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Pharmacological and Genetic Modulation of Adult Neurogenesis in Animal Models Relevant to Neuropsychiatric Disorders

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About the cover: The image is of a cross section of the mouse hippocampus demonstrating the large number of cells undergoing neurogenesis seen in red. Red represents immature neurons (DCX) while green represents interneurons (parvalbumin) and blue are nuclei of cells (DAPI). The image is representative of the proposed mechanisms in paper I where p11 in interneurons is suggested to regulate the response to antidepressants. As visible, interneurons along the dentate gyrus have processes throughout the SGZ which are know to modulate aspects of adult neurogenesis.

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To Mom and Dad

ABSTRACT

During the past decade, the modulation of adult neurogenesis has been an intensively studied area of neuroscience due to the implications for understanding of physiological mechanisms in the adult brain and the potential clinical applications for neuropsychiatric disorders. This research has resulted in countless discoveries during a relatively short period of time elucidating mechanistic details about where adult neurogenesis takes place, how the process of neurogenesis occurs and how this process can be regulated at several different steps by, not only endogenous mechanisms which normally maintain a homeostasis of adult neurogenesis, but also by exogenous regulation using genetic and pharmacological modulations to manipulate steps of the process. The modulation of adult neurogenesis has been demonstrated to notably occur as a result of chronic antidepressant treatment which affects several stages of this process resulting in increased adult neurogenesis. A consensus of studies examining the importance of this modulation agree that this increase could be an integral and important part of the behavioral effects of antidepressant, indicating that increased neurogenesis is a part of the therapeutic process in the majority of treatment methods. Questions remain though regarding how neurogenesis is involved in modulating mood as a consensus on this matter finds that decreases in adult neurogenesis *per se* do not induce depression. However, recent studies indicate that adult neurogenesis is important in the regulation of stress, suggesting that a consequence of decreases in adult neurogenesis may play a role in the dysregulation of this endocrine system in combination with severe or chronic stress which may eventually result in depression. These findings highlight the potential significance of treatments which have the potential to increase adult neurogenesis during pathological states to reach stable levels.

Current findings indicate that one of the most important and accessible systems in modulating neurogenesis is the serotonergic system, as exemplified by the potent ability of serotonin enhancing drugs such as the antidepressant fluoxetine to increase neurogenesis. A first set of studies present in this thesis investigate the neurogenic potential in the hippocampus of proteins of the S100 family associated with the serotonergic system including p11 and S100B. The first of these studies uses a genetic deletion of p11 in mice. Results from these experiments demonstrate that mice lack a neurogenic and behavioral response to fluoxetine, seen in normal mice. This finding indicates that p11 is involved in the antidepressant mechanism of fluoxetine. Further examination into potential mechanisms revealed that p11 is highly expressed in interneurons which also express low levels of 5-HT1B and 5-HT4 receptors, of which p11 is a known adaptor protein. Interneurons are known to regulate aspects of adult neurogenesis indicating a possible mechanism through which p11 may modulate the neurogenic and furthermore behavioral effects of this antidepressant. A subsequent study identifies other areas of the brain potentially involved in depression which express p11 and 5-HT1B and 5-HT4 receptors. The last of these S100 studies uses a genetic amplification of S100B in mice to investigate its potential role in adult neurogenesis and revealed that S100B mice have an increased baseline level of cell proliferation which however did not translate into an increase in total neurogenesis. Furthermore, these mice display a normal neurogenic and behavioral response to fluoxetine. These results indicate that S100B is involved in cell proliferation though not other aspects of neurogenesis. Furthermore, S100B may be partially involved in aspects of neurogenesis enhancing drugs and highlight the potential benefits of modulation of this protein.

Besides the serotonergic system, other neurotransmitter systems have been implicated in the regulation of adult neurogenesis, including the dopaminergic system. Altered dopamine levels are associated with several disorders of the brain with neuropsychiatric complications. Furthermore this system, in similarity to the serotonergic system, is a primary target of pharmacological therapies for neuropsychiatric disorders. A second set of studies therefore further investigated effects of pharmacological and genetic modulation of the dopaminergic system on adult neurogenesis. The first of these studies investigated the neurogenic and behavioral effects of the drug sarizotan which targets both the serotonergic and dopaminergic system. This drug has previously been shown to have potential antidyskinetic beneficial effects against involuntary movements seen in Parkinson's disease and therefore we investigated effects of this drug in an animal model of Parkinson's disease in which dopaminergic afferents are lesioned unilaterally. In the lesioned hemisphere, sarizotan increased cell proliferation in two neurogenic regions of the lateral ventricles and the hippocampus. Sarizotan in combination with the anti-Parkinsonian drug L-DOPA, also increased ongoing neurogenesis in the hippocampus. Furthermore, sarizotan had antidepressant-like activity in the forced swim test in lesioned animals. These findings indicate that targeting of both the serotonergic and dopaminergic systems may be an effective modulator of aspects of neurogenesis and behavior in certain pathologies. For example sarizotan may, in addition to antidyskinetic effects, have antidepressant potential in the frequently seen subgroup of Parkinson's disease patients who also suffer from depression.

The numerous studies regarding purely dopaminergic regulation of adult neurogenesis in either the lateral ventricles or hippocampus have resulted in conflicting data suggesting a complex regulation in which several receptors may be involved. Currently available data suggest expression of the D3 receptors in the proliferative zone of the hippocampus indicating a role in adult neurogenesis. The role of the D3 receptor using a genetic deletion of this receptor in mice was therefore investigated. A robust increase was found in baseline levels of cell proliferation and ongoing neurogenesis in these mice, though not in cell survival. Furthermore, pharmacological modulation using the preferential D3 antagonist S33138 had a similar effect on cell proliferation, although less robust. Thus, in the hippocampus, the D3 receptor appears to act inhibitory on cell proliferation. Previous indicating that D3 is expressed in proliferating cells indicates that this may be a direct effect of dopamine whereas expression of D3 and D2 receptors on niche astrocytes may in contrast indirectly stimulate cell proliferation. This study further highlights how modulation of the dopaminergic system affects adult neurogenesis and may ultimately have significance for pathologies in which adult neurogenesis is affected.

In summary, these findings exemplify the numerous different ways in which adult neurogenesis can be modulated which is also indicative of the situations in which adult neurogenesis can be defective, potentially contributing to disease. Studies presented in this thesis have via the use of genetic manipulation as well as pharmacological compounds highlighted specific proteins and pharmacological targets which can be used to modulate aspects of neurogenesis, having potential clinical significance for neuropsychiatric disorders in which adult neurogenesis is affected.

LIST OF PUBLICATIONS

I. **Martin Egeland**, Jennifer Warner-Schmidt, Paul Greengard & Per Svenningsson. (2010) Neurogenic effects of fluoxetine are attenuated in p11 (S100A10) knockout mice. *67*(11), 1048-56.
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II. **Martin Egeland**, Jennifer Warner-Schmidt, Paul Greengard & Per Svenningsson. (2011) Co-expression of serotonin 5-HT (1B) and 5-HT (4) receptors in p11 containing cells in cerebral cortex, hippocampus, caudate-putamen and cerebellum. *61*(3), 442-50.
Neuropharmacology

III. **Martin Egeland**, Vasco Sousa & Per Svenningsson. (2012)
Changes in basal and fluoxetine-induced adult neurogenesis and depression-related behaviors in transgenic S100B over-expressing mice.
Manuscript

IV. Xiaoqun Zhang*, **Martin Egeland*** & Per Svenningsson. (2011)
Antidepressant-like properties of sarizotan in experimental Parkinsonism. *218*(4),621-34.
Psychopharmacology

*shared first-authorship

V. **Martin Egeland,** Xiaoqun Zhang, Mark J. Millan & Per Svenningsson. (2012)
D3 receptor blockade via genetic deletion or the novel atypical antipsychotic S33138 increases adult hippocampal neurogenesis and Delta FosB expression.
Manuscript

Additional publication during PhD study

i. Mark J. Millan, Per Svenningsson, Charles R. Ashby, Jr., Michael Hill, **Martin Egeland**, Anne Dekeyne, Mauricette Brocco, Benjamin Di Cara, Francoise Lejeune, Nitza Thomasson, Carmen Munoz, Elisabeth Mocaer, Alan Crossman, Laetitia Cistarelli, Sylvie Girardon, Loretta Iob, Sylvie Veiga, and Alain Gobert (2008).
S33138, A Preferential Dopamine D 3 versus D 2 Receptor Antagonist and Potential Antipsychotic Agent. II. A Neurochemical, Electrophysiological and Behavioral Characterization in Vivo. *324*(2), 600-611.
Journal of Pharmacology and Experimental Therapeutics

CONTENTS

LIST OF ABBREVIATIONS

5,7 DHT	5,7-dihydroxytryptamine	L-DOPA	L-3,4-dihydroxyphenylalanine
6-OHDA	6-hydroxydopamine	MAM	methylazoxymethanol
AIMS	abnormal involuntary movements	MDMA	3,4-Methylenedioxymethamphetamine
AN	adult neurogenesis	MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
ANOVA	analysis of variance	NGF	nerve growth factor
ANP	amplifying neural progenitors	NIH	Novelty induced hypophagia
BAC	bacterial artificial chromosome	NMDA	N-methyl-D-aspartate
BDNF	brain-derived neurotrophic factor	NSF	novelty suppressed feeding
BrdU	5-bromo-2'-deoxyuridine	PCNA	proliferating cell nuclear antigen
CCK	cholecystokinin	PD	Parkinson's disease
CNS	central nervous system	PHH3	phospho-Histone H3
CNTF	ciliary neurotrophic factor	PSA-NCAM	poly-Sialated Neural Cell Adhesion Molecule
D3R	dopamine D3 receptor	QNP	quiesentSHH neural progenitors
DCX	doublecortin	qPCR	real-time PCR
DNA	deoxyribonucleic acid	RMS	rostral migratory stream
ECT	electroconvulsive therapy	SFN	society for Neuroscience
EGF	epidermal growth factor	SGZ	Subgranular zone
FACS	fluorescence Activated Cell Sorting	SHH	sonic hedgehog homolog
FGF-2	fibroblast growth factor	SSRI	selective serotonin reuptake inhibitor
FST	forced swim test	SVZ	subventricular zone
GABA	γ-Aminobutyric acid	TCA	tricyclic antidepressants
GFP	green fluorescent protein	TG	transgenic
HPA	hypothalamic-pituitary-adrenal	TST	Tail suspension test
IL-6	interleukin-6	VEGF	vascular endothelial growth factor
KO	Knock-out	WT	Wild -type

INTRODUCTION

BACKGROUND INFORMATION ABOUT ADULT NEUROGENESIS

Historical Perspective

Adult neurogenesis (AN) is one of the fastest growing areas in neuroscience so far in the 21st century. This topic has gone from being an idea that was relatively disregarded before the end of the last century to having presently thousands of publications. This topic has also been riveted with controversy since its introduction, controversy which has to date shown no signs of dissipating. Even the suggested existence of adult neurogenesis was controversial due to the long standing dogma that neurogenesis does not occur after development. Studies published by Altman and colleges in 1960s began to change this dogma which however did not fall easily due to technical limitations which left room for doubt in these studies (Altman & Das 1965). Several decades later, the technical breakthrough of BrdU labeling allowed the positive identification of AN in first birds followed by mammals (Goldman & Nottebohm 1983; Gould et al. 1992). These findings were later confirmed in primates as well as finally in humans, putting a definitive end to the century old dogma that neurogenesis does not occur in adulthood (Eriksson et al. 1998; Gould et al. 1999).

The Process of Adult Neurogenesis

It is now generally accepted by the scientific community that neurogenesis continues in adults in two regions, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, which together make the neurogenic niches (Fig. 1). Adult born neurons have also been found in other areas of the brain, albeit only under pathological circumstances, including the striatum, neocortex, amygdala and hypothalamus (see Balu & Lucki 2009 for review). Adult neurogenesis is a complex process which is remarkably similar in both of the neurogenic niches although there are differences. The process begins within the neurogenic niches with the stem cells, the identity and lineage of which has been an area of much research (see Ming & Song 2011 for review). These cells are currently thought to be radial glia-like cells in both niches.

In the SGZ of the hippocampal dentate gyrus, these cells, due to their low proliferative rate, are also known as quiescent neural progenitors (also Type I cells) and divide either symmetrically, giving rise to identical progeny, or asymmetrically giving rise to cells which eventually differentiate into cells known as amplifying neural progenitors (also known as type IIa cells) (Encinas et al. 2006). These amplifying neural progenitors divide actively thus increasing rapidly the proliferative pool from which adult neurons will be derived. At this point, new cells reach a critical point (1-4 days) at which they become apoptotic or continue to differentiate (Sierra et al. 2010). Those cells which are neuronal-lineage fate-determined are called neuroblasts (also known as D2 and 3 cells or Type IIb and III cells). These neuroblasts or immature neurons continue to mature and over a period of several weeks. Immature neurons go through several developmental changes during this time and display interesting properties different from mature cells including a transient low membrane capacitance (Ge 2006).

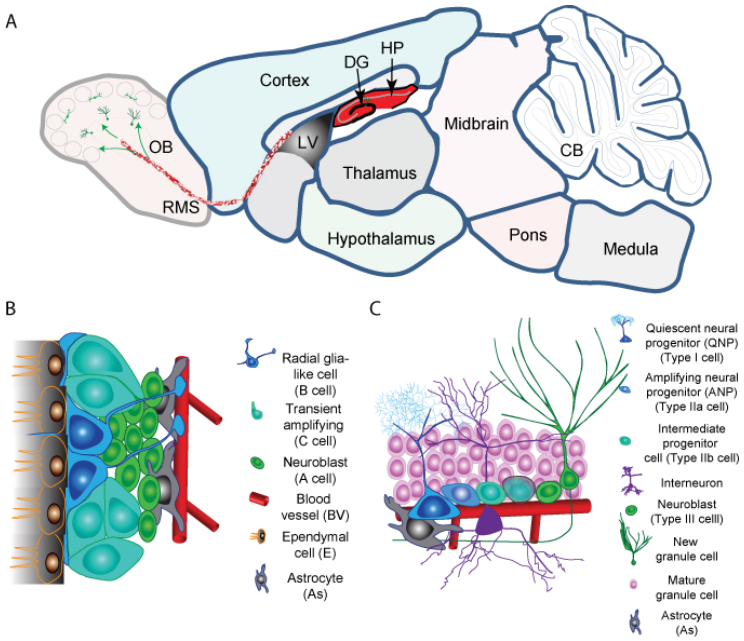

Figure 1 A schematic representation of the neurogenic niches in the rodent brain.
(A) A sagittal section view of an adult rodent brain highlighting the two restricted regions that exhibit active adult neurogenesis: dentate gyrus (DG) in the hippocampal formation (HP), and the lateral ventricle (LV) to the rostral migratory stream (RMS) to the olfactory bulb (OB).
(B) A schematic illustration of the neural stem cell niche in the subventricular zone (SVZ).
(C) A schematic illustration of the neural stem cell niche in the subgranular zone (SGZ) in the dentate gyrus.
Modified from (G.-L. Ming & Song 2011)

Eventually these cells migrate into the granular layer and integrate into the pre-existing circuitry of the dentate gyrus (van Praag et al. 2002).

In the SVZ of the lateral ventricles, the process is similar with the radial glia-like cells (also known as B cells) giving rise to the transient amplifying progenitor cells (C cells). These in turn give rise to the neuroblasts (A cells) which migrate through the rostral migratory stream (RMS) to the olfactory bulb where they differentiate into functional interneurons (Lois & Alvarez-Buylla 1993).

Endogenous Regulation

Endogenous regulation of AN can occur at various stages in the process. The complexity of AN and the possibility for modulation at numerous of these steps has meant that the list of known factors which regulate AN has increased immensely since the re-discovery of AN during the past decade. These factors can affect a single or several aspects of AN in a bi-directional manner, thus acting either inhibitory or stimulatory on aspects of AN. For detailed reviews of specific details of these factors see Ming & Song 2011 and Balu & Lucki 2009. Although these many of these factors affect both neurogenic niches, the majority of studies have been performed in the SGZ and therefore the following references refer to this niche unless otherwise stated.

Among the many endogenous factors which affect neurogenesis are those which regulated the early stages of the process, the morphogens. These factors regulate maintenance, activation, and fate choice of adult neural precursors and include well known and characterized members such as Notch, Shh, Wnt and Ephrins (see Balu & Lucki 2009).

Some of the most studied regulators of AN are growth factors and neurotrophins (reviewed by Zhao et al. 2008 and Balu & Lucki 2009). A variety of growth factors are potent modulators of cell proliferation and some cell survival, inducing increased total neurogenesis. These include Fibroblast-growth factor-2 (FGF-2), Insulin-like growth factor-I (IGF-I), and Vascular endothelial growth factor (VEGF) (see Balu & Lucki 2009). Similar to the growth factors, several neurotrophic factors are also known be

potent regulators of aspects of AN including neurotrophins, such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF), as well as neurotrophic cytokines, such as ciliary neurotrophic factor and interleukin-6 (IL-6) (Zhao et al. 2008). BDNF is one of the extrinsic factors most associated with depression and therefore much focus has been placed on this neurotrophic factor, specifically with its regulation of aspects of AN. Studies of this factor have revealed that BDNF is involved in the effects of many other factors which promote AN, for example increasing the cell survival of new neurons during antidepressant treatment (Sairanen et al. 2005). However the effects of BDNF on cell proliferation remain unclear with results from transgenic animal models giving mixed results (see Balu & Lucki 2009). NGF is similarly thought to increase cell survival (Frielingsdorf et al. 2007).

