

# Brain Regeneration

*in vitro* and *in vivo* studies of exercise-related effects on brain plasticity

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2008

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*Cecilia Bull*

*Skandia Tryckeriet*

*Göteborg 2008*

*ISBN: 978-91-628-7483-4*

*Cover illustration by Cecilia Bull & Andrew Naylor*

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**ABSTRACT**

Neural stem and progenitor cells in the central nervous system provide a source of new neurons, astrocytes, and oligodendrocytes during development, as well as during adulthood. Germinal regions of the adult brain, such as the hippocampus, subventricular zone, and the subcallosal zone, are of great interest, because they provide the possibility for enhancing brain plasticity or contributing to endogenous cell replacement after injury or disease.

Voluntary exercise has been recently shown to robustly induce cellular and structural plasticity, thereby contributing to overall brain health. This thesis focuses on exercise-related effects on cell genesis of neurons and oligodendrocytes *in vitro* and *in vivo*. In Paper I, we demonstrated that exercise-induced, endogenously released opioid peptide  $\beta$ -endorphin enhanced oligodendrogenesis in adult hippocampal progenitors *in vitro*. Adult hippocampal progenitors were further used as a model system to study the signaling pathways that lead to  $\beta$ -endorphin-induced oligodendrogenesis. Results revealed a requirement for the helix-loop-helix transcriptional regulator "Inhibitor of Differentiation" (Id)-1 in opioid-induced oligodendrogenesis, and concomitant decreased expression of the proneural transcriptional activator Mash-1.

In Paper II, we study the effects of voluntary exercise during adulthood on neurogenesis and behavior, subsequent to irradiation in the young mouse brain. The immature brain is extremely vulnerable to irradiation, including long-lasting detrimental effects to hippocampal neurogenesis and behavior. The brains of young mice were irradiated, and the acute effects of irradiation were measured, as well as the effects of voluntary running on hippocampal neurogenesis three months after irradiation. Voluntary exercise following irradiation restored the hippocampal stem cell pool and increased neurogenesis. Additionally, voluntary exercise ameliorated irradiation-induced alterations in behavior. Moreover, the orientation of immature neurons in the dentate gyrus of the hippocampus was perturbed after irradiation; however, voluntary exercise restored the proper orientation.

In Paper III, we proceeded to investigate potential effects of voluntary exercise on oligodendrogenesis. By irradiating the brains of young mice, we have demonstrated an efficient reduction in the total number of Olig2-positive cells, which are considered to be mainly oligodendroglial cells, of the corpus callosum without affecting the number of newborn glial progenitor cells. We determined that, with time, the irradiation effects on the number of Olig2-positive cells in the corpus callosum was reduced, probably due to an overproduction of oligodendrocytes during the juvenile stage. Voluntary running had no effect on cell survival, oligodendrogenesis or myelin density in the corpus callosum. These results suggest that a neurogenic niche, such as the subgranular zone of the hippocampus, might be more responsive to exercise-induced signals regulating cell genesis.

In conclusion, this thesis demonstrates the usefulness of physical exercise for functional and structural brain recovery, with special emphasis on insults to the juvenile brain. In addition, these results highlight the capacity of the adult brain to regenerate through activation of endogenous neural progenitors and stem cells.

Keywords: brain, neural progenitor cells, stem cells, hippocampus, corpus callosum, rat, mouse, regeneration, neurogenesis, oligodendrogenesis, irradiation, exercise

## POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Vi har en fantastisk förmåga att motstå det slitage som det innebär att leva – varje dag förnyas celler i vår kropp, allt efter behov. Det är stamcellerna och deras dotterceller, progenitorcellerna, som sköter denna förnyelse. Även hjärnan har områden där nya celler produceras som kan ersätta celler som skadats eller dött, vare sig det skett som ett led i en naturlig process eller vid sjukdom. Den relativt nya kunskapen om dessa hjärnans stamceller har revolutionerat möjligheterna att utveckla nya strategier för att behandla olika neurologiska tillstånd. Transplantation av stam- och progenitorceller, eller utvecklande av nya mediciner som påverkar dessa, är två alternativ som det forskas på. Men hjärnan kan också reagera starkt på enklare, yttre stimuli. Ett sådant stimulus som har visat sig ha enorm effekt på hjärnans hälsa är fysisk aktivitet. Denna avhandling baseras på tre artiklar som alla på ett eller annat sätt rör fysisk aktivitet och dess effekter på nybildningen av celler från stam- och progenitorceller. I den första artikeln (Paper I) har vi studerat hur  $\beta$ -endorfin, en av produkterna av fysisk aktivitet, påverkar stam- och progenitorceller tagna från rått hippocampus och odlade i kultur. Vi fann att  $\beta$ -endorfin stimulerade dessa celler, som vanligtvis helst blir neuron, att bli oligodendrocyter, en sorts gliaceller som är viktiga för bildandet av myelin i hjärnan. Vi fann också att detta skedde genom aktivering av Id-1, ett protein som påverkar vilken celltyp en omogen cell slutligen blir.

I de två andra artiklarna har vi tittat på hur fysisk aktivitet påverkar nybildningen av både neuron (Paper II) och oligodendrocyter (Paper III) efter strålning av den omogna hjärnan. Strålning, som används vid olika cancerbehandlingar, orsakar nämligen utbredd celdöd hos stam- och progenitorceller. Detta syns inte bara i mikroskopet utan orsakar allvarliga biverkningar hos patienter i form av nedsatt kognition och minnesproblem, särskilt om man genomgått en behandling som barn då vi har extra många och känsliga stamceller. I den första av dessa två artiklar visar vi att möss, som strålats tidigt i livet, kan återfå sitt naturliga beteende efter strålning om de får springa i hjul. Vi visar också att den av strålning minskade stamcellspopulationen kan återställas av fysisk aktivitet, och att neurogenesen ökar av fysisk aktivitet efter strålning. Vi visar också att nervcellernas integrering i hippocampus, ett område rikt på stamceller och som är viktigt för minne och inlärning, förstörs vid strålning men kan återställas av fysisk aktivitet.

I den tredje och sista artikeln har vi tittat på hur fysisk aktivitet efter strålning påverkar nybildningen av celler i corpus callosum, ett område där det normalt sett finns en hög produktion av oligodendrocyter i den omogna hjärnan. Vi fann att trots att en mild dos strålning orsakade en stor förlust av gliaceller, som resulterade i ett långvarigt underskott, påverkades varken själva nybildningen av dessa celler eller mängden myelin negativt av strålningen. Nuvarande data från våra försök pekar också på att fysisk aktivitet i vuxen ålder inte nämnvärt ökar nybildningen av oligodendrocyter eller mängden myelin i corpus callosum, till skillnad från de tydliga effekterna av fysisk aktivitet på nervceller i hippocampus.

## LIST OF ORIGINAL PAPERS

This thesis is based on the following papers:

- I. **Persson A.I.\* , Bull C.\* and Eriksson P. S.** Requirement of Id-1 in opioid-induced oligodendrogenesis in cultures of adult hippocampal progenitors. *Eur. J. Neurosci.* 23, 2277-2288 (2006).
- II. **Naylor S. A.\* , Bull C.\* , Nilsson M., Zhu C., Björk-Eriksson T., Eriksson P. S., Blomgren K., and Kuhn H. G.** Voluntary running rescues adult hippocampal neurogenesis after irradiation of the young mouse brain. *Submitted for re-revision to Proceedings of the National Academy of Science, (PNAS).*
- III. **Bull C., Naylor A., Lindahl V., Grandér R., Alborn A. Persson A. I., Björk-Eriksson T., Blomgren K., and Cooper-Kuhn C.** Effects of physical activity on oligodendrocytes in the corpus callosum after irradiation of the postnatal mouse brain.

\*these authors contributed equally

Additional papers of relevance not included in the thesis:

**Persson A.I., Thorlin T., Bull C. and Eriksson P.S.** Opioid-Induced Proliferation through the MAPK Pathway in Cultures of Adult Hippocampal Progenitors. *Mol. Cell. Neurosci.* 23, 360-372 (2003).

**Persson A.I., Thorlin T., Bull C., Zarnegar P., Ekman R., Terenius L. and Eriksson P.S.** Mu- and delta-opioid receptor antagonists decrease proliferation and increase neurogenesis in cultures of rat adult hippocampal progenitors. *Eur. J. Neurosci.* 17, 1159-1172 (2003).

**Åberg N. D.\* , Johansson U.E.\* , Åberg M.A.I., Hellström N.A.K., Lind J., Bull C, Isgaard J., Anderson M.F., Oscarsson J., and Eriksson P.S.** Peripheral infusion of insulin-like growth factor-I increases the number of newborn oligodendrocytes in the cerebral cortex of adult hypophysectomized rats. *Endocrinology.* 148, 3765-3772 (2007).

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## LIST OF ABBREVIATIONS

NSC = Neural stem cell  
CNS = Central nervous system  
VZ = Ventricular zone  
RA = Retinoic acid  
Pax = Paired box  
Wnt = Wingless type  
FGF = Fibroblast growth factor  
BMP = Bone morphogenic protein  
Shh = Sonic hedgehog  
EGF = Epidermal growth factor  
SVZ = Subventricular zone  
Mash = Mammalian achaete-scute  
Olig = Oligodendrocyte transcription factor  
bHLH = Basic helix-loop-helix  
HLH = Helix-loop-helix  
Hes = Hairy and enhancer of split  
Id = Inhibitor of differentiation  
NG = Neuron glia  
PDGF = Platelet derived growth factor  
OPC = Oligodendrocyte progenitor cell  
APC = Adenomatous polyposis coli  
MBP = Myelin basic protein  
GCL = Granule cell layer  
SGZ = Subgranular zone  
Sox = sex determining region of Y-chromosome-related high motility group box  
GFAP = Glial fibrillary acidic protein  
BLBP = Brain lipid binding protein  
PSA-NCAM = Polysylated neural adhesion molecule  
DCX = Doublecortin  
NeuN = Neuronal nuclei  
AHP = Adult hippocampal progenitor  
IGF= Insulin-like growth factor  
SCZ = Subcallosal zone  
ECM = Extracellular matrix  
GABA = Gamma-aminobutyric acid  
VEGF = Vascular endothelial growth factor  
LTP = Long-term potentiation  
POMC = Pro-opiomelanocortin  
ACTH = Adrenocorticotrophic hormone  
MAPK = Mitogen-associated protein kinase  
BDNF = Brain-derived neurotrophic factor  
GST pi = Glutathione S-transferase pi  
Egr-1 = Early growth response  
ERK = Extracellular signal-regulated kinase  
HPA = hypothalamic-pituitary-adrenal  
BrdU = Bromodeoxyuridine  
Gy = Gray

*"Nature appears to have endowed stem cells with many of the properties that we all seek, eternal youth and a whiff of immortality."*

P. Balaram 2001

## INTRODUCTION

### History of stem cell science

Without too much thought, we trust our bodies to supply us with new "spare parts" throughout our daily lives. Even our birthday suit is renewed an impressive number of times throughout life, although it may have a more worn look over the years. We require high degree of maintenance, and it is the stem cells that are doing this job.

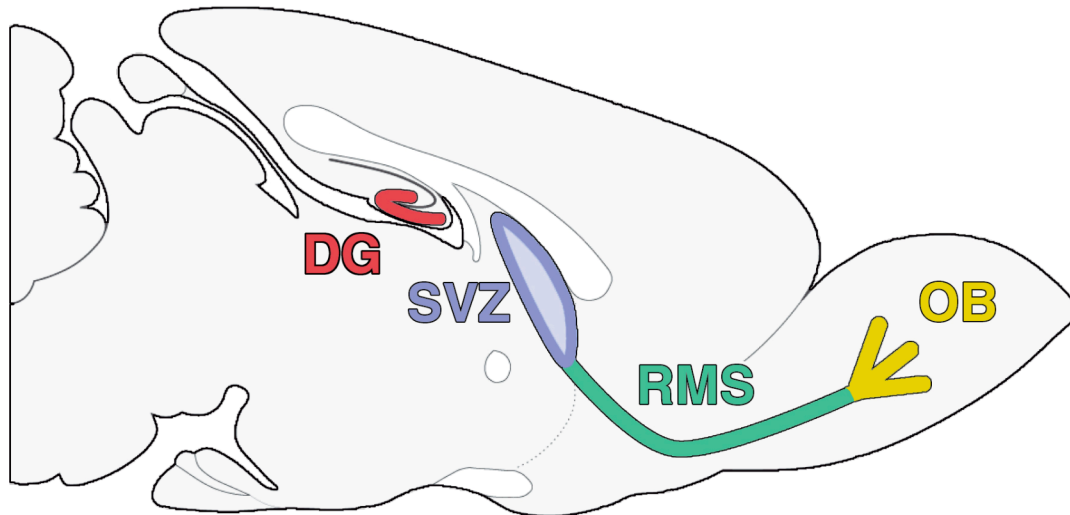
The history of stem cell science can be traced back to 1855, when German physiologist Rudolph Virchow established that all cells arise from pre-existing cells ("All cells come from cells"). Prior to Virchow's statement, life was thought to arise from "nothingness", or the nonliving (Shier, 2004).

A cell's turnover can be days or decades, but every cell type in the body will be renewed at least once during the human life span. However, until recently, the common understanding has been that neurons from the human adult brain no longer had the capacity for renewal. It has been thought that plasticity, by means of new cells, occurs *only* during embryogenesis and the perinatal period. For many years, plasticity in the adult brain has referred to the development and increased complexity of new synapses and dendrites. This belief was only half of the truth, as we now know that stem cells reside in a multitude of tissues, perhaps even in all tissues, of the body. Nevertheless, the brain's obviously poor, self-repair capacity, and the lack of appropriate techniques, has delayed the discovery of adult brain regeneration.

With the emergence of a method using radioactive [<sup>3</sup>H]-thymidine, which is incorporated in dividing cells and can be visualized by autoradiography, Altman and his colleague Das published several papers during the 60's demonstrating the formation of new brain cells in rats, guinea pigs, and cats (Altman, 1962b, a, 1963; Altman and Das, 1965a; Altman and Das, 1965b; Altman, 1966; Altman and Das, 1966, 1967). However, these papers were met with skepticism and received little attention on the whole. Altman and Das's work was re-evaluated in 1977, when Kaplan and Hinds utilized electron microscopy to reveal neurogenesis in the dentate gyrus and olfactory bulb of adult rats (Fig 1) (Kaplan and Hinds, 1977). This work was followed by several publications on the same topic, showing regeneration in the adult brain of both mammals and birds (Goldman and Nottebohm, 1983; Kaplan, 1983; Kaplan et al., 1985; Nottebohm, 1985, 1989).

During the mid-80's, because Rakic failed to detect neurogenesis in juvenile and adult monkeys, he drew the conclusion that adult primates, including humans, were devoid of neuronal brain regeneration, although he observed "a slight turnover of glial cells" (Rakic, 1985). Fortunately, the interest in adult neural regeneration was re-kindled, when Kuhn et al. (1996) used bromodeoxyuridine (BrdU) to co-label cells with mature neuronal markers and demonstrated the presence of newly formed neurons in the dentate gyrus of adult rats. In fact, with follow-up studies, they were able to show that neurogenesis is retained even in older animals, although the levels are somewhat reduced (Kuhn et al., 1996) It didn't take long for these results to also be confirmed in the adult human brain. In the late 90's, Eriksson and colleagues were

able to study the brains of terminal cancer patients that had received BrdU to trace tumor growth. Remarkably, although many were elderly, newly formed neurons were detected in the hippocampus of these patients (Eriksson et al., 1998). Since then, several other studies indicating a regenerative capacity for the adult human brain have been performed (Kukekov et al., 1999; Roy et al., 2000; Weickert et al., 2000).



*Figure 1 – Regions of adult neurogenesis: neuronal progenitors reside in the dentate gyrus (DG) of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricle. Progenitors migrate through the rostral migratory stream (RMS) and reach final destination, the olfactory bulb (OB), where they differentiate into mature granular neurons and periglomerular interneurons.*

Today, stem cell science is running at full speed, and amazing achievements are being made, particularly in the medical field. Nevertheless, the factors and mechanisms that stimulate stem/progenitor cells to proliferate, remain undifferentiated, or to commit to a certain lineage, as well as the influence of different environments on these cells, is still largely unknown. We have not yet grasped the full capacity, as well as the possible limitations, of stem/progenitor cells. Further studies in this exciting field will hopefully help to develop new techniques and effective therapeutic treatments.

## BACKGROUND

### What is a stem cell?

The criteria defining a stem cell are:

- (I) a cell that has an unlimited (or prolonged) capacity for self-renewal, and
- (II) a cell that is multipotent, *i.e.*, can differentiate into cells of multiple lineages.

Rudolph Virchow's statement in 1855, "All cells come from cells", describes in an extremely simplified way how over 200 different cell types in the human body ultimately arise from stem cells - starting with the fertilized egg. With the ability to differentiate into any cell type, the fertilized egg is referred to as totipotent. Later in development, when the egg is in the blastocyst stage and is implanted in the uterus, repeated divisions form an outer layer of cells and an inner cell mass. The outer layer develops into the placenta and embryonic membranes, while the inner cell mass develops into the embryo. The cells of the inner cell mass are pluripotent, as they can give rise to any cell type except those forming the placenta and embryonic membranes. Cells derived from the inner cell mass can also give rise to embryonic stem cell lines that are the typical embryonic stem cells normally propagated in culture (Gage, 2000).

In the adult, some of the cells remain undifferentiated and maintain their ability to self-renew, hence the name "adult stem cells". Their full potential is not yet completely understood; however, adult stem cells that remain in their natural environment give rise only to cells of the organ from which they were derived. Studies have shown, however, that they most likely possess the ability to develop into other lineages as well (Mezey et al., 2000). In the natural environment, however, adult neural stem cells give rise to the three main cell lineages of the brain: neurons, astrocytes, and oligodendrocytes (Morshead et al., 1994; Palmer et al., 1997; Whittemore et al., 1999).

Stem cells do not generate fully differentiated cell types directly, but progress through intermediate cell stages, first generating progenitor, or precursor, cells. Progenitor cells descend from stem cells, but have a restricted potential, usually more or less committed to a certain cell type and limited in their capacity to self-renew. Both daughter cells from a progenitor cell may stop dividing and start to differentiate, while at least one of the daughter cells from a stem cell will remain undifferentiated and continue to divide. Nevertheless, progenitor cells are usually more proliferative than stem cells. The term "precursor cell" simply refers to any cell that is earlier in a developmental pathway than another (reviewed in McKay, 1997).

### Mammalian brain development during embryogenesis

The central nervous system, as well as the skin, develops from the ectodermal layer. Potent morphogens and cascade pathways of downstream transcription factors orchestrate the precise and delicate machinery termed *embryogenesis*. According to their position in space, cells respond to different concentrations of morphogens with transcriptional activation of certain homeogenes. In the dorsal bone morphogenic protein (BMP)-rich region of the telencephalon, the transcription factors Paired homeobox (Pax) 6 and Emx1/2 are activated prior to neurogenesis. At later stages, the dorsal telencephalon gives rise to mainly projection neurons, ultimately becoming the cerebral cortex and hippocampus (reviewed in Campbell, 2003). The morphogenic glycoprotein Wingless type (Wnt)-3a is expressed in the medial part of

the dorsal telencephalon and is required for correct hippocampal development (Lee et al., 2000).

Conversely, the transcription factors *Dlx1/2* and *Gsh1/2* are activated in the *Shh*-rich ventral parts of the telencephalon. These ventral regions later give rise to the basal ganglia neurons, oligodendroglia, and interneurons, some of which migrate to populate dorsal regions (Fig 2). The ventral regions are also the origin of the olfactory system (Campbell, 2003).

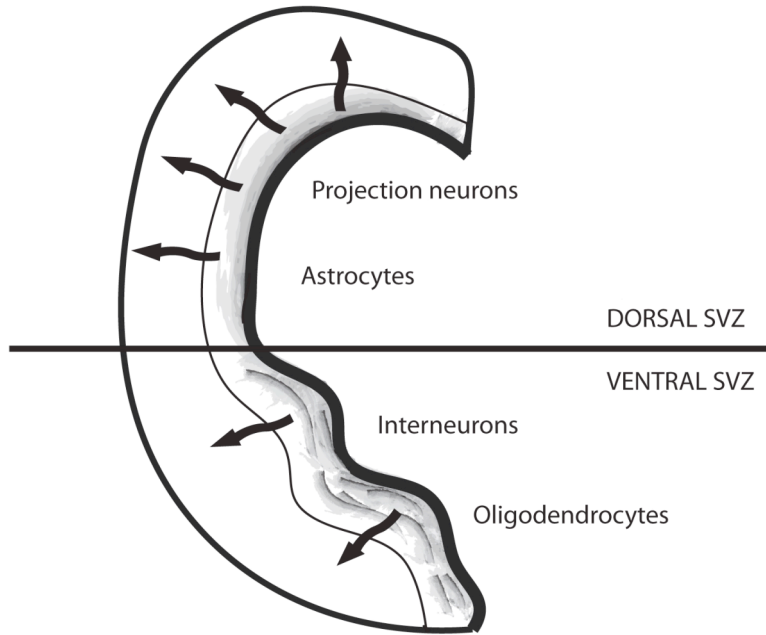


Figure 2 – Neural populations originate from different regions of the anterior neural tube during development.

#### Expansion of the precursor pool

During the expansion phase, before the onset of neurogenesis, secretion of mitogens, many of them functioning as morphogens as well, drives the expansion of precursor cells through symmetric division. Some of the mitogens identified to be responsible for the expansion phase include FGF, bone morphogenic protein (BMP) and epidermal growth factor (EGF) (Tropepe et al., 1999; Lillien and Raphael, 2000).

#### Neurogenic and gliogenic phases

The expansion phase is followed by the neurogenic phase, which begins around E12, peaks around E13, and ceases by E16 in mice (Fig 3). At E11, preceding the neurogenic phase, the VZ evolves into the subventricular zone (SVZ), which overtakes the role of neuronal production (Smart, 1976). Neurons arise from asymmetrically dividing radial glia in the VZ, as well from symmetrical division of intermediate precursors

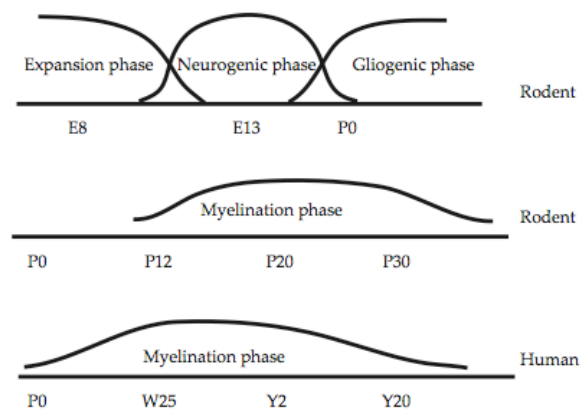


Figure 3 – Phases during mammalian brain development

in the SVZ (Noctor et al., 2004). Developmental neurogenesis, as well as the transition from neuroepithelial cells to radial glia and finally astrocytes, is thought to be partly regulated by Notch signaling (Artavanis-Tsakonas et al., 1999; Gaiano et al., 2000; Mizutani and Saito, 2005). However, the gliogenic phase does not truly begin until around P0, when astrocyte and oligodendroglial progenitors arise from the SVZ. Interestingly, new findings suggest that all three major cell types of the CNS also arise from the outer first layer during development, beneath the pial surface (the marginal zone) (Costa et al., 2007).

*Differentiation is directed by basic helix-loop-helix proteins*

Although counterintuitive, neurons produced by the SVZ form the cortical structures of the brain in an inside-out manner; the inner layers are formed first, and newly generated cells use the radial glia processes, extending to the pial surface, to migrate past older cells (Rakic, 1974; Noctor et al., 2004). What are the codes instructing newborn cells to take on a certain cell fate, whether it be neuronal, astroglial, or oligodendroglial? Downstream of the morphogens and homeoproteins, which are involved in regionalization during development, are transcription factors that are involved in cell fate determination. These cell fate determinants all belong to the family of basic helix-loop-helix (bHLH) proteins, such as Mammalian achaete-scute (Mash)-, mammalian atonal homolog (Math)-, Olig-, Neurogenins (Ngn)-, Nkx, and NeuroD. The proneural activity of these bHLH proteins is counteracted by the bHLH proteins that belong to the family Hairy and enhancer of split (Hes), as well as by the helix-loop-helix (HLH) proteins Inhibitor of Differentiation (Id) (reviewed in Kintner, 2002; Brandt et al., 2003). Id proteins act by sequestering E-proteins, the binding partners of bHLH proteins. Because Id proteins lack the basic domain that binds to DNA, transcriptional activity is repressed. Hes proteins act by either sequestering E-proteins or bHLH proteins. Additionally, Hes proteins form homo- or heterodimers, resulting in DNA remodeling, which blocks transcriptional activity of bHLH proteins (reviewed in Brandt et al., 2003).

In addition to the orchestra of cell fate determining signals, it also appears as if the timing affects the response of neural precursor cells to specific signals, probably due to interactions between previously expressed patterning genes and bHLH proteins (Kintner, 2002).

