the antidepressant effect of running and escitalopram are mediated partly by different mechanisms. In addition, the environment seems to influence the behavioral and biochemical response to escitalopram. This is consistent with findings that the long-term outcome for depressed patients is better for those treated with a combination of antidepressant drugs and psychotherapy compared to drug alone (178, 179).

MECHANISMS ASSOCIATED WITH ANTIDEPRESSANT-LIKE EFFECT OF RUNNING AND ESCITALOPRAM (PAPERS I-IV)

In papers I-IV we studied the involvement of hippocampal plasticity mechanisms, and molecules in the brain reward system in the antidepressant-like response of wheel-running, escitalopram and a combination of these two treatments.

HIPPOCAMPUS

One hypothesis of depression is that it is caused by reduced neuronal plasticity and disturbed hippocampal neurogenesis. Therefore we examined whether cell proliferation was altered in our animal model of depression, and also if running and escitalopram would modulate hippocampal cell proliferation. In paper I we found that male adult FSL rats have a lower cell proliferation level than FRL. To our knowledge this was the first study to demonstrate reduced cell proliferation in a rodent model of depression. Moreover, recently Husum and co-workers (180) demonstrated that age differentially influences the level of cell proliferation in the two strains. For instance, 3 month-old rats FSL actually had more BrdU labeled cells than FRL. However, aging caused an exacerbated loss of BrdU labeled cells in the FSL compared to FRL (180). In our study the animals were between 4 and 5 months old when injected with BrdU, suggesting a decline in hippocampal plasticity in FSL at a relatively early stage.

The lower level of proliferated cells in the dentate gyrus of FSL rats was normalized following running. Thus, it is possible that one underlying neurobiological cause of “depression” in the FSL strain is a decreased cell proliferation in the dentate gyrus, that is normalized by running, which then also consequently relieves depression.

The neurotrophic factor BDNF is suggested to play an important role in the effect of antidepressive treatments and in regulating neurogenesis (85, 181). Running elevated BDNF mRNA levels in the dentate gyrus in the FRL rats, but the FSL had unchanged BDNF mRNA levels. The BDNF mRNA expression correlated positively to running distance and might explain why we only found an increase in FRL rats that exhibited a higher running activity than the FSL. Thus, it seems as if the antidepressant effect of running in our model is not dependent on hippocampal BDNF regulation.

Clinical and experimental evidence suggests a role for NPY in the pathophysiology of depression (106, 107, 182-184). Moreover, NPY has been demonstrated to promote neuronal proliferation (110). In paper II we found that basal NPY mRNA levels in the dentate gyrus were lower in FSL compared to FRL rats, consistent with previous findings (182, 185). Following running there was a marked increase in NPY mRNA levels in the CA4 and the dentate gyrus selectively in the FSL strain, suggesting that NPY might have a role in the antidepressant-like effect of running. This is consistent with clinical and preclinical data where antidepressants and ECT are found to elevate NPY in human CSF and in selected brain regions, predominantly in the hippocampus (27, 182, 183, 186). Moreover, NPY mRNA levels in the CA4 and dentate gyrus were strongly correlated to cell proliferation, thus supporting a role for NPY in promoting proliferation of dentate precursor cells.

In paper III we compared the adaptive response of different antidepressant treatments on cell proliferation, BDNF, NPY and the NPY Y1 receptor in hippocampus. To better understand the possible involvement of these factors in neuronal plasticity and in mediating antidepressant-

like effect, we analyzed correlations between these factors and behavioral parameters in the PST. The running mediated increase in cell proliferation in paper I were one day-old proliferated cells. To elucidate whether the proliferated cells survive, the animals in paper III were sacrificed two weeks after a 10-day BrdU treatment, thus measuring proliferated cells that have survived for 14-24 days, the most critical period where the majority of the proliferated cells die (59, 173). Running as well as escitalopram and the combined treatment increased the number of 14-24 days cells, suggesting increased hippocampal plasticity after all treatments. BrdU immunoreactive cells did not correlate with antidepressant-like behavior in the PST.

Treatment	BrdU IR	BDNF	NPY	Y1 rec	Climbing	Swimming
<i>Esc</i>	↑	-	-	(↑)	-	-
<i>Run</i>	↑	↑*	↑	↑	↑	-
<i>Esc+Run</i>	↑	-	↑	↑	-	↑

Table 1. Summary of how different antidepressant treatments regulate PST behavior and factors involved in hippocampal plasticity with putative antidepressant property in the FSL strain. BrdU immunoreactive cells (IR), mRNA levels of BDNF, NPY and the NPY Y1 receptor and swimming and climbing behavior in the PST was analyzed after long term treatment with escitalopram (ESC), wheel-running (Run) and escitalopram and access to running-wheels (Esc+Run).