Another major endogenous group of factors regulating AN are neurotransmitters. This group has been widely studied due to the ease of using specific pharmacological tools and also due to the fact that many prescribed clinical drug treatments and potential drugs target neurotransmitter receptors. Neurotransmitters which have been indicated to regulate AN include glutamate, noradrenaline, acetylcholine, γ-amino-butyric acid (GABA), dopamine and serotonin. Early on in the study of neurogenesis, it was demonstrated that afferents releasing glutamate in the dentate gyrus have an inhibitory effect on cell proliferation (E Gould et al. 1992). However later studies indicated that the regulation of glutamate is complex and has differential effects via different receptors (see Ming & Song 2011). The role of noradrenaline is less well understood and less studied although it appears that it regulates both cell proliferation and cell survival (Rizk et al. 2006; Kulkarni et al. 2002). The role of acetylcholine on the other hand has been widely studied and data from numerous studies indicates a likely role in proliferation, differentiation and integration with further data also indicating a role in survival (for review see Bruel-Jungerman et al. 2011). GABA acts normally as an inhibitory neurotransmitter and is released by interneurons of which, in the adult hippocampus, there are various types (Parra et al. 1998). In both the SGZ and SVZ, GABA appears to interact specifically in the neurogenic process and actually stimulates immature neurons rather than inhibiting via GABA-A receptors expressed during the first 2-3 weeks of neural progression (Tozuka et al. 2005). This stimulation has furthermore been demonstrated to promote progenitor differentiation and also has a role in synaptic integration ultimately affecting survival (Ge 2006; Tozuka et al. 2005). It has also been suggested that GABA may play a role in the migration of adult born neural progenitors (Ge et al. 2007). Results from specific studies modulating GABA receptors indicate that GABA does not affect cell proliferation (Earnheart et al. 2007). Due to the pertinent relevance to neuropsychiatric disorders, serotonergic and dopaminergic modulation has been studied widely and is also of particular focus to this thesis and therefore elaborated upon in following sections.

The preceding section briefly introduces a much larger area of study. Indeed there are numerous other endogenous regulators including intracellular factors and not to mention epigenetic regulation which play important roles in the regulation of AN. A whole other area is the study of exogenous regulators such as environmental regulation including physical exercise, enriched environment and learning, all which have beneficial effects on AN. In addition, exogenous factors can also have a negative effect on AN, for example stress which is further explored in later sections. Exogenous compounds and treatments which potentially regulate AN have also been an area of

great interest, particularly with regards to clinical applications. These are explored further in the section regarding AN and disease.

Functional Importance

The function of AN has been a critical question since the re-discovery of AN in the 1990's with many labs using various techniques to ablate AN and assess the consequences on behavior. This investigation has however not been without hurdles. Investigators have used several different methods with which to ablate AN including chemical anti-mitotic agents such as methylazoxymethanol (MAM), global and focused radiation and more recently the development of various genetic and molecular biologically driven ablation of neurogenic populations at specific time points allowing more spatial and temporal resolution. However, these methods each have individual disadvantages making the results and interpretations precarious. This has also led to data which are often conflicting. Despite this, several patterns have emerged giving a consensus to specific functions of AN. The general functions of the two structures in which AN occurs, the hippocampus and olfactory bulb, give clues to potential roles of the AN.

The olfactory bulb is strongly associated with olfaction and although this structure has been much less studied than the hippocampus, data thus far indicate a role in olfactory learning (see Lledo et al. 2006 for review). This is supported by data indicating that olfactory experience can actually regulate AN in the SVZ (see Lledo et al. 2006). Conclusive results however remain elusive and require the application of ablation techniques with spatial specificity for the olfactory bulb. It has also been suggested that in pathological states which result in neurodegeneration the SVZ, new neurons can have a restorative function(Höglinger et al. 2004). However, this remains controversial, though the vast clinical implications of harnessing this potential for diseases such as Alzheimer's, stroke and Parkinson's disease have led to much focus on this research area.

One of the main functions of the hippocampus is of learning and memory prompting even early studies of AN to speculate on the function of AN in learning and memory (Altman & Das 1967). Although not without controversy, studies have revealed patterns of results indicating involvement in specific functions of AN in the hippocampus - extensively reviewed by Deng et al. (Deng et al. 2010). Unsurprisingly most of these functions involve types of learning, particularly involving spatial aspects of learning including long-term spatial memory retention, spatial pattern discrimination and spatial navigation learning. Several studies also reveal an importance in modulation of input processing as demonstrated by involvement in contextual fear conditioning and trace conditioning. Emphasis has been placed on dentate gyrus dependent functions, in particular the critical involvement of AN in pattern separation- a process by which similar stimuli are discriminated (Clelland et al. 2009).

Recently several findings have begun to describe an even more intricate relationship with AN and stress than previously thought and demonstrate that not only does stress regulate AN but that AN actually can modulate stress responses (see commentary by Anacker & Pariante 2011). In particular it has been suggested that a function of neurogenesis is to buffer stress (Snyder et al. 2011). Adaptive responses to stress are indeed an important function in animals with benefits for survival which have been

conserved (Joëls et al. 2006; Lagace et al. 2010). The importance of a proper stress response is highlighted in situations in which excess amounts of stress can cause a dysregulation of the mechanisms regulating stress responses, including AN, and lead to pathological conditions such as depression, a topic explored further in subsequent sections.

Although scientific innovation and dedication have revealed much about these described functions of AN over the past years, many questions remain unanswered and much is left to be discovered. However, continued effort, particularly with the implementation of new genetic models and techniques will undoubtedly lead to a more comprehensive understanding.

Relevance to Disease

An indication of the functional significance of AN are the changes seen in various pathologies of the central nervous system (CNS) including, among others, neurodegenerative disorders and neuropsychiatric diseases. Several specific neurodegenerative disorders have been associated with changes in AN including Huntington's, Alzheimer's and Parkinson's diseases (PD). AN has been suggested to be a part of the pathology of Huntington's disease as large decreases in hippocampal AN are seen in several animal models of the disease before the onset of motor symptoms (reviewd by Gil-Mohapel et al. 2011). Similarly, it has also been suggested that changes in AN might be associated with part of the pathology of Alzheimer's disease (reviewed by Marlatt & Lucassen 2010). This hypothesis is based on patients as well as a transgenic mouse model of Alzheimer's which display changes in AN (reviewed in Kuhn et al. 2007). With regards to these neurodegenerative diseases, preclinical evidence indicates that treatments aimed at increasing deficits in AN in neurodegenerative disorders may have clinical impact (MacMillan et al. 2011).

An initial study of AN in PD revealed that animal models of PD including 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning lead to a decrease in cell proliferation in both the SGZ as well as SVZ (Höglinger et al. 2004). Further studies of AN in postmortem PD patients, using proliferating cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation, revealed that this aspect of AN was decreased in the ependymal zone, the human equivalent to the SVZ (Höglinger et al. 2004). Subsequent publications have however questioned the validity of both decreases in animal models of dopamine lesions as well in PD patients (see table 1). Specifically, a recent publication re-examined AN in postmortem PD patients and demonstrated, using two established proliferative marker and on a larger sample size than the Höglinger study, that PD patients did not display decreases in cell proliferation and in fact displayed no change (van den Berge et al. 2011). Thus the effect of this disease on AN in the lateral ventricles is a matter of controversy as is the effect of dopamine on AN, further described in subsequent sections. Depression is an aspect of PD which has often been overlooked but is frequently seen in patients suffering from this disorder as approximately one third of the patients suffering from PD exhibit clinically significant depressive symptoms (Reijnders et al. 2008). Neither of these human studies examined aspects of AN in the dentate gyrus, however, in animals models, AN in this area is associated with depression as described in detail below. Therefore it could be

speculated that changes might be present in this region in these studies particularly those with symptoms of depression.

The disease group which neurogenesis has been mostly associated with are the neuropsychiatric disorders, particularly depression and stress related pathologies but also more recently schizophrenia.

The strongest association of AN to schizophrenia are the numerous genes associated with the disease which have also been indicated to alter AN in animal models including the genes reelin, neuregulin 1, those genes related to Wnt signaling and retinoid signaling and one of the most associated schizophrenia genes, Disrupted in Schizophrenia 1 (DISC1) (see Toro & Deakin, 2007). In particular, DISC1 has been demonstrated to have a significant role in AN as is was shown to regulate cell proliferation (Y. Mao et al. 2009) as well as to regulate the integration of newborn neurons (Duan et al. 2007). Furthermore, human studies have indicated that cell proliferation is decreased in schizophrenic patients (Reif et al. 2006). Although the association of AN and schizophrenia is far from established, these preliminary data indicate that modulation of AN may be of significance for the treatment of this disorder.

Stress was one of the first exogenous factors shown to affect AN, where it was demonstrated that psychosocial stress decreased cell proliferation (Gould et al. 1997). Subsequent studies have replicated this finding using several different paradigms and it is now well documented that stress, whether chronic or acute, is one of the most potent suppressors of AN (see Lucassen et al. 2010 for review). Data further show that stress can affect different aspects of AN by decreasing cell proliferation as described by Gould et al. but also by decreasing cell survival (B Czéh et al. 2001). Glucocorticoids are implicated in the mechanism through which this suppression occurs, although contradictory evidence exists indicating a deeper complexity in which other regulators may be involved, for example glutamate acting via NMDA receptors (Mirescu & Gould 2006). Evidence in support of a role of glucocorticoids come from studies indicating that glucocorticoids suppress cell proliferation, differentiation and cell survival (Wong & Joe Herbert 2004; Wong & Herbert 2005; Wong & Herbert 2006). A recent study however, has investigated specific aspects of the relationship between stress and AN using a genetic ablation of AN (Snyder et al. 2011). Data from this study has revealed several important clues to this relationship. In particular, ablation of hippocampal AN led to a prolonged elevation of glucocorticoids in response to acute stress as well as a hypersecretion of glucocorticoids in response to prolonged stress indicating an impaired negative feedback. These results indicate that AN regulates endocrine stress reactivity and acts as a buffer to stress responses. The implications of this study are that decreases in AN as a result of an initial stress, can lead to less of a buffer and therefore a magnified response to repeated stress. In restricted incidents of stress, this mechanism may be of adaptive benefit to an animal. However, in a scenario of chronic or severe stress, this loop-mechanism has the potential to develop into an overactive, dysregulated system with the potential consequences such as symptoms which reflect those of depression.

The first direct associations of AN and depression arose with the findings that a decreased serotonergic tone, as also seen in clinical depression, led to a decrease in AN (Brezun & Daszuta 1999). This and subsequent studies grounded the base for a

neurogenic theory of depression in which decreases in AN are proposed to be the causal factor in the pathophysiology of depression and furthermore that antidepressants ameliorate this decrease. Indeed it was subsequently demonstrated that several different types of antidepressant treatments increase AN (Malberg et al. 2000;Madsen et al. 2000). In the absence of established depressed animal models, these experiments were performed in animals under normal physiological conditions and despite this, antidepressant treatment display a neurogenic effect. A subsequent publication examined the correlation of this response with behavioral effects using the novelty suppressed feeding (NSF) test; a behavioral paradigm in which normal animals which have been chronically but not acutely treated with antidepressants display a positive behavioral response. However, in animals with ablated neurogenesis, this behavioral response was absent from fluoxetine treated animals indicating that neurogenesis was necessary for a response (Santarelli et al. 2003). This study appeared to cement the neurogenic theory of depression. Several other aspects correlated well with this theory including the possibility that a decrease in neurogenesis might explain the decrease in hippocampal brain volume seen in depressed patients and furthermore explain the time-lag between treatment and response seen in the clinic. The neurogenic theory as formalized by Jacobs et al. hypothesized that the "waning and waxing of neurogenesis in the hippocampal formation are important causal factors, respectively, in the precipitation of, and recovery from, episodes of clinical depression" (Jacobs et al. 2000). Indeed, with the publication by Santarelli and colleagues, this theory appeared appealing, gaining much support but also causing much controversy. The excitement generated by these studies created a substantial interest in this area and resulted in numerous subsequent publications revolving around this topic.

Thus, time has allowed advances as well as perspective into these matters though unfortunately it would appear that definitive answers remain elusive. However, studies regarding decreases in hippocampal volume implicate factors beyond AN indicating that a decrease in AN is not the reason for this change (Czéh & Lucassen 2007). In an elegant review by Petrik, Lagace and Eisch, these authors categorically and systematically dissect the many studies produced over the past decade in an attempt to obtain a perspective on the current understanding of AN and depression (Petrik et al. 2011). This review examined the postulates of the neurogenic theory investigating individually the consensus on the relevance of decreased AN for the pathophysiology of depression and accordingly the consensus on relevance of AN for the effects of antidepressants. These were furthermore divided into non-stressed and stressed groups. Thus, a consensus thus on various common depression-like behavioral tests indicates that neurogenic ablation does not induce depressive-like states in 76% of all tests in non-stressed animals and furthermore do not induced this state in 79% of tests stressed animals. With regards to the relevance of AN for antidepressant effects, investigations indicate that in 57 % of all tests in non-stressed, AN is required for antidepressant induced alterations in behavior whereas AN is required in 53% of tests of stressed animals. The NSF test alone, as described above, is one of the only tests (besides the modified, NSF, the NIH discussed later) which responds to chronic treatment rather than acute and therefore is postulated to better reflect mechanisms relevant to the clinic in which only chronic treatment is effective. Examination of this test alone reveals that 12% of studies on non-stressed animals indicate that ablation of AN induces a depressive response in this test whereas 50% of studies in stressed animals indicate that

ablation of AN induces a depressive response. Similarly examination of the relevance of AN for antidepressant response in this test reveals that 86% of tests in unstressed animals indicate a requirement of AN whereas 70% of tests in unstressed animals require AN for an antidepressant response. Of course, numerous factors differ between many of the above studies making interpretations difficult and therefore should be made carefully. Several factors appear to affect these studies greatly including basal anxiety of animals. Therefore strain, in which this varies greatly is also a factor to be considered. Secondly ablation method should be considered as well as antidepressant treatment type. Indeed several antidepressants have been shown to have antidepressant effects independent of AN (Sahay & Hen 2007) indicating that AN is perhaps a step within a mechanism.

One pattern which appears more clear than the others is that a decrease in AN does not induce a depressive like phenotype in unstressed animals although NSF data cast doubt on this postulate in stressed animals. There is furthermore a strong case, judging from these studies that in both stressed and non-stressed, AN is necessary to observe an antidepressants effect. Therefore a summary of the presently available data indicate that the original neurogenic theory of depression appears to be contradicted with regards to AN being an important causal factor but AN does appear important for the effect of antidepressant. This second postulate is indeed further supported by the fact that almost all currently prescribed antidepressant treatments also stimulate AN in animal models including SSRIs, TCAs, ECT, exercise and behavioral therapy (Malberg et al. 2000; Madsen et al. 2000; Bjørnebekk et al. 2005; review by Sahay & Hen 2007). However, a key point which is brought up in the first postulate is the question of the important causal factors of depression; Indeed a trend, which began with the lack of the accurate model of depression, was to use animals in normal physiological conditions thus neglecting aspects of stress. Results from these studies have provided conflicting evidence as just described. However, this controversy prompted scientist to dig deeper into the mechanisms involved, in particular forcing the confronting of aspects of stress. Indeed clinical studies have long since implicated stress as a probable co-factor in the development of depression (see Kendler et al. 1999) as further demonstrated by dysregulation of the hypothalamic–pituitary– adrenocortical (HPA) axis, present in roughly half of depressed patients (see Swaab et al. 2005). More recent studies have begun to turn this trend to once again involve aspects of stress in the study of AN and depression (Schloesser et al. 2009; Anacker et al. 2011; Surget et al. 2011; Snyder et al. 2011). Ironically, controversy in procedure and subsequent results in these studies demonstrate that despite a decade of research into “depression” and AN, the study of aspects of stress are still in their infancy. Nonetheless, these studies give important insight into the importance of this aspect of depression and indicate that a potentially crucial mechanisms by which antidepressants work is via the reversal of glucocorticoid resistance and normalizing HPA axis hyper- activity (Anacker et al. 2011; Surget et al. 2011).

Thus, a promising outcome of these recent publications will hopefully be an even more integrated study of the different aspects of depression including both AN and the stress response system. In particular, this advancement will hopefully enable experimental application of newly discovered modulators of neurogenesis in optimized animal models, in the hopes of accurately identifying clinically relevant treatments for disorders in which AN is affected.