*Neuronal maturation and cell survival during embryogenesis*

Secreted neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), glial-derived neurotrophic factor (GDNF), leukemia-inhibiting factor (LIF), and ciliary neurotrophic factor (CNTF), promote key events, such as proliferation and survival, as well as axonal growth and dendritic arborization (reviewed in Davies, 2003). It has been estimated that over 250 000 new neurons are produced in the human brain each minute during the most proliferative stage of development (Purves, 2004). Cells are, however overproduced; survival or apoptosis selection are not only dependent on neurotrophic factor competition, but also on activity-dependent processes (reviewed in Mennerick and Zorumski, 2000). In some areas of the rodent brain, up to 80% of the newborn neurons are eliminated after establishing contact with their targets (Davies, 2003).

*Oligodendrocyte origin and the replacement of embryonic oligodendrocytes*

Ivanova and colleagues (Ivanova et al., 2003) suggested that telencephalic oligodendrocytes arise from the ganglionic eminences during embryogenesis and populate the cortex, while a second wave of oligodendrocytes are produced postnatally in the SVZ. In 2006, Kessar and coworkers used Cre-lox fate mapping



methods in transgenic mice and convincingly demonstrated that oligodendrocyte progenitors initially arise in the medial ganglionic eminence (MGE) and the anterior entopeduncular area (AEP), and are later produced from the lateral and/or caudal ganglionic eminences (LGE and CGE). Furthermore, they showed that although ventrally arising oligodendrocytes spread throughout the brain during embryogenesis, these are replaced by postnatally produced oligodendrocytes from dorsal regions of the cortex (Kessaris et al., 2006). Dorsal oligodendrogenesis, such as in the postnatal rodent cortex, does not seem to take place in avian species (Olivier et al., 2001). In mammals, however, the appearance of oligodendrocyte proliferation waves, beginning ventrally and continuing towards the cortex, could perhaps reflect the need for dorsal sources, because the mammalian cortex expanded during evolution (Kessaris et al., 2006).

In the adult brain, cycling progenitors in the cortex, as well as precursors in the SVZ and the subcallosal zone, generate oligodendrocytes. This will be discussed further in the sections about cell genesis in the adult brain and oligodendrogenesis in non-neurogenic areas.

#### *Common markers used for oligodendrocyte identification*

One of the greatest challenges in stem cell biology is to understand the sequential steps that are taken by a stem cell during maturation into a functional, specialized cell type. There are several, more or less specific, markers for various oligodendroglial developmental stages (Fig 4). Because an exclusive oligodendrocyte progenitor-specific marker has not yet been identified, co-labeling with more than one marker is required to be certain. In brief, oligodendrocyte progenitors express early markers, such as Olig2, Neuron-Glia (NG) 2, and the platelet-derived growth factor alpha receptor (PDGF- $\alpha$ R), the only PDGF receptor expressed by oligodendrocytes (Schnitzer and Schachner, 1982; Nishiyama et al., 1996). When oligodendrocyte progenitors (OPCs) have migrated and settled in regions of myelination, the early markers are downregulated. The OPCs then turn into pre-oligodendrocytes and express O4 {Sommer, 1981 #100}, before becoming immature oligodendrocytes and producing more mature markers, such as RIP, and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) (Zalc et al., 1981; Friedman et al., 1989; Sprinkle, 1989). At this stage, the oligodendroglial cell loses its capacity to self-renew, and will also express the marker for adenomatous polyposis coli (APC) tumor suppressor protein (Bhat et al., 1996) and personal communication with Prof. Magdalena Götz, Institute for Stem Cell Research, Neuherberg/Munich, Germany). Fully mature oligodendrocytes additionally express robust myelin component markers, such as myelin proteolipid protein (PLP), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and myelin-associated glycoprotein (MAG) (Monge et al., 1986; Brunner et al., 1989; Trapp et al., 1989).

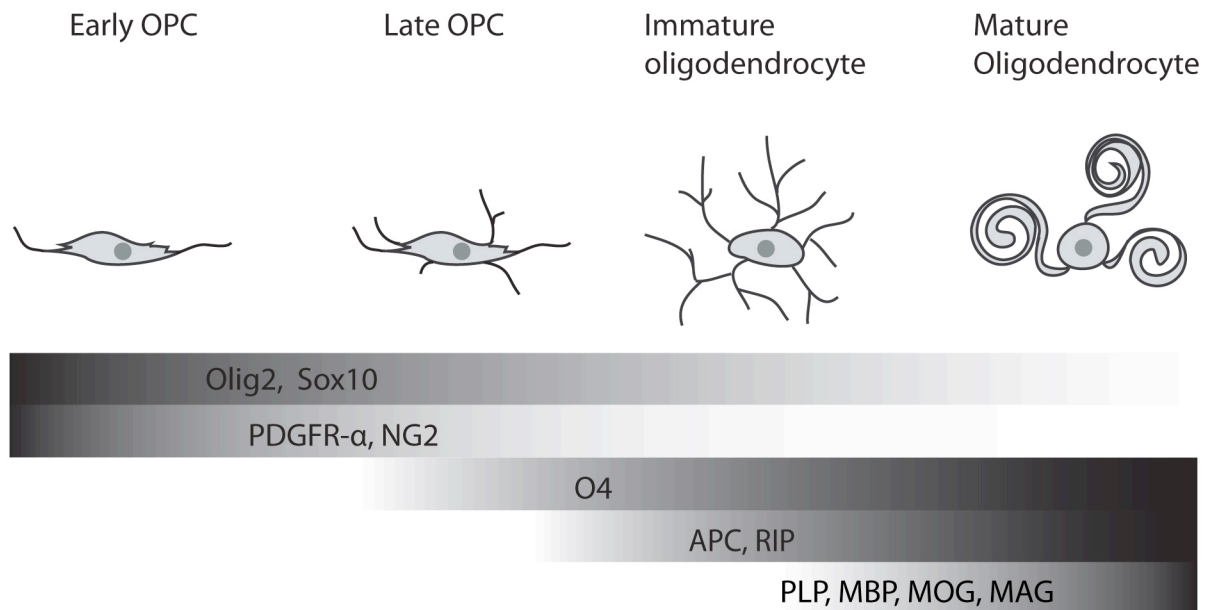


Figure 4 – Marker expression during oligodendrocyte maturation

## Formation of the neurogenic zones during embryogenesis

### *The subgranular zone of the hippocampus*

Hippocampal development is initiated in the dorsal part of the cortex. Around E18, Mash-1 expressing precursors (Pleasure et al., 2000a), from a dorsally located germinal zone in the SVZ, begin to migrate inward towards the future site of the dentate gyrus to form a secondary germinal zone (Altman and Bayer, 1990; reviewed in Kempermann, 2006). Pre-mature neurogenesis in the primary germinal zone and migratory pathway is prohibited by expression of Notch-1 and anti-neurogenic bHLH-proteins, such as Id2, Id3, and Hes-5 (Pleasure et al., 2000a). The secondary germinal matrix forms the outer layer of the dentate gyrus, but is dissolved postnatally to allow for a tertiary germinal matrix (Altman and Bayer, 1990). Between P3 and P10, this tertiary matrix produces the inner layer of granule neurons between P3-P10 and then transforms into the SGZ, continuing to produce new neurons throughout adulthood. Anti-neurogenic factors are downregulated in the postnatal tertiary matrix and SGZ, while Mash-1 expression persists (Pleasure et al., 2000a; Uda et al., 2007). The persistent expression is thought to maintain an undifferentiated state in neuronal precursors (Lo et al., 1991; Sommer et al., 1995; Torii et al., 1999; Tomita et al., 2000). Radial glia already reside in the embryonic dentate gyrus anlage at E13, prior to arrival of migrating precursors (Rickmann et al., 1987). Radial glia initially guide precursor cells to the correct positions by extending processes across the structure (Rickmann et al., 1987; Altman and Bayer, 1990; Sievers et al., 1992). Postnatally, radial glia are restricted to the SGZ and their morphology changes; the long processes retract and end in the molecular layer. In the adult, radial glia-like cells, presumably derived from embryonic radial glia (although this has yet to be proven), divide asymmetrically to produce progenitors for the overlying granule cell layer, where they become excitatory granule cell neurons (Seri et al., 2001). The adult production of neurons is further described in the section cell genesis in the adult brain.

In contrast to hippocampal excitatory granule cell neurons, hippocampal inhibitory interneurons arise from ventral areas; immature interneurons migrate tangentially to the hippocampus from the lateral and medial ganglionic eminences in the basal telencephalon (Pleasure et al., 2000b).

*The subventricular zone of the lateral ventricles*

The neuroepithelial cells of the VZ form the SVZ, a one- to two-cell body thick inner layer that is visible around E11 in the rodent (Smart, 1976). Prior to the onset of neurogenesis, neuroepithelial cells that line the ventricles acquire radial glia characteristics and divide asymmetrically to produce neurons, as well as intermediate precursors residing in the SVZ (Rakic, 1971, 1978; Noctor et al., 2001; Noctor et al., 2002; reviewed in Gotz, 2003; Malatesta et al., 2003; Anthony et al., 2004). The SVZ produces neurons initially (E12-E16 in mice), followed by glia during development (starting at E18) (Hartfuss et al., 2001). The embryonic SVZ radial glia eventually become astrocytes and radial glia-like astrocytes postnatally (reviewed in Gotz and Huttner, 2005) [Alvarez-Buylla, 2001 #689], while VZ neuroepithelial cells become the ependymal lining of the ventricles (Sturrock and Smart, 1980).

**Cell genesis in the adult brain – neurogenic zones of the adult brain**

*The subgranular zone of the hippocampus*

In the late 60's, the subgranular zone (SGZ) of the hippocampus was shown to contain dividing cells (Altman, 1962a, 1963; Altman and Das, 1965a; Altman and Das, 1965b; Altman and Das, 1965c). Stretching to each side of the temporal lobes, the hippocampus is part of the evolutionary limbic system and is involved in memory and learning, as well as emotion processing (reviewed in Squire, 1992; Tulving and Markowitsch, 1998). Declarative memory, the memory of facts and events, as well as spatial memory, are hippocampal-dependent. The hippocampus does not store memories, but rather processes and prepares incoming information before sending it back for long-term storage in the neocortex. Therefore, the hippocampus acts like a spider in a web, sensing informational cues and relating them to each other. It is easy to imagine that the addition of new cells in this area, under normal conditions or due to various factors, might affect hippocampal efficacy and the ability of the organism to adapt to the environment.

The hippocampus consists of two major cell layers folded closely around each other. One layer is called 'Ammon's horn' and is divided into four regions; CA1, CA2, CA3, and CA4 (CA4 is also part of the hilus). The other layer is the dentate gyrus and consists of granule cell neurons and the underlying subgranular zone (SGZ), a two- to three-cell body thick layer. The main circuitry is trisynaptic; incoming excitatory signals from the association cortex travel the perforant path, where fibers synapse on dendritic trees of granular cell neurons in the granular cell layer. Subsequently, the granule cell neurons send information, via the mossy fiber tract, to CA3 pyramidal neurons. Transmission proceeds via the schaffer collateral pathway to CA1 pyramidal, and finally to the neocortex (Fig 5) (reviewed in Kempermann, 2006). The trisynaptic pathway signals through the glutamate, alpha-amino-3-hydroxy-5-methyl-isoxazolepropionic acid (AMPA), and N-methyl-D-aspartate (NMDA) receptors (Knowles, 1992). Other input to the hippocampus outside the trisynaptic circuitry and local inhibitory neurons primarily modulate information processing (Marchetti et al., 2004; Widmer et al., 2006; Goto and Grace, 2007; Mockett et al., 2007; Glickfeld et al., 2008).

The granule cell layer (GCL) produces an overabundance of new neuronal progenitor cells; most of these are selectively eliminated by apoptosis (Biebl et al.,

2000) and never reach full maturity. The GCL volume is related to overall cell number and is, therefore, dependent on cell division and removal (Peirce et al., 2003). In young, adult mice, nearly 5000 new granule cells are produced daily (Kempermann, 2006), and the generation of these new neurons leads to increased GCL volume during the first postnatal year in rodents (Peirce et al., 2003). Nevertheless, although neurogenesis continues through the life of the animal, it decreases considerably with age (Kuhn et al., 1996). Interestingly, the overall number and phenotype of stem cells seems to be unaffected by aging, but the rate of proliferation increasingly diminishes (Kuhn et al., 1996; Hattiangady and Shetty, 2008).

*Architecture and cell markers of the SGZ*

The radial glia-like type 1 cells, those highest in the stem cell hierarchy, constitute the stem cell population within the SGZ (Alvarez-Buylla et al., 2001; Seri et al., 2001; Garcia et al., 2004). The morphology of these cells resembles embryonic radial glia cells (Eckenhoff and Rakic, 1984; Seri et al., 2001), triangular in shape, with a single vertical process. In addition to GFAP (Cameron et al., 1993), radial glia-like type-1 cells express nestin (Fukuda et al., 2003), brain lipid binding protein (BLBP) (Steiner et al., 2006), and the transcription factor Sox2 [sex determining region of Y-chromosome (SRY) related high motility group (HMG) box] (Komitova and Eriksson, 2004). Interestingly, Sox-2 overexpression inhibits neurogenesis and induces astrogenesis during mouse development (Bani-Yaghoub et al., 2006). In contrast to common astrocytes, radial glia-like type 1 cells do not express S-100 $\beta$  (Steiner et al., 2004).

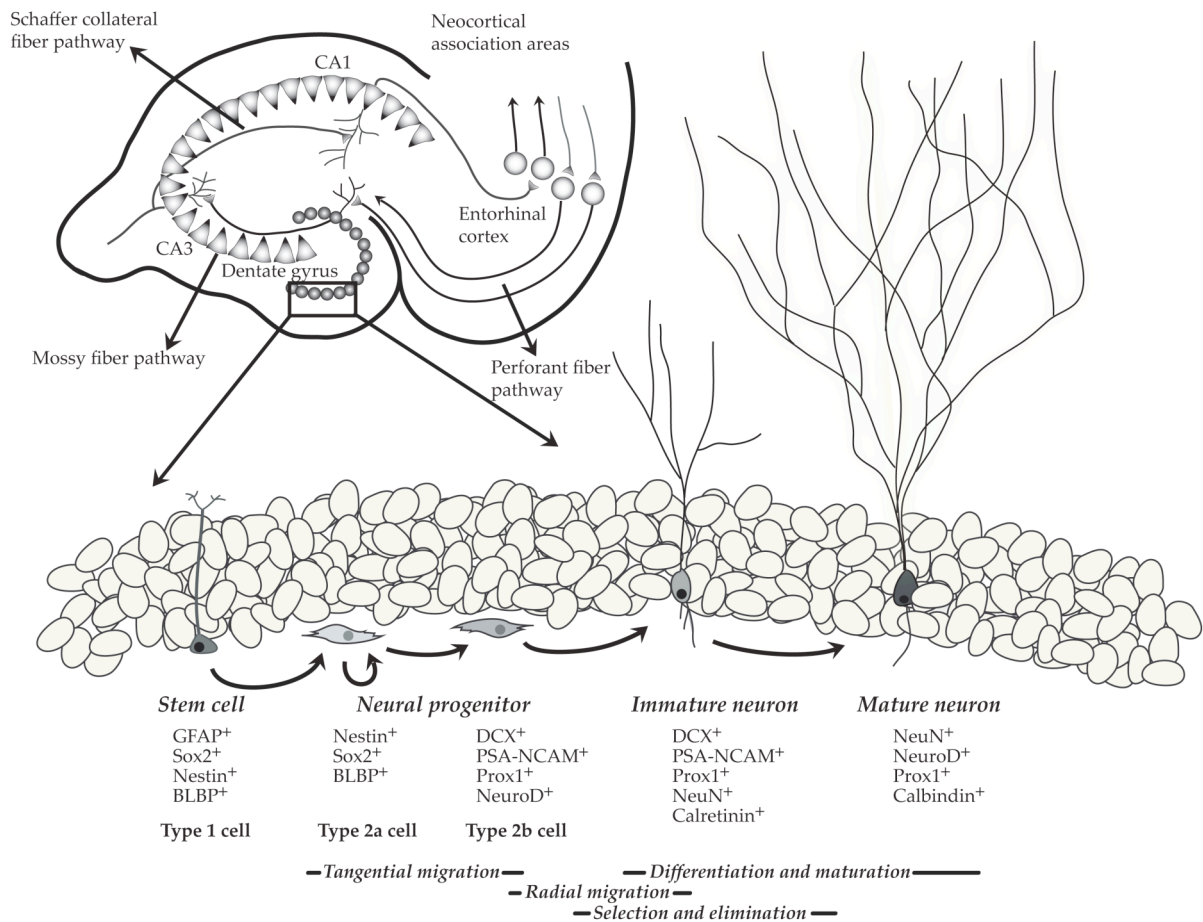


Figure 5 – Stem and neuronal progenitor cells differentially express various markers during maturation in the hippocampal granular cell layer.

The type 1 cells divide asymmetrically to give rise to type 2 cells, which orient along the SGZ with an elongated morphology and short, immature processes. At this stage, the cells are most likely committed to a neuronal lineage, tangentially migrate short distances, and are highly proliferative (also called transiently amplifying progenitor cells) (Kronenberg et al., 2003). Initially, they express nestin, Sox2, BLBP, and NeuroD (Seki, 2002) (and possibly also GFAP). The cells then develop into a secondary phenotype, the type-2b cell (Kronenberg et al., 2003). BLBP and Sox2 expression, but continues to express nestin (to a certain extent), NeuroD, and additionally acquires expression of the transcription factor Prox-1, the immature neuronal marker doublecortin (DCX), as well as the polysialated form of the neural cell adhesion molecule (PSA-NCAM) (Kronenberg et al., 2003). Both DCX and PSA-NCAM are involved in migratory properties (Seki and Arai, 1991; Francis et al., 1999) and immature neuronal cells that DCX also exhibit signs of maturation with regard to electrophysiological properties (Ambrogini et al., 2004).

With acquisition of DCX and PSA-NCAM expression, transition from tangential migration along the SGZ to radial migration into the GCL begins. During the type-3 stage, cells migrate to the GCL and continue to express Prox-1, NeuroD, DCX, and PSA-NCAM, but are nestin-negative (Fig 5) (Kronenberg et al., 2003).

The expression of the calcium-binding protein calretinin marks the transition to postmitotic, immature neurons (Brandt et al., 2003), and the cells begin to integrate axonal and dendritic processes (Kempermann et al., 2004). The postmitotic cells also express NeuN (Neuronal Nuclei) in the nuclei and cytoplasm (Lind et al., 2005), which is preserved throughout maturation, while expression of calretinin is transient and exchanged by calbindin in the fully matured granule cell neuron (Brandt et al., 2003). It takes several days for newborn cells to become postmitotic, but several weeks are required for immature postmitotic neurons to become fully integrated into the neuronal network (Jessberger and Kempermann, 2003).

Approximately 30% of the newborn hippocampal cells differentiate into cells other than granule neurons {Cameron, 1993 #197}. The majority of these cells are astrocytic, and only a small percentage is NG2 positive (Steiner et al., 2004).

#### *Adult hippocampal stem and progenitor cells in vitro*

Cell cultures are a valuable resource for testing hypotheses prior to *in vivo* experimentation, as well as for studying basic cellular, physiological, and biochemical processes. Although cell culture conditions strive to produce artificial environments similar to *in vivo* situations, results from *in vitro* experiments seldom reflect the entire *in vivo* condition. Additionally, species cell types respond differently to various culture conditions (Ray and Gage, 2006). Carefully conducted *in vitro* studies can nonetheless provide valuable information. Adult hippocampal progenitors (AHPs) have been isolated from the adult rat hippocampus and successfully propagated, both as neurospheres and as adherent monolayers (Palmer et al., 1997). These cultures display stem cells properties, probably as a heterogeneous mix of progenitors and stem cells. Rat AHPs are cultured in serum-free medium, supplemented with N2, which contains progesterone, sodium selenite, glutamine, insulin, transferrin, and putrescine (Bottenstein and Sato, 1979; Ray et al., 1993). With the addition of 20 ng/ml human basic fibroblast growth factor (bFGF), AHP cultures can be passaged up to 30 times without losing their initial properties (Palmer et al., 1997) and express immature cell markers, such as microtubule associated protein (Map) 2c, O4, and nestin. Once bFGF is withdrawn, AHPs spontaneously differentiate primarily into neurons, which is in accordance with their normal *in vivo* fate. Approximately one-fifth of the cells differentiate into astrocytes, and very few become oligodendrocytes (Palmer et al., 1997).

In addition to spontaneous differentiation, AHPs can be manipulated to differentiate into specific cell types. To stimulate the generation of neurons, forskolin (Palmer et al., 1997), valproic acid (Watterson et al., 2002), low doses of IGF-I (Aberg et al., 2003), or retinoic acid in combination with BDNF can be added to the cultures (Takahashi et al., 1999). BMP-2, leukemia inhibiting factor (LIF) (Hsieh et al., 2004), ciliary neurotrophic factor (CNTF), and fetal bovine serum (FBS) (Johe et al., 1996) stimulate the generation of astrocytes. Insulin (Hsieh et al., 2004), triiodothyronine (T3) (Johe et al., 1996), and high doses of IGF-I or IGF-II (Hsieh et al., 2004) provoke differentiation towards the oligodendroglial lineage. High numbers of oligodendrocytes can also be achieved by expanding AHPs in bFGF-2 until the cultures reach density arrest (Palmer et al., 1997).

#### *The subventricular zone of the lateral ventricles- architecture and cell markers*

The subventricular zone (SVZ), a component of the olfactory system, is the second known area to harbor neuronal progenitors and stem cells that produce neurons and glia in the adult brain (Levison et al., 1993; Lois and Alvarez-Buylla, 1993, 1994).

The radial glia-like cells of the adult SVZ are commonly referred to as “B-cells”, according to nomenclature suggested by Doetsch and colleagues (Doetsch et al., 1997). The SVZ B-cells correspond to the type-1 cells in the hippocampus, functioning as neural stem cells (Doetsch et al., 1999), although they differ in several aspects. For example, radial glia-like B-cells of the adult SVZ retract their basal process, probably because they do not need to guide newly generated neurons (Merkle et al., 2004; Malatesta et al., 2008). In addition, B-cells have a single, long cilium that extends from the ependymal cell wall to the cerebrospinal fluid, a feature that might be important for regulatory functions of the neurogenic niche (Tramontin et al., 2003). Moreover, type-1 cells generate excitatory granule cell neurons, while B-cells generate inhibitory interneurons of the olfactory bulb (Luskin, 1993; Lois and Alvarez-Buylla, 1994).

SVZ B-cells can be identified by expression of GFAP (Doetsch et al., 1999), BLBP (Sundholm-Peters et al., 2004), Sox-2 (Komitova and Eriksson, 2004), nestin (Doetsch et al., 1997), and the neurogenic transcription factor Pax-6 (Heins et al., 2002).

B-cells divide asymmetrically to give rise to “C-cells” (transiently amplifying progenitor cells) that proliferate in abundance, generating clusters of cells. C-cells can be distinguished by expression of the proneuronal transcription factor Dlx2 (Doetsch et al., 2002; Petryniak et al., 2007). Many C-cells also express Pax-6 or Olig2 (Hack et al., 2005).

C-cells generate “A-cells”, neuroblasts destined for the olfactory bulb. At this stage, the progeny downregulate nestin expression and begin to express PSA-NCAM and DCX (Rousselot et al., 1995; Doetsch et al., 1997; Yang et al., 2004), and chain migration along the rostral migratory stream is initiated (Lois and Alvarez-Buylla, 1994). During migration, A-cells progressively mature, although they also continue to proliferate (Menezes et al., 1995). Once the A-cells reach the olfactory bulb, they differentiate into one of three kinds of inhibitory interneurons: the majority differentiates into calretinin-positive GABAergic granule neurons, and the remaining population becomes calretinin-negative GABAergic periglomerular interneurons. A smaller fraction of the latter population differentiates into dopaminergic periglomerular interneurons (Gall et al., 1987; Kosaka et al., 1987). Similar to the hippocampus, the olfactory system produces an overabundance of new cells, where cells not properly integrated into the circuits are removed by apoptosis (Winner et al., 2002).

### *SVZ oligodendrocytes*

Ivanova and colleagues (Ivanova et al., 2003) have suggested that oligodendrocytes arise in two distinct “waves” - one arising from the ganglionic eminences during embryogenesis, spreading out in the cortex, and a second arising from the postnatal SVZ. The origin of oligodendrocyte precursors in the postnatal SVZ has been difficult to trace, because these cells do not express common oligodendrocyte progenitor markers, such as PDGF-R $\alpha$  or NG2 until they migrate away from the SVZ (Pringle et al., 1992; Staugaitis et al., 2001). However, with the emergence of new candidate oligodendrocyte progenitor markers, such as Olig2 (Marshall et al., 2005) and mammalian achaete-scute homologue 1 (Mash-1) (Parras et al., 2004), new information regarding oligodendrocyte origin in the adult brain has been revealed. For example, some SVZ-generated oligodendrocytes have been shown to arise from radial glia-like B-cells, producing Olig2-positive transiently amplifying C-cells (Menn et al., 2006). The progeny, identified by the expression of Olig2, PDGF-R $\alpha$ , and PSA-NCAM, are capable of migrating long distances and remyelinating in response to demyelination.

### *The concept of the neurogenic niche*

Why do neural stem and progenitor cells, when transplanted into non-neurogenic regions, not generate neurons, but rather die or differentiate into glial cells? Why do progenitors from non-neurogenic regions give rise to neurons when transplanted into neurogenic regions? With increased knowledge about mechanisms regulating cell genesis, it has become clear that neurogenic permissiveness depends largely on the microenvironment surrounding resident stem and progenitor cells. With increased awareness of a specialized microenvironment as a prerequisite for successful neurogenesis, the concept of the “neurogenic niche” has developed. Currently, the composition of the neurogenic niche is being extensively studied, and it seems as if all the constituents of the neurogenic niche - endothelial cells lining the blood vessels, ependymal cells, extracellular matrix, astrocytes, mature neurons, and even neural stem and progenitor cells - contribute to neurogenic permissiveness.