Interestingly, animals in the combined treatment group (Esc+Run) barely ran at all.

↑ indicates a significant increase of the measured variable by the treatment ($p < 0.05$). (↑) indicates a tendency to an increase. * BDNF data are taken from paper IV, and are different from the data obtained in paper I (where no change was found).

Note that this table is a simplified summary of data to give a brief overview of the results. For more detailed description about the regulation in hippocampal subregions, see papers I & IV.

NPY mRNA was markedly increased in the hilus and dentate gyrus by the treatments that had an antidepressant-like response in the PST, which is running and the combined treatment. Moreover, NPY levels were correlated to climbing behavior and to number of BrdU immunoreactive cells. This is in line with our previous finding and other reports suggesting a role for NPY in antidepressant action (106, 107, 182) and in stimulating proliferation (110, 187, 188), and possibly promoting survival of the newly proliferated cells. The largest elevation of NPY mRNA was found after running. The strong correlation to climbing behavior indicates that it is possible that noradrenergic activation is mediating the increased NPY mRNA levels in the dentate gyrus. Interestingly, norepinephrine is increased in several brain regions after physical activity (38, 39). Moreover, NPY is present in noradrenergic neurons originating in the locus coeruleus and nucleus of dorsal tegmentum. The hippocampal formation is innervated by noradrenergic nerve fibers, and NPY is also present in the noradrenergic nerve terminals terminating in the polymorphic layer of the dentate gyrus (189).

It is suggested that the anxiolytic and the proliferative effects of NPY are mediated via activation of the Y1 receptors (110, 190). High levels of the Y1 receptor are expressed in the molecular layer of the dentate gyrus (113), containing the dendrites of the subgranular progenitor cells. Thus, a hypothetical mechanism underlying the antidepressant-like effect of running is increased production and activation of NPY in response to noradrenergic activation, and activation of dentate NPY Y1 receptors upon release. This hypothesis is consistent with the findings of NPY elevation after ECS (27, 108, 191, 192), that after repeated administration

produces a long-lasting increase in activity of noradrenergic neurons (193). However, NPY is also prominently expressed by inhibitory GABAergic interneurons of the hilus (194), implying that NPY in hippocampus can be released upon activation of different pathways.

Expression of the Y1 receptor mRNA was elevated in the dentate gyrus after running, and in the CA1 and dentate gyrus after the combined treatment. Also, a trend to increase after escitalopram was found. Interestingly, the only factor that was correlated to swimming behavior was the NPY Y1 receptor, and it is possible that the antidepressant-like effect of escitalopram could be mediated by the NPY Y1 receptor.

In contrast to paper I, BDNF mRNA levels were up-regulated by running in the CA1, CA3 and CA4 in FSL rats in paper IV. We replicated the finding of a strong correlation between BDNF mRNA levels and the amount of running, thus the absence of BDNF mRNA regulation in FSL rats in paper I can most likely be explained by the lower running behavior in this experiment. Moreover, BDNF mRNA levels were correlated to climbing behavior in the PST, thus suggesting a link between norepinephrine activation and BDNF induction in hippocampus. Interestingly, a lesion of the norepinephrine system followed by one week of voluntary wheel-running eliminates the exercise induced increase of hippocampal BDNF mRNA (40). In contrast, a lesion of the 5-HT system does not alter exercise induced BDNF increase, suggesting that norepinephrinergic activation is necessary for the enhancement of BDNF following exercise, and possibly also for the exercise induced climbing response in the PST. However, the absence of a BDNF increase in “depressed” rats after voluntary running in paper I, indicates that BDNF probably is not required for the antidepressant-like response to running. Escitalopram treatment did not affect BDNF mRNA levels implying that BDNF is also not necessary for the antidepressant-like response of escitalopram.

STRIATAL DOPAMINE PATHWAYS

Most depressed patients exhibit loss of motivation and reduced ability to experience pleasure (anhedonia) (7), and the striatal dopamine system has been proposed to be involved in the pathophysiology of depression. Therefore we investigated factors in the striatal dopamine system that affects dopamine neurotransmission with a possible role in the anhedonia symptoms of depression.