POTENTIAL MODULATORS OF ADULT NEUROGENESIS RELEVANT TO STUDIES

Serotonergic modulation

One of the main reasons for the study of AN to shift towards the study of its effect in depression was the original finding that decreases in serotonin using a serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) in the raphe nucleus reduced cell proliferation and ongoing neurogenesis (Brezun & Daszuta 1999). It was quickly postulated that increasing serotonin would increase AN and soon thereafter it was reported that SSRI's which increase serotonin levels also increased AN (Malberg et al. 2000). Although the effects of fluoxetine on AN may potentially involve extra-serotonergic mechanism, studies of serotonin receptors indicate that serotonin is involved. Early studies suspected the 5-HT1A receptor in the proliferative effects of fluoxetine and results from experiments demonstrated that pharmacological antagonism of this receptor had detrimental effects on cell proliferation (Radley & Jacobs 2002). Furthermore,5-HT1A agonists increased cell proliferation in both neurogenic niches (Santarelli et al. 2003; Banasr et al. 2004) while this effect or the effects of fluoxetine were no longer present in 5-HT1A KO mice (Santarelli et al. 2003). However, no effects of these agonists upon cell survival have been reported indicating that the survival enhancing effects seen during treatment with fluoxetine (Sairanen et al. 2005) are mediated through a separate mechanism. Although the exact cellular distribution of 5-HT1A is unclear, it does not appear that they are expressed in cells undergoing neurogenesis at any stage thus indicating an indirect mechanism by which cell proliferation is affected. This mechanism however remains unknown. In a further examination of serotonergic receptors, Banasr et al. examined several other serotonin receptors including the 5-HT1B receptor. Data from this experiment demonstrated that while in the SGZ, 5-HT1B modulation did not affect baseline levels of the different aspects of AN, stimulation of this receptor in serotonin depleted, neurogenesis impaired mice increased cell proliferation indicating that this receptor may be of interest in pathological scenarios. Data from the SVZ in contrast revealed that 5-HT1B stimulation decreased baseline proliferation indicating differences between these niches. Further data from 5-HT2A and 5-HT2C revealed that only 5-HT2A affected AN with stimulation having a negative effect indicating and inhibitory activity in the SGZ which again was opposite in the SVZ. In another study, 5-HT4 was shown to increase cell proliferation after remarkably sub-chronic treatment in the SGZ although effects were not studied in the SVZ (Guillaume Lucas et al. 2007). Further analysis of serotonergic effects via the 5-HT3, 5-HT6 and 5-HT7 receptors remains to be performed.

Potential modulation of adult neurogenesis via p11

p11, also known as S100A10, is a part of the S100 protein family – a group of proteins which consists of small acidic calcium binding proteins with diverse intracellular and extracellular functions. p11 is widely distributed in the body and brain where it is specifically expressed in numerous regions, including the cerebral cortex, cerebellum, striatum, hippocampus, hypothalamus and raphe nucleus. Several publications have recently demonstrated a strong correlation of p11 to depression (Svenningsson et al.

2006; Alexander et al. 2010; Warner-schmidt et al. 2010; Warner-schmidt et al. 2009). Initially in these studies, this correlation was demonstrated by the discovery that p11 mRNA levels were decreased in a well-validated genetic mouse model of depression, the helpless/Rouen mice (Svenningsson et al. 2006). This finding was found to have a translational relevance as it was found in addition that postmortem brains of depressed individuals also had decreased p11 mRNA levels (Svenningsson et al. 2006). Furthermore, data from p11 knock-out (KO) mice indicate that these mice have a depressive-like phenotype measured with two antidepressant tests and were less sensitive to the behavioral effects of the tricyclic antidepressant imipramine. Alternatively, mice that overexpressed p11 showed the opposite behavior with a less depressive phenotype and a normal response to antidepressants (Svenningsson et al. 2006).

The correlation to depression was originally demonstrated to have an association with the serotonergic system as it was demonstrated that p11 binds to the 5-HT1B receptor. As with other S100 proteins which translocate proteins, p11 was also demonstrated to partially regulate this receptor by increasing localization to the cell surface (Svenningsson et al. 2006). A subsequent publication, further supported this association of p11 with the serotonergic system as it was demonstrated that p11 not only binds to 5-HT1B but also binds to the 5-HT4 receptor and similarly increases cell surface localization (Warner-Schmidt et al. 2009). This cell surface localization was also demonstrated to increase serotonergic signaling through the 5-HT4 receptor, measured by changes in intracellular protein concentration and phosphorylation levels (Warner-Schmidt et al. 2009). Furthermore, p11 was shown to be required for the 5-HT4 regulated- antidepressant behavioral effects as p11 KO mice did not respond to the 5-HT4 partial agonist, RS67333, a drug previously shown to have antidepressant effects (Warner-Schmidt et al. 2009). p11 has also been shown to have effects independent of serotonergic modulation as it for example interacts with multiple ion channels, though no direct correlations to depression regarding these has been investigated (Svenningsson & Paul Greengard 2007). However, a serotonergic independent link for p11 to depression is its' interaction with BDNF- a neurotrophic factor known to be highly correlated to depression. This is interaction is highlighted in a study in which p11 expression is shown to be regulated by BDNF (Warner-Schmidt et al. 2010). Subsequently 5-HT1B expression was also regulated by BDNF indicating a potential BDNF/p11 mediated modulation of serotonergic signaling. Furthermore, p11 KO mice were shown to be insensitive to the antidepressant actions of BDNF (Warner-Schmidt et al. 2010). p11 potentially also regulates BDNF via its' extracellular expression on the surface of cells where it binds tissue plasminogen activator (tPA) (Kwon et al. 2005). tPA regulates several important structural and neurochemical aspects of hippocampal functions including the activation of the protease plasmin which cleaves the precursor pro–BDNF to mature BDNF (Pang et al. 2004). Therefore p11 may alter mature BDNF levels via this mechanism though the implications of this for depression remains unexplored.

These publications highlight several potential pathways through which p11 might be involved in the pathophysiology and possible treatment of depression. As described, changes in AN are another proposed component of depression and its' treatment. The correlation of p11 with depression warranted therefore examination of a possible involvement of p11 in neurogenesis.

Potential modulation of adult neurogenesis via S100B

S100B is a widely studied member of the S100 protein family, having been discovered before p11. In the brain, these functions include regulation of protein phosphorylation, calcium homeostasis, enzyme activity, protein scaffolding, and participation in inflammatory processes (see Donato, 2001 for review). S100B is found both intracellular and extracellular. As an extracellular protein it can thus act in both an autocrine and paracrine fashion (Liu & Lauder 1992)**.** S100B is primarily secreted by astrocytes, although it can also be secreted from microglia, oligodendrocytes and neurons (Baudry et al. 2010; Shashoua et al. 1984) and S100B can act as neurotrophin in small amounts but also acts as a neurotoxin inducing apoptosis in higher amounts (Fanò et al. 1993; Ahlemeyer et al. 2000). S100B has several specific associations to the serotonergic systems starting already during development where in the developing hippocampus serotonin mediates the release of S100B which in this case acts as a neurotrophin, promoting dendritic development (Mazer et al. 1997). Further support of a neurotrophic effect comes from a study demonstrating that S100B overexpressing mice have increased axonal sprouting and neurite proliferation in the hippocampus (R. Reeves et al. 1994). This neurotrophic affect appears to be serotonergic system specific as S100B has been shown to inhibit dopaminergic neuron growth (Azmitia et al. 1990; Liu & Lauder 1992; Kligman & Marshak 1985). Further involvement with serotonin is demonstrated by the fact that stimulation of 5-HT1A receptors mediates the secretion of S100B in rat astrocyte cultures (Whitaker-Azmitia 1994; Ahlemeyer et al. 2000). Although this 5-HT1A mediated release has been disputed in vivo (Tramontina et al. 2008), drugs which increase serotonin levels including 3,4-Methylenedioxymethamphetamine (MDMA) and fluoxetine (SSRI) are shown to increase levels of S100B in the intact hippocampus (Kindlundh-Högberg et al. 2009; Manev 2001; Akhisaroglu et al. 2003). Furthermore, in another study, S100B levels in vivo were demonstrated to be positively correlated with levels of serotonin further indicating a serotonin dependent secretion of S100B in vivo (Haring et al. 1993).

S100B levels have been shown to be altered in various pathologies of the nervous system including Alzheimer's disease and Down syndrome (see Shapiro et al. 2010 for review) as well as several affective disorders including schizophrenia (Rothermundt et al. 2001) bipolar disorder (Machado-Vieira et al. 2002), and depression. In particular, increases in S100B plasma levels of depressed patients has been described in several studies (Grabe et al. 2001; Rothermundt et al. 2001; Arolt et al. 2003; Yang et al. 2008; Bernard et al. 2011). A further clinical study indicates that S100B may have a role in antidepressant treatment as there was a positive correlation to positive antidepressant response and high S100B plasma levels , measured upon admission, in depressed patients (Arolt et al. 2003). A recent paper has also identified fluoxetine induced raphe nuclei expression of S100B acting on noradrenergic neurons, which in turn induces a serotonergic phenotype which ultimately contributes to antidepressant-like behavioral effects (Baudry et al. 2010).

As described in the background information, AN is also associated with certain aspects of depression and antidepressant response. Furthermore, fluoxetine increases secretion of S100B in the hippocampus (Manev 2001) and fluoxetine is known to increase cell proliferation as well as cell survival though the mechanism through which this occurs is unknown although it is shown to be dependent on the 5-HT1A receptor

(Encinas et al. 2006; J.-W. Wang et al. 2008; Malberg et al. 2000; Santarelli et al. 2003). 5-HT1A receptor stimulation increases the release of S100B as just described. Thus, these pieces of evidence convene, indicating involvement of S100B in neurogenesis. This is further supported by a study in which intra-ventricular S100B infusion induced increases in cell proliferation and subsequent neurogenesis in the dentate gyrus of the hippocampus after an experimental traumatic brain injury (Kleindienst et al. 2005). This evidence prompted thus further studies into the involvement of S100B in AN.

Dopaminergic modulation

Potential modulation of adult neurogenesis via modulation of dopamine signaling

Dopaminergic modulation of AN has recently been an area of increasing interest and has been the focus of numerous recent publications. From an anatomical perspective, dopaminergic afferents are in position to regulate neurogenesis (Freundlieb et al. 2006; Höglinger et al. 2004; Lennington et al. 2011). Specifically, initial anatomical study by Höglinger et al. used confocal microscopy combined with immunohistochemistry to reveal that in the SVZ midbrain dopaminergic afferents innervate the SVZ close to the rapidly dividing neurogenic cells (Höglinger et al. 2004). Using, a genetic model for dopaminergic neuron loss in the substantia nigra, the aphakia mouse, a more recent study specified that afferents which innervate the SVZ primarily originate from the ventral tegmental area, an area associated with motivation and reward processing(Lennington et al. 2011). Dopaminergic projections have also been shown to have afferents in the dentate gyrus of the hippocampus which contains the SGZ (Gasbarri et al. 1997; Gasbarri et al. 1994; Höglinger et al. 2004). However it is unknown whether these afferents originate from the ventral tegmental area or the substantia nigra. Of the two neurogenic niches, dopaminergic modulation of neurogenesis in the SVZ has been widely studied whereas few publications have addressed the possible dopaminergic modulation of neurogenesis in the SGZ. Therefore the majority of data regarding dopaminergic regulation of AN comes from studies of the SVZ. Studies using pharmacological tools and specific dopamine depletion strategies indicate that in this region, dopaminergic innervation acts to strongly regulate cell proliferation (see Borta & Höglinger 2007 for review). The details however of whether dopamine acts to increase or decrease cell proliferation in the SVZ are still far from clear as exhibited by conflicting results in the literature as to which receptors are involved and whether dopamine acts ultimately to increase or decrease cell proliferation. A valuable tool to examine the effect of dopamine has been dopamine denervation models. While, results from several of these studies of lesion models have indicated that in the SVZ, dopamine depletion decreases cell proliferation (Höglinger et al. 2004; O'Keeffe et al. 2009; S. Baker et al. 2004), equally as many studies have revealed the opposite, that depletion increases cell proliferation in these models (Aponso et al. 2008; B. F. Liu et al. 2006; Peng et al. 2008). Furthermore, additional studies describe no change in cell proliferation in lesion models (Winner et al. 2009; van den Berge et al. 2011). There are of course numerous factors which may have important implications for the outcome and interpretation of these results including

species, lesioning method and neurogenesis analysis method. In Table 1, a summary of the different lesioning studies is presented for comparison of some of these factors. Examination of this table reveals the numerous differences found between these studies and highlights the need for caution when comparing studies. This summary demonstrates that there is little consensus of these lesion studies as results appear to speak equally for both an inhibitory effect and a stimulatory effect of dopamine on cell proliferation (Table 1). This controversy is indicative of a complex regulation.

An obvious possibility is that dopamine is acting on more than one of the five known dopamine receptors, possibly having differential effects on each receptor and/or even differential effects on different cell populations involved in the control of aspects of AN. Knowledge regarding the actual expression of dopamine receptors is a crucial factor in discerning the mechanism through which dopamine affects AN, however, this knowledge is currently very limited due to technical limitations, in particular the lack of successful antibodies to identify and characterize the distribution of dopamine receptors in specific cell populations. Cell specific expression of dopamine receptors has been studied on a limited scale using alternative methods in SVZ. In one study, Höglinger et al. show using SVZ derived neurosphere cultures that both D1-like receptors as well as D2-like receptors are expressed in cells undergoing neurogenesis in the rat (Höglinger et al. 2004). Kim et al. used yet another alternative technique in which they used mice which express GFP under either GFAP or DCX promoters. GFP positive cells from the adult SVZ region were then sorted using fluorescence activated cell sorting (FACS) into specific cell populations after which qPCR was used to determine dopamine receptor expression in these populations. Results were in agreement with Höglinger et al and demonstrated that in the SVZ of the mouse, adult born neuroblasts express multiple dopamine receptors including D1, D2 and D5 but do not express the D3R (Kim et al. 2010). D3R expression was subsequently found in the rapidly dividing transit amplifying progenitor cells, indicating a direct involvement of this receptor in

Table 1 Effects of dopaminergic denervation on adult neurogenesis in the SVZ

Author	Year	Species	Lesion type	Effect	Investigation point	Aspect of AN	Method
Höglinger	2004	rat	6-OHDA	↓	n.a./n.r.	proliferation	PCNA
		rat	6-OHDA	↓	n.a./n.r.	proliferation	PCNA
		mouse	acute MPTP	↓	1,2,7 days	proliferation	BrdU 2 days + 0days
		mouse		-	21,70 days	proliferation	BrdU 2 days + 0days
		mouse	acute MPTP	↓	n.a./n.r.	cell survival	BrdU 2 days + 21days
		humans	PD	↓	n.a./n.r.	proliferation	PCNA
O'Keefe	2009	rat	6-OHDA	↓	27 days	proliferation	BrdU 6days + 1day
		rat	6-OHDA	↓	58 days	cell survival	BrdU 6days + 21days
Baker	2004	mouse	6-OHDA	↓	29 days	proliferation	BrdU 1day+2hrs
		mouse	6-OHDA	↓	29 days	proliferation	Ki-67
Freunlieb	2006	macaques	acute MPTP	↓	5 weeks	proliferation	PCNA
		macaques	acute MPTP	↓	5 weeks	ongoing neurogenesis	PSA-NCAM
Liu	2006	rat	6-OHDA	↑	14 days	proliferation	BrdU 4 days + 2days
		rat	6-OHDA	-	28 days	proliferation	BrdU 4 days + 2days
		rat	6-OHDA	↑	n.a./n.r.	ongoing neurogenesis	PSA-NCAM
van den berge	2011	mouse	chronic MPTP	-	n.a./n.r.	proliferation	PCNA
		mouse		↑	n.a./n.r.	proliferation	PHH3
		Humans	PD	-	n.a./n.r.	proliferation	PCNA
		Humans		-	n.a./n.r.	proliferation	PHH3
Peng	2008	mouse	acute MPTP	↑	14 days	proliferation	BrdU 3 days + 4 days
		mouse	acute MPTP	↑	14 days	cell survival	BrdU 3 days + 11 days
Aponso	2008	rat	6-OHDA**	-	21,70 days	proliferation	BrdU 1day+2hrs
Winner	2009	rat	6-OHDA	- *	n.a./n.r.		BrdU (10days)+ 0 hrs
		rat	6-OHDA	- *	n.a./n.r.		BrdU (10days) + 4 weeks

** partial progressive intra-striatal 6-OHDA lesion, * indirect comparison with non- lesioned animals
n.a./n.r. information not available or not relevant due to biomarker

cell proliferation in the SVZ as well as in niche astrocytes (Kim et al. 2010). Further information regarding the role of these specific receptors in the SVZ comes from studies using pharmacological tools such as agonists and antagonists for specific dopamine receptors. A summary of the studies examining dopaminergic modulation of neurogenesis using pharmacological tools is presented in table 2. Examination of these results indicates again numerous differing factors stressing the need for caution upon comparison of studies. Despite an added specificity of dopamine subtype stimulation, a summary of data again gives no consensus with regards to the effect of dopamine as it appears that both antagonists and agonists of D2 either stimulate or have no change on aspects of AN. Furthermore D3
receptor stimulation appears also to either stimulate or have no changes upon AN. Thus, the role of dopamine in the SVZ remains a complex issue warranting further study.