In culture, endothelial cells release soluble factors that promote neuronal proliferation and generation from neural stem cells (Shen et al., 2004), which seems to take place *in vivo* as well (Ramirez-Castillejo et al., 2006). In addition, endothelial cells upregulate neurotrophic factors and increase angiogenesis in response to injury (Gotts and Chesselet, 2005; Ohab et al., 2006), features that are thought to strongly influence the neurogenic niche. Furthermore, neurogenic niches of dividing progenitors are in close proximity to vessels (Palmer et al., 2000), suggesting a role for the vasculature in promoting neurogenesis. In addition, circulating VEGF has a survival-promoting effect on neuronal cells (Schanzer et al., 2004). Radial glia, such as astrocytes in the subgranular zone, contact blood vessels with their vascular endfeet and are in direct communication with endothelial cells (Filippov et al., 2003).

Multiciliated ependymal cells of the SVZ constitute the barrier between the CSF-filled ventricles and the SVZ and are potent regulators of the neurogenic niche. In accordance with this, ependymal cells have been shown to produce the BMP antagonist noggin, thereby promoting neurogenesis (Lim et al., 2000). Recently, ependymal cells have also been demonstrated to harbor the capacity to de-differentiate and act as neural stem cells, contributing to the production of neurons (Zhang et al., 2007; Coskun et al., 2008). Further studies confirming these findings are needed to establish the function of ependymal cells as neural stem cells in the SVZ neurogenic zone.



The extracellular matrix of the neurogenic niche is enriched with molecules that influence a neurogenic environment, such as Tenascin C and Reelin (Garcion et al., 2004; Zhao et al., 2007). Laminins not only provide anchorage, but also influence proliferation, differentiation, and migration of stem and progenitor cells (Campos, 2005).

Local astroglia secrete cytokines and chemokines, thereby modulating the neurogenic environment (Barkho et al., 2006). Astrocytes also produce basic morphogenic signals in the neurogenic niches, such as sonic hedgehog (Jiao and Chen, 2008) and the Wingless type (Wnt) glycoprotein. Sonic hedgehog can even increase neurogenic permissiveness in the otherwise non-neurogenic neocortex (Jiao and Chen, 2008). In the hippocampus, Wnt 3a is expressed by astrocytes and supports neuronal production (Lie et al., 2005). In both the subventricular zone and hippocampus, astrocytes express the cell surface receptor Notch, which maintains an undifferentiated state and capacity for self-renewal in neural stem cells (Tanaka et al., 1999; Alexson et al., 2006; Givogri et al., 2006; Breunig et al., 2007).

Interaction between stem cells and their progeny also constitutes a part of the neurogenic niche. Neuroblasts in the niche function within a negative feedback loop, where inhibitory GABA signals from neuroblasts on radial glia-like astrocytes reduce the production of additional neuroblasts (Liu et al., 2005). Synaptic activity from mature neurons strongly affects the production of and integration of new cells within the neurogenic niche, especially in the hippocampus (reviewed Ming and Song, 2005).

Perhaps most interesting, in the context of regenerative medicine, is the ability of the brain, under certain circumstances, such as injury, to produce local neurogenic niches in non-neurogenic regions. This has been demonstrated in the case of stroke (Ohab et al., 2006).

### **Oligodendrogenesis in non-neurogenic areas of the brain - the subcallosal zone**

During hippocampal development and expansion, the ventricular walls between the hippocampus and the corpus callosum collapse. In the adult, the resulting lamina of cells between the corpus callosum and the hippocampus brain is referred to as the subcallosal zone (SCZ) (Seri et al., 2006). The SCZ is considered to be an extension of the SVZ that is separated from the large ventricles. Cavities filled with “trapped” cerebrospinal fluid, are dispersed throughout the structure. The architecture of the SCZ is similar to the SVZ, with ependymal cells, astrocytes (possibly similar to B-cells, but this has not been shown), C-cells, and A-cells. The SCZ has fewer C- and A-cells than the SVZ, as well as a lower proliferation rate; however, proliferation exceeds that of the hippocampal SGZ (Seri et al., 2006). Neurospheres derived from the SCZ exhibit multipotency; however, *in vivo* the progeny mainly differentiate into oligodendrocytes that migrate into the overlying corpus callosum. These oligodendrocytes are thought to arise from A-cells (Seri et al., 2006). Interestingly, A-cells in the SCZ form contacts with myelinated fibers in the corpus callosum. This feature could be important for the recruitment of oligodendrocyte progenitors to the corpus callosum (Seri et al., 2006). To what extent the SCZ contributes to the pool of myelinating oligodendrocytes under normal or diseased conditions is still poorly understood.

### **Oligodendrogenesis in non-neurogenic areas of the brain - the neocortex**

Under normal conditions, endogenous precursors in the adult neocortex produce cells of astro- or oligodendroglial cell lineages (Kempermann, 2006). Postnatally, all oligodendrocyte progenitors do not differentiate into mature, myelin-forming oligodendrocytes during the gliogenic phase, or upon leaving the SVZ. It has been



shown using [3H]-thymidine incorporation that quiescent populations of oligodendrocyte progenitors reside in the adult white matter and neocortex of the mammalian brain (Smart and Leblond, 1961; Reynolds and Hardy, 1997). These populations are often referred to as “cycling cells” and can proliferate, but do not migrate long distances, and only occasionally differentiate (Gensert and Goldman, 1996, 2001). For instance, under certain neurological conditions, the cells are activated and form new myelinating oligodendrocytes (see the section Functional importance of oligodendrocytes and myelination and Gensert and Goldman, 1997).

The major population of cycling cells expresses the oligodendroglial marker NG2 (Nishiyama et al., 1996; Dawson et al., 2003). This population is heterogeneous, both in expression of additional markers as well as morphologically (reviewed in Nishiyama, 2007). Many NG2-expressing cells in the CNS also co-express O4 and PGDF-R $\alpha$  (Hart et al., 1989; Gensert and Goldman, 1996; Nishiyama et al., 1996; Gensert and Goldman, 1997; Reynolds and Hardy, 1997). Also, about 90% of the NG2 positive cells in the adult rodent brain express Olig2 (Ligon et al., 2006). Although most oligodendrocyte progenitors in the adult brain express NG2, some cells express O4 and/or A2B5 only (Reynolds and Hardy, 1997). The relationship between these different populations is not clear.

NG2-positive cell populations are heterogeneous; some cells have a small, bipolar migratory appearance resembling oligodendrocyte progenitors, while others have an elaborated, multi-branched morphology (Berry et al., 2002). The multi-branched phenotype (sometimes referred to as “synantocyte”) is suggested to be a mature, highly specialized cell type with regulatory functions (Ong and Levine, 1999; Butt et al., 2002; Greenwood and Butt, 2003). Also, NG2-positive cells have been shown to produce astrocytes in the gray matter (Zhu et al., 2008). Interestingly, a neocortical subpopulation of NG2-positive cells expresses the neuronal marker DCX, although expression is mainly transient and the cells have not been shown to produce mature neurons (Tamura et al., 2007). Despite this, when transplanted to the hippocampus, NG2-positive cells give rise to granular neurons. Furthermore, NG2-positive cells that reside in the hippocampus can produce neurons (Belachew et al., 2003), and NG2-positive cells in the SVZ can produce neurons in the olfactory bulb (Aguirre and Gallo, 2004). Although it is possible that NG2-positive cells from non-neurogenic and neurogenic regions are distinct populations (Aguirre and Gallo, 2004), these findings indicate that there is an intrinsic potential for NG2-positive cells to generate neurons. However, in non-neurogenic regions such as the neocortex, this potential appears to be suppressed.

Cells expressing NG2 and Olig2 represent the population that primarily responds to brain injury (Buffo et al., 2005; Kronenberg et al., 2005b; Tatsumi et al., 2005; Magnus et al., 2007); these cells are also found in the healthy human brain and within lesions of multiple sclerosis (MS). This suggests that NG2-positive cells participate in the remyelination process (Chang et al., 2000; Wilson et al., 2006), although remyelination by oligodendrocyte precursors often fails (Blakemore et al., 2000 ; reviewed in Franklin, 2002; Reynolds et al., 2002).

### **Effects of exercise on adult hippocampal neurogenesis *in vivo***

A wide range of molecules has been shown to affect proliferation, survival, and neuronal differentiation of adult neural stem and progenitor cells *in vivo*. However, most of these molecules are involved in the larger context of certain environmental or physiological influences (Yoshimura et al., 2003; Heine et al., 2005; Shetty et al., 2005; During and Cao, 2006; Ohab et al., 2006; Rossi et al., 2006; Thakker-Varia et al., 2007; Koo and Duman, 2008). Increased neurogenesis takes place during voluntary exercise (Van Praag et al., 1999b), enriched environment (Kempermann et al., 1997),

and learning (Gould et al., 1999) conditions, as well as under certain pathological conditions, such as after stroke (Takagi et al., 1999; Komitova et al., 2002), seizures (Bengzon et al., 1997; Jiang et al., 2003), and traumatic brain injury (Dash et al., 2001).

Certain conditions have also been shown to decrease adult neurogenesis, such as stress (Gould et al., 1998; Oomen et al., 2007), aging (Seki and Arai, 1995; Kuhn et al., 1996), and inflammation (Ekdahl et al., 2003; Monje et al., 2003). Depression is thought to negatively influence hippocampal neurogenesis, although this has yet to be proven (reviewed in Schmidt and Duman, 2007). On the other hand, antidepressant medications increase neurogenesis, probably a key mechanism for successful amelioration of depression (Malberg et al., 2000). CNS irradiation induces neural cell dysfunction (Monje et al., 2002; Limoli et al., 2004), resulting in a profound reduction of hippocampal neurogenesis (Parent et al., 1999; Peissner et al., 1999; Tada et al., 2000) in a dose-dependent manner (Mizumatsu et al., 2003). In this thesis, two major regulators with opposite effects on cell genesis in the adult CNS will be addressed: voluntary exercise, including the exercise-induced opioid peptide  $\beta$ -endorphin, and irradiation.

#### *Voluntary exercise – a positive regulator of neurogenesis in the CNS*

Physical exercise is a very powerful influence on overall brain health, including brain plasticity. The beneficial effects of physical exercise extend beyond microscopic levels; enhanced cognitive functions, such as improved learning and memory, are observed in animals and humans after exercise (Carles et al., 2007; Pereira et al., 2007). In humans, physical exercise is associated with reduced risk of developing dementia and Alzheimer's disease (Rovio et al., 2005; Andel et al., 2008), as well as a reduction in age-related cognitive decline (Rogers et al., 1990; Laurin et al., 2001). Physical exercise also ameliorates anxiety and depression; some cases have reported that it is as effective as pharmacological anti-depressants (Blumenthal et al., 1999; Frazer et al., 2005; Manger and Motta, 2005; Blumenthal et al., 2007); reviewed in Byrne, 1993 #871]. Moreover, physical exercise increases the resistance of the brain to insult, as shown in rodents (Stummer et al., 1994; Carro et al., 2001; Luo et al., 2007).

#### *Voluntary exercise and neurogenesis*

The effects of physical exercise on brain health and function is linked to enhanced plasticity, proliferation, and survival of newly formed cells (Vaynman et al., 2004; Bjornebekk et al., 2005; Duman et al., 2008). On cellular levels, the most striking effects of physical exercise are seen in the hippocampal neurogenic region. Voluntary exercise has been demonstrated to increase proliferation, survival, and neurogenesis in the hippocampus in a multitude of studies (Van Praag et al., 1999b; Van Praag et al., 1999a; Trejo et al., 2001; Kim et al., 2002; Ra et al., 2002; Kronenberg et al., 2003; Kronenberg et al., 2005a). Consistent with these results, the neurogenic beneficial effects of exercise have also been shown in young (Van Praag et al., 1999b) and old age (van Praag et al., 2005), as well as during development (Bick-Sander et al., 2006). Increased neurogenesis after exercise has been implicated in enhanced long-term potentiation (LTP) in rodents (Van Praag et al., 1999b; Farmer et al., 2004; O'Callaghan et al., 2007) and improved learning (Van Praag et al., 1999b). In an animal model of chronic stress, voluntary exercise during stressful circumstances has been shown to attenuate stress-induced suppression of LTP by counteracting rising glucocorticoid levels (Ma et al., 2002), which is typically detrimental to neurogenesis and memory. It is important to note, however, that excess running counteracts the running-induced hippocampal proliferation, probably due to interference by stress mechanisms (Naylor et al., 2005).

The antidepressant effects of exercise in rodent models of depression have been suggested to relate to hippocampal neurogenesis (Bjornebekk et al., 2005), and successful antidepressant medication requires hippocampal neurogenesis (Santarelli et al., 2003; Wang et al., 2008). Furthermore, exercise-promoted recovery of spatial memory after cerebral ischemia is associated with increased neurogenesis in the brain (Luo et al., 2007).

#### *Voluntary exercise and gliogenesis*

Voluntary exercise stimulates proliferation of astroglia and microglia in the mouse neocortex (Ehninger and Kempermann, 2003). These effects are locally restricted to the various cortical layers. The authors did not observe similar positive effects on oligodendrogenesis, but several regions, such as the corpus callosum and striatum, were not studied. It has been suggested that increased oligodendrogenesis might take place in the spinal cord of rats subjected to exercise (Skup et al., 2002), and genes involved in oligodendrocyte development are upregulated in the rat spinal cord after exercise (Perreau et al., 2005). However, there are very few studies investigating the effect of exercise on oligodendrogenesis and myelination. At the start of this thesis, there was no clear evidence for exercise-induced oligodendrogenesis. Recently, however, one paper was published demonstrating that voluntary exercise indeed produced a three-fold increase in newborn NG2-positive cells in the medial prefrontal cortex of adult rats (Mandyam et al., 2007).

#### *Molecules involved in exercise-induced brain plasticity*

In humans, exercise increases cerebral blood volume in the hippocampus (Pereira et al., 2007), suggesting increased cellular activity in this neurogenic region and the concomitant augmented need for supporting molecules. The effects of physical exercise on brain plasticity are thought to be mediated by increased production of dynamic neurotrophic factors, such as nerve growth factor (NGF) (Neeper et al., 1996) and BDNF (Neeper et al., 1995; Vaynman et al., 2004), as well as growth factors, such as FGF-2, vascular growth factor (VGF) (Hunsberger et al., 2007), VEGF (Fabel et al., 2003). Hormones, such as insulin-like growth factor-1 (IGF-1) (Trejo et al., 2001; Trejo et al., 2008), and opioids, especially  $\beta$ -endorphin (Persson et al., 2004; Koehl et al., 2008), have also been shown to influence adult neurogenesis.

#### *Opioid peptides*

The term opioid is applied to a substance that interacts and mediates its effect through the major classes of opioid receptors, and whose action is antagonized by naloxone or naltrexone. The analgesic morphine is perhaps the best-known opioid substance, responsible for the effects of opium. However, there are several other classes of opioids. Prior to the early 1970's, it was not known that the body produces its own opioid peptides. Today, four classes of endogenously produced opioids have been discovered: endorphins, enkephalins, dynorphins, and endomorphins (for a historical review, see Corbett et al., 2006). The three former categories arise from three different classes of prohormones: pro-opiomelanocortin (POMC), proenkephalin, and prodynorphin, respectively. The precursor(s) of endomorphins is (are) still unidentified (Zadina et al., 1997; Sainz et al., 2008).  $\beta$ -endorphin is produced by cleavage of POMC (Hadley and Haskell-Luevano, 1999), which is also the precursor for a group of hormones with non-opioid effects, including adrenocorticotrophic hormone (ACTH) and melanocyte stimulating hormone (MSH) (reviewed in Raffin-Sanson et al., 2003).

Opioids are thought to play an important role as neuromodulators and neurotransmitters. During stressful circumstances, corticotrophin-releasing hormone

(CRH) from the hypothalamus cleaves POMC from the pituitary gland, thereby producing ACTH, which results in stress hormones glucocorticoid release from the adrenal cortex, as well as the production of  $\beta$ -endorphin (Przewlocki and Przewlocka, 2001). The release of  $\beta$ -endorphin results in a rapid and effective analgesic effect (Hargreaves et al., 1990). In addition to typical analgesic effects, opioids are also involved in a variety of physiological functions, such as respiration, renal and cardiovascular function, gastrointestinal function, temperature regulation, metabolism, hormonal secretion, reproduction, and immune function (reviewed in Herz, 1993).

#### *Opioid receptors*

The characterization of opioid receptors, using ligand-binding techniques, actually preceded the discovery of endogenously produced opioids. Three major classes of opioid receptors have been characterized: mu ( $\mu$ ), delta ( $\delta$ ), and kappa ( $\kappa$ ) receptors (for a historical review, see Snyder and Pasternak, 2003). There are subtypes for each receptor, and other less well-characterized opioid receptors also exist ( $\epsilon$ ,  $\lambda$ ,  $\iota$ ,  $\zeta$ , and ORL receptors). Opioid receptors belong to a subfamily of the rhodopsin receptor and are G-protein coupled so-called “seven transmembrane receptors” (Singh et al., 1997).  $\beta$ -endorphin has a high affinity to  $\mu$  and  $\delta$ -receptors, whereas the enkephalins preferentially bind to the  $\delta$ -receptor. Dynorphins usually bind to the  $\kappa$ -receptor (Grossman and Clement-Jones, 1983), and endomorphins have a very high affinity to the  $\mu$ -receptor (Zadina et al., 1997). Antagonists commonly used are naltorphimine, which blocks the  $\kappa$ -receptor,  $\beta$ -funtaltrexamine, which blocks the  $\mu$ -receptor, naltrindole, which blocks the  $\delta$ -receptor, and naloxone, which blocks all three receptors but most effectively the  $\mu$ -receptor (Minami and Satoh, 1995). The characteristics and actions of opioid receptors are thoroughly reviewed by Minami and Satoh (1995).

#### *$\beta$ -endorphin – the physical exercise ally in the stimulation of hippocampal neurogenesis*

Endogenously produced opioids are released during exercise and contribute to the feeling of well being (“runners high”) following exercise (Boecker et al., 2008). Exercise-induced release of endogenous  $\beta$ -endorphin is one plausible explanation for the “addiction” of some people to running (Appenzeller, 1981).  $\beta$ -endorphins have recently been shown to be essential for exercise-induced hippocampal cell proliferation in running mice (Koehl et al., 2008). Blockage of  $\beta$ -endorphin in sedentary mice does not affect neurogenesis, but completely blocks running-induced cell proliferation in the hippocampus of physically active mice. However, Koehl and colleagues demonstrated that the effect was compensated by increased survival of newly born cells and decreased cell death. The authors concluded that running-induced  $\beta$ -endorphin production is a key factor for exercise-induced cell proliferation; however, other factors regulate the final number of new neurons. In contrast, other studies claim that endogenously released opioids could hamper neuronal cell survival in the hippocampus (Harburg et al., 2007; Kolodziej et al., 2008). In any case, it is clear that opiate modulation of hippocampal neurogenesis is highly dose- and time-dependent. Several studies demonstrated that chronic exposure and/or high opiate doses (reminiscent of drug abuse) have detrimental effects on rodent hippocampal neurogenesis (Eisch et al., 2000; Mandyam et al., 2004; Kahn et al., 2005).

#### *Opioids and the proliferation and maturation of AHPs*

$\beta$ -endorphin expression (Fig 6) in the developing rodent hippocampus peaks during critical periods of hippocampal proliferation and differentiation (Loughlin et al.,

1985), and expression of  $\mu$ -receptors correlates with regions and times of neurogenesis (Tong et al., 2000). This suggests a natural regulatory role for endogenous opioids in hippocampal neurogenesis during development. In the adult rodent SVZ, all three opioid receptors are expressed (Stiene-Martin et al., 2001).  $\mu$ -receptors are expressed on radial glia in the late embryonic mouse SVZ, but not on proliferating neuroblasts, suggesting a regulatory effect of  $\mu$ -receptors in neurogenesis (Sargeant et al., 2007). In the dentate gyrus, mainly  $\mu$ - and  $\delta$ -receptors have been detected (Kornblum et al., 1987b), although  $\kappa$ -receptors are expressed in other areas of the hippocampal formation (Halasy et al., 2000; Racz and Halasy, 2002). We have previously shown that AHPs express  $\mu$ - and  $\delta$ -receptors *in vitro*, but not  $\kappa$ -receptors (Persson et al., 2003b). In the same study,  $\beta$ -endorphin stimulated AHP proliferation via activation of the mitogen-associated protein kinase (MAPK) pathway. Furthermore, we showed that antagonists directed towards  $\mu$ - and  $\delta$ -receptors increased neurogenesis and reduced proliferation in AHPs (Persson et al., 2003b). To date, there are no previous studies addressing opioid stimulation of AHP cells, with respect to oligodendrogenesis.

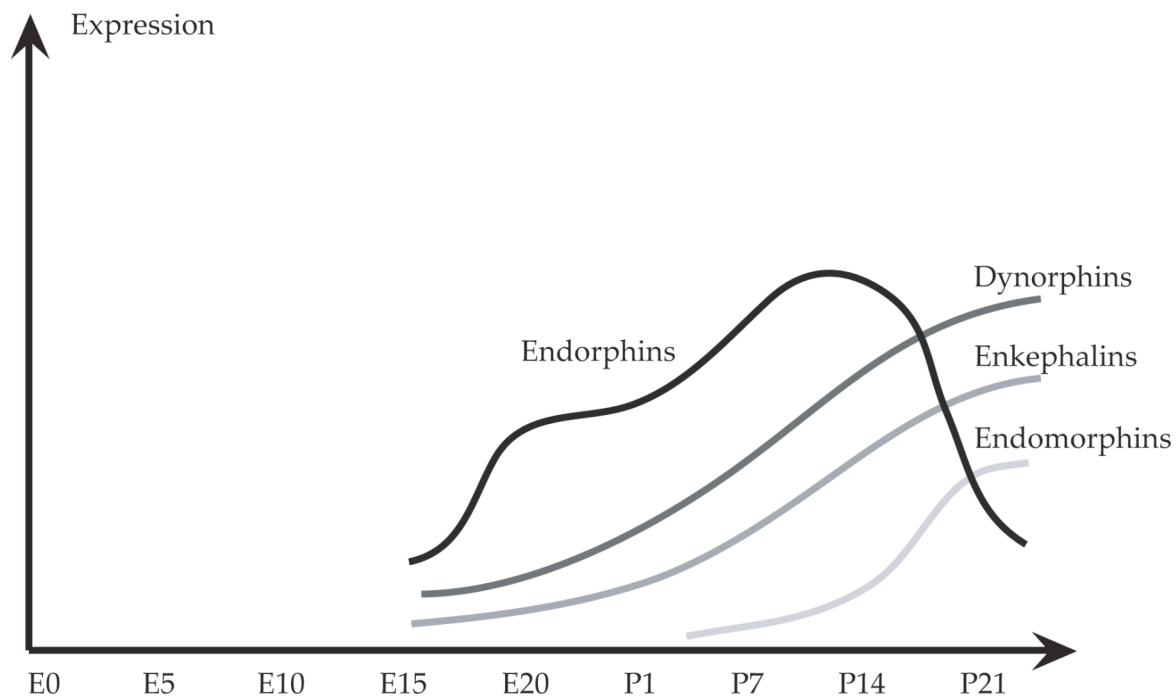


Figure 6 – Opioid expression in the hippocampus during development. Endorphin expression peak correlates with granule cell production in the hippocampus

An anti-proliferative effect of opioids has been reported in astrocytes (Stiene-Martin and Hauser, 1993; Gurwell et al., 1996). Another study showed that opioids could have apoptotic effects on neurons in culture (Hu et al., 2002a). Immature O4-positive oligodendrocytes express  $\mu$ -receptors and proliferate in response to opioids (Hauser et al., 1993; Knapp and Hauser, 1996; Knapp et al., 1998; Tryoen-Toth et al., 2000). Mature oligodendrocytes downregulate  $\mu$ -receptors, and upregulate the expression of  $\kappa$ -receptors.  $\delta$ -receptors are not expressed on immature and mature oligodendrocytes *in vitro* (Knapp et al., 1998). Interestingly, blocking  $\kappa$ -receptor signaling on oligodendrocytes increases the size of myelin-like membranes (Knapp et al., 1998).

In contrast to proliferative effects of opioids on immature oligodendrocytes from the neonatal mouse brain, glial-restricted precursors (GRPs) isolated from embryonic spinal cord and oligodendrocyte-type 2 astrocyte (O-2A) progenitors, isolated from neonatal mouse striatum, react to morphine exposure with increased cell death (Khurdayan et al., 2004; Buch et al., 2007). It is, however, necessary to consider the importance of the overall context of opioid-induced cell responses, as they largely depend on factors such as time, dose, cell type, and developmental stages.