In paper I running induced an increase of dynorphin mRNA in all striatal subregions analyzed in the FRL and in the accumbens shell and mediate caudate putamen in the FSL strain. This finding in the FSL was replicated in paper III. An increased dynorphin tone is suggested to underlie a negative mood state, and a kappa receptor agonist increases immobility behavior in the PST (120, 195). Dynorphin acting on presynaptic kappa receptors on dopamine nerve terminals in the accumbens has an inhibitory control over dopamine release, whereas the uptake is increased (125). Moreover, D1 receptor agonists and drugs of abuse all up-regulates striatal dynorphin levels (196-200). Most likely the increased level of dynorphin following running is a response to the running induced dopamine activation, and might constitute a mechanism to prevent the development of a compulsive reinforcing running behavior.

Treatment	D1	5HT2A	DYN	ENK	Climbing	Swimming
<i>Esc</i>	-	-	-	-	-	-
<i>Run</i>	-	↑	↑	-	↑	-
<i>Esc+Run</i>	↓	↑	-	↓	-	↑

Table 2. Summary of how different antidepressant treatments regulate PST behavior and mRNA levels of receptors and neuropeptides in the brain reward system with a putative role in depressive illness in the FSL strain.

↑ indicates a significant increase in mRNA levels or time engaged in swimming/climbing in the PST ($p < 0.05$). ↓ indicates a significant decrease in mRNA levels. Note that this table is a simplified summary of data to give a brief overview of the results. For more detailed description about the regulation in hippocampal subregions, see paper I & III.

In paper III the combination of escitalopram and running-wheel access significantly increased swimming behavior in the PST as well as decreased levels of D1 receptor and enkephalin mRNA in accumbens shell, and increased the overall expression of 5HT2A receptor mRNA. It has previously been shown that different antidepressant compounds (201) differentially regulate the D1 receptor level. The dopamine D1 receptor in the striatum is essential for mediation of reward and motor activity, and the decrease found on D1 receptor mRNA is probably not involved in the antidepressant-like effect of escitalopram. More likely, as described in more detailed in the next section, the D1 decrease following escitalopram is involved in the regulation of motor activity.

The decrease in enkephalin mRNA in the nucleus accumbens after the combined treatment is in line with findings that chronic dietary with different classes of antidepressants decrease met-enkephalin immunoreactivity in the accumbens of rats (202), and suggests that decreased enkephalin in brain reward pathways can contribute to the mechanisms of antidepressant action. However, based on preclinical findings it has been suggested that decreased enkephalin tone is associated with depression-like symptoms and that increase in enkephalin signaling could have a therapeutic value in the treatment of depression (203, 204). Interestingly, animals lacking enkephalin do not display a depressive-like phenotype in the PST or tail suspension tests, and enkephalin does not seem to be necessary to achieve an antidepressant-like effect (205). The lack of consensus regarding the involvement of enkephalin in depression implies that more investigations are needed to elucidate that possible involvement of enkephalin in the antidepressant-like response of SSRIs.

The 5HT2A receptor is suggested to be involved in the pathophysiology of depression, however the results are conflicting considering the direction of the 5-HT2A receptors regulation, and which brain regions that are implicated (206-208). 5-HT2A receptor mRNA levels were increased in the nucleus accumbens shell following running, and in all areas beside the accumbens shell after the combined treatment with escitalopram and running-wheel access. Thus, it is possible that this receptor, in striatal subregions, might be important for the antidepressant action of escitalopram and possibly also running.

ESCITALOPRAM ATTENUATES RUNNING BEHAVIOR (PAPER III)

In paper III we surprisingly found that chronic treatment with escitalopram markedly decreased running behavior, or actually totally inhibited running in six out of eight rats. Projections from

the dorsal striatum are critically involved in control of voluntary movements. Interestingly, there was an overall decrease of dopamine D1 receptor mRNA in animals administered escitalopram and with access to running-wheels. In striatum the D1 receptors are expressed on medium size spiny neurons that project directly to the substantia nigra. Activation of the “direct” striato-nigral pathway facilitates locomotion by disinhibiting thalamocortical neurons. One possible explanation of the decreased running after escitalopram could be that the D1 receptors are decreased, which would lead to less activation of the “direct” striato-nigral pathway.