Dopaminergic regulation of the dentate gyrus is much less studied but the data available is equally filled with conflicting results. Anatomical studies in the SGZ have documented dopaminergic afferents to the dentate gyrus while denervation experiments have demonstrated both increases as well as decreases of cell proliferation indicating again both a stimulatory as well as an inhibitory effect of dopamine (Höglinger et al. 2004; Peng et al. 2008; Park & Enikolopov 2010) These studies indicate again the

Table 2 Effects of pharmacological modulation of dopamine on adult neurogenesis in the SVZ

Author	Year	Species	Drug	Primary Pharmacology	Dose	Admin. method	Duration of Treatment	Effect	Aspect of AN	Method	Timing
Baker	2005	mouse	7-OH-DPAT	D3 preferential ag.	3 ug /day	i.i	14 days	-	cp/on	BrdU	14 days + 0hrs
		mouse	U-99194A	D3 antag.	4 ug /day	i.i	14 days	-	cp/on	BrdU	14 days + 0hrs
Kim	2010	mouse	U-99194A	D3 antag.	2mg/kg	i.p.	3 days	↓	cs	BrdU*	3 days + 5 days
		mouse	U-99194A	D3 antag.	20mg/kg	i.p.	3 days	↓	cs	BrdU*	3 days + 5 days
		mouse	U-99194A	D3 antag.	2mg/kg	i.p.	3 days	-	cp	BrdU	3 days + 2 hrs
		mouse	U-99194A	D3 antag.	20mg/kg	i.p.	3 days	↓	cp	BrdU	3 days + 2 hrs
Van Kampen	2004	rat	7-OH-DPAT	D3 preferential ag.	2ug /day	i.i . or i.p	14 days	↑	cp/on	BrdU	14 days + 0hrs
		rat	SKF82958	D1 ag.	2ug /day	i.i .	14 days	-	cp/on	BrdU	14 days + 0hrs
		rat	SB 277011-A	D3 antag.	1.5ug /day	i.i .	4 days	-	cp/on	BrdU	14 days + 0hrs
Yang	2008	mouse	quinpirole	D2 ag.	2 mg/kg	i.p.	3 days	↑	cp	BrdU	1 dose + 2 hrs
Kippin	2005	rat	Haloperidol	D2-like antag.	2mg/kg	i.p.	14 days	↑	cp	BrdU	1 day + 1 hr
		rat	Haloperidol	D2-like antag.	2mg/kg	i.p.	acute 6 hrs before BrdU	-	cp	BrdU	1 day + 1 hr
		rat	Haloperidol	D2-like antag.	2mg/kg	i.p.	acute 24 hrs before Brdu	-	cp	BrdU	1 day + 1 hr
Höglinger	2004	rat	ropinirole	D2 ag.	3mg/kg	o.m.	acute 45 mins before death	↑	cp	PCNA	
Wakade	2002	rat	Haloperidol	D2-like antag.	0.4mg/kg	d.w.	21 days	-	cp	BrdU	1 dose + 24 hrs
		rat	risperidone	D2-like antag.	0.5mg/kg	d.w.	21 days	↑	cp	BrdU	1 dose + 24 hrs
		rat	olanzapine	D2-like antag.	2mg/kg	d.w.	21 days	↑	cp	BrdU	1 dose + 24 hrs
Green et	2006	rat	olanzapine	D2-like antag.	2mg/kg	d.w.	21 days	↑	cp	Ki-67	
		rat	risperidone	D2-like antag.	0.5mg/kg	d.w.	21 days	-	cp	Ki-67	
Wang	2004	rat	olanzapine	D2-like antag.	10mg/kg	d.w.	35 days	↑	cp+cs	BrdU**	1 day + 14 days
			olanzapine	D2-like antag	10mg/kg	d.w.	21 days	↑	cs	BrdU**	1 day + 14 days
			Haloperidol	D2-like antag.	2mg/kg	d.w.	35 days	-	cp+cs	BrdU**	1 day + 14 days
			Haloperidol	D2-like antag.	2mg/kg	d.w.	21 days	-	cs	BrdU**	1 day + 14 days

cp cell proliferation, cs, cell survival, on ongoing neurogenesis
i.p intra-peritoneal, i.i. intra-ventricular infusion, d.w. drinking water o.m. osmotic mini-pump
*measured in olfactory bulb, **measured in striatum

likely involvement of several dopamine receptors similar to the SVZ. A lack of successful antibodies again limits the interpretations of these studies and unfortunately the studies using alternative methods were limited to the SVZ and did not investigate the hippocampus. However, another study examined the dopamine receptor expression in the entire hippocampus using qPCR, which although lacking in cellular resolution, is able to give a quantitative measurement of receptor levels in relatively defined regions (Mu et al. 2011). This study demonstrated that several dopamine receptors are expressed in the dentate gyrus, including the D3R, albeit at low levels. Interestingly their data regarding other receptors is very similar to data from the SVZ found in Kim et al. with both studies revealing high expression of D1 and D5 receptors, suggested in both studies to be expressed by neuroblasts, indicating that the dopamine receptors expression profile of the SGZ and SVZ are indeed similar. A solution to this caveat of a lack of proper visualization method is the use of the recently developed technique which uses BAC transgenic mouse lines which allow the expression of EGFP under specific promoters enabling reproducible visualization of expression of a specific gene (Gong et al. 2003). The publicly available gene expression atlas, GENSAT (www.gensat.org), has mapped many proteins including the D3R using this technique. Data from confocal analysis which is found on this online database reveal that the D3R is expressed in the dentate gyrus in numerous cells along the SGZ (Figure 2). This *in silico* finding prompted us to question whether expression of D3Rs in this region has a role in regulation of aspects of AN. This novel information regarding D3R expressing cells in the SGZ combined with previous experiments describing dopaminergic modulation of cell proliferation indicate a possible relevance of this receptor for cell proliferation. Previous experiments in the SVZ indicate that D3R stimulation in this niche increases cell proliferation as exemplified by D3R specific agonists which increase cell proliferation in the rat SVZ (Van Kampen et al. 2004) whereas correspondingly, specific D3R antagonists decrease cell proliferation in the mouse SVZ (Kim et al. 2010). However modulation of this receptor in the SGZ has not yet been examined.

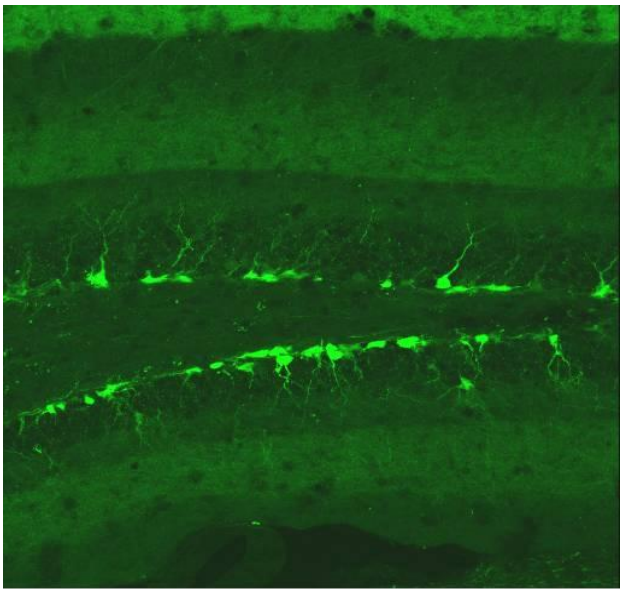

Figure 2 DRD3-EGFP mice generated by GENSAT indicate that D3 receptors are expressed in cells along SGZ of the dentate gyrus in the hippocampus. (Image obtained from the GENSAT database www.gensat.org)

AIMS

The general aim of this thesis was to use pharmacological tools in combination with genetic animal models to identify potential drugs and drug targets which could be used to modulate aspects of adult neurogenesis in the ultimate aim of finding better understanding and treatments for neuropsychiatric disorders. As a result of preliminary investigations and previously published data described in the introduction we developed the following specific aims:

I. Investigate the potential involvement of p11 in the process of adult neurogenesis by examining this process in mice which have a genetic deletion of the p11 gene.

II. Further examine p11 expression in the brain to determine areas of p 11 expressions potentially relevant to depression or modulation of adult neurogenesis.

III. Investigate the potential involvement of S100B in the process of adult neurogenesis by examining this process in mice which genetically overexpress the S100B gene.

IV. Examine the potential behavioral and neurogenic effects of the mixed dopaminergic/serotonergic drug Sarizotan in an animal model of Parkinson's disease.

V. Investigate the potential involvement of the D3R in the process of adult neurogenesis by examining this process in mice which have a genetic deletion of the D3R and/or mice treated with the D3 preferential antagonist, S33138.

METHODOLOGICAL CONSIDERATIONS

Adult neurogenesis has only relatively recently become a general area of study in the field of neuroscience due in part to the rapid development of new methods making investigations into aspects of this complex technically reasonable. The speed of this development is exemplified in this thesis which has been completed over a few years during which this rapid development has meant that the methods used to study neurogenesis have evolved across the studies. The individual procedures and methods used for each study are described in detail the materials and methods of the associated paper. The main topic of AN is explored throughout the thesis and therefore the following is a short summary about methods chosen to study different aspects of this topic and a short discussion of the rationale behind these choices, as well as the disadvantages.

Cell proliferation

The earliest techniques with which to measure AN used [H^3]-thymidine to label dividing cells which could then be visualized using autoradiographic methods (Altman & Das 1965). However it was only upon the advent of BrdU to label dividing cells that investigations into neurogenesis began gaining momentum (Gratzner 1982; Kuhn et al. 1996). BrdU is an exogenous thymidine analog which, similarly to [H^3]-thymidine, integrates into DNA of dividing cells during S-phase when injected into animals. The advantage of BrdU is that it can be visualized using immunohistochemical techniques, allowing simple quantification of labeled cells as well as characterization of BrdU labeled cells with double labeling techniques. Therefore, BrdU quickly became the preferred techniques with which to investigate AN. A major disadvantage of BrdU labeling as well as other techniques currently available is that AN can only be examined in post-mortem tissue. Therefore, an important factor to consider when using BrdU is timing of the BrdU injections in relation to the time of sacrifice. Depending on this timing, BrdU can be used to examine different aspects of AN including cell proliferation, but also cell survival as discussed below. The process of proliferation is the point at which new cells integrate BrdU into their DNA. Already at an early point, 1-4 days after cell division, these cells reach a critical point at which they either undergo apoptosis or develop into neuroblasts (Sierra et al. 2010). Therefore, to accurately examine cell proliferation, animals must be sacrificed within this time point. In the literature, the point of sacrifice varies widely in different protocols, often from 2 hours post injection up to several days and interpretations of these data must be made carefully with regards to this timing. In our initial neurogenesis experiments investigating cell proliferation which are included in this thesis (Paper IV), we used BrdU and chose to inject at four time points during an 8 hour period in order to label a large cohort of cells. The animals were then sacrificed 24 hours after the last injection. In the infancy of the study of AN, this was the preferred method of measuring cell proliferation; however, already at an early stage, other method of measurement became evident including the use of endogenous proteins expressed during proliferation, particularly Ki-67 (Kee et al. 2002). Ki-67 is expressed during all stages of the mitotic process making it a reliable and, since its discovery, a much used marker of cell proliferation in the study of AN. Cell proliferation was measured using BrdU at the

beginning of the development of paper IV, however, the development of the paper was prolonged over a long period. During this time, it became increasingly popular in the literature to use Ki-67 for the investigation of cell proliferation and therefor it was decided to test this new method and complement the BrdU cell proliferation data with Ki-67 cell proliferation data. Results from these experiments were comparable with regards to tendencies although BrdU positive cell numbers were much lower (see paper IV) most likely due to the fact that BrdU only labels cells in S phase whereas Ki-67 is expressed during the entire mitotic process. The fact that Ki-67 labels such a large cohort is also useful for detecting subtle changes in cell proliferation and furthermore, successful visualization of the Ki-67 antibody does not require several pretreatment steps as BrdU staining. The successful application of this method in paper IV in addition to the benefits discussed resulted in the use of Ki-67 in all subsequent studies to study cell proliferation (Papers I, III and V).

Ongoing Neurogenesis

One of the first endogenous markers of AN to be widely used was doublecortin (DCX), a protein expressed almost exclusively in immature neurons in the neurogenic niches (Brown et al. 2003). Immature neurons expressing this protein are past the proliferative critical point and are therefore defined as true neuroblasts ceasing to express markers for these early cell types (Couillard-Despres et al. 2005). An initial study of the dynamics of this protein demonstrated that expression of DCX in cells reflect increases in AN induced by physical activity and seizure induction (Couillard-Despres et al. 2005). DCX expression has since been used in numerous studies as a method with which to examine the levels and modulation of AN. In our studies of neurogenesis it was decided that, in addition to other aspects of neurogenesis, DCX measurements would also be used to examine possible changes in neurogenesis (Papers I,III,IV and V). Despite the utility of this protein, several aspects of it must be noted in order make interpretations from these measurements. There appears to be no consensus in the literature defining with regards to nomenclature what specifically DCX is measuring. DCX is expressed during several weeks of the process of neurogenesis where cells have already undergone cell proliferation but have not yet become neurons as defined by the expression of mature neuronal markers (Brown et al. 2003). Furthermore DCX-expressing cells are not a homogenous group but rather a complex population of cells at different stages of maturation. Therefore several publications including paper I and IV in this thesis refer to changes in DCX simply as changes in *neurogenesis*. However upon further consideration, neurogenesis is a complex process involving many other steps and this nomenclature is not entirely accurate. Neither can DCX expression be referred to correctly as *maturation* as DCX expression is not only affected by maturation but also the size of the pool of proliferating cells present as well as the degree of apoptosis of these maturing cells. Therefore in order to give a more accurate description for this measurement we referred to it thereafter as *ongoing neurogenesis* in subsequent publications (papers III and V).

Cell Survival

In terms of AN, cell survival is defined as ability of new cells to avert apoptosis and mature into new neurons. Under normal physiological conditions, 50% of proliferating cells undergo apoptosis before becoming mature neurons (Cameron & McKay 2001). Cell survival is a crucial aspect for the development of new neurons which can be decreased under pathological conditions for example during stress (Thomas et al. 2007). Antidepressant have on the other hand been shown to increase cell survival (Nakagawa et al. 2002; Sairanen et al. 2005). In particular antidepressant-mediated increases in cell survival is known to be independent from antidepressant effects on cell proliferation (Sairanen et al. 2005). Thus, this aspect of neurogenesis represents another point of this process which can be modulated and therefore is an important measurement of AN. Currently there are no known endogenous markers of new cells which have become mature neurons and therefore tracing methods using exogenous substances must be employed for the detection and characterization of cells which have survive. BrdU, due again to the fact that it can be visualized using immunohistochemical techniques has been very useful and greatly used in the literature for the purpose of quantifying cell survival. Differently to measuring cell proliferation, cell survival requires a different experiment design in which BrdU is injected, in rodents for example, several weeks before sacrifice. The second critical point in the development of new neurons, after cell proliferation, occurs before their integration into the neuronal circuitry and expression of the mature neuronal marker NeuN (Tashiro et al. 2006). Therefore cell survival experiments must be timed so that cells have had the chance to have passed this critical point, typically after 3 weeks. A drawback to this is that in each animal, BrdU can only be used to investigate one aspect, either cell proliferation or survival requiring separate animals for each experiment and introducing further variation. However, with the advent of endogenous markers such as Ki-67 to examine cell proliferation, these experiments can be performed in the same animals reducing variation, animals sacrificed as well as workload. For this however, careful experimental design must ensure that BrdU is only measuring survival and not cell proliferation; thus, drug injection must begin first after injections of BrdU. In our studies we thus employed this combination in order to investigate cell survival and cell proliferation (paper I, III and V). A drawback of BrdU is that, in contrast to most antigens, BrdU is tightly packed within DNA and therefore requires a harsh treatment using high acid concentration to denature the DNA and gain access to the epitope which ultimately may compromise the quality of the tissue and complicate double staining protocols. In initial experiments using BrdU, a neutralization step was used, as this was standard protocol for BrdU antibodies available (papers I, IV) adding further stress to the tissue. However, more newly available antibodies no longer required this step and therefore this step was omitted in subsequent experiments (papers III and V). BrdU is thus a useful method and due to lack of alternatives a currently essential method with which to trace and quantify new cells leading to its wide use in neuroscience. However it should also be noted that BrdU has several limitations including potential toxic or cell behavior- modulating properties (Lehner et al. 2011) and potentially also labels cells undergoing DNA repair and duplication (Taupin 2007). Therefore, interpretations of BrdU analysis must be done taking these aspects into consideration.

RESULTS AND DISCUSSION

The following sections briefly summarize the results obtained in papers I-V. Each paper summary is followed by an extended discussion of the results in the perspective of the thesis as a whole, particularly the translational relevance of these findings. The reader is asked to refer to the individual articles for the actual results including additional data as well as detailed images and statistics.