### **Irradiation effects on neurogenesis and oligodendrogenesis**

Proliferating cells suffer DNA damage through ionization or release of free radicals when irradiated, subsequently leading to mitotic catastrophe, growth arrest, and apoptosis (Monje et al., 2002; Kanzawa et al., 2006). Brain irradiation early in life targets proliferating cells, resulting in severe cognitive impairment in humans (Oi et al., 1990; Abayomi, 1996; Mulhern et al., 2004). Depletion of cells in the SGZ by ionizing irradiation is hypothesized to contribute to these cognitive impairments (Raber et al., 2004). The neurogenic regions of the adult brain are highly sensitive to irradiation, due to high proliferative activity in these regions (Mizumatsu et al., 2003). Even low to moderate doses can result in persistent negative effects on hippocampal neurogenesis (Tada et al., 2000; Mizumatsu et al., 2003; Fukuda et al., 2004). Because of the extreme sensitivity of the neurogenic regions to irradiation, compared with surrounding tissue, irradiation can advantageously be used as a tool to study adult neurogenesis and its functional importance in the brain (Wojtowicz, 2006).

Because CNS sensitivity to irradiation is dependent on proliferative activity, postnatal irradiation during the peak of gliogenesis is likely to eliminate a large number of proliferating OPCs residing in the white matter. Children subjected to irradiation for treatment of malignant brain tumors indeed display hypomyelination (Oi et al., 1990). Loss of oligodendroglial precursors after irradiation in the rat neonatal brain has been demonstrated (Sato et al., 2003), and early postnatal irradiation to the rodent brain has been shown to reduce MBP density to less than half one week post-irradiation (Fukuda et al., 2004; Fukuda et al., 2005). However, high doses of irradiation to the mature brain and spinal cord induces apoptosis in residing mature oligodendrocytes. Why mature oligodendrocytes are sensitive to irradiation is not entirely known. One plausible reason is the vulnerability of mature oligodendrocytes to oxidative stress (Jana and Pahan, 2007). Additionally, irradiation to the brain causes inflammation, and oligodendrocytes are sensitive to inflammatory cytokines (Nakazawa et al., 2006). Regardless of the exact underlying mechanisms, the lethal effects of irradiation to oligodendrocytes have been used by scientists to study myelination processes (Hinks et al., 2001; Blakemore et al., 2002; Chari and Blakemore, 2002; Sato et al., 2003).

### **Why do we need new neurons in the brain?**

The preservation of neurogenesis in the adult brain must serve a biological function. We know that the rate of hippocampal neurogenesis correlates with learning and memory; learning itself enhances survival of newborn cells. When subjected to factors that decrease hippocampal neurogenesis, memory performance and learning is decreased. This suggests that the addition of new neurons to the hippocampus must contribute to reconstruction of the hippocampus, as an adaptation of the brain to new challenges in the process of memory and learning (Brüel-Jungerman et al., 2007). Although plasticity in this region, and subsequent improvement in memory and performance, intuitively makes sense, the mechanisms of how new neurons lead to functional improvement are still unknown. In addition, other exciting functions of neurogenesis, beyond memory and learning are beginning to surface. One example

is the involvement of hippocampal neurogenesis in depressive disorders (reviewed in Kempermann et al., 2008).

Sensory experience from odor molecules is thought to target adult-generated neurons in the olfactory system to appropriate regions, and to direct differentiation and affect survival. Adult olfactory bulb neurogenesis is thought to provide a means for adaptation; an organism must navigate between thousands of odor stimuli throughout a lifetime (reviewed in Lledo and Saghatelian, 2005).

### **What is the importance of new oligodendrocytes and myelination in the postnatal brain?**

The wrapping of insulating oligodendrocyte myelin sheets around nerve cell axons allows considerably faster transmission than in non-myelinated axons (Fig 7) (Pellegrino and Ritchie, 1984). Thereby, regulation of oligodendrogenesis and myelination is a process that has a great impact on brain functional performance. Development of white matter structures in children correlates with increased development of motor skills, reading ability, and increased cognitive function (Paus et al., 1999; Casey et al., 2000). Interestingly, increased myelination has been observed in professional piano players (Bengtsson et al., 2005) and cortical thickening has been observed in humans that practice meditation on a regular basis (Lazar et al., 2005). A function of preserved oligodendrogenesis in the adult brain is thought to increase plasticity in the brain by regulating the speed of signal transduction through myelination (for a review over the functional importance of myelin, see Fields, 2005). In addition, NG2-positive cells in the adult brain seems to have other, *e.g.*, regulatory, functions beyond the formation of myelin (Nishiyama, 2007). In accordance with this theory, proliferating NG2-positive cells produce primarily other NG2-positive cells, rather than myelinating oligodendrocytes (Horner et al., 2002).

In multiple sclerosis, the fundamental importance of myelin becomes obvious. The autoimmune activation of T-cell activity results in the loss of oligodendrocytes and, subsequently, myelin. The disruption of effective neuronal transduction can lead to severe physiological, as well as cognitive, dysfunction. However, demyelination also induces regenerative attempts in the brain (Nait-Oumesmar et al., 2007). Cells belonging to the early oligodendrocyte lineage are detected in human multiple sclerosis lesions, although they fail to effectively remyelinate (Chang et al., 2000).

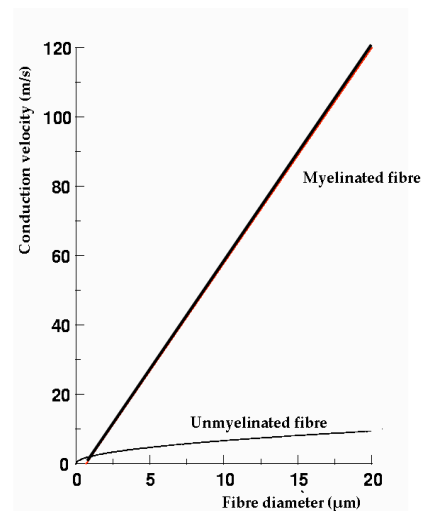


Figure 7 - Myelination of nerve fibers markedly improves conduction velocity (modified from Rushton, 1951)

### **Concluding introductory remarks**

In summary, regulated production of neurons and glial cells is not only a key mechanism of the developing brain, but also an important feature of adult brain function. Proliferating stem/progenitor cells produce new neurons in two brain regions, the hippocampus and the olfactory bulb. New neurons are integrated into pre-existing circuits and initial studies indicate that new neurons improve respective functions of both brain regions. In contrast, oligodendrocytes are produced throughout the brain parenchyma from dividing progenitor cells. It is, therefore, likely that local environmental signals control proliferation and differentiation of these cells. Regulatory signals for generation of oligodendrocytes and neurons

## Brain regeneration: *in vitro* and *in vivo* studies of exercise-related effects

overlap to some extent, but more restricted signals for glial and neuronal lineages are also present. The current project will focus on both neurogenesis and oligodendrogenesis and will analyze three different mechanisms that influence the microenvironment of stem cells:  $\beta$ -endorphin stimulation, physical exercise, and ionizing radiation.



## GENERAL AIM OF DISSERTATION

The projects of this dissertation aimed to address the exercise-related effects on neural stem and progenitor cell plasticity, both *in vitro* with adult stem and progenitor cells, as well as *in vivo* following irradiation-induced reduction of the endogenous pool of stem and progenitor cells.

### Specific aims

- I. To disclose the signaling pathways activated in AHPs by  $\beta$ -endorphin, with special emphasis on opioid-induced oligodendrogenesis.
- II. To determine the effects of voluntary exercise on adult neurogenesis and behavior following irradiation to the young mouse brain.
- III. To determine the effects of voluntary exercise during adulthood on oligodendrogenesis and myelination following irradiation to the young mouse brain.

## METHODS AND METHODOLOGICAL CONSIDERATIONS

### Cell culture (Paper I)

The AHP cell population in Paper I was received at passage 4 as a gift from Dr Fred Gage at the Salk Institute, La Jolla, USA. Differentiation of AHPs in our laboratory using 10 mM forskolin increased the number of neuronal and glial cells expressing differentiated markers in accordance with earlier experiments (Palmer et al., 1997).

The cells are expanded as adherent cultures in 75cm<sup>2</sup> polyornithine coated flasks in Dulbecco's MEM/Nut Mix F12 plus 2.5 mM L-glutamine and N2 supplement (N2 medium, GIBCO Invitrogen Corp., Scotland, UK). Twenty ng/ml human basic FGF-2 (Peprotech Inc., NJ, USA) is used for propagation of AHPs in an undifferentiated state. The culture medium is exchanged every two days. Cells for *in vitro* experiments were used between passages 10 and 20 postcloning.

#### Comments:

AHPs can be isolated and expanded *in vitro* with FGF-2 or other growth factors, retaining their original characteristics for at least 35 passages. Isolated subclones, marked with a retroviral vector, are similar to the parental culture with respect to morphology and expression on cell type specific markers even after 10 population doublings. The cultures used here are clonal; originally descending from one single stem cell with the ability to produce all three major CNS cell types. The AHP cells produce these cell types *in vitro*, and when transplanted back into the hippocampus they generate new granule cells (Gage et al., 1995; Palmer et al., 1997). However, it cannot be excluded that when propagating the AHPs, a certain cell type is selected for. Additionally, over time genetic mutations can emerge that might lead to morphological changes, loss of growth control or changes in the normally diploid karyotype (Yosida, 1983; Kerler and Rabes, 1994). Therefore, it is desirable to use a passage as low as possible when performing experiments.

Cultured without FGF-2 in N2 medium until density arrest is reached, AHPs will produce neurons and astrocytes more or less exclusively. If cultured in a low concentration of FGF-2 (5ng/ml), or in a high concentration (20ng/ml) until density arrest is reached, they will mainly differentiate into oligodendrocytes. AHPs differentiating into oligodendrocytes in culture will go from expressing O4, nestin, Map2c and NSE to an O4-negative/A2B5-positive intermediate stage, and finally express mature markers such as MBP (Palmer et al., 1997).

### Immunocytochemistry (Paper I)

In Paper I, quantitative analysis was performed using immunocytochemistry. Cells were plated onto polyornithine/laminin-coated cover glass slides in 24 well plates at a density approximately 1500 cells/cm<sup>2</sup>. For all experiments, cells of four different passages were used. The cells were incubated overnight in N2 medium containing 20 ng/ml FGF-2 that was replaced with N2 medium alone (control cultures) or N2 medium containing 1  $\mu$ M  $\beta$ -endorphin, 10 nM  $\beta$ -endorphin, or co-incubation of 1  $\mu$ M  $\beta$ -endorphin with 10  $\mu$ M naloxone. The N2 medium was replaced every 2 days. These experiments were repeated four times. After 10 days *in vitro*, the cells were washed in PBS and fixed in 4% paraformaldehyde. After fixation, cells were rinsed in PBS and incubated in room temperature for 30 minutes in blocking solution containing PBS, 0.05% saponin (Sigma-Aldrich Sweden AB) and 3% bovine serum albumin (BSA, Sigma-Aldrich Sweden AB). Primary antibodies in blocking solution with PBS and 1% BSA and 0.05% saponin were added to the glass slides during 1 hour in room temperature, in order to bind to the epitope. When staining for the

transcription factor Mash1, blocking and incubation steps were performed using 3% donkey serum instead of bovine serum albumin (Jackson ImmunoResearch Laboratories Ltd., Cambridgeshire, UK) and 0.2% Triton-X. After rinsing thoroughly three times with PBS, secondary antibodies were incubated for an additional hour. Glass slides were then rinsed to remove excess antibody, and mounted upside down on microscope slides (SuperFrost Plus, Menzel GmbH & CO KG, Braunschweig, Germany) with mounting medium (Dako fluorescent mounting medium, Dakopatts, Älvsjö, Sweden).

A minimum of 1500 cells were counted in at least 15 non-overlapping fields on two cover glass slides for each treatment. Counting was performed using 40 times magnification in a Nikon Diaphot microscope, equipped with a cooled CCD camera (Hamamatsu, Hamamatsu, Japan). For quantification of Mash1-positive cells, approximately 1000 cells in 5 non-overlapping fields were counted for each treatment and experiment (at least 4000 cells/treatment). Antisense experiments were performed three times for all treatments. Approximately 500 cells were systematically observed in total at least 10 non-overlapping fields for each treatment. Staining of individual nuclei with Hoechst 33258 (Sigma-Aldrich, Sweden) was used to discriminate total number of cells per field of view. All cell-counting procedures were performed blindly with the microscope slides coded.

#### *Comments:*

The possibility of unspecific binding has been taken into account when performing immunocyto- and histochemistry. The antibodies used to identify different cell types or proteins have been used in previous studies and have been shown to be antigen-specific and the localization of the immunoreactivity in our experiments was in accordance with previous literature.

When performing immunocytochemistry for cell membrane or cytoplasmic antigens, saponin is often used as a detergent to allow the primary antibodies to penetrate the cell membrane. Triton-X is a stronger detergent and is used here to facilitate penetration into the nucleus, where Mash1 is expressed.

#### **Antisense technology (Paper I)**

For our Id1 antisense and sense construct, we used the following sequences of oligonucleotides, purchased from Cybergene AB (Sweden):

Id1 Antisense 5'-GCGACCTTCATGATCCTG AG-3'

Id1 Sense: 5'-CTCAGGATCATGAAGGTCGC-3';

It is necessary to verify that Id1 antisense indeed penetrates the cell membrane and causes a reduction of intracellular Id1 protein levels. Therefore, AHPs were seeded onto polyornithine/laminin coated glass slides in 6-well plates at a density of approximately 1500 cells/cm<sup>2</sup> and cultured with or without 100 µg/ml Id1 antisense or sense oligonucleotides for 48 h in N2 medium. Cells were then stained with antisera raised against Id1 and nestin. Nestin-positive cells incubated with antisense towards Id1 expressed lower levels of Id1 compared with sense-treated cells, or cells cultured in plain N2 medium, suggesting that Id1 antisense had penetrated the cell membrane and bound to its target.

To evaluate if Id1 expression regulates gliogenesis in AHPs, we incubated cells seeded in 6-well plates onto polyornithine/laminin coated glass slides (approximately 1.5 x 10<sup>3</sup> cells/cm<sup>2</sup>) in N2 medium without FGF-2 (control cultures) or in N2 medium with 1 µM β-endorphin or 100 µg/ml Id1 sense or 100 µg/ml Id1 antisense. Additionally, some cultures were incubated with β-endorphin together with Id1 sense or antisense oligonucleotides, respectively. Sense or antisense

oligonucleotides were added 2 h prior to incubation with  $\beta$ -endorphin. The medium was changed every second day. After ten days, cells were stained with markers for Id1, nestin and the oligodendrocyte marker RIP and the coverslips mounted upside down on microscope slides (SuperFrost Plus, Menzel GmbH & CO KG, Braunschweig, Germany) with mounting medium (Dako fluorescent mounting medium, Dakopatts, Älvsjö, Sweden). These experiments were repeated three times.

*Comments:*

Antisense technology is a method where synthesized, highly selective oligonucleotides inhibit the production of a functional protein by binding to its mRNA. Antisense technology can be used to study events following elimination of a protein both *in vitro* and *in vivo*. *In vitro*, short single stranded sequences (often 15-20 bp), complementary to a target sequence of mRNA nucleotides, are added to the culture medium and incorporated into the cell by diffusion or endocytosis. Bound to its target mRNA, it is thought to cause disruptions of the translational process or enhance degradation of the mRNA (Shi and Hoekstra, 2004). The exact mechanisms of antisense technology are still unclear. Due to the small size and low abundance of the oligonucleotides, it is difficult to confirm their exact target and intracellular method of action. The major issues affecting the results using antisense are their specificity, affinity to the target, stability, toxicity and uptake. Previous studies, using identical Id1 antisense oligonucleotides in primary astrocyte cultures, have been shown to inhibit Id1 protein expression (Tzeng and de Vellis, 1997).

The Id1 antisense and sense oligonucleotides were modified with phosphorothioate, which increases stability, makes them water-soluble and increases penetration efficiency (Weiss et al., 1997). The sense is a non-functional sequence that is added as a control to rule out non-specific effects caused by the addition of oligonucleotides to the cell medium.

### **cDNA array (Paper I)**

*Isolation and purification of RNA and protein*

In paper I, we cultured cells seeded onto polyornithine/laminin coated 6-well plates with/without 1  $\mu$ M  $\beta$ -endorphin or in combination with 1  $\mu$ M naltrindole or 10  $\mu$ M  $\beta$ -funaltrexamine for 48 h. We isolated RNA using a strong denaturant containing 4 M guanidiumthiocyanate, 0.1 M 2-mercaptoethanol, 25mM sodium citrate pH 4.0, 0.5% sarcosyl and H<sub>2</sub>O-saturated phenol. Chloroform-isoamylalcohol (24:1) was additionally added to the sample, and RNA could be collected in the top water phase after centrifugation (Chomczynski, 1993). RNA was then precipitated with isopropanol, followed by centrifugation and washed in ethanol (70%). After the last centrifugation, the remaining RNA pellets were dissolved in a small amount of H<sub>2</sub>O and the total RNA yield from each sample was determined spectrophotometrically. The quality of the total RNA was tested on a formaldehyde/agarose/ethidium bromide gel. Two bands at 4.5 and 1.9 kb, representing 28S and 18S ribosomal (r)RNA, should appear on the gel if the RNA has not been degraded.

*Labeling*

To label our RNA we used CLONTECH's Atlas<sup>TM</sup> Pure Total RNA labeling system (Catalog #: K1038-1, Becton-Dickinson Biosciences, Clontech Laboratories Inc., CA, USA) according to the user manual (PT3140-1).

Total RNA was converted into cDNA and labeled with radioactive [ $\alpha$ -P<sup>32</sup>]dATP (Amersham Pharmacia Biotech, IL, USA) before hybridization to array membranes. In brief, total RNA samples first underwent enrichment for PolyA<sup>+</sup>-RNA by using

biotinylated Oligo(dT) and streptavidin-coated magnetic beads included in the kit. PolyA<sup>+</sup> -RNA attach to the biotinylated Oligo(dT), which in turn attach to streptavidin on the beads, making separation possible. PolyA<sup>+</sup> -RNA was then converted to radioactive labeled cDNA by incubation with a primer mix containing primers for genes included on the membrane, reverse transcriptase, nucleotides and [ $\alpha$ -P<sup>32</sup>]. Using column chromatography, labeled cDNA was separated from excess [ $\alpha$ -P<sup>32</sup>] nucleotides and small cDNA fragments and radioactivity of the samples was measured using a scintillation counter.

#### *Hybridization and autoradiography*

Unspecific binding caused by impurities was first tested for by hybridizing some of the labeled cDNA to a blank membrane. Each probe was then hybridized to a separate array membrane, at 68°C overnight. To avoid unspecific binding, DNA from sheared salmon testes was added. After extensive washes, the membranes were wrapped in plastic wrap and exposed to x-ray film, here Kodak BioMax MS film (Eastman Kodak Co., Rochester, NY, USA), in -80 °C for varying lengths of time (hours to days) to visualize both strongly and weakly expressed genes (Fig 8).

#### *Data handling and interpretation*

To analyze data, we used the software AtlasImage<sup>TM</sup> 1.5 (Clontech). Arrays were aligned to a grid template. Spots exhibiting extremely strong signals were excluded in the analysis, and only spots that were regulated more than two-fold were singled out for further investigation.

#### *Comments:*

cDNA array is an efficient method to simultaneously screen the expression of a large number (hundreds to tens of thousands) of genes in a sample, creating a large amount of highly quantitative data (Taniguchi et al., 2001). *Focused* arrays are designed to hold only genes expressed from a specific area of interest, such as genes expressed in the CNS, or a smaller number of well-known genes. The Atlas<sup>TM</sup> rat cDNA expression array (Catalog #: 7738-1, Becton-Dickinson Biosciences, Clontech Laboratories Inc.) includes 1176 genes with a known function, arranged into functional classes on the membrane. Each cDNA fragment is about 200-600 bp long and arranged in spots on a nylon membrane, where each spot corresponds to a gene. The manufacturer has verified the size and identity of all cDNAs by PCR amplification, gel electrophoresis and sequencing. According to the manufacturer, the arrays can detect mRNA at as low of levels as 10-20 copies per cell. Comparisons between Affymetrix and cDNA microarrays show that both arrays produce comparable and reliable data (Yuen et al., 2002).

When adding chloroform-isoamylalcohol to the sample, three phases will form after centrifugation. Proteins will be trapped in the bottom phenol phase, DNA in the middle chloroform phase and RNA in the top water phase (Chomczynski, 1993). When working with cDNA array technology, it is important that equal amounts of RNA are added to the membranes. The most important factor influencing the quality of data generated with cDNA array, however, is the purity of the RNA yield. Precipitating with a strong denaturant containing phenol will not only extract RNA and DNA out of the cell but also inactivate RNases. However, before proceeding to the labeling step, the quality of the total RNA has to be tested (here on a formaldehyde/agarose/ethidium bromide gel) to visualize any possible degradation.

Included on the membrane are nine housekeeping control cDNAs and 3 plasmids and bacteriophage DNAs as negative controls. These are used to normalize the

signal intensity and to assess unspecific binding, respectively. The housekeeping control cDNAs that are used for normalization should be unaltered between the samples and then serve as positive controls. By adjusting the signal intensity between the housekeeping control cDNAs on each membrane, the overall signal intensity between spots will be normalized. If comparing unrelated tissue or cells, the signal intensity between the same housekeeping genes on two arrays can vary greatly. In such cases, normalization can be executed by choosing cDNA signals from the surrounding area of each spot. Alternatively, the average intensity of all nine housekeeping cDNAs can be used. We normalized by comparing the spot intensity of the housekeeping genes between the arrays.

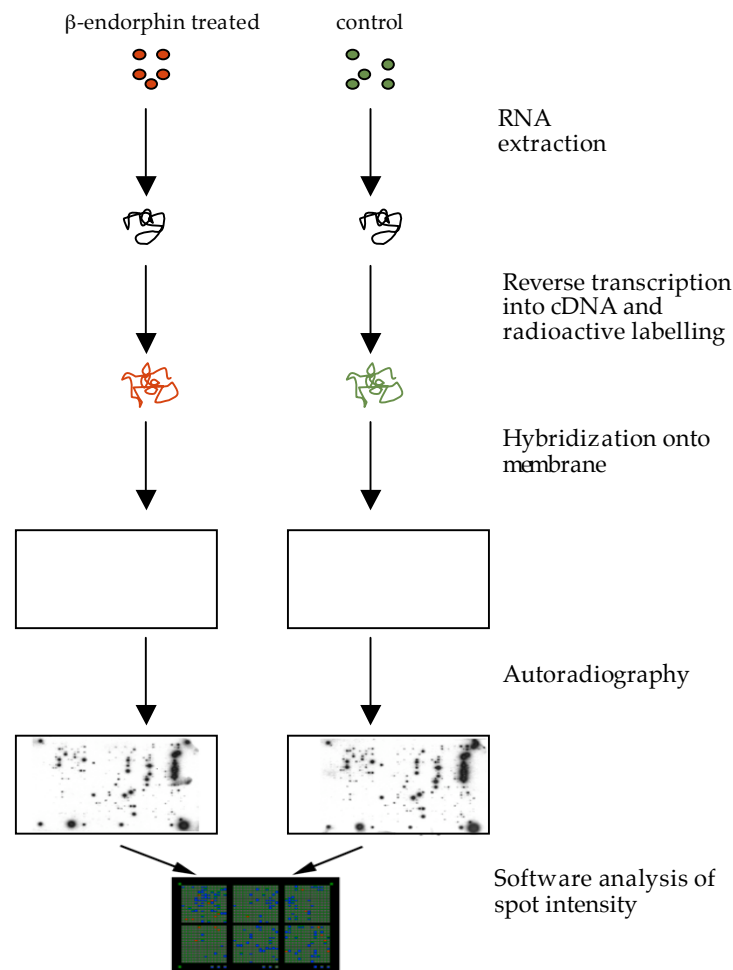


Figure 8 - Principal steps when analyzing gene regulation using Atlas™ rat cDNA expression array.

### Western blot (Paper I)

AHPs were plated at a density of approximately 1000 cells/cm<sup>2</sup> onto 6-well plates and propagated in N2 medium with FGF-2 for 4 days. Cells were then cultured overnight in N2 medium without FGF-2. Next day, 100 µl β-endorphin was added to a final concentration of 1 µM for 1 h, whereas control cultures received the same amount of medium. Cells were lysed in RIPA-buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium desoxycholate, 0.1% sodium dodecyl sulfate (SDS)) and a protease inhibitor cocktail was added (Sigma-Aldrich Sweden AB, pH 7.5).

After mixing and a brief centrifugation, protein concentration was determined in the supernatant fraction using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA).

#### *Protein separation*

For the protein separation procedure, we loaded about 20 µg of protein from each sample, leaving one lane for a molecular marker (MultiMark, Invitrogen, CA, USA) on the SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel; 10% acrylamide separation gel with Tris HCl pH8.8, 4% acrylamide stacking gel with Tris HCl pH 6.8, both containing 0.1% SDS).

#### *Incubation with antibodies*

After separation, proteins are transferred from the gel to a membrane, again by electrophoresis. The membranes are usually made from nitrocellulose or PDVF (polyvinylidene fluoride). Here, PDVF membranes (Immobion-P, Millipore, MA, USA) were used. In this step some proteins can be lost; very large proteins (>80 kDa) might not bind well to the membrane, and very small proteins could migrate through (Westermeier and Marouga, 2005), although our protein of interest did not belong to any of these categories.

Our PVDF membranes were first prepared by 30-min incubation with 5% milk powder in Tris-buffered saline (TBS) solution. This prevents unspecific binding of the primary antibody. Overnight incubation at 4°C with primary antibody Egr-1 (diluted 1:2000, Santa Cruz Biotechnology Inc., CA, USA) was also done in blocking buffer. Membranes were then washed in TBS with 0.05% Tween-20 (TTBS), having a somewhat stronger denaturing effect, and blocked for 30 min in TTBS with 5% milk powder. Horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (1:1000; Amersham Biosciences Europe GmbH) was used as secondary antiserum in blocking buffer for 30 min at room temperature.