Serotonergic and dopaminergic systems in striatum interact and such interactions might be important for respective modes of action. Interestingly, agonist of dopamine receptors inhibit the expression of 5-HT_{2A} receptors located on striato-nigral medium size spiny neurons (209), which are those that also express the D1 receptor. Moreover, increase in 5HT_{2A} receptor mRNA levels after 6-OHDA-lesions has been reported (209, 210), and both D1 receptors and 5HT_{2A} receptors are suggested to be involved in some of the motor abnormalities exhibited by 6-OHDA lesioned rats. Conceivably, the chronic escitalopram diet modulates dopamine transmission, and the 5-HT_{2A} receptor mRNAs are up-regulated in response to decreased dopamine activity. We propose that decreased dopaminergic activity and a decreased expression of D1 receptors are nominators of the attenuated running behavior of escitalopram treated rats.

ENVIRONMENTAL INFLUENCE ON ANTIDEPRESSANT-LIKE EFFECT OF ESCITALOPRAM (PAPER III)

Escitalopram treatment did not increase swimming behavior in the PST, nor did escitalopram treatment regulate mRNA expression of any candidate molecule of investigation. In contrast, the addition of a running wheel, barely used for running, facilitated swimming behavior, and as previously described markedly influenced the mRNA expression of enkephalin, NPY, Y1, D1 and 5-HT_{2A} receptors (paper III & IV). Since most of the rats in this group did not run at all or displayed a minimal running activity, it seems plausible that the effects of escitalopram are not due to running activity, but rather to the enriched housing situation, which facilitated the effects of escitalopram. This is consistent with findings that a combination of antidepressant drugs and psychotherapy is associated with a better outcome for depressed patients than antidepressant medication alone (22, 178, 179). Presumably, the interaction observed between an animals environment and the effect of escitalopram could be true for other types of pharmacotherapy as well.

ENVIRONMENT AND GENDER INFLUENCES (PAPER III, V & VI)

As previously described the antidepressant-like effect of escitalopram was dependent on a running-wheel in the cage, but not actual running. Moreover, the environmental condition cannot only influence drug response but also induce changes in the dopamine system (141, 211). Social isolation is stressful and increases anxiety-like behavior in animals (165). Moreover, genetic background influences the responsiveness to stress and plays a crucial role in the pathophysiology of depression.

The purpose of paper V was to investigate the effect of social isolation in adulthood in FSL rats and SD controls on mRNA regulation in the brain reward pathway. We found a strain specific response to social isolation on the regulation of dopamine D2 receptors. Whereas the

levels of striatal dopamine D2 receptor mRNA did not differ between group housed FSL and SD rats, socially isolated FSL had lower expression of D2 receptor mRNA in all subregions for analyze compared to SD. Interestingly, a line of evidence suggest a role for the D2 receptor in depression (140, 212), and that the social environment can influence the expression of the D2 receptor (141, 211). Data from rodent models suggest that chronic stress results in decreased dopamine release (213), and D2 receptor levels in the accumbens (214), and stress induced anhedonia is thought to result from changes in the D2 receptor function in the nucleus accumbens (214). Furthermore, some characteristics of the FSL strain suggest that this strain is prone to stress induced anhedonia (215). Stress is an important factor for the development of affective disorders. It is possible that individuals with a genetic predisposition to depression respond to stress by decreasing D2 receptor levels in the accumbens, which could be a factor involved in the anhedonia symptoms of depression. Low levels of the dopamine D2 receptor has been shown to be associated with vulnerability to develop addictions (141, 145, 146), and high D2 receptor levels to be a protective factor against developing addictive behavior (144). Thus, low dopamine D2 receptor levels might be a factor in depression that could affect a vulnerable subpopulation of depressed patients to develop alcoholism.

Hippocampal neurons are sensitive to stress and preclinical studies indicate that stress may cause atrophy and death of pyramidal neurons in hippocampus as well as decreased neurogenesis. In paper VI we investigated how social isolation during seven weeks would affect survival of newly proliferated cells in the dentate gyrus, as well as key receptors and neuropeptides involved in neuronal plasticity and mood disorder. Possible differences in the response to social isolation in FSL and SD rats on these factors were examined. Since the majority of the depressed populations are females, and females react differentially to stress than males, female rats were used in this study.