GENETIC AND PHARMACOLOGICAL MODULATION OF ADULT NEUROGENESIS

Genetic Modulation of S100 Proteins

Genetic ablation of p11 attenuates the neurogenic response to fluoxetine (Papers I & II)

Previous publications demonstrated that the S100 protein, p11 (S100A10) has a strong association with depression and antidepressant treatment (see introduction for more detailed description) (Svenningsson et al. 2006). Furthermore, as described in the introduction, AN is strongly associated with depression and hence, the potential involvement of p11 in aspects of AN was a pressing question. We therefore designed experiments to examine AN by a specific genetic deletion of p11 using p11 KO mice which had been treated chronically with the antidepressant fluoxetine. Results from our experiments, presented in paper I, demonstrate that several distinct aspects of AN and neurogenic response to fluoxetine were altered in p11 KO mice.

As described in literature, mice which are treated chronically with fluoxetine exhibit an increase in cell proliferation, ongoing neurogenesis as well as cell survival (Encinas et al. 2006;Wang et al. 2008; Sairanen et al. 2005). To examine cell proliferation we used the cell cycle marker Ki-67 and results from these experiments revealed an expected significant increase in WT mice upon chronic (21 day) treatment with

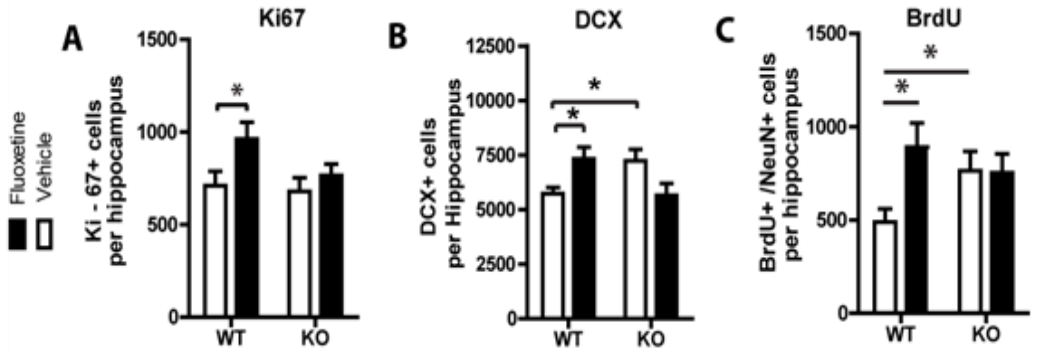

Figure 2 Regulation of aspects of adult neurogenesis in the SGZ by fluoxetine in WT and p11 KO mice**. (A)** Histogram showing the quantification of Ki-67 positive cells representing cell proliferation. Fluoxetine increased cell proliferation ($p<0.05$) in WT but not KO mice. **(B)** Histogram showing the quantification of DCX positive cells representing ongoing neurogenesis. Fluoxetine increased ongoing neurogenesis ($p<0.05$) in WT but not KO mice. p11 KO mice had an increased baseline level ($p<0.05$) of ongoing neurogenesis. **(C)** Histogram showing the quantification of BrdU positive cells representing cell survival. Fluoxetine increased cell survival ($p<0.05$) in WT but not KO mice. p11 KO mice had an increased baseline level ($p<0.05$) of cell survival. Data are reported as mean values +/- SEM. *p □ .05; two-way analysis of variance followed by Bonferroni's t test for pairwise comparisons.

fluoxetine (10mg/kg). This cell proliferative effect of fluoxetine was no longer present in the p11 KO mice as these mice displayed levels of cell proliferation not significantly different to vehicle treated mice (Fig. 2A). Ongoing neurogenesis, measured using the immature neuronal marker DCX displayed results similar to cell proliferation with an increase in ongoing neurogenesis upon fluoxetine treatment in WT mice which was no longer present in p11 KO mice (Fig. 2B). Finally, survival was measured using the thymidine analog BrdU (3 days, 2x 75mg/kg) which was injected 3 weeks before sacrifice after which the number of labeled mature neurons (BrdU/NeuN positive) were quantified (Fig. 2C). Data from these survival experiments revealed that although fluoxetine increased cell survival in WT mice compared to vehicle, this cell survival effect does not occur in p11 KO mice as numbers of mature neurons are not significantly different between chronic fluoxetine and vehicle treated mice. Thus it appears from these experiments that the neurogenic response to fluoxetine is attenuated in p11 KO mice indicating involvement of p11 in the neurogenic response to fluoxetine.

Another altered aspect of AN was found in the p11 KO mice when looking at baseline levels of both ongoing neurogenesis and cell survival in comparison to WT mice. A comparison of levels of ongoing neurogenesis in vehicle treated WT and p11 KO mice revealed a significant increase in the p11 KO mice indicating an endogenous increased level of ongoing neurogenesis in these mice (Fig. 2B).. Similarly, a comparison of BrdU/NeuN positive cells in vehicle treated WT and p11 KO mice also revealed a significant increase in levels of cell survival in the p11 KO mice (Fig. 2C). No difference however was found in comparing baseline levels of cell proliferation between WT and KO mice (Fig. 2A)..

In an attempt to elucidate the underlying mechanisms through which p11 regulates aspects of AN observed we performed several immunohistochemical experiments to determine if this mechanism was direct or indirect. Results from these experiments demonstrated that p11 did not appear to be expressed in either proliferating cells (Ki-67 positive cells) or immature neurons (DCX positive cells) indicating an indirect regulation. It was however observed that p11 was highly expressed in cells displaying a large soma, characteristic of interneurons. Indeed, further staining with parvalbumin, a GABAergic interneuron marker revealed in fact that this marker was co-expressed by many of the cells highly expressing p11 indicating that a proportion of these p11 positive cells are in fact interneurons. Further staining using markers for other types of interneurons, namely calbindin 28K and CCK demonstrated that highly expressing p11 cells also co-expressed these markers. As p11 has been demonstrated to regulate trafficking and signaling of both the 5HT-1B and 5-HT4 receptors (Svenningsson et al. 2006; Warner-schmidt et al. 2009), we also wanted to investigate if these receptors were co-expressed in these highly expressing p11 cells. Initial experiments using specific antibodies demonstrated that both 5HT-1B and 5-HT4 receptors were co-expressed in these cells (paper I). Although the 5HT-1B antibody appeared to have a strong staining, the 5-HT4 antibody was less robust and therefore this co-expression of p11 and 5-HT4 in the dentate gyrus was confirmed using a 5-HT4-GFP mouse line in a subsequent article (paper II). In addition to being expressed in p11 positive cells, co-expression of both 5HT-1B and 5-HT4 receptors was found in parvalbumin as well as calbindin interneurons in the dentate gyrus (see fig 6). Results demonstrating expression of both p11 and specific receptors in interneurons are interesting as they fit

well with previous literature which demonstrate that GABA-ergic interneurons regulate the differentiation, development and integration of newborn neurons (Tozuka et al. 2005; Ge 2006) (see fig 6). As further discussed in paper I, serotonergic afferents are known to innervate the dentate gyrus and therefore potential modulation of signaling to these interneurons in p11 KO mice indicate a highly plausible mechanism through which p11 could affect both fluoxetine induced neurogenesis as well as basal aspects of maturation and survival. However, p11 has several known interactions with other pathways which could ultimately affect AN including the fact that p11 interacts with several ion channels, and binds tissue plasminogen activator (tPA) (Svenningsson & Greengard 2007; Kwon et al. 2005). The interaction with tPA is particularly pertinent to regulation of AN as tPA/plasmin is important for the cleavage of proBDNF to BDNF, a well characterized regulator of AN (Pang et al. 2004; Sairanen et al. 2005) (see fig 6). The degree to which p11 affects each of these proposed mechanisms, further affecting AN, has yet to be definitively described. In our results p11 affects both proliferative response as well as the cell survival response to fluoxetine. As described in the introduction these are two separate aspects of AN which are often regulated by different factors. Whether these differential responses to fluoxetine in the different aspects of AN in p11 KO mice are the result of p11 related changes in a single common mechanism or multiple mechanisms has yet to be determined. Furthermore, results from baseline levels of aspects of AN also demonstrated differences in p11 KO mice, particularly in ongoing neurogenesis and cell survival. One possible explanation of the observed increase in DCX is a decreased maturation rate resulting in a larger cohort of cells expressing DCX creating an increased maturation window, as has been suggested to occur in the modulation of aspects of AN (Wang et al. 2008). This increase in ongoing neurogenesis as well as in the increase seen in baseline cell survival could also be result of an increased promotion of survival from different factors which may be the result from compensatory mechanisms in p11 KO mice. For example, in S100B KO mice, in which there is a genetic deletion of the neurotrophic factor S100B, there is a compensatory 53 % increase of another neurotrophic factor, namely BDNF (Schulte-Herbrüggen et al. 2008). It is therefore possible that the potential modulation of BDNF resulting from a genetic deletion of p11 may result in altered and even increased levels of other neurotrophic factors which may affect, or specifically increase survival. In summary paper I demonstrated that genetic deletion of p11 affects the neurogenic response to fluoxetine indicating that p11 may be involved in this response.

Genetic amplification of S100B expression increases cell proliferation (Paper III)

Previous publications have indicated the involvement of an additional S100 protein, S100B in both the pathophysiology of depression but also its involvement in antidepressant actions (Grabe et al. 2001; Manev & Manev 2001). Furthermore, it has been suggested that S100B can also affect AN in the SGZ of the hippocampus under certain conditions (Kleindienst et al. 2005). To further examine this potential modulation of AN, in paper III, we examined different aspects of hippocampal AN in S100B TG mice which overexpress this protein. To examine cell proliferation we quantified Ki-67 positive cells in the SGZ. Results from this experiment demonstrated that S100B TG mice had significantly increased baseline levels of cell proliferation in comparison to WT mice (Fig. 3A). We also examined ongoing neurogenesis using

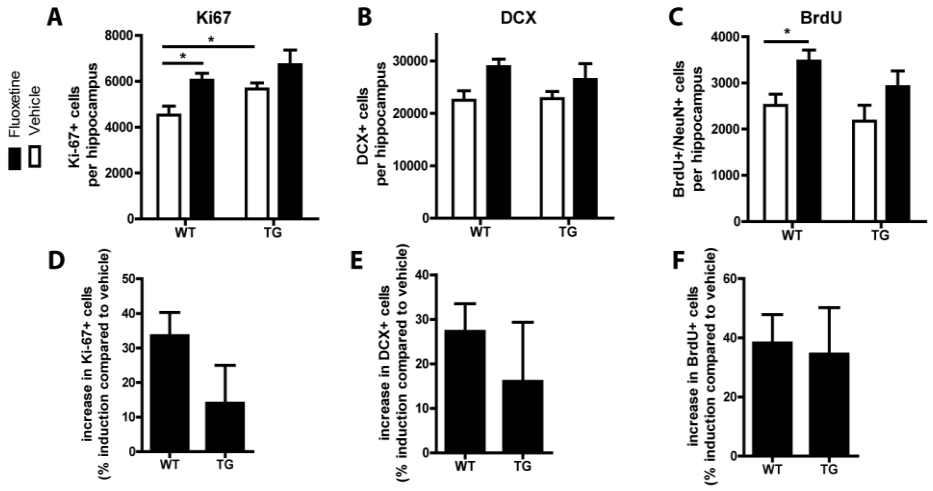

Figure 3 Regulation of aspects of adult neurogenesis in the SGZ by fluoxetine in WT and S100B TG mice**. (A-C)** Histogram showing the quantification of Ki-67(A), DCX (B) and BrdU (C) positive cells representing cell proliferation, ongoing neurogenesis and cell survival respectively. **(**A) Two-way ANOVA demonstrates a highly significant ($p<0.01$) difference in treatment and a significant difference ($p<0.05$) in genotype but no significant interaction ($p>0.05$). Post hoc analysis revealed a significant increase ($p<0.05$) upon chronic treatment with fluoxetine in WT mice and a significant increase ($p<0.05$) in baseline levels of cell proliferation in the S100B TG mice compared with WT. **(B)** Two-way ANOVA demonstrates a significant difference ($p<0.05$) in treatment but not for genotype or interaction ($p>0.05$). Post-hoc analysis revealed no significant individual increases upon treatment with fluoxetine despite induction (E). Two-way ANOVA demonstrates a highly significant difference ($p<0.01$) in treatment but not for genotype and no significant interaction. Post-hoc analysis revealed a significant difference in cell survival upon treatment with fluoxetine in WT mice ($p<0.05$). **(D-F)** Data presented as a percent of induction with fluoxetine in comparison to vehicle reveals no significant difference between fluoxetine induction in the WT and TG mice with regards to cell proliferation **(D)**, ongoing neurogenesis **(E)** or cell survival **(F).** Data are reported as mean values +/- SEM. *p < .05 Newman-keuls.

DCX and finally cell survival by quantifying BrdU positive cells (3 days, 2x 75mg/kg-3 weeks post injection). Levels of both ongoing neurogenesis and cell survival in S100B TG mice were similar to WT mice indicating that there was no difference in baseline levels of either of these parameters (Fig. 3B,C).. As S100B and p11 both belong to the S100 family, it could be speculated that they have related functions. p11 KO mice, as described in the preceding section (data from paper I), have an attenuated response to fluoxetine in several aspects of the neurogenic process. As described in the introduction, S100B is similarly to p11 related to several aspects of serotonergic signaling, though these are distinctly different from p11. We therefore also analyzed the neurogenic response of S100B TG mice after chronic treatment with fluoxetine (10mg/day for 21 days). Cell proliferation in these treated WT mice displayed a significant increase which was seen as a 33 % induction in comparison to vehicle (Fig. 3A,D). S100B TG mice displayed a 14 % induction in response to fluoxetine which was not as robust as in the WT mice and does not reach significance in post hoc analysis (Fig. 3A,D). This could possibly be the result of a lack of effect of fluoxetine in the S100B TG mice or as a result of the described baseline increase leading to a maximum ceiling effect being reached upon further increases with fluoxetine. Statistical analysis of induction levels demonstrates however no significant difference in response to fluoxetine between S100B and WT mice (Fig. 3D). The response to fluoxetine was also similar in S100B TG and WT mice when examining ongoing neurogenesis where there was an overall significance of treatment using two-way ANOVA as seen by the 27 % and 16 % induction in WT and S100B TG mice respectively (Fig. 3B,E). Individual post-hoc analysis did not reveal significance in either group, however, analysis of induction displayed no differences between S100B

TG or WT mice indicating no difference in induction as a result of fluoxetine treatment (Fig. 3E). Finally analysis of the effect on cell survival in response to fluoxetine demonstrated a highly significant effect of treatment and no significant difference in induction with 38 % in WT mice and 34 % induction in S100B TG mice (Fig. 3C,F). Post hoc analysis revealed significant increases in WT mice which did not reach significance in S100B mice despite the large induction. Thus, analysis of induction in response to fluoxetine reveals that there are no significant differences in any of the aspects of AN measured indicating that the neurogenic response to fluoxetine is unchanged in S100B TG mice.

The difference thus in S100B TG mice in comparison to WT is that these mice have an increased baseline level of cell proliferation while their neurogenic response to fluoxetine appears unaltered. These results indicate that a S100B can stimulate cell proliferation in the adult hippocampus. Indeed it is interesting to speculate whether the documented neurogenic effects of fluoxetine are at least in part mediated by S100B. This possible mechanism is supported by several lines of evidence in the literature. This includes the facts that S100B is primarily expressed in the hippocampus by astrocytes which are also known to be the source of the majority of the 5-HT1A expression in the hippocampus, while stimulation of this receptor induces the release of S100B in rat astrocyte cultures (Whitaker-Azmitia et al. 1990). Furthermore, acute stimulation with 5-HT1A agonists and chronic treatment with fluoxetine increases cell proliferation; both effects of which are ablated in 5-HT1A KO mice (Santarelli et al. 2003). Additional support of S100B mediated effects of fluoxetine on AN comes from the facts that fluoxetine increases S100B in the hippocampus and furthermore that fluoxetine specifically targets cell proliferation by targeting the amplifying progenitor cells (Encinas et al. 2006; Manev 2001).

Several interesting questions regarding this proposed mechanism remain to be answered including whether or not S100B has a role in the regulation of AN during physiological conditions or is only involved during increased expression for example during fluoxetine treatment. Our observation that an overexpression of S100B increases cell proliferation suggests that S100B acts to increase cell proliferation during when stimulated to for example during treatment. However, whether or not blockade of normal endogenous levels of S100B would also affect cell proliferation must be determined in order to answer this. Questions also remain regarding the further mechanism through which increased expression of S100B affects cell proliferation. Proliferative effects of S100B could be the result of indirect involvement but could also be the result of a direct effect on progenitor cells such as the amplifying progenitor cells described by Encinas et al. (Encinas et al. 2006) (see fig 6). Interestingly, the S100B receptor RAGE has been demonstrated to be expressed on newly divided cells in the adult SGZ indicating a direct involvement via this receptor (Manev et al. 2003) (fig 6). In a further publication, in vitro treatment of adult neural progenitors derived from the SVZ with S100B stimulated cell-proliferation adding further support to this proposed direct mechanism (Meneghini et al. 2010). An interesting study to further confirm this would be to examine the effects of fluoxetine in S100B KO mice which have been studied with regards to several other processes but not AN (Nishiyama et al. 2002; Schulte-Herbrüggen et al. 2008). It could thus be hypothesized that if S100B was important for baseline cell proliferation or antidepressant induced proliferation, AN would be correspondingly decreased in one or both of these parameters. However, it

has also been suggested that KO mice have increased levels of BDNF, suggested as a compensatory mechanism, which could perhaps complicate this experiment and subsequent interpretations (Schulte-Herbrüggen et al. 2008).