#### *Visualization of protein bands and analysis*

After thorough washes of the membranes in TTBS, proteins were visualized with a BM Chemiluminescence Blotting Substrate kit (Roche Diagnostics GmbH, Germany). For autoradiography, we used Kodak XAR-5 film (Eastman Kodak Co.), scanning and processing with Adobe Photoshop. Analysis was carried out with Scion Image 4.0 (Scion Corp., MA, USA). To adjust the signal strength of the bands according to the amount of protein loaded in each lane, we stained the gels with Coomassie Brilliant Blue (Merck Eurolab, Germany). The value of the integrated density for protein bands in each lane was then divided by the integrated density value of the Coomassie staining in the same lane.

#### *Comments:*

The principle of western blot is to separate proteins on a one-dimensional gel by electrophoresis, followed by immunodetection using antibodies. When adding SDS to the protein sample, the proteins unfold and acquire a uniform negative charge. During electrophoresis, the negatively charged proteins will be drawn through the gel matrix towards the positive end. The gel itself acts as a sieve, the smaller proteins moving faster than the larger ones. In this way, proteins are separated only according to their size.

The BM Chemiluminescence Blotting Substrate kit contains luminol and H<sub>2</sub>O<sub>2</sub>. HRP will, in the presence of H<sub>2</sub>O<sub>2</sub>, catalyze the oxidation of luminol, thus forming a reaction product. This reaction product decays to ground state by emitting light. Using autoradiography, the signal intensity and protein amount can be measured.

## **Reverse Transcription Polymerase Chain Reaction (RT-PCR) (Paper I)**

On the cDNA arrays, interesting genes, displaying a 2-fold or greater change in mRNA levels in response to  $\beta$ -endorphin treatment, were chosen and confirmed by Reverse Transcription Polymerase Chain Reaction (RT-PCR). These were Id1, Id3, myelin basic protein (MBP), c-JunD, glutathione S-transferase pi (GST pi) and rab16. All RT-PCR experiments were performed three times for each treatment and each sample was tested twice. Reagents in RT-PCR experiments in Paper I were purchased from Promega (Promega, Madison, WI, USA). The PCR amplification was performed using a T3 thermocycler (Biometra, Göttingen, Germany).

### *The RT reaction*

To produce cDNA, random hexamers were added to 4  $\mu$ g of total RNA (previously prepared for the cDNA array experiments) for each sample. The mixture was heated to 70°C for 5 min and then quickly transferred to ice. Thereafter, nucleotides (deoxynucleotides triphosphates; dNTPs), Moloney murine leukemia virus reverse transcriptase (MMLVRT), MMLVRT buffer and RNase inhibitor were added and the mixture heated to 37°C for one hour.

### *The PCR reaction*

In the PCR-reaction, gene-specific primers, which coded for the same sequences that produced the cDNA fragments on the array membranes, were used. To verify equal starting amount of cDNA in each sample, we used primer pairs coding for the housekeeping gene ribosomal protein L27 (RPL27).

After the gene-specific primers were added, nucleotides, Taq polymerase and magnesium chloride (MgCl<sub>2</sub>) were introduced to the cDNA mixture. The samples were pre-heated for 2 min at 94°C, followed by an optimized number of cycles at 94°C for 30 seconds, 57°C or 55°C for 30 seconds, and 72°C for 30 seconds. The programs were finished by a 5 min extension step at 72°C followed by 4°C. The PCR amplification was performed using a T3 thermocycler (Biometra, Göttingen, Germany).

### *Visualization of mRNA bands and analysis*

Twenty microliters of each sample were run on a 2% agarose gel and bands were detected using the transilluminator Ti 3, Biometra. Pictures of the gels were taken using KODAK DC290 Zoom Digital Camera (Eastman Kodak Company) and processed using KODAK ID image analysis Software (Eastman Kodak Company). The integrated density of each band of amplified mRNA products was calculated using the software Scion Image 4.0 (Scion Corp) and divided by integrated density of the housekeeping gene RPL27. Only bands at the expected molecular weights for respective primer pairs were detected after optimizations. When reverse transcriptase was omitted in the RT-step, or when cDNA was omitted in the PCR-step, no mRNA product for any gene was detected.

### *Comments:*

Using RT-PCR, extremely low levels of mRNA transcripts can be detected in a sample. The concept is to convert total RNA into single stranded cDNA, using it to amplify the mRNA transcripts in the initial sample to such amounts that it can be visualized on a gel.

To produce cDNA, random hexamer primers, nucleotides (deoxynucleotides triphosphates; dNTPs) and Moloney murine leukemia virus reverse transcriptase are added to the total RNA. Reverse transcriptase is a retroviral enzyme that will



produce cDNA by combining the dNTPs, when the primers have annealed to the mRNA. The different steps during RT-PCR amplification (*e.g.*, denaturation, annealing, and elongation) are highly dependent on temperature. In the RT step leading to cDNA synthesis, 70°C will allow the annealing of random hexamer primers to total RNA. Lowering the temperature to 37°C will then activate the reverse transcriptase.

Following the RT step, the cDNA is amplified in the PCR step using additional components. Instead of random hexamers, gene-specific primers are added leading to amplification of the gene transcripts of interest only. Nucleotides and Taq polymerase, a heat stable bacterial DNA polymerase, are also added to produce complementary strands after the annealing of the primers. Magnesium chloride (MgCl<sub>2</sub>) enhances the binding strength of the primers and is included in the mixture as well. In the PCR step, an initial heating of the sample to 94°C will cause denaturation of the mRNA-cDNA complex, allowing gene-specific primers to access the cDNA strand. Lowering the temperature (approximately 55°C, depending on primer) will permit the primers to anneal to the cDNA strand. Increasing the temperature to 72°C will activate the polymerase to begin replicating the cDNA. Each cycle of denaturation, annealing and elongation of the strands will exponentially yield more transcripts. An initially low number of a certain mRNA will require more cycles. Temperature and reaction mixtures also affect the outcome. If the temperature is too high in the annealing phase, there is a risk of unspecific binding by the primers, while a temperature that is too low may result in no binding at all. MgCl<sub>2</sub> can, at exaggerated concentrations, also result in unspecific binding of the primers. Furthermore, components, such as primers and dNTP, can become limited after a number of cycles, leading to a plateau phase where differences between samples are lost. The primer design itself is also of importance, and can affect the efficacy of the amplification. To make sure that we were in the linear range, we optimized with different number of cycles and different amounts of DNA.

After the PCR step, the end product can be visualized by gel electrophoresis and UV-light. Ethidium bromide is added to the gel and will be incorporated into the DNA, illuminating it under UV-light. By omitting reverse transcriptase in the RT step, possible DNA contamination of the samples can be assessed. If RT is omitted in a pure sample, an end product should not be detected. If cDNA is omitted in the PCR step, contamination from the surroundings can be excluded.

### **Experimental set up (Paper II and III)**

Male C57/BL6 mice (Charles River Breeding Laboratories, Germany) were used for all experiments. All animals were housed at a constant temperature (24°C), with 50-60% relative humidity. A 12-h dark/light cycle was maintained with lights from 19:00 to 07:00, and food and water was available *ad libitum*. All animal experimental procedures were approved by the Göteborg committee of the Swedish Animal Welfare Agency (application number 407-2004).

To investigate the effect of irradiation on cell proliferation in the young postnatal mouse (Paper II and III), pups (n=4 in each group) were subjected to irradiation (Irr) or sham-irradiation (sham; anaesthetized but not subjected to irradiation) at P9. To label proliferating cells, mice were injected with a single injection of BrdU (100mg/kg) at P10, and sacrificed 12 hours later at P11.

To investigate the effect of irradiation on cell proliferation and oligodendroglial numbers in the young mouse (Paper III), pups (n=6 in each group) were subjected to irradiation (irr) or sham-irradiation (sham) at P9. The animals were then housed with their biological dams and subsequently sacrificed at P23.

Brain regeneration: *in vitro* and *in vivo* studies of exercise-related effects

For experiments including voluntary exercise (Paper II and III), twenty-four male C57BL/6 mice pups at nine days of age (P9) were randomly divided into four groups; sham-irradiated (sham), irradiated (irr), runners (run) and irradiated runners (irr/run). The mice were anaesthetized 10 minutes before irradiation by an intraperitoneal injection of tribromoethanol (Sigma, Stockholm Sweden). Animals belonging to the control and the runner group were anesthetized but not subjected to irradiation. Following cranial irradiation at P9, pups were returned to their biological dams and allowed to rest for 7 weeks. At the start of week 7, the animals were transferred to individual cages for acclimatization to individual housing (P55). Mice in the running groups had free access to a running wheel that was mounted in the cage, and wheel revolutions were automatically registered with customized computer software. At P62, the running wheels were unlocked, and all groups received BrdU, 50 mg/kg per day for 5 consecutive days. Animals remained in their relevant housing conditions and were sacrificed four weeks after the first day of BrdU injections (P93) (Fig 9). All mice were weighed weekly during the running experiment to monitor potential weight loss.

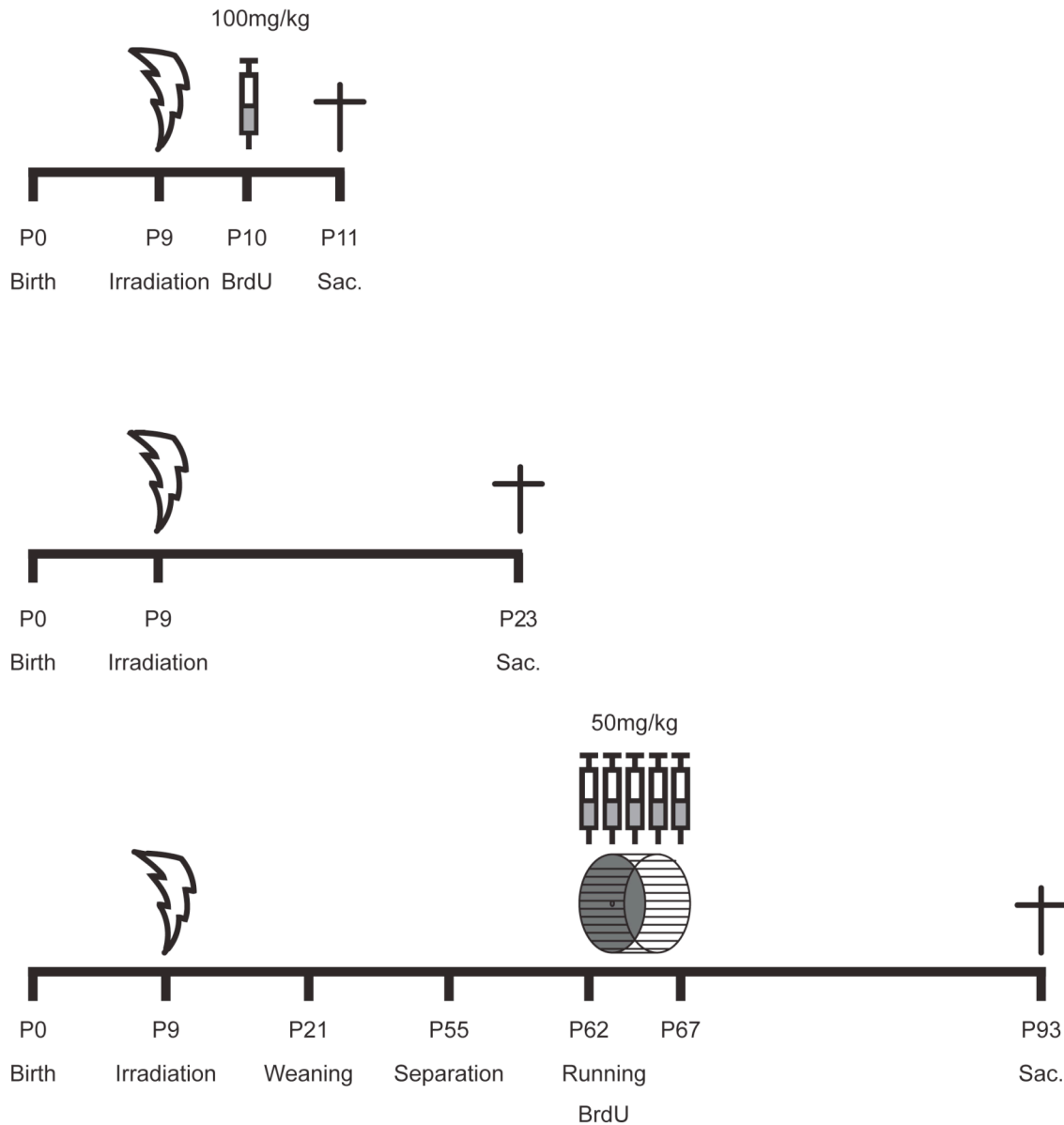


Figure 9 – Experimental set-up for Paper II and III

### **Irradiation procedure (Paper II and III)**

Mice were exposed to cranial irradiation, using a linear accelerator (Varian Clinac 600 CD; Radiation Oncology Systems LLC, San Diego, CA, USA) with 4 MV nominal photon energy, 2.3 Gy/min, and a radiation field of 2x2 cm. The irradiation source was placed 99.5 cm away from the skin, and the head was covered with 1-cm thick bolus material to obtain even irradiation throughout the underlying tissue. Each mouse received a total dose of 6 Gy. Dose variation within the target volume was estimated to be  $\pm 5\%$ . The entire procedure was completed within 3 minutes for each animal. After irradiation, the pups were housed with their biological dams.

#### *Comments:*

Proliferating cells are particularly vulnerable to irradiation due to exposed DNA during mitosis. Therefore, CNS sensitivity to irradiation is especially high in young subjects, due to extensive proliferation early in life. Postnatal proliferation in the hippocampus peaks at P9, and during this time, the proliferation of oligodendrocyte precursors in the corpus callosum accelerates. In humans, this period corresponds to children less than three years of age. Using the linear quadratic model and a  $\alpha/\beta$  of 3 for late effects in normal brain tissue, the acute exposure of 6 Gy in this study corresponds to 12 Gy when delivered in 2 Gy fractions. Children treated for leukemia relapse are subjected to 18 Gy whole brain irradiation, although the side effects of the treatment are so severe so that children under 4 years of age are normally not treated with radiation therapy.

### **BrdU injections (Paper II and III)**

Intraperitoneal injections of BrdU (P10; 100mg/kg for and for P62; 50 mg/kg once per day for 5 consecutive days) were used to label dividing cells at the time of injection.

#### *Comments:*

Bromodeoxyuridine (BrdU) is a thymidine analogue that is incorporated into DNA during S-phase of the cell cycle, labeling proliferating cells (Miller and Nowakowski, 1988) Incorporated BrdU can be detected years after administration (Komitova, 2005), but excess BrdU (or unincorporated) will be metabolized by liver enzymes within 2 hours after administration (Penit, 1986). Therefore, only cells that undergo division at the time of BrdU administration will be labeled. Endogenous proliferation markers such as phosphohistone-H3, Ki-67 and PCNA are, in contrast to BrdU, transient and will be downregulated when cells proceed to differentiate or return to a quiescent state. For this reason, endogenous cell proliferation markers are not used when tracing the fate of cells that have undergone division.

Another method to label newborn cells is [<sup>3</sup>H]thymidine autoradiography. However, as radioactive compounds such as [<sup>3</sup>H]thymidine can inhibit proliferation and induce apoptosis, this method has been criticized (Hu et al., 2002b). BrdU labeling also has its drawbacks though – firstly, it is carcinogenic and must therefore be handled with care, and it may also be toxic to animals and cells at large doses. Secondly, there is a risk of BrdU incorporation in cells during DNA repair, although this should only result in very weak labeling.

### **Open field test (Paper II)**

Open field test was conducted for one hour on three consecutive days, starting at P90. On the day of the experiment the animals were individually introduced into an unfamiliar, open field arena and videotaped for 60 minutes with a CCD monochrome video camera connected to an S-VHS videocassette recorder. The

arenas were made of black plexiglass (l, w, h: 46 x 33 x 35 cm), rubbed with sandpaper and indirectly illuminated to avoid reflections and shadows. The floors of the arenas were covered with grey gravel that had earlier been exposed to other mice.

The video tracking was performed at a sampling frequency of 12.5 Hz. After completion of the experiment, the videotapes were analyzed with the video-tracking program EthoVision Color-Pro 2.3.19 (Noldus Information Technologies, the Netherlands). 108 variables describing different aspects of motor activity, movement path shape and exploratory activity were generated and summarized into 10-minute bins. Animals were sacrificed immediately after testing (P93).

*Comments:*

Open field test is a method for movement pattern analysis and has been described in detail (Nilsson, 2005). Briefly, when a naïve mouse is introduced to a new environment it will display an active behavior, including exploration, grooming, digging etc. Different treatments or events may change the behavior of the animal. During open field testing, a camera records the behavior of the animal in an arena over a period of time. The behavior is then analyzed using a computer program. The program records the position of the mouse for each sampling occasion, together with the animal's body area detected from the overhead camera. The analysis results in a track record that describes the animal's behavioral pattern, measuring and comparing more than a hundred different parameters, such as velocity, rearing, stops, and so forth. The variables in Paper II were calculated on the entire arena as well as in a middle zone (m), defined as the central part located 6 cm or more away from the border of the arena. The reason for performing separate analyses on activities performed in the middle zone is that the spatial variables, e.g. turning, sinuosity and meander, as well as the number of stops, can be regarded as spontaneous movements in the middle zone as they are not influenced by the restrictions of the walls of the arena. This may not be true if the animal's behavior is highly stereotyped though; therefore, the gross appearances of the animals are also studied.

In Partial Least Square (PLS) analysis, the relationship between the measured variables is explored and the co-variances are used to reduce the original set of variables to a smaller set, commonly referred to as latent variables or principal components. The principal components are not correlated to each other and they show significant relation between the original variables. The objective with PLS-DA (Partial Least Square Discrimination Analysis) is to find a model that separates classes of observations on the basis of their X-variables. The X-matrix in this case consists of the measured behavioral variables (Table 1, Paper II). In order to encode a class identity, we used a Y-data matrix of dummy variables describing the class membership of each observation. A dummy variable is an artificial variable that assumes a discrete numerical value for the class description, in this case denoting the treatment group.

The results of these analyses are presented as low dimensional projections or loading plots, where the relations between the X and Y variables are visualized. The loading plot shows how the measured variables are correlated and how they are influenced by the treatment. Variables close to each other have similar characteristics and a location far from origin indicates high discriminating importance.

### **Perfusion procedure and tissue preparation (Paper II and III)**

Animals were anaesthetized at P93 with pentobarbital sodium (60 mg/ml) 2ml/100 g body weight) and perfused with 0,1M phosphate buffer followed by 4%

paraformaldehyde in 0,1M phosphate buffer. The brains were removed and post-fixed with 4% paraformaldehyde in 0,1M phosphate buffer for 24 hours at +4°C before being transferred to 30% sucrose in 0,1M phosphate buffer and stored at +4°C.

The brains were cut frozen in consecutive series of 25µm thick sections on a sliding microtome (Leica SM 2000R). Sections were stored in tissue cryoprotectant solution containing 25% ethylene glycol, 25% glycerol, and 0.1 M phosphate buffer at +4°C.

#### *Comments:*

Perfusion forces intravascular contents out of the tissue, resulting in a cleaner staining and less autofluorescence- a common problem when using other methods for tissue preparation. A disadvantage of perfusion is that some antigens, such as NG2, are sensitive to paraformaldehyde treatment and that fixation is not suitable for protein or mRNA analysis.

Common ways to perform sectioning of tissue are fresh-frozen, fixed-frozen, or paraffin embedded. Fresh-frozen tissue is usually cut with a cryostat; however, we use a technique in our lab of cutting fixed-frozen tissue with a sliding microtome, resulting in free-floating sections. If the target antigen is sensitive, brains can be fresh-frozen and cut on a cryostat without any preceding fixation, directly transferring sections onto slides. However, the production of serial sections for stereology is difficult with this method. To obtain reproducible serial sections, cutting with a sliding microtome and subsequent storage of the sections in TCS is more suitable. Paraffin embedding of the tissue into a solid form can be necessary when, for example, handling damaged brains that easily fall apart. Paraffin embedded tissue also allow for very thin sectioning (*e.g.*, 5µm), which allows the antibody to penetrate the tissue better and to avoid multiple layers of cells when quantifying. Fixed-frozen tissue yields fewer sections, since it is usually sectioned thicker (*e.g.*, 20-40 µm) to avoid ruptures and breakage. This is, however, not a disadvantage when producing serial sections and also allows for three-dimensional reconstruction of the tissue using confocal microscopy. Three-dimensional reconstruction with confocal microscopy facilitates the evaluation of co-localization of markers, and permits discrimination between cell bodies (Kuhn and Cooper-Kuhn, 2007).

#### **Immunohistochemistry (Paper II)**

For BrdU, DNA denaturation was conducted by incubation for 30 min in 2 N hydrochloric acid at 37°C, followed by 10 min in 0.1 N borate buffer (pH 8.5). After washing, sections were incubated for 30 min in 0.6% H<sub>2</sub>O<sub>2</sub>, blocked with 3% normal donkey serum in 0.1% Triton X-100, then incubated with monoclonal anti-BrdU (1:500; Nordic Biosite, Sweden) overnight at 4 °C. Sections were then washed in TBS, placed in the secondary antibody (biotinylated donkey anti-mouse; 1:1000; Jackson ImmunoResearch Laboratories, PA, USA) followed by amplification with avidin-biotin complex (Vectastain ABC Elite, Vector laboratories, CA, USA) and then visualized using a detection solution (0.25 mg/ml diaminobenzidine, Saveen Biotech AB, Sweden).

For DCX, after antigen retrieval (sodium citrate, pH 9.0), sections were washed, incubated for 30 min in 0.6% H<sub>2</sub>O<sub>2</sub>, blocked with 3% normal donkey serum in 0.1% Triton X-100, then incubated in polyclonal goat-anti DCX (1:250; Santa Cruz Biotech., CA, USA) overnight at 4 °C. Sections were then washed in TBS, placed in the secondary antibody (biotinylated donkey anti-goat; 1:1000; Jackson ImmunoResearch Laboratories, PA, USA) followed by amplification with avidin-biotin complex (Vectastain ABC Elite, Vector laboratories, CA, USA) and then visualized using a detection solution (0.25 mg/ml diaminobenzidine, Saveen Biotech

AB, Sweden). For double-immunolabeling, free floating sections were incubated in a mixture of primary antibodies, anti-BrdU (1:250) and anti-NeuN (1:125; Chemicon Int., CA, USA) or GFAP (ms anti-GFAP 1:500; Chemicon Int., CA, USA) and Sox-2 (rb anti Sox-2 1:200; Chemicon Int., CA, USA) or DCX (gt anti DCX 1:125, Santa Cruz Biotech., CA, USA) and phosphoHistone-H3 (rb anti H3 1:400, Upstate, CA, USA) raised in different species for 48h at 4 °C. Sections were then washed and visualized using appropriate Alexa Fluor conjugated secondary antibodies (1:1000; Molecular Probes, OR, USA). Sections were mounted on slides in fluorescent medium containing DAPI (DAPI Pro-Long Gold anti-fade reagent, Molecular Probes, OR, USA).

### **Stereological quantification of cells (Paper II)**

Every 8th (P11) and 12th (P93) section throughout the hippocampus was used to determine the total number of BrdU and DCX-labeled cells in the dentate gyrus (SGZ and GCL) under light microscopy in each animal. The percentage number of newborn neurons (60 BrdU-positive cells per animal) was assessed using a confocal microscope (Leica TCS SP2, Leica Microsystems, Germany). The resulting percentages of NeuN positive cells were multiplied with the absolute number of BrdU-positive cells to give the absolute number of newly generated neurons. Double labeled GFAP/Sox-2 and DCX/phosphoHistone-H3 positive cells were counted exhaustively in every 12<sup>th</sup> serial section containing dorsal hippocampus. Cell counts were then multiplied with the series factor (12) and represent the total number of cells per dentate gyrus. The volume of the GCL in P93 mice was measured in every 6th section throughout the hippocampus and the total sum of the traced area was multiplied by section thickness and series number to give the total GCL volume.

### **Assessment of DCX-positive cell orientation (Paper II)**

To determine the orientation of the DCX-positive cells in the SGZ of the DG, we used a compass mapping system. The orientation of the leading process from the DCX-positive cell body was used to determine the angle of the cell in relation to its position within the SGZ. A normal leading cell process from a DCX-positive cell in the SGZ protrudes directly through the GCL into the perforant pathway of the molecular layer. We defined the angle of a perfect process leaving the cell body perpendicular to the SGZ (defined as 0°). We then measured the leading process on at least 60 DCX-positive cells in each animal in several random sections of the hippocampus (or to the maximum number available if necessary) and calculated a mean average degree of the orientation of all the cells.

### **GCL volume measurements (Paper II)**

For area quantification of GCL, every sixth section equally distributed throughout one hemisphere was collected from each animal and mounted with mounting medium containing DAPI to stain the nucleus of all cells (DAPI Prolong Gold antifade reagent, Molecular Probes, OR). The area of the GCL in each section was measured by tracing contours, using stereology computer software, StereoInvestigator 6 (Microbrightfield Inc., Colchester, VT, USA), and Leica microscope.