We demonstrated that FSL and SD had a differential response to the chronic mild stress of social isolation on proliferated cells (17-26 day old). Whereas housing did not affect the number of BrdU immunoreactive cells in the SD strain, social isolation surprisingly increased the number of BrdU positive cells in the FSL. This is in contrast to a number of findings where acute and chronic stress reduce cell proliferation (63, 216-219). Whereas most studies on stress and neurogenesis use male animals, female rats were used in this study. Consistent with our finding, a number of reports have shown that stress exposure have no impact or increases cell proliferation and neurogenesis in females (220-224). The isolation induced increase in number of BrdU immunoreactive cells, are not in favor of the neurogenesis hypothesis of depression. Hypothetically, the newly formed cells migrate and form functional connections, which could be involved in spatial memory formation. Interestingly, chronic stress improves spatial memory, which is a hippocampal dependent task, in females (163), whereas it impairs spatial memory and reduces neurogenesis in males (225, 226). It is possible that sex differences in hippocampal plasticity and spatial memory in response to chronic stress have evolved in response to differential evolutionary demands on males and females regarding reproduction and taking care of offspring.

A number of findings indicate that reduced hippocampal BDNF and NPY are related to depressive illness. NPY is decreased in CSF from depressed patients and in genetic and environmental models of depression, and both compounds are elevated by antidepressant treatment modalities (27, 87, 105, 108, 183, 186, 191, 227). Previously, we and others have

demonstrated that male FSL rats have lower NPY mRNA expression in the dentate gyrus than FRL (182, 185, 228). Now we also demonstrate that hippocampal NPY mRNA expression is reduced in group housed female FSL rats compared to SD. Thus, decreased NPY mRNA levels seem to be a robust feature of the “depressed” FSL phenotype. Moreover mRNA levels of BDNF, and the 5HT2A receptor were also lower in group housed FSL compared to SD, which is consistent with human post mortem data of reduced hippocampal levels of these molecules (207) . In the SD strain mRNA levels of NPY, BDNF and 5HT2A receptors were decreased upon social isolation, whereas housing had no additional change on mRNA regulation of these compounds in the FSL strain. This result is in line with the recent finding that the stress of maternal separation reduces NPY in the control FRL but had no additional effect in the genetically “depressed” FSL (229).

Our results support the suggestion that clear gender differences exist in how stress affects hippocampal neurogenesis. Moreover we demonstrated strain specific responses to social isolation on mRNAs encoding the neurotrophic factor BDNF, neuropeptide Y (NPY) and the serotonin 5HT2A receptor, factors suggested to be important for neuronal plasticity and mood disorders. Conceivably, the genetic loading in FSL is so prominent that some systems are no longer influenced by the mild environmental stress of social isolation.

CONCLUSIONS

- Wheel running has an antidepressant norepinephrine-like effect in the PST in FSL rats. The SSRI-type drug escitalopram has a serotonergic-like antidepressant response in the PST in single housed FSL rats with a running wheel as a cage enrichment. However, escitalopram does not have an antidepressant-like effect in single housed FSL rats without access to running-wheel. Thus environmental factors can be important for the antidepressant action of escitalopram.
- The “depressed” FSL strain has lower adult hippocampal cell proliferation than the “non-depressed” FRL strain. Interestingly, wheel running normalizes this mismatch. Moreover, wheel running, and escitalopram with or without access to running wheels increase the survival of newly proliferated cells.
- NPY mRNA is markedly increased in hilus and dentate gyrus by treatments that have antidepressant-like responses in the PST, which is running and the combined treatment. Moreover, NPY levels are correlated to climbing behavior and to BrdU immunoreactive cells, suggesting a role for NPY, which involves norepinephrinergic mechanisms, in the antidepressant actions and in stimulating proliferation.
- The Y1 receptor is elevated by treatment with antidepressant-like responses, and is correlated to swimming behavior. It is possible that the antidepressant-like effect of escitalopram could involve regulation of the NPY Y1 receptor.
- Wheel running and escitalopram treatment of FSL rats with or without running wheel access has differential effect on mRNAs in brain reward pathways. These effects are most likely not important for the antidepressant effect but rather for the control of the motor performance in the running wheel.
- Social isolation increases the number of BrdU positive cells in the dentate gyrus in female FSL whereas it has no affect in SD rats. This is in contrast to numerous findings of decreased cell proliferation and neurogenesis in response to stress. In contrast, the few other studies performed on female animals support our findings that stress has no impact or increases cell proliferation and neurogenesis in females, thus suggesting clear gender differences in how stress affects adult neurogenesis.
- In humans, stress is a factor that can cause depression in vulnerable individuals. Single housing is a mild stress in rodents, which down regulates the dopamine D2 receptor in depressed FSL but not in SD rats. Thus, a stress induced down-regulation of the dopamine D2 receptor in reward pathways can be a factor that can contribute to depression.

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- Lesson 5: Going to the doctor after being bitten by a rat
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