A comparison of AN in S100B overexpressing and p11 KO mice gives a clue to the similarities and differences in function on AN of these S100 proteins. The neurogenic response to fluoxetine of S100B TG mice appeared similar to that of WT mice which is in contrast to what was observed in the p11 KO mice where there was a clear lack of induction in all aspects of AN. Examination of baseline levels further highlight differences where S100B TG mice display increased levels of cell proliferation, p11 KO mice have normal levels. However, examining ongoing neurogenesis and cell survival reveals that baseline levels are normal in S100B TG mice while p11 KO mice have increased levels in both parameters. When comparing these results it must be kept in mind that while one mouse line lacks a protein, the other line has an excess of a protein. Interpretations of these result indicate that S100B to acts to stimulate cell proliferation in physiological conditions and/or further increase proliferation in response to antidepressant treatment. Results from p11 on the other hand suggest that p11 may inhibit cell survival in physiological conditions but acts to increase cell proliferation, ongoing neurogenesis and cell survival in response to antidepressant treatment. Thus, although there are some similarities in the functions of these proteins with respect to AN, their mechanisms and overall function appear quite distinct.

Pharmacological Modulation of Dopaminergic and Serotonergic Signaling

Sarizotan increases cell proliferation in neurogenic niches (Paper IV)

Sarizotan is a drug which was developed as a potential atypical antipsychotic, similarly to S33138 (paper V), but was at an early stage described to be of benefit against extrapyramidal motor symptoms seen in Parkinson's disease (Bartoszyk et al. 1997; Rabiner et al. 2002; Bibbiani et al. 2001). Sarizotan has an interesting albeit complicated pharmacological profile with demonstrated affinities for the 5-HT1A receptor but also for D2-like receptors, binding highly to D2 receptors but even higher to D3 and D4 receptors. Specifically, at the 5-HT1A receptor, sarizotan has been proven to act as a pronounced agonist whereas its dopaminergic profile appears more complicated (Bartoszyk et al. 2004). Studies have indicated that at D2 receptors, sarizotan may act as a partial agonist, depending on the dopaminergic impulse flow whereas at D3Rs, it might act as a competitive antagonist to endogenous dopamine (Bartoszyk et al. 2004; Gerlach et al. 2011).

There are several associations of these receptors with depression, AN and PD. In particular both 5-HT1A as well as dopamine receptors are known to regulate aspects of AN and, furthermore, the 5-HT1A receptor is important for the behavioral effects of antidepressants (Banasr et al. 2004; Borta & Höglinger 2007; Santarelli et al. 2003).With regards to PD, there has recently been increasing understanding that PD patients often also experience depression (Chaudhuri et al. 2006; Aarsland et al. 2011).

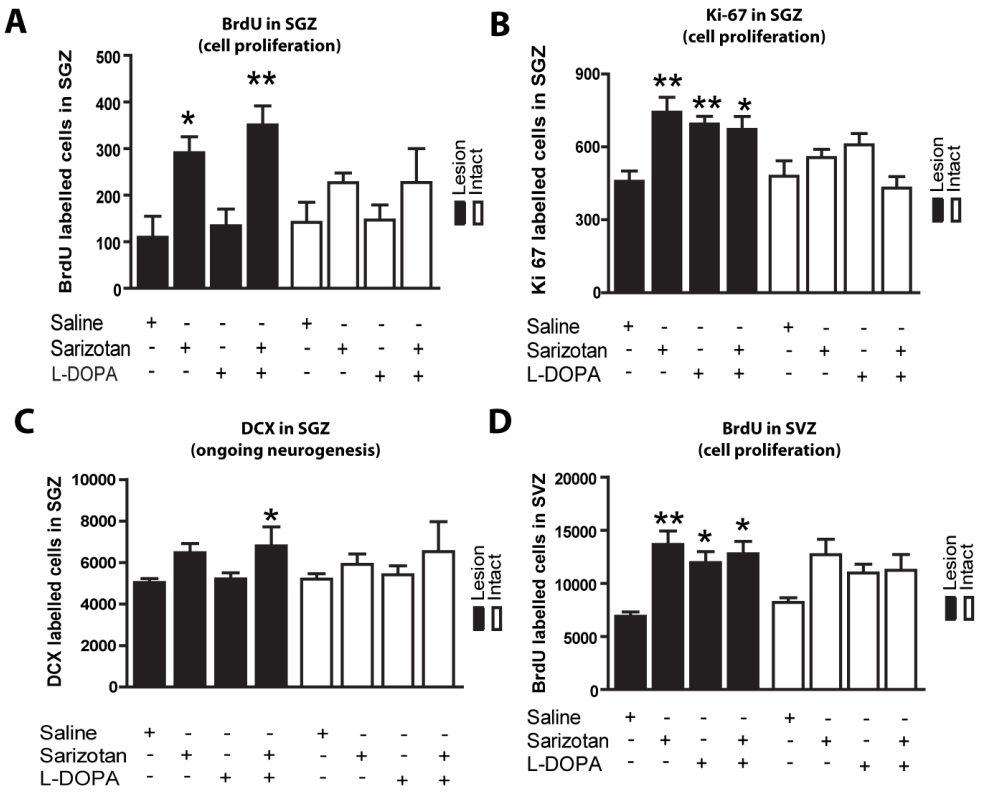

Figure 4 Sarizotan induces increases in aspects of neurogenesis in an animal lesion model **(A-C)** Histogram of data from the SGZ showing the quantification of Ki-67(A), BrdU (B) and DCX (C) positive cells with Ki-67 and BrdU both representing cell proliferation and DCX representing ongoing neurogenesis. Results indicate a significant increase in cell proliferation in the lesioned side with sarizotan alone or in combination with L-DOPA **(A,B)**. L-DOPA alone increased cell proliferation measured with Ki-67 but not BrdU **(B)**. Sarizotan in combination with L-DOPA also increased ongoing neurogenesis in the SGZ **(C)**. **(D)** Histogram of data from the SVZ with BrdU positive cells representing cell proliferation indicating that in the lesioned side sarizotan alone or in combination with L-DOPA increases cell proliferation as does L-DOPA alone. *p<0.05, **p<0.01 versus saline in the lesioned side.

Finally, decreased AN has been demonstrated to be associated with both depression and PD (Boldrini et al. 2009; Höglinger et al. 2004). These points thus motivated investigations into the possible effects of sarizotan on AN in an animal model of PD which are presented in paper IV.

The animal model employed in this study was the 6-0HDA lesion model in rats. In addition to sarizotan, rats were also treated with the anti-parkinsonian drug L-DOPA/benserazide either alone or in combination with sarizotan. To examine potential changes in AN in response to these treatments, lesioned and intact hemispheres were quantified for two aspects of AN including cell proliferation and ongoing neurogenesis. Both of these aspects were examined in the SGZ. However due to technical impossibilities, only cell proliferation was examined in the SVZ. To investigate cell proliferation in the SGZ and SVZ, BrdU-labeled cells were quantified in these regions (1 day, 4x 50mg/kg- 24 hrs. post injection). Results using this method were confirmed, in the SGZ, by quantification of cell proliferation using endogenous Ki-67 positive cells. Analysis of BrdU positive cells in SVZ of the differently treated groups revealed that in the lesioned hemisphere, sarizotan treated rats had significantly increased levels of proliferation as did L-DOPA/benserazide treated rats (Fig. 4D). Furthermore, combined treatment with Sarizotan and L-DOPA/benserazide induced increases although there was no additive effect of these drugs. In the intact hemisphere, these treatments induced similar though less robust increases which were insignificant upon

statistical analysis (Fig. 4D). In vehicle treated rats, no significant difference was found between hemispheres in baseline levels of cell proliferation despite a slight decrease on the lesioned side. In the SGZ, analysis of BrdU positive cells on the lesioned side revealed that sarizotan significantly increased levels of cell proliferation as did combined treatment with L-DOPA/benserazide (Fig. 4A). L-DOPA/benserazide alone however did not increase cell proliferation measured using BrdU. Results from Ki67 analysis in the SGZ confirmed these results with significant increases found with both sarizotan alone and in combination with L-DOPA/benserazide(Fig. 4B). Furthermore, Ki-67 analysis also found an increase in cell proliferation upon treatment with L-DOPA/benserazide alone. Ongoing neurogenesis was quantified using DCX and analysis of DCX positive cells in the SGZ revealed similar tendencies to proliferation data, with a trend to increase upon treatment with sarizotan. However, only a combination of sarizotan and L-DOPA/benserazide induced a significant increase (Fig. 4C). Results from the non-lesioned hemisphere displayed similar tendencies in both cell proliferation and ongoing neurogenesis though in comparison to vehicle, none of these differences were found to be significantly different. No significant differences were found between hemispheres in baseline levels of cell proliferation or ongoing neurogenesis in the SGZ.

As described in the introduction, the effects of dopamine on AN in the SVZ, is a matter of controversy. Results from several studies of lesion models have indicated increases in cell proliferation while equally as many have indicated decreases and furthermore a few studies have indicated no change (see table 1). In paper IV, the lack of change in baseline levels of cell proliferation between hemispheres is in finding with the latter of these studies describing no change. Numerous differing factors in these studies could affect the outcome and interpretation of the results including differences in strain and lesion method. However in close analysis of these studies (table 1), differences in strain or method do not seem to be the decisive factor. However, the elapsed time between the time of lesioning and the time of cell proliferation investigation appears to be an important factor as all the studies indicating an increase, seem to be around the same period (~14 days post lesion), a time point that it not present in the other studies. This time point is identical to another study, albeit in the hippocampus, which describes a transient increase in proliferation 14 days post lesion as further described in paper IV(Park & Enikolopov 2010). This coincidence indicates a prevalent mechanism in both the SGZ and SVZ which warrants further investigation. As described regarding the transient increase in the SGZ in paper IV, this mechanism could be speculated to involve compensatory increases in dopamine receptor subtypes as a result of abrupt dopamine depletion and further dynamics which may further alter cell proliferation.

Pharmacological manipulations of the dopamine system in paper IV further highlight the importance of dopaminergic regulation of AN. In paper IV, the increase in cell proliferation observed in the SVZ upon treatment with L-DOPA/benserazide is in agreement with previous findings in this region (Höglinger et al. 2004; O'Keeffe et al. 2009) and indicates that dopamine acts to stimulate cell proliferation in the adult SVZ. As with the lesioning studies, studies of dopaminergic involvement in AN using pharmacological tools also often present with conflicting results as described in the introduction and table 2. Even though the details of how dopamine regulates cell proliferation are unclear, one consensus of these results is that dopamine appears to be a

potent modulator of cell proliferation. Sarizotan, as indicated above also has affinity to D3 and D2 dopamine receptors. D2 receptors, as described above, have been demonstrated to be central to dopamine related increases in cell proliferation in the SVZ, particularly through their release of the neurotrophin, CNTF, from D2 expressing niche astrocytes (Yang et al. 2008). Niche astrocytes are also known to express the D3R (Kim et al. 2010) and D3R stimulation has been demonstrated to increase cell proliferation in the SVZ (Kim et al. 2010; VanKampen et al. 2004), indicating that D3 may also regulate the expression on CNTF and affect proliferation through this mechanism. Furthermore, stimulation of dopamine also regulates the release of EGF, another neurotrophin which is has been demonstrated to regulate cell proliferation (O'Keeffe et al. 2009). It is likely that the proliferative effects of sarizotan described in paper IV are at least partially mediated by its interaction with either or both of these receptors. Interestingly, these increases in proliferation were only observed to be significant in the lesioned side whereas, despite trends, there were no significant effects in the non-lesioned side. This difference is surprising considering there were no deficits in cell proliferation in either side where an actual deficit might otherwise facilitate a proliferative response upon pharmacological treatment. It could be speculated, that this difference is due to the fact that the dopamine denervation in the lesioned side leads to an up-regulation of dopamine receptors, inducing an increased sensitivity to dopamine modulating aspects of sarizotan and a subsequent increased proliferative response via the mechanisms described. Furthermore, the pharmacodynamics of dopaminergic aspects of sarizotan may be altered in the presence of endogenous dopamine, as has been previously suggested (Bartoszyk et al. 2004), in the non-lesioned side. This idea might further explain the fact that L-DOPA and sarizotan together were not observed to have additive effects. The proliferative effects of sarizotan observed could also be partially mediated via the 5-HT1A agonistic properties of this drug. Indeed the importance of serotonin stimulation in the maintenance of AN in both neurogenic niches has been demonstrated and furthermore, that this maintenance is most likely mediated via the 5HT1A receptor (Brezun & a Daszuta 1999; Banasr et al. 2004).

Lesion experiments in the SGZ have, similarly to that of the SVZ, also presented controversial results regarding the effects of dopamine denervation with both increases and decreases and no changes being described (see introduction). Results from the SGZ in paper IV thus demonstrating no differences in the lesioned and non-lesioned side are not outstanding and are furthermore similar to what was also observed in the SVZ in this paper. Data on the effects of L-DOPA demonstrated using BrdU indicate that there was no effect of this drug on cell proliferation, however using Ki-67, a significant effect was observed. This discrepancy could be the result of numerous factors including differences in representation of cell proliferation from each method, counting strategies using each antibody and human error. Although little has been published on the effects of L-DOPA in the SGZ, at least one previous publication described no effects of L-DOPA, similar to the BrdU data (Park & Enikolopov 2010). Furthermore, analysis using DCX to examine ongoing neurogenesis also indicated no effects of L-DOPA in the SGZ. The fact that chronic treatment with sarizotan had significant effects on cell proliferation in the lesioned side is interesting due to sarizotans' high affinity and agonist properties on the 5-HT1A receptor. Previous literature regarding effects of other 5-HT1A agonists also have indicated a potent effect on cell proliferation in the SGZ and furthermore that these agonists no longer have a

proliferative effect in 5-HT1A KO mice (Banasr et al. 2004; Santarelli et al. 2003). These data indicate that cell proliferative effects of sarizotan are at least partially mediated via this receptor. SSRI's are also known to increase cell proliferation, an effect which is dependent on 5-HT1A receptors (Santarelli et al. 2003). Therefore it could also be speculated that the mechanism of action through which sarizotan increases cell proliferation in the lesioned side is partially related to that of fluoxetine. Interestingly, 5-HT1A stimulation is also known to stimulate the release of S100B, as described in paper III (Whitaker-Azmitia et al. 1990). It would therefore also be interesting to know if the cell proliferative effects of sarizotan involve S100B (see fig 6). Despite effects on cell proliferation, results from ongoing neurogenesis using DCX in the lesioned side indicate that these effects of sarizotan were not robust enough to induce a significant increase, although co-treatment with L-DOPA was significant indicating that the dopaminergic affinity of sarizotan is also involved. Interestingly, the effects of sarizotan on cell proliferation in the SGZ were only significant in the lesioned side, whereas in the non-lesioned side, this effect, although present, was not robust enough to be significant. The reason for this difference is perhaps due to the affinity of sarizotan for dopamine receptors which may also affect its proliferative effects in the SGZ, similarly to the SVZ. As described in the introduction, dopamine appears to regulate AN in the SGZ, though the details of which and how different receptors regulate the different aspects is unclear. Therefore speculation on which dopamine receptors sarizotan is affecting are even more unclear than the SVZ, though the mechanism could be similar. However, the difference in effect on lesion in comparison to non-lesioned side indicates that dopaminergic aspects of sarizotan do modulate its effect on cell proliferation. Although cell survival was not examined in this study, results from the ongoing neurogenesis experiments demonstrating no significant increase in sarizotan alone on ongoing neurogenesis indicate that sarizotan did not have effects on these maturing cells. This indicates that the effects of sarizotan on AN are primarily on cell proliferation and do not affect cell survival.