### **Statistical analysis (Paper II)**

Values are expressed as mean  $\pm$  standard error of the mean (SEM). Data were analyzed using a relevant ANOVA followed by post-hoc comparisons using a Newman-Keuls test to determine differences between groups.

For statistical analysis of Open Field data, data were evaluated using both multivariate and univariate statistical methods. For multivariate evaluation, Partial

Least Square Discriminant Analysis (PLS-DA) was applied. The significance of the components was evaluated by means of cross-validation and only significant components are shown in the figures. The pre-processing of all data consisted of unit variance scaling and mean-centering (Jackson, 1991). The Simca-P program, version 11.0.0.0, UMETRICS, was used for the calculations.

Univariate comparisons between groups for individual variables were performed with the non-parametric Kruskal-Wallis test followed by Mann-Whitney U-test.

### **Immunohistochemistry (Paper III)**

Free floating sections were stained for MBP, Olig2, and phospho-H3 immunoreactivity; the sections were rinsed three times in TBS buffer and incubated in blocking solution, containing 3% donkey serum (Jackson ImmunoResearch Laboratories Ltd., UK) and 0.1% Triton-X/TBS, for 30 min. Sections were subsequently incubated overnight at 4°C with the following primary antisera: rabbit anti-MBP (1:500, DakoCytomation, Denmark), goat anti-Olig2 (1:200, R&D Systems, MN, USA) or rabbit anti-phosphoH3 (1:200, Upstate, CA, USA) in 3% donkey serum/0.1% Triton-X/PBS. Following another 3 washes in TBS, sections were incubated for 2 hours at room temperature in secondary antisera: donkey anti-mouse Alexa 555, donkey anti-goat Alexa 488, and donkey anti-rabbit 555 (1:1000, Molecular Probes, The Netherlands). All secondary antibodies were diluted 1:500 in 3% donkey serum/0.1% Triton-X/PBS. The sections were rinsed five times in TBS and mounted in 0.1% phosphate buffer on microscope slides (SuperFrost Plus, Menzel GmbH & CO KG, Germany) with fluorescent mounting medium (Prolong Gold antifade reagent, Molecular Probes, The Netherlands).

For double-labeling of APC, Olig2 and BrdU, sections were rinsed three times in TBS buffer and incubated in 2N HCL for 30 minutes at 37°C. Sections were then rinsed three times in borate buffer (pH 8.5), three times in TBS, and incubated in blocking solution containing 3% donkey serum/0.1% Triton-X/TBS for 30 minutes at room temperature. Subsequently, the sections were incubated overnight at 4°C, shaking, in blocking solution containing primary antisera rat anti-BrdU (1:500, Nordic Biosite, Sweden), goat anti-Olig2 (1:200, R&D Systems, MA, USA) and mouse anti-APC (1:200, Calbiochem, Germany). On day two, sections were washed three times in TBS and incubated in blocking solution containing secondary antisera donkey anti-rat Alexa CY3, donkey anti-goat Alexa 488 (1:1000, Molecular Probes, The Netherlands) and donkey anti-mouse Alexa 647 (1:500, Molecular Probes, The Netherlands). Incubation was followed by an additional five washes in TBS, and sections were mounted with fluorescent mounting medium as previously described.

### **Volume measurements (Paper III)**

For volume measurements of the corpus callosum, a 1:12 series of all sections throughout one hemisphere was collected from each animal and stained for MBP, according to the previously described protocol. Using a semi-automated stereology system (Stereoinvestigator 6, Microbrightfield Inc., Colchester, VT, USA), the corpus callosum area was measured in each section by tracing the MBP-stained contours. The total sum of area measurements was multiplied with the section thickness and the series (12) to calculate corpus callosum volume for each hemisphere.

### **MBP density measurements (Paper III)**

MBP density measurements were performed according to the following method: in sections stained for MBP with fluorescent immunohistochemistry, the corpus callosum area of two mid-sagittal sections was measured by tracing in 5X magnification, followed by the placement of a grid to cover the traced area. The

computer software (Stereo Investigator 6, Microbrightfield Inc., VT, USA) then randomly chose six squares in the grid. Each square was photographed in grayscale using 40X magnification. Evaluations of MBP intensity were performed by creating a circle with a constant diameter and aligning the circle in the center of each picture (illustrated in Fig. 8A). Using the software ImageJ (ImageJ, National Institutes of Health, Maryland, USA), a mean gray value from each circle was calculated. Background intensity was measured by choosing an area where myelin is sparse, such as area between the molecular layer and CA3 of the hippocampus. The background mean gray value from each section was subtracted from the mean gray value of each measurement. The total mean gray value of circles from 12 pictures and from two slices of each brain was calculated. Myelin density was finally obtained by dividing the resulting mean gray value with the corpus callosum volume.

*Comments:*

The brightness of each pixel in a digitized image constitutes the grey value of the pixel. A brightness of zero corresponds to black and a brightness of 255 corresponds to white. The mean grey value is the sum of the grey value of each individual pixel divided with the total number of pixels (Oberholzer et al., 1996). Uniformity of illumination when acquiring the image is therefore of great importance and consequently, the camera and microscope settings must be fixed. Nevertheless, background noise, possible shadows and variations in tissue handling between sections can be adjusted for by subtracting the mean grey value of a background image from the mean grey values of the original images.

**Assessment of proliferating cells and oligodendrocyte cell numbers (Paper III)**

Every 12<sup>th</sup> section of the corpus callosum was analyzed between two well-defined landmarks (from the separation point between dorsal and ventral dentate gyrus to the mid-sagittal section). The corpus callosum borders were defined by contours as described above. Using computer software (Stereo Investigator 6, Microbrightfield Inc., VT, USA), a grid was placed over the traced area, and approximately 40 squares in the grid were randomly chosen at 20X magnification to count cells. All cell counts were performed blinded. For BrdU and phospho-H3 quantification, all positive cells in a traced area were exhaustively counted.

**Statistical analysis (Paper III)**

Values are expressed as mean  $\pm$  %; % = (standard error of mean/mean). Comparisons between two groups were performed using an unpaired t-test. Comparisons between multiple groups were performed using ANOVA, followed by Tukey post-hoc test.  $P < 0.05$  was considered statistically significant. \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ .



**Antibody List**

Primary antibody	Dilution	Company
Rat anti-BrdU	1:500;1:250	Nordic Biosite
Goat anti-DCX	1:125	Santa Cruz Biotech.
Mouse anti-RIP	1:50	Dev. Studies Hybridoma Bank
Rabbit anti-Egr-1	1:2000	Santa Cruz Biotech.
Rabbit anti-GFAP	1:500	Chemicon Int.
Mouse anti-GFAP	1:500	Chemicon Int.
Rabbit anti-Id1	1:200	Santa Cruz Biotech.
Mouse anti-Mash1	1:100	BD Pharmingen
Rabbit anti-MBP	1:500	DakoCytomation
Mouse anti-Nestin	1:500	BD Pharmingen
Mouse anti-APC	1:200	Calbiochem
Goat anti-Olig2	1:200	R&D Systems
Rabbit anti-phosphoH3	1:400 ;1:200	Upstate
Rabbit anti-Sox-2	1:200	Chemicon Int.
Mouse anti-NeuN	1:125	Chemicon Int.
Secondary antibody	Dilution	Company
Goat anti-rabbit AlexaFluor 594	1:3000	Molecular Probes
Goat anti-mouse AlexaFluor 488	1:4000	Molecular Probes
Donkey anti-mouse AlexaFluor 555	1:1000	Molecular Probes
Rabbit anti-mouse AlexaFluor 555	1:1000	Molecular Probes
Donkey anti-goat AlexaFluor 633	1:500	Molecular Probes
Donkey anti-rabbit HRP	1:1000	Amersham Biotech.
Donkey anti-mouse biotinylated	1:1000	Jackson Immunoresearch Lab.
Donkey anti-goat biotinylated	1:1000	Jackson Immunoresearch Lab.
Donkey anti-goat AlexaFluor 488	1:1000	Molecular Probes
Donkey anti-rabbit AlexaFluor 555	1:1000	Molecular Probes
Donkey anti-rat AlexaFluor Cy3	1:1000	Molecular Probes
Donkey anti-mouse AlexaFluor 647	1:500	Molecular Probes

## SUMMARY OF RESULTS

### Paper I

#### *β-endorphin induced changes in gene expression*

Incubation with β-endorphin, as well as blockade with opioid antagonists, induced gene expression changes in cultured AHPs. Altered mRNA transcript levels were detected and measured by cDNA array (Table 1, Paper I). Several genes, which were previously shown to be downregulated by opioid antagonists (Persson et al., 2003b), were upregulated by β-endorphin in the present study (Table 1, Paper I). Specific genes, which were upregulated by 2-fold or greater in the cDNA array and were also blocked by opioid antagonists, were confirmed by RT-PCR. The selected genes were *Id1*, *c-junD*, *GSTpi*, *MBP*, and *rab16* (Fig. 1, Paper I).

Incubation with opioid receptor antagonists to different opioid receptors revealed the receptors that mediated regulatory effects on the specific genes. Stimulation with β-endorphin induced changes in the expression of *Id1* and *GSTpi* via δ-receptor activation, while the effect on *c-JunD* was primarily mediated through stimulation of the μ-receptor. Both the μ-receptor and the δ-receptor were involved in increased expression of *Rab16* and *MBP* mRNA (Table 1, Paper I).

#### *β-endorphin induced oligodendrogenesis*

In a previous study, we observed that opioid blockade reduced AHP proliferation, increased neurogenesis, and reduced gliogenesis (Persson et al., 2003b). In this experiment, cDNA arrays revealed decreased *MBP* and *GSTpi* mRNA levels. Both *MBP* and *GSTpi* are expressed by oligodendrocytes in the rat brain and are used as oligodendrocyte markers (Tansey and Cammer, 1991; Qi et al., 2001). Therefore, we investigated whether incubation with β-endorphin induces gliogenesis in AHPs. When AHPs were cultured for ten days in the presence of 1 μM β-endorphin, immunocytochemistry revealed an approximately three-fold increase in the number of RIP-positive cells displaying oligodendroglial morphology. This effect was completely blocked by 10 μM naloxone. A lower concentration (10 nM) of β-endorphin had a weak effect on oligodendrogenesis compared to the higher concentration (Fig. 5A and B, Paper I). β-endorphin did not induce any significant change in the number of GFAP-positive astrocytes (Fig. 5C, Paper I).

#### *Id1 is required for opioid-induced oligodendrogenesis*

Helix-loop-helix proteins of the *Id* family have been implicated in the regulation of cell fate in neural stem and progenitor cells (Cai et al., 2000). Because *Id1* mRNA was upregulated by β-endorphin in our cultures, we hypothesized that *Id1* plays a role in opioid-induced oligodendrogenesis and, therefore, used antisense oligonucleotides to decrease *Id1* expression in AHP cultures. Antisense (100 μg/ml) added to the medium for 48 hours reduced immunoreactivity of nestin-positive AHPs to *Id1*, compared to control cultures or cultures treated with 100 μg/ml *Id1* sense. These results confirmed the effectiveness of *Id1* antisense oligonucleotides in blocking *Id1* protein production (Fig 6, Paper I).

After co-culturing the cells with 1 μM β-endorphin and 100 μg/ml *Id1* antisense for ten days, opioid-induced oligodendrogenesis was completely blocked, as seen by RIP immunoreactivity, demonstrating a requirement for *Id1* in opioid-induced oligodendrogenesis of AHPs. The number of GFAP-positive astrocytes was,

however, slightly increased. Interestingly, Id1 was not detected in RIP-positive oligodendrocytes (Fig 7, Paper I).

*Egr1 - a mediator of Id1 activation?*

We have previously demonstrated that  $\beta$ -endorphin stimulates AHPs proliferation via the mitogen-activated protein (MAPK) pathway (Persson et al., 2003a). This pathway has also been shown to increase transcription of the zinc finger protein early growth response 1 (Egr-1), also referred to as NGFI-A, Krox-24 or zif 268 (Revest et al., 2005), which is involved in protein complex triggering transcription of the Id1 gene via the Id1 promoter (Tournay and Benezra, 1996). Because Id1 was upregulated by  $\beta$ -endorphin on the cDNA arrays, Egr-1 immunoblotting was performed to investigate whether the protein was also altered in the opioid-treated AHP cultures. Immunoblotting revealed an approximately three-fold increase in Egr-1 protein levels after 4 hours of stimulation with  $\beta$ -endorphin (Fig 3, Paper I).

*$\beta$ -endorphin reduced the number of Mash1-positive cells*

A subpopulation, constituting 25% of the total AHP population, was immunoreactive for Mash1, a bHLH protein that has been implicated as a proneural transcription factor (Fig 8A, Paper I). When AHPs were cultured in 1  $\mu$ M  $\beta$ -endorphin for ten days, Mash1 was expressed in 5% of the total AHP population. This effect was antagonized by 10  $\mu$ M naloxone (Fig. 8B, Paper I).

## **Paper II**

*Moderate dose irradiation dramatically reduced precursor cell proliferation in the young mouse brain*

Irradiation has been shown to decrease the number of proliferating cells in the DG of rodent through mechanisms of apoptotic cell death; this method is often used as a tool to study neurogenesis (Wojtowicz, 2006). Whole brain irradiation of the young mouse brain (P9, n=4), using a moderate dose (6 Gy), reduced the pool of proliferating cells in the dentate gyrus (SGZ and GCL) by 85%, as seen 36 hours post-irradiation (supporting information – SI Fig 1A and Fig. 2A). The reduction of proliferating cells was not confined to the dentate gyrus, but was observed throughout the entire hippocampal formation (SI Fig 2B and C).

*Voluntary running increased the stem cell pool in the dentate gyrus of irradiated mice*

Voluntary exercise is a strong inducer of hippocampal neurogenesis and affects overall brain health (Van Praag et al., 1999b; van Praag et al., 2005; Olson et al., 2006). We hypothesized that voluntary running could promote recovery of dentate gyrus neurogenesis following irradiation to the young mouse brain. Therefore, running wheels were introduced to half of the animals (n=6 per group, SI Fig. 1B) at 7 weeks post-irradiation, and running wheel activity was recorded. There was no difference in the average daily running distance in irradiated mice compared to non-irradiated mice (data not shown). To evaluate whether the effects of irradiation on proliferating cells was transient or permanent, and whether the number of proliferating cells and neurogenesis was altered by voluntary exercise, BrdU was injected at the start of the running period.

To assess changes in the stem cell pool, immunohistochemistry co-labeling of glial-fibrillary acidic protein (GFAP) and the transcription factor Sox-2 was performed. The combination of GFAP and Sox-2 markers has been used to identify radial glia-like stem cells (Komitova and Eriksson, 2004; Seri et al., 2004; Lagace et al., 2007). A significant decrease in the number of GFAP/Sox-2-positive cells was observed in

irradiated animals compared to sham-irradiated controls (irr:  $769 \pm 120$  vs. sham irr:  $1558 \pm 150$ ; Fig. 1A and B;  $P < 0.01$ ). Voluntary running significantly increased the number of GFAP/Sox-2-positive stem cells after irradiation (irr/run:  $1216 \pm 51.6$  vs. irr:  $769 \pm 120$ ;  $P < 0.05$ ; Fig. 1B). Similar results were observed when Sox-2 was quantified as a single marker (SI Fig. 3).

*Voluntary running increases neurogenesis in the dentate gyrus of irradiated mice*

To determine whether voluntary running during adulthood affects neurogenesis in the dentate gyrus of mice subjected to irradiation early in life, we analyzed BrdU in combination with the neuronal marker NeuN using confocal microscopy. There was a significant increase in the percentage of new neurons in the sham-irradiated running mice compared to sham irradiated non-running controls (run:  $81 \pm 1.2\%$  vs. sham:  $72 \pm 3.1\%$ ; Fig. 2A;  $5P < 0.05$ ). Irradiation alone had no effect on the percentage of cells that became neurons (Fig. 2A). However, voluntary running after irradiation increased the percentage of neurons among the newborn cells (irr/run:  $79 \pm 1.7\%$  vs. irr:  $71 \pm 1.2\%$ ; Fig 2A;  $P < 0.05$ ). Using these percentages, and the total number of stereologically counted BrdU-labeled cells (SI Fig. 3A-D), the level of neurogenesis in these mice was determined. The total number of new neurons (BrdU+/NeuN+) significantly increased (117%) in the sham-irradiated running compared to sham-irradiated non-running mice (Fig. 2B, D-G;  $P < 0.01$ ). There was a significant increase (275%) in the number of newborn neurons in the irradiated running mice compared to irradiated non-running mice (Fig 2B, D-G;  $P < 0.01$ ).

*Irradiation-induced negative effects on DG volume attenuated by voluntary running*

Moderate dose irradiation to the young mouse brain resulted in long lasting reduction of GCL volume; a significant difference was observed three months after irradiation (Fig. 2C;  $P < 0.05$ ). However, animals subjected to voluntary running exhibited no significant difference in GCL volume compared to the other groups.

*Moderate dose irradiation resulted in long lasting reduction of immature neurons in the DG*

Doublecortin (DCX) was used as a marker to distinguish neuronal precursors and immature neurons in the dentate gyrus (Brown et al., 2003; Couillard-Despres et al., 2005). Moderate dose irradiation to the young postnatal mouse brain resulted in a markedly reduced pool of DCX-positive cells in adult mice (Fig. 3A;  $P < 0.01$ ). We quantified an increase in the number of DCX-positive cells in the sham-irradiated running animals compared to sham-irradiated non-running mice, which confirmed the strong effect that running has on hippocampal neurogenesis (Fig. 3A). However, irradiated mice subjected to voluntary running exhibited an apparent increase in DCX numbers that was not statistically significant (Fig. 3A). DCX double-labeling with the proliferation marker phospho-histone-H3 revealed very few double-positive cells (maximum of 8 cells) in the dentate gyrus from each group (data not shown).

*Voluntary running after irradiation reversed DCX cell process orientation within the DG*

Survival and maturation of newborn neurons in the dentate gyrus is dependent on successful integration into the neuronal network (Dayer et al., 2003; Deisseroth et al., 2004; Marques-Mari et al., 2007). To discern whether irradiation and/or running altered integration of immature cells into the dentate gyrus, we analyzed the orientation of the leading apical processes in DCX-positive cells, which later develop into a single dendritic tree in the mature granule cell (Fig. 3D). We employed a compass system (see *SI Methods*) to determine the angle of the leading process in relation to the cell position along the subgranular zone (SGZ) border (Fig. 3B). We determined that DCX-positive cells in sham-irradiated mice had an average leading

process angle of  $24 \pm 3^\circ$ . This angle was significantly altered to  $56 \pm 5^\circ$  after irradiation (Fig. 3C-G;  $P < 0.001$ ). Interestingly, we found that the orientation of the leading process in the irradiated running mice was reversed to the sham-irradiated angle ( $25 \pm 3^\circ$ ; Fig. 3C-G).

#### *Voluntary running ameliorated irradiation-induced behavioral changes*

The open field test has been used to measure alterations in a wide range of behavioral features, including locomotor behavior, spatial variability, and exploratory behaviors (Krischke and Petermann, 1995). The data from mice subjected to irradiation were clearly separated from the sham-irradiated mice, indicating that these two groups of mice exhibited different behavioral patterns (Fig. 4A; each point represents an individual mouse). Irradiated mice revealed increased exploratory rearing frequency, increased numbers of stops, and increased meandering after irradiation (Fig. 4B – D;  $P < 0.01$ ). These irradiation-induced behavioral alterations, other than meandering, were reversed by voluntary running (Fig. 4B – C;  $P < 0.05$ ). Our findings indicate that running alleviates irradiation-induced behavioral changes.

### **Paper III**

#### *Mice subjected to voluntary exercise exhibit greater brain weight compared to irradiated mice*

The brains of running mice were significantly larger ( $0.43 \text{ g} \pm 1.6\%$ ) than irradiated mice ( $0.39 \text{ g} \pm 1.8\%$ ). However, there was no difference between running mice and sham-irradiated mice (Fig. 2;  $P < 0.05$ ).

#### *Transient effects of corpus callosum volume after moderate irradiation dose to the postnatal mouse CNS*

Volume changes after irradiation have been previously reported in rodents, as well as humans (Panagiotakos et al., 2007). In accordance with this, corpus callosum volume changes of irradiated mice were compared to controls; however, these changes were transient and only significant when evaluated two weeks after irradiation ( $P_{23}$ , Fig 3,  $P < 0.01$ ).

#### *Moderate dose irradiation immediately reduced the pool of Olig2-positive cells, but did not affect the generation of new Olig2-positive cells*

The effects of a moderate, yet clinically relevant, radiation dose of 6 Gy on Olig2-positive cells in the immature brain were evaluated. P9 mice ( $n=4$  per group) were subjected to irradiation or sham irradiation. After 24 hours, mice were injected with BrdU and sacrificed 24 hours later at P11 (Fig. 1A). The number of proliferating cells (BrdU- and phospho-H3-positive cells), and cells of the glial cell lineage (Olig2-positive cells), were stereologically counted. There was a dramatic loss (68%) of BrdU-labeled proliferating cells in the corpus callosum after irradiation (Fig 4B;  $51338 \pm 5\%$  vs.  $16237 \pm 19\%$ , sham-irradiated and irradiated, respectively.  $P < 0.001$ ). However, there was no significant difference in the number of phospho-H3-positive cells two days after irradiation (Fig. 5A;  $7992 \pm 30\%$  vs.  $7838 \pm 18\%$ , sham-irradiated and irradiated, respectively). Nevertheless, the total number of Olig2-positive cells in the corpus callosum decreased significantly to 61% of control values after irradiation (Fig 4C;  $155734 \pm 5\%$  vs.  $95367 \pm 4\%$ , sham-irradiated and irradiated, respectively.  $P < 0.001$ ).

The number of newly born glial progenitors (BrdU/Olig2-double-labeled cells) was not altered shortly after irradiation (Fig 4D;  $16858 \pm 13\%$  vs.  $13749 \pm 25\%$ , sham-irradiated and irradiated, respectively). This finding was confirmed by no change in the number of proliferating glial progenitor cells (Olig2/phospho H3-double-labeled

cells) at the time point of sacrifice (Fig 5B;  $2848 \pm 38\%$  versus  $5090 \pm 21\%$ , sham-irradiated and irradiated, respectively). In summary, radiation killed nearly two thirds of the BrdU-positive cells 24 hours after irradiation in the corpus callosum, but did not affect the proliferation rate of dividing Olig2-positive cells.

*Stable production, but no recovery of Olig2-positive cell numbers in the juvenile brain after postnatal moderate dose irradiation*

To investigate whether the Olig2-positive cell population recovers in juvenile animals after radiation to the immature brain, P9 mice (n=8 per group) were exposed to irradiation or sham-irradiation and sacrificed two weeks later (P23; Fig. 1B).

The total number of proliferating cells was similar between sham-irradiated and irradiated animals (Fig 6B;  $6039 \pm 13\%$  compared to  $5463 \pm 10\%$ , respectively).

Two weeks after irradiation, the number of Olig2-positive cells were still not recovered, but were less than half of the sham-irradiated values (Fig 6C; 46% of sham-irradiated;  $165678 \pm 5\%$  vs.  $76234 \pm 8\%$ , sham-irradiated and irradiated, respectively.  $P < 0.05$ ). Interestingly, the total number of Olig2-positive cells was similar in P23 and P11 sham-irradiated animals (Fig 4C and 6C;  $165678 \pm 5\%$  vs.  $155734 \pm 4\%$ , respectively). Thus, the number of Olig2-positive cells in the corpus callosum remained constant during the first weeks of postnatal development. Furthermore, at three weeks of age, neither irradiation nor age affected the production of phosphoH3/Olig2-positive cells. The number of phosphoH3/Olig2-positive cells was similar between sham-irradiated and irradiated animals (Fig 6D;  $2227 \pm 14\%$  compared to  $1972 \pm 10\%$ ).

*Voluntary running did not increase survival or maturation of newborn cells in the corpus callosum*

At P93, after four weeks of running, no significant changes in the number of BrdU-positive cells was detected in the corpus callosum (Fig. 7A; sham-irradiated =  $7199 \pm 17\%$ , irr =  $7418 \pm 9\%$ , irr/run =  $9154 \pm 9\%$ , run =  $8145 \pm 14\%$ ). Additionally, there were no longer any significant differences in the number of Olig2-positive cells between the groups (Fig 7B; sham-irradiated =  $67902 \pm 17\%$ , irr =  $51035 \pm 15\%$ , irr/run =  $81823 \pm 11\%$ , run =  $79031 \pm 13\%$ ). Approximately 10% of the BrdU-labeled cells expressed Olig2 four weeks after BrdU injections (Fig. 7C; sham-irradiated =  $5525 \pm 13\%$ , irr =  $4081 \pm 17\%$ , irr/run =  $4655 \pm 17\%$ , run =  $5824 \pm 19\%$ ). The number of BrdU-positive cells surviving into a more mature phenotype expressing APC was unaffected by irradiation, as well as by voluntary exercise (Fig. 7D; sham-irradiated =  $47 \pm 6\%$ , irr =  $55 \pm 4\%$ , irr/run =  $51 \pm 4\%$ , run =  $47 \pm 5\%$ ).

*Myelin density was altered in the juvenile CNS after postnatal irradiation, but returned to normal by adulthood in mice*

At P11, two days post-irradiation, there were no differences in myelin density (Fig. 8B;  $25 \pm 2\%$  vs.  $26 \pm 2\%$ , sham-irradiated and irradiated, respectively). Surprisingly, by P23, during the peak of myelination in rodents, irradiated mice displayed increased MBP density in the corpus callosum (Fig. 8C;  $66 \pm 4\%$  vs.  $85 \pm 9\%$ , sham-irradiated and irradiated, respectively.  $P < 0.05$ ).

No significant difference was observed between the four adult groups, indicating a transient effect of moderate dose irradiation on myelin density (Fig. 8D; sham-irradiated =  $55 \pm 7\%$ , irr =  $67 \pm 7\%$ , run =  $57 \pm 14\%$ , irr/run =  $61 \pm 14\%$ ).

## ASPECTS ON THE PRESENTED FINDINGS

### Paper 1 – a summary

Voluntary exercise has, time and again, proved to be a major regulator of brain plasticity, especially in the neurogenic niche of the adult hippocampus (Van Praag et al., 1999b; Van Praag et al., 1999a; Trejo et al., 2001; Kim et al., 2002; Ra et al., 2002; Kronenberg et al., 2003; Kronenberg et al., 2005a).  $\beta$ -endorphin has been shown to be one of the mediators of the beneficial effects of exercise (Koehl et al., 2008) and is also a member of the notorious opioid family, primarily recognized to be responsible for addictive behaviour in humans.  $\beta$ -endorphin levels increased in the hippocampus during the postnatal proliferative period (P8) and persisted, albeit at lower levels, throughout adulthood (Angelogianni et al., 2000). Endogenously produced  $\beta$ -endorphin is required for exercise-induced proliferation of neural stem and progenitor cells in the hippocampus *in vivo* (Koehl et al., 2008), and also induces proliferation of AHPs *in vitro* (Persson et al., 2003a). When the opioid receptors were blocked, cell genesis in the hippocampus (Persson et al., 2004), and in AHP cultures (Persson et al., 2003b), was hampered. However, the role of  $\beta$ -endorphin in cell fate determination of AHPs is unclear. Although it is a proliferative agent,  $\beta$ -endorphin does not appear to be neurogenic, as shown in *in vitro* (Persson et al., 2003b) and *in vivo* studies (Koehl et al., 2008). In fact, we have previously shown that opioid antagonists increase neuronal differentiation in AHP cultures, while decreasing the number of RIP-positive oligodendrocytes (Persson et al., 2003a). Based on these findings, we investigated the role of  $\beta$ -endorphin in AHP-derived gliogenesis. We found that 1  $\mu$ M  $\beta$ -endorphin induced upregulation of genes involved in oligodendrogenesis, a finding that was confirmed by RT-PCR. Differentiation experiments revealed a three-fold increase in the number of RIP-positive oligodendrocytes, without altering the generation of GFAP-positive astrocytes. Moreover, we found that Id-1, an HLH-protein upregulated by  $\beta$ -endorphin on the cDNA arrays, and known to be involved in cell fate decisions, was required for  $\beta$ -endorphin-induced oligodendrogenesis. Blocking Id-1 with antisense completely blocked opioid-induced oligodendrogenesis. We have previously shown that AHP proliferation is triggered by phosphorylation of ERK 1/2, a subgroup of the MAPK-pathway. Phosphorylation of ERK1/2 is known to activate the transcription factor Egr-1, which translocates to the nucleus for transcriptional activation. In the nucleus, Egr-1 induces expression of Id-1. Indeed, in our AHP cultures, we found elevated levels of Egr-1 one hour after incubation with  $\beta$ -endorphin.

Id-1 was specifically expressed in immature, nestin-positive AHPs, and not in mature oligodendrocytes, indicating that  $\beta$ -endorphin regulates cell fate during early stages of maturation. In addition, the number of cells that expressed Mash-1, a bHLH protein expressed in multipotent, nestin-positive AHPs, was reduced. Taken together, we suggest a mechanism where  $\beta$ -endorphin, via phosphorylation of ERK1/2 and the subsequent activation of the transcription factor Egr-1, increased the transcriptional repressor Id-1. Augmented levels of Id-1 in turn altered the balance of transcriptional activators and repressors, possibly leading to suppression of Mash-1, and favouring oligodendrogenesis.

### *Diverging effects of opioid signaling on proliferation*

The influence of opioids on cell cycling, differentiation, survival, and death of CNS cells is still poorly understood.  $\mu$ -,  $\delta$ -, and  $\kappa$ -receptors individually direct proliferation and maturation, depending on cell type, and the relative abundance of the three receptors varies depending on aspects, such as species, age, cell cycle stage, and brain

region (Loughlin et al., 1985; Stiene-Martin et al., 1998; Hauser et al., 2000; Stiene-Martin et al., 2001). We have previously shown that AHPs co-express  $\mu$ - and  $\delta$ -receptors, but lack  $\kappa$ -receptors (Persson et al., 2003a). Opioid signaling, especially via agonists acting on the  $\mu$ -receptor, is considered to inhibit cell proliferation *in vitro*, as well as *in vivo* (Kornblum et al., 1987a; Mandyam et al., 2004) and in the hippocampus (Eisch et al., 2000). In contrast to these findings, we have previously reported increased proliferation by treating AHP cultures with 1  $\mu$ M  $\beta$ -endorphin. This effect was mediated by phosphorylation cascades of the MAPK pathway (Persson et al., 2003a; Persson et al., 2003b). The discrepancy between studies describing that opioids inhibit proliferation and our study might be contributed to the fact that exogenous opioid peptides *in vivo* are likely to induce systemic effects, in addition to influencing the opioid system. For example, one system known to be affected by opioids is the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the secretion of the stress hormone corticosterone (Domokos et al., 2008). Chronic exposure to stress and increased corticosterone levels is a potent inhibitor of cell proliferation in the hippocampus (reviewed in Joels, 2007). A recent paper demonstrated that morphine regulates hippocampal proliferation negatively only if chronically administered (Fischer et al., 2008). It is possible to exclude many secondary systemic effects, such as prolonged exposure to increased levels of stress hormones, by acute administration, or, as in Paper I, performing *in vitro* studies. Acute administration of morphine does not have the same detrimental effects on cell proliferation (Eisch et al., 2000). In addition, administration of naloxone has been demonstrated to reduce cell proliferation in the rat dentate gyrus (Holmes and Galea, 2002). Nevertheless, mice deficient in  $\mu$ -receptors displayed unaltered cell proliferation levels in the dentate gyrus, but increased neuronal cell survival (Harburg et al., 2007). The authors suggested that  $\mu$ -receptors exert effects on the survival of newly generated neurons, without affecting cell proliferation of precursors. However, we have determined that opioid-induced cell proliferation in AHPs is regulated by  $\mu$ - and  $\delta$ -receptors (Persson et al., 2003a; Persson et al., 2003b), so it is plausible that endogenous opioid regulatory mechanisms are counter-balanced by  $\delta$ -receptors in the absence of  $\mu$ -receptors. Also, species differences between rat and mice, with regard to opioid regulation of hippocampal proliferation, are possible. The same authors reported that blocking opioid receptors with naltrexone, which is not entirely  $\mu$ -receptor specific, did not alter cell proliferation in the dentate gyrus of mice, while another group of researchers found that the  $\mu$ - and  $\delta$ -receptor agonist naloxone decreased cell proliferation in the rat dentate gyrus (Holmes and Galea, 2002). In line with this notion,  $\mu$ -receptor mice knockouts performed better in the hippocampal-dependent Morris water maze task (unpublished results and Meilandt et al., 2004), while blocking the  $\mu$ -receptor in rats decreased performance in the same task (Meilandt et al., 2004).

#### *How does $\beta$ -endorphin induce proliferation in AHPs?*

Various extra-cellular stimuli direct intracellular events by initiating phosphorylation cascades. The MAPK pathway comprises the entire superfamily of signaling cascades, including extra-cellular signal regulated kinases (ERKs), the c-jun N-terminal kinase (JNKs), and the p38 stress activated protein kinases, activated by mitogens or stress stimuli. JNK and p38 mediate stress or cytokine-related effects, while ERK1/2 is coupled to mitogenic or differentiation stimuli (Yang et al., 1998; Sweatt, 2004). We have previously shown that the MAPK pathway is activated in opioid-induced cell proliferation of AHPs via ERK1 and 2 (Persson et al., 2003a). Elevated intracellular levels of PI3K and calcium initiate phosphorylation of the ERK1/2 MAPK-pathway, and we measured elevated levels of intracellular calcium in AHPs treated with opioids. Moreover, G-protein coupled receptor signaling often



involves PI3K; blocking either calcium or PI3K in opioid-treated AHP cultures results in the inhibition of opioid-induced AHP proliferation (Persson et al., 2003a).

*What is the mechanism underlying  $\beta$ -endorphin-induced oligodendrogenesis?*

The transcription factor Elk-1 is a nuclear substrate target for phosphorylation of ERK1/2, which can in turn cause transcriptional activation of c-fos that influences cell proliferation and early growth receptor-1 (Egr-1; also referred to as Krox-24, NGFI-A or zif 268) (Hodge et al., 1998; Chen et al., 2004). C-fos is involved in mitogenic effects of the MAPK-pathway, while Egr-1 is a member of a multiprotein complex that promotes transcription of Id1 protein (Tournay and Benezra, 1996). In addition to increased Id1 mRNA levels on the arrays, Egr-1 was upregulated after 4 hours of stimulation with  $\beta$ -endorphin, probably reflecting activation of the MAPK-pathway.

Elevated levels of Id1 mRNA, together with increased mRNA of proteins involved in the mitogen-activated protein kinase (MAPK) pathway, have previously been reported in association with enhanced oligodendrogenesis (Scarlato et al., 2000). We, therefore, hypothesized that increased Id1 mRNA on the arrays was a result of opioid-induced oligodendrocyte differentiation, as measured by quantification of RIP-positive cells. Id2 mRNA was not expressed in our AHP cultures, and the levels of Id3 mRNA were unchanged after 48 hours of treatment with  $\beta$ -endorphin (Id4 was not included on the arrays). Recently, it has been reported that Id2 expression occurs in oligodendrocytes in the adult rat brain; 70% of all APC-positive oligodendrocytes express Id2. This suggests a role of Id2 in terminal maturation of oligodendrocytes (Chen et al., 2007). A plausible explanation to why Id2 mRNA expression was not detected on our arrays could be that 48 hours of treatment is too short to detect Id2 activity.

Addition of Id1 antisense, which blocks translation of Id1 mRNA to a functional protein, suppressed oligodendrogenesis induced by  $\beta$ -endorphin in AHP cultures. Id1-1 belongs to the family of cell fate regulatory HLH proteins, together with bHLH proteins, such as Mash-1. However, in contrast to the bHLH proteins, Id proteins lack the basic domain that is required for DNA binding. By sequestering bHLH proteins or their binding partners (*e.g.*, E-proteins) Id proteins inhibit transcriptional processes (reviewed in Ross et al., 2003). Although Id proteins are usually discussed in the context of their role as cell fate repressors, the reported inability of Id1 and Id3 to complex with the gliogenic bHLH proteins Olig1 and Olig2 makes Id1 and Id3 weak candidates for repression of glial cell fate (Samanta and Kessler, 2004). Instead, Id1 and Id3, shown to have a similar expression patterns, are during embryogenesis restricted in proliferating neuroblasts and inhibits neuronal cell development (reviewed in Tzeng, 2003). In telencephalic fetal mouse progenitors, ectopic expression of Id1 or Id3 suppressed neurogenic differentiation, with concomitant decreased Mash1 activity (Nakashima et al., 2001). Misexpression of Id1, using a retroviral vector, completely blocked neurogenesis and led to overproduction of glial cells in both embryonic and adult mouse cerebral cortex (Cai et al., 2000). In Id1/Id3-double knockout mice, premature neurogenesis is observed, further supporting a role for Id proteins in regulating neuronal differentiation (Lyden et al., 1999). Id3 seems to work in favour of astroglialogenesis (Gu et al., 2005), and the addition of Id1 antisense oligonucleotides alone not only decreased the number of RIP-positive oligodendrocytes in our cultures but also slightly increased the number of GFAP-astrocytes. Because Id1 and Id3 compete by binding to the same E proteins, the increased number of astrocytes following treatment of AHPs with Id1 antisense, might reflect increased Id3 activity as a result of excess Id1/Id3 binding partners in the absence of Id1.

Id1 does not only induce oligodendroglial fate. In the developing spinal cord, Id1 promotes astrogliogenesis, together with the transcriptional repressor Hes1. However, at least in culture, Id1 and Hes1 stimulated differentiation of astrocytes only in the absence of the patterning factors Pax6, Olig2, and Nkx2.2 (Sugimori et al., 2007). Multiple bHLH and HLH factors are thus expressed simultaneously to direct the final choice of cell fate.

*Mash-1 as a possible co-player in opioid-induced oligodendrogenesis*

As previously mentioned, Id1 represses transcriptional activity of bHLH factors by sequestering them or their binding partners. Mash-1 binds to the E-protein E47, forming an activated heterodimer that promotes a neuronal subtype (e.g. GABAergic neurons) (Parras et al., 2002; Vinals et al., 2004). In addition, Mash-1 has been found to interact with Olig2 to generate oligodendrocytes (Parras et al., 2007), and is also observed in embryonic and postnatal telencephalic progenitors that later differentiate into oligodendrocytes (Parras et al., 2004; Parras et al., 2007). Although GABAergic neurons, as well as many oligodendrocytes, are thought to share a common origin, and are therefore more likely to share expression of certain genes, new findings indicate that Mash1 function expression is highly conserved from embryo to adult. It has been suggested that the conservation of Mash1 function in the postnatal brain indicates that it influences cell fate throughout the life of the organism, and that changes in cell types during development and postnatal periods reflects alterations in the signalling environment. Being a modulator of cell fate, Mash1 is suggested to be a key transcription factor in neuron-glia switch (Parras et al., 2004).

It is known that Id1 binding to the Mash1 heterodimerization partner E47 regulates stability of Mash-1, and increased Id1 levels are sufficient to induce degradation of Mash-1 (Vinals et al., 2004). Nestin-positive cells have been previously shown to express Mash-1, which is downregulated after differentiation (Torii et al., 1999). However, in AHPs, Mash-1 continues to be expressed as cells differentiate into neurons; continued expression might be required for AHPs to produce neurons (Faigle et al., 2004; Elmi et al., 2007). The loss of Mash1 expression after 10 days of incubation with  $\beta$ -endorphin might reflect the progression of Mash1-positive AHPs, with an inherent capacity towards oligodendrogenesis. In support of this, downregulation of Mash1 in  $\beta$ -endorphin-treated cultures approximately corresponded to percentage increase in RIP-positive cells. In our cultures, Id1 was expressed in the nucleus of nestin-positive cells, where the inhibitory activity of Id proteins on transcriptional processes takes place. Mature RIP-positive cells did not express Id1, implicating action on early precursors rather than on immature oligodendrocytes.

Although we demonstrate increased oligodendrogenesis in AHPs after addition of  $\beta$ -endorphin, the majority of cells did not differentiate into oligodendrocytes. AHP cultures are heterogeneous, comprising both stem and progenitor cells. It is, therefore, likely that  $\beta$ -endorphin more effectively induced oligodendrocyte differentiation in a certain population of cells. Because Mash1 was expressed in one-quarter of the total AHP population in the untreated cultures, and it has been previously suggested that 20% of heterogeneous AHP cells are multipotent neural stem cells (Palmer et al., 1999), the actions of  $\beta$ -endorphin might have been directed towards this subgroup.

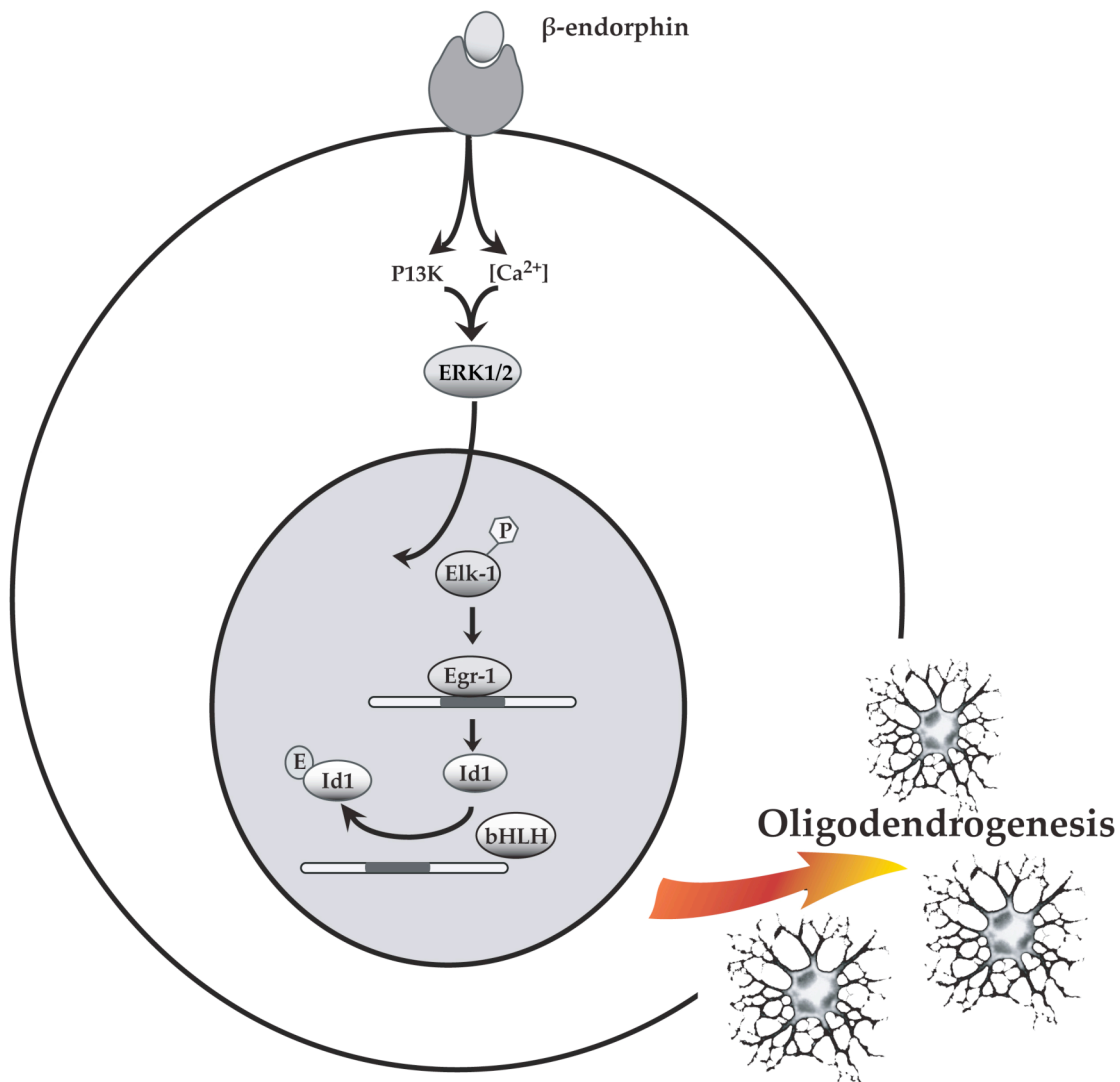


Figure 10 - A possible mechanism for opioid-induced oligodendrogenesis.  $\beta$ -endorphin causes activation of the ERK 1/2 MAPK pathway through elevated levels of intracellular calcium and PI3K. This leads to phosphorylation of Elk-1 that in turn increases transcription factor Egr-1. Egr-1 is known to be part of a multiprotein complex triggering transcription of Id1. Id1 sequesters E-proteins and thereby prevents proneural activation by bHLH, such as Mash1, in favour of oligodendrogenesis.

#### Is opioid signaling actually gliogenic?

Regardless of the discrepancy of the role of opioids and their antagonists on hippocampal proliferation, it is interesting to note that  $\mu$ -receptor knockout mice display increased survival of neurons in the dentate gyrus (Harburg et al., 2007). In the light of our finding of opioid-induced decreased neuronal cell numbers and concomitant increased oligodendroglial cell numbers, this might suggest that opioid signaling is gliogenic, when it comes to progression towards a specific cell fate. It is now widely accepted that the properties of stem- and progenitor cells depend largely on the surrounding environment. The gliogenic effect of opioid signaling might be masked in the environment of a neurogenic niche, such as the hippocampus, suppressing oligodendrocyte differentiation. By removing cells from their natural neurogenic environment, such as *in vitro* experiments, one might disclose the gliogenic actions of opioids that are not apparent *in vivo*. In accordance with this theory, enriched oligodendrocyte precursors cultures from neonatal mouse brains

proliferate in response to  $\mu$ -receptor opioid signalling, but only when co-cultured with astrocytes (Knapp and Hauser, 1996). Oligodendrocytes release opioids in an autocrine/paracrine manner, and may thereby regulate opioid-induced effects in a local manner (Knapp et al., 2001). Additional information on the subject has been obtained from studies of the SVZ. The SVZ is an area that, despite producing large numbers of neurons destined for the olfactory bulb, also generates astrocytes and oligodendrocytes postnatally, which continues throughout life. In this regard, the SVZ can be considered to be more permissive to gliogenesis than the hippocampus. In the SVZ, the overall expression of  $\mu$ -receptor decreases during postnatal development. Interestingly, this does not hold true for expression of the  $\mu$ -receptor on immature SVZ oligodendrocytes, which remains constant (Stiene-Martin et al., 2001). If opioid signalling via the  $\mu$ -receptor induces oligodendrogenesis in progenitors from the SVZ, as it does in the AHPs, persistent expression of  $\mu$ -receptors in the SVZ might contribute to the ability of the SVZ to generate new oligodendrocytes during adulthood.

Although not reported to be expressed on oligodendrocyte progenitors *in vitro*, oligodendrocyte progenitors do express  $\delta$ -receptors *in vivo*, albeit at lower levels. We have previously demonstrated that AHPs express both  $\mu$ - and  $\delta$ -receptors *in vitro*, but seem to lack  $\kappa$ -receptors (Persson et al., 2003a). The upregulation of oligodendroglial genes, as seen on the cDNA arrays, was mediated via  $\mu$ - and  $\delta$ -receptors, implicating reciprocal action of both  $\mu$ - and  $\delta$ -receptors in opioid oligodendrogenesis. We also detected expression of  $\mu$ - and  $\delta$ -receptors, but not  $\kappa$ -receptors, in the rat dentate gyrus *in vivo* (unpublished data). Mature oligodendrocytes downregulate  $\mu$ -receptors and are unaffected by  $\mu$ -receptor agonists. Instead, mature MBP-positive oligodendrocytes express  $\kappa$ -receptors, suggested to promote their survival. Although speculative, it is possible that the lack of  $\kappa$ -receptors in the hippocampus reflects the non-permissiveness of the hippocampus towards oligodendroglial differentiation. Also, it is possible that as AHPs progress down an oligodendroglial pathway, they begin to express  $\kappa$ -receptors, although this was not analyzed in the present studies. If so, it would be interesting to see whether a selective  $\kappa$ -receptor agonist, such as norbinaltorphimine, could affect the numbers of mature oligodendrocytes in opioid-treated cultures.

## **Paper II – a summary**

Free access to running wheels for laboratory animals has been successfully used to study exercise-related effects on brain (Van Praag et al., 1999b; Naylor et al., 2005; Bick-Sander et al., 2006). Most studies, with a few notable exceptions (Van Praag et al., 1999b; Naylor et al., 2005; Bick-Sander et al., 2006), reveal beneficial effects of exercise on brain plasticity and health, including increased cell proliferation and survival, especially in the hippocampal neurogenic niche (Van Praag et al., 1999a; Kim et al., 2002; Chen et al., 2007). Another useful tool to study cell plasticity in the brain is cranial irradiation. In contrast to voluntary exercise, cranial irradiation is detrimental to brain health and particularly affects neurogenic regions, such as the hippocampus, and immature brains undergoing extensive proliferation (Mizumatsu et al., 2003; Schindler et al., 2008). Proliferating cells are extremely vulnerable to irradiation due to DNA exposure. DNA damage through irradiation-induced ionization and/or release of free radicals leads to mitotic catastrophe, growth arrest, and (Monje et al., 2002; Kanzawa et al., 2006). The brains of C57/Bl6 mice were irradiated on postnatal day 9 with a clinically relevant, yet moderate dose, and the acute effects of irradiation two days later, as well as the effects on hippocampal neurogenesis and behavior after four weeks of voluntary running were evaluated three months after irradiation. Irradiation significantly decreased proliferation and

the dentate gyrus stem cell pool and the number of immature neurons, as well as reduced GCL volume. These effects were long lasting and still present in adulthood. Interestingly, the orientation of neuronal processes into the GCL was perturbed, indicating improper integration. By monitoring behavior with the open field test, irradiation-induced behavioral alterations were observed in the adult mice. Voluntary exercise significantly increased cell survival, as well as restored the stem cell pool after irradiation. Additionally, voluntary exercise increased the number of new mature neurons in irradiated mice. Interestingly, voluntary exercise resulted in a reversal of the perturbed orientation of immature neurons. Furthermore, irradiation-induced hyperactivity in the open-field test was ameliorated.