Genetic and Pharmacological Modulation of D3 Receptor Signaling

Genetic ablation and pharmacological blockade of the D3 receptor increases hippocampal cell proliferation (Paper V)

To investigate possible changes in baseline levels AN in the D3R KO mice we compared different aspects of AN including cell proliferation, ongoing neurogenesis and cell survival in D3 KO and WT mice as presented in paper V. Half of these animals were treated with a preferential D3 antagonist, the results of which are presented and discussed in the section regarding pharmacological modulation of D3 signaling whereas presented here results solely describe results from investigation of the effects of the genetic modulation of D3 signaling. To examine cell proliferation in these mice we used Ki-67 as a proliferative marker and demonstrated that in comparison to WT mice, the D3 KO mice displayed a significantly increased level of cell proliferation which was an average 48 % more than WT mice (Fig. 5A). To further investigate AN we also looked at ongoing neurogenesis using DCX to quantify the number of immature neurons. This experiment revealed a highly significant increase in the number of immature neurons in the D3 KO mice with an average 72 % more than WT mice (Fig. 5B). Finally we looked at the potential changes in cell survival in D3 KO

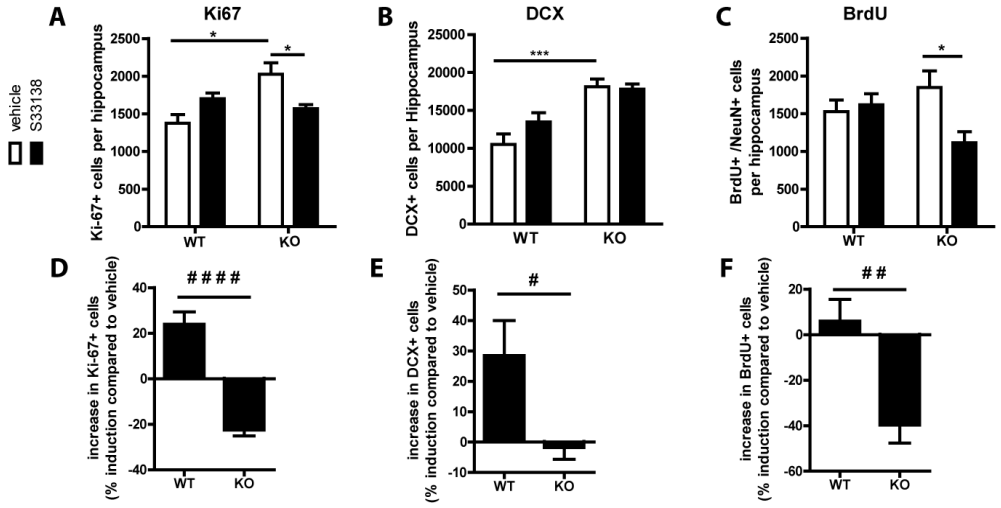

Figure 5 (A) Bar graph of the number of Ki-67(A), DCX (B) and BrdU (C) positive cells representing cell proliferation, ongoing neurogenesis and cell survival respectively in WT and D3R KO mice treated chronically with vehicle or S33138. **(A)** Results demonstrating a significant increase ($p<0.05$) in baseline levels of cell proliferation in the D3R KO mice and significant decrease ($p<0.05$) of proliferation upon treatment with S33138 in the D3R KO mice. **(B)** Results demonstrating a highly significant increase ($p<0.001$) in baseline levels of ongoing neurogenesis in the D3R KO mice. **(C)** Results demonstrating no significant difference in baseline levels of cell proliferation in the D3R KO mice or with treatment with S33138 in WT mice but significant decrease ($p<0.05$) of proliferation upon treatment with S33138 in the D3R KO mice. **(D-F)** Data presented as a percent of induction in comparison to vehicle reveals a highly significant difference between induction of cell proliferation **(D)**, ongoing neurogenesis **(E)** and cell survival **(F)** in the WT and KO mice. Two-way analysis of variance followed by Bonferroni's t-test * - $p<0.05$. #### - $p<0.0001$, ## - $p<0.01$, # - $p<0.05$; Students t-test.

mice using BrdU/NeuN co-expression as a marker for cell survival (3 week post injection). Results from these experiments revealed only slightly higher (18 %), although not significantly different, levels of cell survival in D3 KO mice in comparison to WT (Fig. 5C).. In summary, results from these experiments demonstrate that D3 KO mice have a robust increase in levels of cell proliferation and ongoing neurogenesis but no significant differences in cell survival.

In addition to examining the effects of genetic modulation of D3R signaling using D3R KO mice, D3R signaling was also examined in paper V via pharmacological modulation. In particular, the drug S33138, a preferential D3 vs. D_2 dopamine receptor antagonist, was used to chronically treat WT mice. To investigate non-D3 related effects, D3R KO mice were also treated with the drug. In parallel with the study above, effects of this drug on several aspects of AN were examined including cell proliferation, ongoing neurogenesis and BrdU as described. In the cell proliferation experiment, WT mice had a 24 % increase in comparison to vehicle which was however not significant (Fig. 5A,D). Effects on D3 KO mice revealed a significant interaction of this genotype with S33138 as, in opposition to the increase seen in the WT mice, S33138 treated mice had a significant 22 % decrease in comparison to vehicle (Fig. 5A,D). S33138 induced a 28 % increase in ongoing neurogenesis in WT mice but this increase was again found to be insignificant (Fig. 5B,E). This induction was no longer present in D3 KO mice (-2%). Finally, S33138 did not have a profound of an effect on survival with a 6 % induction in WT mice which was not significant (Fig. 5C,F). However, S33138 had a significant effect on treated D3 KO mice which had a 40% decrease in levels of cell survival. Overall, S33138 did not have any robust, significant effects in WT mice although despite this lack of significance, inductions

levels indicate that there was a slight effect of the drug. Interestingly these effects correlate well with baseline data from the KO mice discussed above. Particularly, the robust increase in baseline cell proliferation seen in the D3 KO mice is partially mirrored by use of a D3 antagonist in WT mice which also led to a mild increase - both represent a blockade of D3 signaling which resulted in an induction of cell proliferation. This trend was also repeated when examining ongoing neurogenesis in D3 KO mice and S33138 treated WT mice giving further support to a correlation. Effects of S33138 which are not specific to the D3R are prominent as exhibited by significant decreases seen in both cell proliferation and cell survival upon treatment with S33138 in D3 KO mice. Effects seen on these mice can largely be attributed to the D2 receptor to which S33138 most likely has a high affinity in the absence of the D3R. S33138 acting as an antagonist to D2 receptors thus appears to decrease cell proliferation and cell survival. This observation correlates well with several studies which indicate that in the SVZ as well as in the SGZ, D2 receptor stimulation increases cell proliferation (Höglinger et al. 2004; Yang et al. 2008). Overall, this experiment is an indication of a possible opposite nature of D3 vs. D2 receptors in the SGZ. On the one hand results with S33138 on WT mice, though not robust support the idea that D3Rs inhibit cell proliferation and subsequent aspects of AN in the SGZ. On the other hand results with S33138 in D3 KO mice further exemplify the complex nature of dopamine regulation on AN, demonstrating an opposite effect of probable D2 receptors, whose mechanism is to stimulate cell proliferation and subsequent aspects of AN in the SGZ.

The finding that genetic and pharmacological modulation of the D3R has direct consequences on cell proliferation correlates well with the consensus of previous publications that modulation of dopamine via different methods, using both *in vitro* and *in vivo* experiments, consistently alters specifically cell proliferation in both neurogenic niches rather than other aspects of AN (see Borta & Höglinger 2007 for review). It remains however unclear whether the observed effects on ongoing neurogenesis are also the direct result of a D3R modulation *per se* or an indirect result of an increased proliferative pool. Indeed, the robust increased level of proliferation observed would be expected to increase the number of immature neurons before they either undergo apoptosis or continue to develop into mature neurons. It is also possible that in addition to the mechanism through which proliferation is increased, D3Rs also regulate aspects of ongoing neurogenesis. For example it has been suggested that either decreasing the maturation rate or increasing survival of maturing neurons can both increase the levels of ongoing neurogenesis (Sairanen et al. 2005; Wang et al. 2008). Literature regarding dopaminergic effects on AN beyond cell proliferation indicate that dopamine primarily negatively modulates the activity of young neurons, particularly by inhibiting aspects of synaptic plasticity (Mu et al. 2011). This modulation could in theory alter the maturation and survival of immature neurons but this however remains to be determined. Therefore, the question as to whether or not there is a post-proliferative mechanism causing the observed increased level of ongoing neurogenesis in the D3R KO mice remains unanswered.

Knowledge regarding the actual expression of dopamine receptors in the SGZ, particularly the D3R, is a crucial factor in discerning the mechanism through which dopamine affects AN, however, this knowledge is currently very limited due, as discussed, to a lack of successful antibodies. However as described in the introduction,

data from the SVZ indicate the presence of the D3R in this niche, suggesting that it might also be found in the SGZ. This is supported by qPCR data indicating low levels of D3 expression (Mu et al. 2011). This data demonstrating D3R expression in the dentate gyrus correlates nicely with confocal microscopy images from D3R GFP mice found on GENSAT which show expression of D3Rs in cells which appear to be in the SGZ. The identity of these cells remains currently unknown although their location in the SGZ and amount of expression suggests that they are proliferating cells due to the fact that if a gene was expressed in differentiated maturing neurons it would be expected that there would be greater expression which was deeper into the granular layer. Furthermore, the morphology of these cells with few processes differs to what is seen in immature neurons which already have rather developed processes. Data from D3R expression in the SVZ demonstrating expression of D3R in transit amplifying progenitor cells in this niche supports the idea that the D3R positive cells in the SGZ are the SGZ equivalent, namely the ANPs (amplifying neural progenitors) (see fig 6). Although, it cannot be assumed that the case is identical to the SVZ, previous data regarding expression of other receptors indicate that expression of D3R might be similar. Another possibility is that these cells are QNPs (quiescent neural progenitors) which are less abundant than the ANPs and have furthermore been suggested to be activated upon neurodegeneration, fitting the idea of an inhibitory activity of D3 (Park & Enikolopov 2010) (fig 6).

If indeed, D3Rs are expressed in the ANP or QNP cells then our results demonstrating that a genetic ablation of the D3R results in an increase in cell proliferation would indicate that these receptors act directly to inhibit cell proliferation. Literature to date regarding dopaminergic control of hippocampal AN is as described limited and despite this, is conflicting as to whether dopaminergic control is stimulatory or inhibitory. The first paper to investigate dopaminergic control of AN in the SGZ used MPTP lesioning of dopaminergic projections and noticed a decrease in cell proliferation, indicating a stimulatory effect of dopamine (Höglinger et al. 2004). However, two subsequent papers demonstrate the opposite, that dopamine afferents have an inhibitory effect. One of these studies demonstrates that MPTP lesioning induces increases in both cell proliferation and ongoing neurogenesis (Peng et al. 2008). The other study also demonstrates that MPTP lesioning induces an increase in cell proliferation which was shown to be transient (Park & Enikolopov 2010). Several differing factors between these studies might explain the conflicting results including differences in the timing of post-lesion quantification, the concentration of MPTP used and the age of the animals. Furthermore, the results are not necessarily conflicting on all points as the first study, indicating a stimulatory effect of dopamine, did not investigate the time-point at which the last publications describe their observed inhibitory effect of dopamine (14 day post-lesion) (see paper IV for further description).

Recently it has also been described that these dopaminergic afferents produce other factors, as exemplified by the production of sonic hedgehog (SHH) by these neurons, which have an impact on aspects of AN (presentation SFN). Therefore it cannot be excluded that these conflicts in the literature are a result of factors other than dopamine which are ultimately affecting the neurogenic niches in lesioning experiments. The conflicts seen in these studies thus highlight potential shortcomings of lesioning experiments and demonstrate the need to also look at other methods with which to study dopamine modulation, using for example pharmacological tools and genetic models. Studies using pharmacological tools particularly agonists and antagonist, have

demonstrated the importance of D2 receptors found on niche astrocytes in the SVZ and SGZ which indirectly stimulate cell proliferation via the release of CNTF (Yang et al. 2008). Although there is no data on pharmacological D3 modulation in the SGZ, several studies on the SVZ indicate that D3R stimulation in this area stimulates cell proliferation (Coronas et al. 2004; Kim et al. 2010). However this is contrary to what was found in other studies where no change was found upon stimulation of the D3R and in D3 KO mice which displayed no change in the cell proliferation in the SVZ(Baker et al. 2005). This discrepancy is perhaps partially resolved by mechanisms discussed below regarding distribution of D3 in different populations. In apparent contrast to these studies, in the SGZ, we observe that a lack of D3Rs results in an increase in cell proliferation indicating that D3 inhibit stimulation. A careful examination of the literature though indicates a likely explanation involving D3Rs which as mentioned are also expressed on niche astrocytes in the SVZ, in addition to transit amplifying progenitors (Kim et al. 2010) (see fig. 6). This study implies that it is expression on the progenitors which is involved in the D3 mediated stimulation of cell proliferation. However, data from Yang et al. indicate that this is highly unlikely due to the fact that CNTF KO mice have a reduced cell proliferation which is not further reduced as a result of dopaminergic lesions (Yang et al. 2008). This experiment demonstrates that dopamine stimulation of cell proliferation in the SVZ is dependent on CNTF expression found in niche astrocytes indicating that effects of D3R stimulation in the SVZ are via these cells and not directly via the proliferating cells themselves. Therefore the actual function in the SVZ of D3Rs found on transit amplifying progenitors remains unknown and could possibly have an inhibitory function, similar to what we describe in the SGZ. Despite the fact that D3Rs are known to be expressed in niche astrocytes in the SVZ, it is not known if they are also expressed in niche astrocytes in the SGZ. D3R GFP expressing cells visualized in confocal images from GENSAT do not however resemble astrocytes with regards to size and morphology, indicating that SGZ niche astrocytes, in contrast to SVZ astrocytes, do not express D3Rs. Therefore it could be speculated that the overall effects of dopamine might also be differentially regulated in the SGZ in comparison to the SVZ. In summary the increased cell proliferation in the D3R KO mice, together with the knowledge of D3R expression in the SGZ might prove to be valuable clues to the regulation of dopamine in this neurogenic niche. Future experiments will however have to determine the precise relevance of these receptors to definitively determine their role in AN.

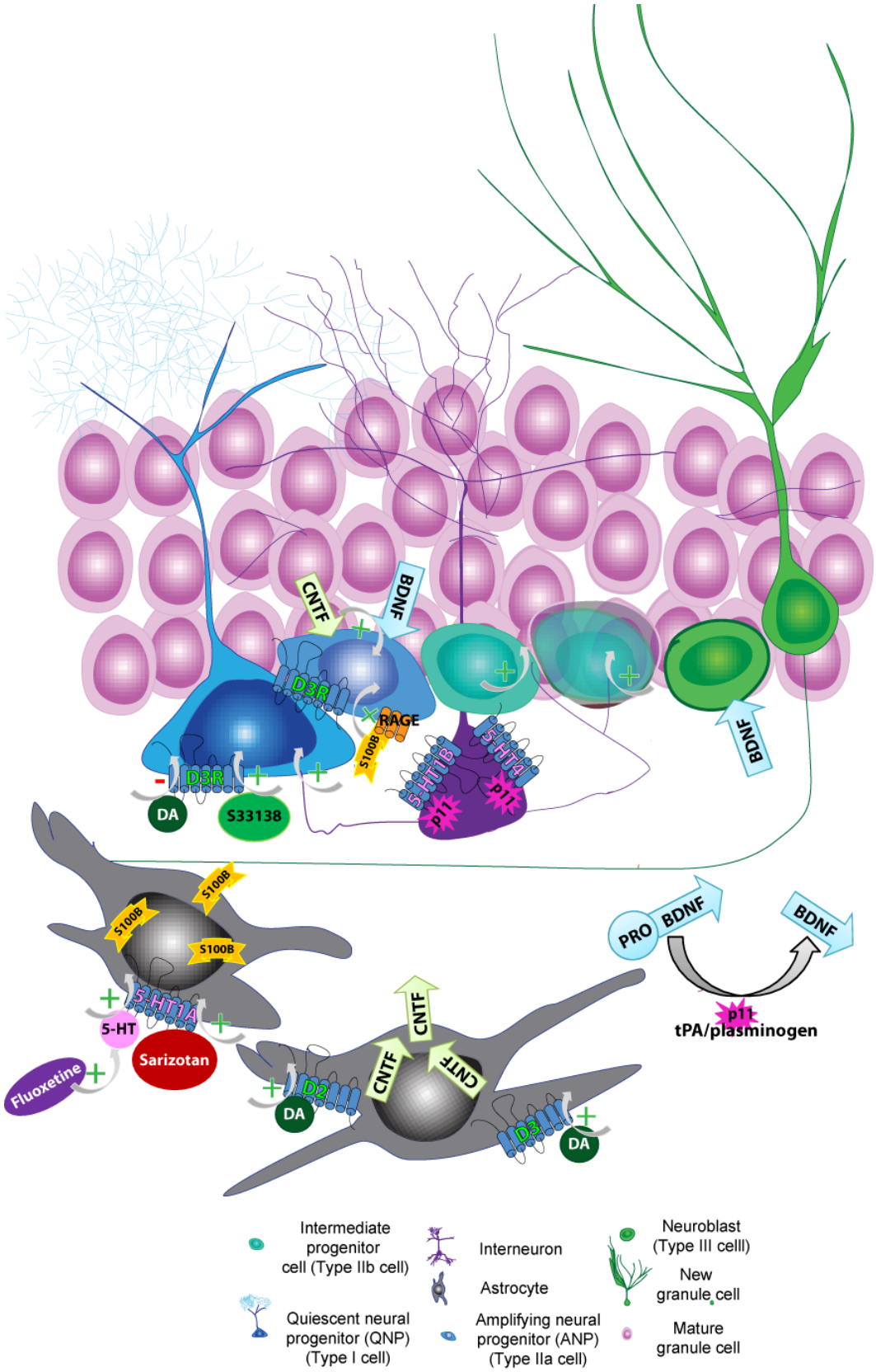

Figure 6 Summary figure of the speculative mechanisms described in papers I-V. In response to fluoxetine p11 is increased in interneurons where it potentially indirectly modulates the proliferation of ANPs and development of neuroblasts. Furthermore, p11 may interact with tPA/plasminogen, increasing cleavage of proBDNF to BDNF thus increasing BDNF which then affects aspects of AN. Fluoxetine also increases 5-HT which may activate 5HT-1ARs on astrocytes causing them to release S100B which then activates rage found on ANPs causing them to proliferate. D3Rs found on QNPs and/or ANPs inhibit cell proliferation when activated. D3R KO mice have an ablation of these receptors and divide uninhibitedly . S33138 antagonizes this receptor causing an increase in proliferation of either QNPs and/or ANPs. D3 and D2Rs found potentially on niche astrocytes release CNTF upon stimulation which modulates aspects of AN. Sarizotan acts on 5-HT1ARs and potentially D2 and D3Rs, thus engaging the mechanisms described above. Graphics used from Ming and Song 2011. GPCR from Niko Stroth.

FUNCTIONAL AND TRANSLATIONAL RELEVANCE FOR NEUROPSYCHIATRIC DISORDERS

Relevance of modulation of p11 for depression (Papers I &II)

As described in the introduction, previous studies of p11 have demonstrated a correlation to depressive-like states in animal models as well as a correlation in humans and in addition p11 is associated with antidepressant treatment as it is increased by treatment with antidepressants (Svenningsson et al. 2006). In particular it has been demonstrated that p11 KO mice have a depressive-like phenotype using the using the tail suspension test (TST) and displayed an increased anxiety-like phenotype when examining thigmotaxis. These results were shown to have clinical relevance as it was shown that levels of p11 mRNA were decreased in post-mortem samples of brain tissue from depressed individuals. Results from studies of AN presented in Paper I mice indicate that p11 KO mice also have an attenuated neurogenic response to fluoxetine. Previous studies have indicated that AN is necessary for certain behavioral responses to antidepressants, in particular the behavioral response in novelty suppressed feeding (NSF) test (Santarelli et al. 2003). The NSF test, as described is interesting as it is one of the few behavioral tests which mimic the time lag seen in the clinic for antidepressant efficacy in that only chronic treatment has significant effects in this test. Furthermore, this test is known to be neurogenesis dependent whereas other experimental behavioral paradigms, for example the TST, are neurogenesis independent (David et al. 2009). To further test the functional consequences of the attenuated neurogenic response to fluoxetine, our collaborators tested p11 KO mice using the NSF test, the results of which are presented in paper I. Comparison with vehicle treated mice revealed no differences between WT and KO mice in the novelty suppressed feeding test suggesting a normal baseline phenotype in the KO mice using this paradigm. WT mice chronically treated with fluoxetine displayed an expected decreased latency to feed mice in comparison to untreated mice - a measure of antidepressant efficacy in this test. However, in the p11 KO mice the latency to feed was statistically similar in vehicle and fluoxetine treated mice, indicating that in p11 KO mice, fluoxetine does not affect the behavioral response using this paradigm. This result suggests that chronic fluoxetine treatment does not have antidepressant effects in p11 KO mice. A lack of neurogenic effect of fluoxetine in p11 KO mice in addition to a lack of anti-depressive effect in p11 KO mice correlate strongly with the previously mentioned study demonstrating that AN is necessary for the antidepressant effect of fluoxetine (Santarelli et al., 2003) and indicate that p11 may be necessary for both the neurogenic and behavioral effects of fluoxetine. These findings further support to the role of p11 in depression. A potential translation relevance of these findings is the indication that p11 may be crucial in being able to respond to both the behavioral as well as neurogenic effects of antidepressants. This role expands the translational relevance of p11 not only as a potential antidepressant target, though which to modulate depressive states, but also as a potential diagnostic target in predicting response to various antidepressants.

As described, further analysis of p11 distribution in paper I indicate that expression of p11 in interneurons which also express 5-HT1B and 5-HT4 receptors near the SGZ of the hippocampus may represent a possible mechanism though which p11 can modulate the neurogenic process, ultimately having behavioral effects and potential translational

relevance. Depression is a complex disorder involving many areas of the brain beyond the dentate gyrus and beyond AN. In paper II, we further investigated the distribution of p11 cells which co-express 5-HT1B and 5-HT4 receptors in other areas of the hippocampus and the brain in an attempt to identify areas in which co-expression of p11 with these receptors may have relevance for depression. In addition to expression in these interneurons as described in paper I, p11 was also found to be co-expressed with 5-HT1B and 5-HT4 receptors in the majority of cells throughout the dentate gyrus, although both 5-HT1BR and p11 were expressed to a lesser extent than these interneurons. In addition, p11 was also co-expressed at moderate levels with these receptors throughout the CA3 and CA1 in the pyramidal cell layer in the hippocampus, although several cells displayed high expression (see figs. 3 and 4. Paper II). The function of p11 in these regions and potential relevance to disease has not yet been examined. Among other areas beyond the hippocampus, p11 was also found to be co-expressed with these receptors in several layers of the cingulate cortex (see fig. 1 Paper II). p11 was expressed throughout layers I-V of the cingulate cortex where it was mainly expressed with 5-HT1BR, particularly in layer V, where numerous double-labeled cells were found though there was a subpopulation of cells expressing triple labeled with p11 and both receptors. Colleagues of ours are currently working a study demonstrating the relevance of expression in specific p11 positive cells in layer V (Schmidt et al., 2011-conference poster). In particular, data from this study demonstrates that specific genetic deletion of p11 in these cells ablates the behavioral response to SSRIs indicating a translational relevance of p11 expression in this region. Another region in which p11 was expressed was in the caudate putamen, where it was particularly highly expressed in both parvalbumin positive and choline acetyl-tranferase, most likely representing GABAergic and cholinergic interneuron, where p11 was co-expressed with 5-HT1B but not 5-HT 4. Expression in this region has particular translational relevance to PD as p11 expressed here has been demonstrated to be involved in the response to L-DOPA (Zhang et al. 2008)**.** Although we did not investigate specific receptor distribution in the nucleus accumbens, a recent study has demonstrated that decreases of p11 in specifically this area induces a depressive-like phenotype which can be rescued by local-gene delivery in global-p11 KO mice (Alexander et al. 2010). This study has particularly interesting ramifications for treatment of depression using gene-therapy techniques and indicates a further translational relevance of p11. Finally, p11 expression was also found to be co-expressed with the 5-HT1BR in the purkinje cells of the cerebellum. The function of p11 in this region and potential relevance to disease remains to be examined. Thus, our results regarding the effect of p11 modulation of neurogenic and behavioral response to fluoxetine as well as the expression of p11 with 5-HT1B and 5-HT4 receptors in other regions represent findings which have potential translational relevance for disease, particularly depression.

Relevance of S100B for depression (Paper III)

Our studies of AN in S100B TG mice in paper III revealed an increase in baseline cell proliferation which did not translate into changes in either ongoing neurogenesis or cell survival and furthermore, neurogenic induction in aspects of AN upon treatment with fluoxetine were similar to WT mice. We further tested the functional relevance of these

changes using the novelty induced hypophagia (NIH) test- a modified version of the NSF which was described in the previous section. Similarly, to the NSF, the NIH test measures the effects of chronic but not acute antidepressant treatment and therefore reflects neurogenesis dependent behavioral effects in certain strains of mice (Gur et al. 2007; David et al. 2009). Testing of S100B TG mice in this paradigm revealed a similar behavioral response to fluoxetine in comparison to WT mice which according to literature (Santarelli et al. 2003) indicates that neurogenesis would be similar in both genotypes which indeed was the case in paper III. Furthermore, there were no significant baseline differences in WT and TG treated with vehicle in this paradigm in that vehicle treated WT and TG mice displayed similar behavioral responses. This result was also somewhat expected with respect to the neurogenesis data demonstrating only a change in cell proliferation and not total neurogenesis. On the other hand if total neurogenesis was increased in TG mice one might expect a behavioral response similar to animals treated with antidepressants. Despite these results demonstrating a lack of effect of S100B on this behavioral paradigm, S100B maintains translational potential. The increase in baseline levels of cell proliferation seen in the TG mice indicates that S100B may be of relevance during times of decreased cell proliferation- for example during stress, a known depressor of cell proliferation (Gould et al. 1997). It could be speculated that in these situations, S100B may be of critical relevance through it effect on cell proliferation to regain baseline levels of neurogenesis. Although it remains to be confirmed, this mechanism may therefore have translational significance in situations in which changes brought about by stress may require additional therapy, for example during depression. Our data however report a similar level of anxiety in S100B TG and WT mice suggesting that S100B does not affect anxiety levels in non-stressed conditions. Interestingly, several studies demonstrate increases in serum levels in depressed patients (see introduction) which are potentially the result of compensatory mechanisms, such as the one described. In particular, one study demonstrated that depressed patients with increased levels of S100B upon admission had a better response to antidepressant treatment (Arolt et al. 2003). A possible translational correlation of this human finding to our animal studies is the finding that the S100BTG mice displayed an increased response to fluoxetine in the tail suspension test. Thus it appears that although the translational relevance of modulation of S100B are not fully explored or understood, it remains an interesting avenue for future developments.

Relevance of treatment with Sarizotan (Paper IV)

As described, one of the proposed clinical applications of sarizotan has been to decrease the extra-pyramidal side effects of L-dopa treatment (Bibbiani et al. 2001). Our experiments, presented in paper IV attempted to examine further potential applications of this drug, specifically its application to modulate neurogenesis and depressive-like behavior. The combination of having anti-dyskinetic properties as well as antidepressive properties would have translational relevance to the sub-population of PD patients who experience depression. Results from these experiments as described demonstrated the ability of sarizotan to increase cell proliferation in both neurogenic niches. In addition to this, functional studies of behavioral consequences of sarizotan treatment were performed by a collaborator, and indicated antidepressant like properties using the forced swim test (FST) but no effect on anxiety like-behaviors,

measured through thigmotaxis and corner time. In addition our results recapitulated the anti-dyskinetic properties of sarizotan, as evaluated using measurements of abnormal involuntary movements (AIMS). These results indicate that sarizotan may be of significant clinical interest due both to its known antidyskinetic properties but also to potential antidepressant activity and therefore paper IV may have particular relevance for those PD patients who experience depression.

Relevance of D3 receptor modulation (Paper V)

In paper V we demonstrated endogenously increased baseline levels of cell proliferation and ongoing neurogenesis in D3R KO mice compared with WT mice, although no increase in levels of mature neurons was observed. Although we did not conduct any functional behavioral analysis in these mice, this lack of increase of mature neurons would indicate, according to literature (i.e. David et al., 2009), that there would be no baseline difference in neurogenesis dependent paradigms associated with depression such as the NSF or NIH tests described in preceding sections. In agreement with this, behavioral testing of D3R KO mice in previous publications have in one study demonstrated baseline levels of locomotion, anxiety and depressive- like behavior in these mice to be comparable with WT mice (Chourbaji et al. 2008). However, a subsequent publication revealed an increased cognitive performance in D3R KO mice measured using the passive avoidance task (Micale et al. 2010). Although increased cell proliferation and ongoing neurogenesis have no established effects on behavior *per se*, it could be deducted from these publications that changes in these aspects of AN do not affect locomotion, anxiety and depressive- like behavior under normal physiological conditions. However, as passive avoidance is known to be a hippocampal dependent task, increases in cell proliferation and ongoing neurogenesis in this region may be involved in improving cognition through this mechanism, which potentially may become even more pronounced during pathological conditions, for example in schizophrenia. Interestingly, in paper V we also saw an inductive response on cell proliferation upon chronic treatment with the D3 preferential antagonist S33138. S33138 has been developed as a potential antipsychotic which has proven pharmacological activity in animal models of schizophrenia (Millan et al. 2008). Therefore, effects of this drug on cell proliferation and ongoing neurogenesis may have potential cognitive enhancing effects for this drug. The ramifications of this could have translational benefits for patients of schizophrenia who often experience cognitive impairments- an aspect of the pathology of which is largely untreated with currently available antipsychotics. Interestingly, the potential translational relevance of the D3R to depression is highlighted in a study demonstrating that D3R KO mice have an increased sensitivity to several types of antidepressants measured using FST. Although it is unclear about how changes in AN might affect the behavioral response to antidepressants, a potential connection is worth investigating. Furthermore, potential modulation of the aspects of AN via the D3R may be potentiated in pathological scenarios during for example extreme stress or depressive like states and indicates that targeting this receptors may have translational relevance for treatment of these disorders. Thus, the effects of D3 modulation on AN presented in paper V highlight the D3R as an interesting target in which to modulate AN, potentially having clinical relevance for neuropsychiatric disorders affected by changes in AN.

CONCLUDING REMARKS

Petrik et al. recently coined the analogy of the hypothesis of depression as a scaffolding for trying to reconstruct the building- what the actual disorder is (Petrik et al. 2011). A portion of both proponents and opponents in the scientific community saw the theory of neurogenesis in depression as a scaffolding which excludes other hypotheses and can support itself- in essence that AN was the answer to depression. However, aspects of the original theory, particularly, the idea that decreased neurogenesis results in a depressive phenotype have been more or less disproven while other aspects suggesting that neurogenesis is required for antidepressants to alleviate depression are still challenged. A summary of studies done so far indicate that a disorder of AN does not explain depression, but they also indicate that it is not irrelevant either. Thus using the analogy, the theory of neurogenesis in depression as a scaffolding within itself does not appear to support the actual shape of the building (the disease). The present reality indicates rather that neurogenesis is part of a much larger scaffolding in which aspects thought to be important to the etiology of depression, represented by different theories such as those of monoamines and stress, combine to create a much more complex scaffolding. This broader view of neurogenesis reveals certain pitfalls which were perhaps made with the original ideas and suggest that scientists adopt a more integrative view of AN as has been done recently with regards to neurogenesis, stress and the endocrine system(a Surget et al. 2011; Anacker et al. 2011; Snyder et al. 2011). These studies present results suggesting that AN acts to regulate stress and therefore indicates less pathological relevance for neurogenic and behavioral outcomes in unstressed animals of which has been the focus of the majority of studies (see table 1 and 2 Petrik et al. 2011).

The numerous different ways in which neurogenesis can be modulated is accordingly indicative of the numerous ways in which AN can be defective and therefore differential methods may be of benefit for diseases with different pathophysiologies. Studies presented in this thesis have via the use of genetic manipulation as well as pharmacological compounds highlighted specific proteins and pharmacological targets which modulate aspects of neurogenesis. These studies have therefore brought potentially valuable information about ways in which AN could be beneficially modified under pathophysiological conditions in which these factors may be defective. Despite potential challenges with suitable animal models on which to test this information, hopefully future studies will be able to overcome these obstacles and use this information to ultimately develop more effective treatments for neuropsychiatric disorders.

CONCLUSIONS

1. p11 is involved in the neurogenic and behavioral response to fluoxetine. In response to chronic treatment with fluoxetine, p11 KO mice lack a neurogenic response with regards to cell proliferation, ongoing neurogenesis and cell survival. This lack of neurogenic response was also reflected in behavior as p11 KO mice also lacked a behavioral response to fluoxetine using the NSF test. These results indicate that p11 is important for the antidepressant effects of fluoxetine.

2. p11 is co-expressed with 5-HT4 and 5-HT1B receptors in different regions associated with depression including several subregions of the hippocampus, cortex and caudate putamen. In the hippocampus, p11 was co-expressed in different types of interneurons which also expressed 5-HT4 and 5-HT1B receptors proximate to the dentate gyrus indicating a likely mechanism through which p11 affects aspects of neurogenesis.

3. S100B has the potential to modulate cell proliferation but not ongoing neurogenesis or cell survival. Mice overexpressing S100B have increased baseline levels of cell proliferation, though normal levels of ongoing neurogenesis and cell survival. These mice also appear to have a normal neurogenic response to fluoxetine which was reflected in a normal behavioral response to fluoxetine using the NIH test. These results indicate that targeting S100B may be of benefit in pathologies in which cell proliferation is decreased.

4. In an animal model of Parkinson's disease, sarizotan has antidepressant-like properties. Chronic treatment with serotonergic/dopaminergic drug sarizotan increased cell proliferation in the experimentally dopamine-lesioned side of both the SVZ and SGZ. Combined treatment with L-DOPA increased ongoing neurogenesis in the SGZ. Furthermore, sarizotan had an antidepressant-like effect in the forced swim test while improving dyskinesia. These results indicate that sarizotan may have useful clinical applications in co-morbid Parkinsonism and depression.

5. D3 receptors inhibit hippocampal cell proliferation. D3 receptor KO mice have robustly increased baseline levels of cell proliferation and ongoing neurogenesis. In correspondence to gene deletion, pharmacological blockade with the preferential D3 antagonist S33138 increased levels of cell proliferation. Collectively, these results indicate that cell proliferation can be modulated by targeting the D3 receptor and be of potential clinical relevance for neuropsychiatric disorders affected by changes in adult neurogenesis.

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