*Irradiation sensitivity of the CNS is closely linked to dose, age and area*

High doses of irradiation during S-phase of cell cycle cause double-stranded DNA breaks, oxidative stress, and disruption of cell membrane integration. This leads to rapid activation of caspase-3 and apoptosis within 4 to 6 hours ("pre-mitotic apoptosis"). With low doses, however, minor DNA damage can be repaired and the cell survives. Nevertheless, delayed apoptosis (after 24 h, so called "post-mitotic apoptosis"), due to oxidative stress, unsuccessful repair of DNA damage, and chromosomal aberrations, as well as radiation-induced protein alterations (reviewed in Shinomiya, 2001), can still take place. Dose-response studies of hippocampal proliferation in rodents have been performed both in adult (Bellinzona et al., 1996; Mizumatsu et al., 2003; Limoli et al., 2004; Rola et al., 2004), as well as in immature, animals (Fukuda et al., 2005). The effects of irradiation on cell proliferation in the dentate gyrus is highly dose-dependent, due to the above-described mechanisms - even low doses are detrimental (Limoli et al., 2004). Pyknotic cell numbers reach a plateau between 3 and 30 Gy in the adult rat dentate gyrus, as described by Tada et al. (Tada et al., 2000), which suggests an apoptotic-sensitive population. Even at 30 Gy doses, mature neuronal apoptosis was not observed and almost all cells undergoing apoptosis were proliferating. Therefore, they suggested that progenitors are the hypersensitive population. At lower doses (0,5 Gy), the numbers of DCX-positive cells are unaltered, yet side-effects on protein expression involving neuronal survival were detected, including decreased levels of ERK1/2 phosphorylation (Silasi et al., 2004). There is a slight difference between mice and rats when it comes to hippocampal sensitivity to irradiation; mice are somewhat more radio-resistant than rats (Mizumatsu et al., 2003). In humans, a near complete inhibition of neurogenesis in the hippocampus is observed following cancer treatment, which is consistent with animal studies (Monje et al., 2007).

So do high levels of cell genesis and plasticity in the immature brain compensate for irradiation-induced CNS damage? In the neurogenic niches, at least, the answer to that question is "no". On the contrary, an inverted relationship between vulnerability to irradiation and age is seen (Fukuda et al., 2005). This seems to be due to greater proliferation of stem/progenitor cells, thereby increased vulnerability to DNA damage, as well as to an altered microenvironment that is somehow more pronounced in the immature brain (Fukuda et al., 2005). The sensitivity of the immature rodent brain to doses similar to 6 Gy used in Paper II and III have been previously characterized in other studies conducted in our lab (Fukuda et al., 2004; Fukuda et al., 2005), showing extensive, dose-dependent apoptosis (activated Caspase-3) in the rodent GCL, as well as a significant dose-dependent reduction in GCL volume (Fukuda et al., 2004). Furthermore, apoptotic cell numbers peak at 6-12 hours post irradiation (Mizumatsu et al., 2003; Fukuda et al., 2004), and at 24 hours post-irradiation, these cells have been cleared from the area (Fukuda et al., 2004). Using 6 Gy, we therefore expected a rapid and profound effect on proliferating cell

numbers, which was the case, because the pool of BrdU-positive cells decreased by 85% two days after irradiation.

What does a reduced number of BrdU-cells reflect? Considering the multitude of studies demonstrating rapid cell death after irradiation, it is quite safe to say that fewer BrdU-labelled cells in the P11 mouse hippocampus reflect irradiation-induced, rapid, pre-mitotic apoptosis. However, it is also interesting to note that in addition to apoptosis, decreased proliferation occurs as a result of irradiation-induced injury (Monje et al., 2002). Because BrdU was injected one day after irradiation (P10), this could contribute to fewer BrdU-labelled cells.

*Radial-glia like stem cells are the primary target of radiation-induced cell loss*

Several papers have described a persistent loss of neurogenesis, even after low to moderate doses of irradiation (Tada et al., 2000; Mizumatsu et al., 2003; Fukuda et al., 2004). Consistent with this, a reduced number of DCX-positive cells was measured at P93, as well as a smaller GCL volume. However, we did not see any irradiation-induced effects on cell survival in the adult animals. Of all cells that divided at the timepoint of BrdU injection, a similar fraction was still present 4 weeks later and had become new neurons, regardless of whether the animals had been irradiated or not. This indicates that, at least when using a moderate dose, irradiation-induced negative impacts on cell survival in the dentate gyrus might not be as long-lasting as effects of irradiation on progenitor proliferation, which has been shown to persist for at least 120 days in the rodent CNS (Tada et al., 2000; Mizumatsu et al., 2003; Fukuda et al., 2004). Still, significantly fewer immature neurons were found in irradiated animals. How can this discrepancy be explained? It is well known that granule cells are overproduced in the dentate gyrus and removed by apoptosis (Biebl et al., 2000). The ratio of immature DCX-positive cells to new mature neurons at a given timepoint (P93) was approximately 20:1 in the control mice, compared to 5:1 in the irradiated animals. This could indicate either an early reduction of neurogenesis, or a loss of neuronal specification in the stem cell progeny. It has been shown that hippocampal irradiation leads to a disrupted neurogenic microenvironment, causing progenitors to adopt a glial fate instead of a neuronal one (Monje et al., 2002). However, we measured a profound (approximately 50%) reduction of radial-glia like astrocytes, considered to be the hippocampal stem cells, a finding that supports the latter theory. To our knowledge, this is the first study that identifies the putative stem cells as a primary target of irradiation. It does not exclude the possibility that radial-glia like stem cells produce glial cells to a greater extent as well, although we did not investigate this possibility. To investigate whether proliferating immature neurons were reduced, we performed double-labeling of phospho-H3 and DCX. In the SGZ and GCL, there were very few double-labelled cells (a maximum of eight cells per dentate gyrus) and variable in all groups, so it was impossible to draw any conclusions. Mizumamtsu and colleagues reported that apoptosis of DCX-positive cells in the dentate gyrus were predominant at doses greater than 2 Gy (Mizumatsu et al., 2003). Because our measurements were made several months after irradiation, it is likely that the reduced radial-glia like stem cell population resulted in the persistent effects of irradiation on neurogenesis.

*How does irradiation affect the microenvironment of the neurogenic niche?*

Irradiation injury to the CNS leading to brain health complications can be broadly divided into four categories: damage to the vascular system, deletion of oligodendrocyte progenitors, as well as mature oligodendrocytes, inflammation and alteration of cytokine expression, and removal of neural stem and progenitor cell populations in the neurogenic niches (reviewed in Belka et al., 2001). Irradiation affects the neurogenic niche in several ways, and while some have been described,

new mechanisms are continuously revealed. It is now clear that irradiation to the neurogenic niche alters the microenvironment, resulting in a loss of support for neurogenesis (Monje et al., 2002; Mizumatsu et al., 2003). Tada *et al.* described transiently increased proliferating astrocytes in the dentate gyrus after irradiation (Tada et al., 2000). Because the concept of radial glia-like astrocytes was not yet established, this was not further discussed. It might have been a regenerative attempt of the stem cells, as this has been described under other pathological conditions. Another explanation might come from studies performed by Monje *et al.*, showing that hippocampal irradiation disrupts the neurogenic microenvironment, causing neural progenitors to adopt a glial fate. Progenitors that survive irradiation readily differentiate into neurons *in vitro*, so the effects are most likely not due to intrinsic mechanisms, but rather to changes in the microenvironment. This was also clearly demonstrated when hippocampal progenitors were transplanted to the irradiated hippocampus – very few differentiated into neurons, while the glial production remained largely unaffected (Monje et al., 2002).

Inflammation and microvasculature changes are two strong factors, resulting in a hostile environment for new neurons. Infiltration of microglia and the release of cytokines are a part of the immune response to the impact of radiation therapy (Vallieres, 2002; Mizumatsu et al., 2003). Inflammatory blockade has been shown to protect neurogenesis, to some extent, following irradiation (Monje et al., 2003). The role of inflammatory mechanisms is unlikely in the present running experiment of irradiated mice for two reasons. Firstly, the time point for running was several months after insult, so inflammation should be reduced by this time. Secondly, the use of pentobarbital, rather than ketamine (or no aesthetic), seems to protect the dentate gyrus from irradiation-induced inflammation, by a yet unknown mechanism (Wojtowicz, 2006).

Changes in the microvasculature due to radiation therapy are well-known phenomena. A functional microvasculature has been proposed to be the foundation of a healthy neurogenic niche (Palmer et al., 2000). Proliferating progenitors tend to form clusters around vessels, supposedly dependent on blood-borne trophic factors and mitogens, such as VEGF (Cao et al., 2004). In addition, neurogenesis seems to be associated with the transient recruitment of endothelial cells, although the function of this is still unknown (Palmer et al., 2000).

#### *Voluntary exercise influences radial glia-like stem cells*

Four weeks of running was sufficient to completely restore the number of radial glia-like stem cells after 6-Gy irradiation. Thus, voluntary running acts by expanding the residual endogenous stem cell pool. But could it also expand the number of immature neurons? It is worth noting that the majority of dividing cells in the GCL were not DCX-positive, independent of treatment, suggesting that there was no exercise-induced expansion of immature neurons.

#### *How does voluntary exercise ameliorate irradiation-induced damage to the neurogenic niche?*

A challenging quest is to understand how voluntary exercise causes changes in the microenvironment, making it more supportive of neurogenesis. A firm relationship between angiogenesis and regional blood volume has been established (Lin et al., 2002; Cha et al., 2003). Intriguingly, exercise increases blood flow specifically to the dentate gyrus (Pereira et al., 2007). Neurotrophic factors, mitogens, and angiogenic factors, such as VEGF might reach target cells easier by travelling through the blood. VEGF improve the neuro-vascular interaction in the neurogenic niches, as a mitogen and angiogenic factor (Jin et al., 2002; Krum et al., 2002), and supported the recovery of a radial glia-like stem cell population. Similar to  $\beta$ -endorphin, VEGF is required for

exercise-induced neurogenesis in mice (Jin et al., 2002; Fabel et al., 2003). These improvements could also account for restoration of the disrupted process orientation in the DCX-positive cells of irradiated animals.

*Irradiation affects behavior of mice and men*

Children subjected to irradiation to treat brain tumours exhibit behavioural deficits, such as attention deficit disorders and memory impairment leading to learning disabilities (Gamis and Nesbit, 1991). The negative consequences to cognition gradually become worse with time (Mulhern et al., 2004) and persist into adulthood (Hall et al., 2004). Irradiation to the rat brain leads to behavioural changes similar to attention-deficit hyperactivity disorder (ADHD) patients, and this is also observed when irradiation is focally limited to the hippocampus, suggesting the involvement of the hippocampus in ADHD (Highfield et al., 1998). Whole brain irradiation to young C57Bl/6 mice results in long-term increased activity, even with low doses (Minamisawa and Hirokaga, 1996). In the present study, the number of rearings, stops, and turns was greater in irradiated mice compared to irradiated runners. It seems also as if irradiated animals were not as quick to habituate to the new environment (open field arena) as the other groups (data not presented in the thesis), possibly indicating impaired memory, because habituation is a form of non-associative memory. Overall, animals subjected to irradiation and subsequent physical exercise were more similar to normal mice in their behavioural pattern than irradiated mice deprived of the possibility to exercise in a running wheel. Noticeably, the significant differences between control and irradiated mice were more consistent over time than between the other groups, indicated a robust and reproducible effect of irradiation on behaviour. In the PLS-DA score plot, the spread was less between individuals in the control group and irradiation group, compared to the other two groups. Although the open field test is not a specific test for hippocampal function, it is highly sensitive (our test compared 108 variables) and well suited to document behavioural changes, such as ADHD-like symptoms (various behavioural tests are reviewed in Sousa et al., 2006).

*How can irradiation and voluntary exercise affect behavior in opposite ways?*

Hippocampal neurogenesis is regulated by proliferation, but also by integration of DCX-cells into a neuronal network. Plumpe and co-workers demonstrated that about 20% of DCX-positive cells proliferate, while more than 70% are postmitotic (Plumpe et al., 2006). Because we detected few proliferating DCX-positive cells and voluntary exercise increased the percentage of new neurons, we investigated whether voluntary exercise altered structural integration of immature cells. If signalling is dysfunctional, synaptic formation and dendritic maturation is disrupted (Ge et al., 2006), and cells that are unable to receive synaptic input correctly are more susceptible to apoptosis (Jessberger and Kempermann, 2003; Kempermann et al., 2003). Following irradiation, the leading process from the majority of DCX-positive cells did not correctly extend from the GCL into the molecular layer. This was normalized after voluntary running.

A previous study reported dramatic changes in the cytoarchitecture of the dentate gyrus following exercise; both dendritic length and complexity were significantly increased, as well as spine density (Eadie et al., 2005). The mechanisms behind these changes are not fully understood, although the authors suggested increased levels of BDNF could be responsible, because BDNF overexpression in mice increased dendritic complexity (Tolwani et al., 2002). Regardless of the underlying mechanism, restoring functional integration of immature neurons in the irradiated hippocampus might have led to behavioural improvements observed in irradiated mice subjected to exercise.



*Voluntary running does more than enrich the life of a socially deprived mouse*

There is an ongoing debate over whether social isolation hampers neurogenesis in rodents or not. It has been suggested that individual housing delays the positive effects of running in rats, with increased levels of the stress hormone corticosterone. In individually housed rats, increased proliferation was not observed until 48 days of running (Stranahan et al., 2006). In contrast to these findings, we show that individually housing does not hamper cell proliferation; after only 28 days access to running wheels, the mice displayed increased proliferation.

Another ongoing debate is whether it is possible to draw conclusions from studies on rodents housed in an impoverished environment (*e.g.*, a standard cage) and compare to humans. The mere access to a running wheel might be sufficient stimulus to result in increased neurogenesis. While it is true that one should be cautious about extrapolating research findings done in laboratory animals and directly compare to humans, other experiments involving stimuli, such as swimming in a water maze, have not demonstrated similar beneficial effects on hippocampal proliferation (Van Praag et al., 1999b; Van der Borght et al., 2005).

**Paper III – a summary**

CNS sensitivity to irradiation is highly age-dependent, and postnatal irradiation during the peak of gliogenesis is likely to eliminate a large number of proliferating OPCs residing in the white matter (Fukuda et al., 2005). In accordance with this, early postnatal irradiation of the rodent brain has been shown to reduce MBP density to less than half at one-week post-irradiation (Fukuda et al., 2004; Fukuda et al., 2005).

Therefore, to examine the effects of voluntary exercise on oligodendrogenesis and myelination, the brains of C57/BL6 male pups were irradiated with a moderate, yet clinically relevant, dose of 6 Gray (Gy) on postnatal day 9. After 7 weeks, bromodeoxyuridine (BrdU) was administered intraperitoneally (*i.p.*) to label proliferating cells, and the animals were subjected to voluntary exercise in a running wheel for a period of four weeks. Stereology was used to measure proliferative changes, as well as changes in the Olig2-positive cell pool and myelin density. The proliferation markers, BrdU and phospho-H3, were used together with Olig2 and myelin basic protein (MBP) to determine the number of newly formed glial progenitors, and the amount of myelin density after irradiation and running. To label newborn oligodendrocytes that survived to a mature phenotype, we also performed confocal analysis using the mature oligodendrocyte marker APC, together with Olig2 and BrdU. We determined that the existing pool of glial cells in the young postnatal brain was very susceptible to a moderate dose of irradiation, but the production of new glial progenitors was unaffected by irradiation. In addition, the production of new glial progenitors in the corpus callosum remained constant during the first postnatal weeks. Early postnatal irradiation induced a transient volume reduction and, unexpectedly, a transient increase in myelin density in the corpus callosum during peak myelin production. Survival of newly produced Olig2-positive cells, as well as the total number of Olig2-positive cells, was unaffected by voluntary exercise in adulthood, regardless of whether the animal was subjected to irradiation. However, using an unpaired t-test, a significant difference was determined between irradiated and non-irradiated runners, suggesting that voluntary exercise positively influenced glial progenitor cell numbers in the corpus callosum after irradiation to the immature brain. Nevertheless, the number of glial progenitors surviving into a more mature phenotype expressing APC was unaffected.

*Radiation therapy and white matter injury*

Demyelination is a serious pathological condition recognized as a late response of the human central nervous system to radiation therapy (Wong and Van der Kogel, 2004). White matter lesions are aggravated by young age (Mulhern et al., 2001), high doses (Nagesh et al., 2008), chemotherapy (Paakko et al., 2000), and a long post-therapeutic interval (Mulhern et al., 2001). Irradiation studies focusing on oligodendrocytes and myelin have been primarily confined to the spinal cord of adult or juvenile animals, and/or using high radiation doses (Franklin et al., 1997; Blakemore et al., 2002; Chari et al., 2003; Irvine and Blakemore, 2007). High dose irradiation to the rat spinal cord profoundly depletes oligodendrocytes, with concomitant myelin loss, and has, therefore, been used to study remyelination mechanisms (Franklin et al., 1997; Blakemore et al., 2002; Chari and Blakemore, 2002). Studies, with regard to irradiation of the rodent brain, are few; however, several studies have shown oligodendroglial depletion and myelin loss in the adult (Kurita et al., 2001; Sato et al., 2003; Irvine and Blakemore, 2007; Panagiotakos et al., 2007) and immature brain (Sano et al., 2000; Fukuda et al., 2004; Fukuda et al., 2005). Two of the latter studies were performed at our lab by Fukuda and colleagues, demonstrating age-dependent sensitivity of the rodent CNS myelin to irradiation (Fukuda et al., 2004; Fukuda et al., 2005).

*Susceptibility of glial progenitors in the immature rodent brain to irradiation*

During the second postnatal week, most proliferating cells in the white matter tracts are oligodendrocyte progenitors (Skoff and Knapp, 1991). Thus, irradiating during this time should target oligodendroglial cells. In accordance with this, Fukuda *et al.* found that a single dose of 8 Gy at P9 resulted in significant myelin loss, while irradiation with the same dose at P23 produced a significantly less pronounced effect (Fukuda et al., 2005). Panagiotakos and co-workers demonstrated that young adult rats irradiated with a single dose of 25 Gy exhibited a 16-fold reduction of proliferating cells in the corpus callosum, followed by a transient increase, without catching up to normal values. Normal values were not reached, and over time the differences were increased. The authors proposed that this was likely due to exhaustion of locally dividing progenitors after the initial attempt to repopulate the area. Loss of myelin was not initially observed, suggesting relative insensitivity of mature oligodendrocytes to irradiation. However, widespread loss began as late as 15 months after irradiation, perhaps due to very slow turnover of mature myelinating cells (Panagiotakos et al., 2007). Other studies have reported acute apoptosis of oligodendrocyte progenitors in the adult rat spinal cord without significantly decreased levels of oligodendrocyte progenitors until 2 weeks later. The authors suggested that irradiation-induced reduction of oligodendrocyte populations is mainly a result of clonogenic cell death, rather than acute apoptosis (Atkinson et al., 2005). While this may be true in the spinal cord, we demonstrate that a moderate dose (6 Gy) of irradiation to the immature rodent brain during the peak of oligodendrogenesis results in an acute extensive loss (nearly 40%) of Olig2-positive cells in the corpus callosum, an effect that persists for several weeks. Acute loss of oligodendroglial precursors in the rat neonatal brain and adult brain has also been previously demonstrated (Sano et al., 2000; Panagiotakos et al., 2007), albeit at higher radiation doses.

*Reaction of surviving oligodendrocyte progenitors to irradiation-induced injury*

Intriguingly, although irradiation greatly reduced the number of Olig2-positive cells in the corpus callosum, an acute reduction in the pool of proliferating Olig2-positive cells was not observed at any time point. Although proliferating Olig2-positive cells might be protected from irradiation by a yet unknown mechanism, Olig2-positive

cells could also be triggered to enter cell cycle as more niches become available. Oligodendrocyte progenitors have the potential to proliferate and remyelinate in response to injury or oligodendrocyte depletion (Redwine and Armstrong, 1998; Chari and Blakemore, 2002; Picard-Riera et al., 2002; Reynolds et al., 2002; Bu et al., 2004; Glezer et al., 2006; Kessaris et al., 2006). Furthermore, oligodendroglial numbers are tightly regulated and compete for available mitogens and trophic factors, and depletion of endogenous oligodendrocytes in white matter is required for successful repopulation by transplanted oligodendrocyte progenitors (Hinks and Franklin, 1999; Irvine and Blakemore, 2007). In the adult rat spinal cord, proliferative responses of oligodendroglial progenitors have been observed, peaking at 2 weeks post-irradiation (Li and Wong, 1998; Atkinson et al., 2005). Studies in the spinal cord of p53-double knockout mice have shown that apoptosis alone is unlikely to trigger compensatory oligodendroglial proliferation after irradiation (Atkinson et al., 2005). However, significantly increased proliferation of Olig2-positive cells at two days or two weeks post-irradiation was not measured in the present study, which does not support a compensatory proliferative mechanism in the immature rodent brain. One possibility is that compensatory mechanisms are activated only after complete, or near elimination, of oligodendrocytes. Such a mechanism has been suggested in other studies (Li and Wong, 1998). Further studies need to be performed to determine whether there is a lower limit of oligodendrocyte numbers after higher doses of irradiation that would stimulate a compensatory mechanism.

#### *Extensive loss of proliferating cells outside the Olig2-positive cell pool*

Contrary to expectations, the majority of proliferating cells that were lost were not Olig2-positive cells. Preliminary data (not presented in the thesis) indicate that these cells are not astrocytes or microglia either. Astrocytes and microglia are also reportedly less prone to undergo irradiation-induced apoptosis (Ferrer et al., 1995; Kurita et al., 2001). Theoretically, the proliferating cells could be DCX-positive neuronal progenitors migrating towards the olfactory bulb, because the corpus callosum and the rostral migratory stream are not clearly separated in very young animals. They could also be more immature cells migrating from the SVZ. Further immunohistological studies with more immature stem/progenitor markers, such as Mash-1 or Sox2, in combination with apoptosis markers, such as active Caspase-3, might provide answers to this discrepancy.

#### *Overproduction of Olig2-positive cells as a “buffer” during postnatal development*

The effect of moderate dose irradiation on glial progenitors persisted two weeks post-irradiation. The production and the pool of glial progenitors remained unchanged between P11, which has been reported to be a highly proliferative period, and P23, where myelination peaks. We determined Olig2 to be downregulated and only diffusely expressed in cells that strongly expressed APC (data not presented in the thesis); APC is a marker expressed in oligodendroglial cells proceeding to a postmitotic stage. This suggests that during the myelination period, the production of new oligodendrocytes is unchanged, and cells proceeding to a more mature stage are replaced by new ones. So why are the cells eliminated by irradiation not replaced? It has been established that oligodendrocyte precursors are overproduced during brain maturation and, as previously mentioned, selectively eliminated by competitive mechanisms (Kessaris et al., 2006). It is plausible that the brain can only support the production of a certain level of mitogens, resulting in an upper limit of proliferation among glial progenitors. In our study, these levels seem to be sufficient for myelination, because we did not observe an irradiation-induced reduction in myelin density. In contrast, a transient increase in myelin density was measured at two weeks post-irradiation. A concurrent, transient, decrease in CC

volume was also measured in the P23 irradiated animals, after correcting for shrinkage. P23 is a developmental stage in the rodent brain of high myelin production, and decreased corpus callosum volume might reflect the lack of immature oligodendrocytes, not myelin. Existing oligodendrocyte populations have been shown to inhibit repopulation of new oligodendrocytes (Chari et al., 2003; Irvine and Blakemore, 2007), which might have resulted in more effective myelination from the surviving oligodendrocytes. In addition, it is possible that reduced cell numbers in irradiated mice results in an excess of trophic factors required for the high metabolic rate of myelin-producing cells. The microenvironment following irradiation might also be more permissive for remyelination, in contrast to the effects of irradiation on the neurogenic niche.

*Does exercise promote oligodendrogenesis?*

Very few studies have evaluated cell proliferation in regions not connected to adult neurogenesis after exercise. Yet, factors that have been shown to be beneficial for oligodendrogenesis and/or myelination are secreted after physical exercise, such as IGF-I (Ding et al., 2006; Aberg et al., 2007) and  $\beta$ -endorphin (Thoren et al., 1990 ; Persson et al., 2006). Ehninger and Kempermann reported that microglia proliferation is also increased after 4 weeks of voluntary running (Ehninger and Kempermann, 2003). In the same study, effects on cortical oligodendrogenesis were not observed; however, the myelin-dense corpus callosum was not investigated. Skup and co-workers (Skup et al., 2002) suggested that increased oligodendrogenesis might occur in the spinal cord of rats subjected to exercise. Furthermore, genes involved in oligodendrocyte development are upregulated in rat spinal cord after exercise (Perreau et al., 2005). Nevertheless, convincing evidence of exercise-induced oligodendrogenesis was not presented at the beginning of this thesis (2003). Recently, however, exercise has been shown to result in a three-fold increase of NG2-positive cells in the medial prefrontal cortex (Mandyam et al., 2007). The authors did not investigate whether these cells gave rise to mature oligodendrocytes. We did not find an effect on the generation of mature oligodendrocytes after four weeks of voluntary running, as measured by the production of APC-positive cells. Voluntary running also did not affect the number of Olig2-positive cells or myelin density. However, there was a large variation of Olig2-positive cell numbers between individual animals. In accordance with this, there were significantly more Olig2-positive cells in irradiated runners compared to irradiated only animals (t-test,  $P < 0,05$ , data not shown). Further studies need to be performed, with a greater number of animals, to determine whether the effect of voluntary running on the generation of Olig-2 positive glial progenitors is significant. If so, these cells do not seem to proceed to a more mature phenotype, or generate an increased myelin density. A greater irradiation dose and/or an earlier running time point could, perhaps, disclose effects of exercise on oligodendrogenesis. However, one must also take into consideration irradiation-induced inflammation when performing studies at an early time-point, because inflammatory mechanisms are known to last for several weeks post-irradiation (Monje et al., 2003).

## GENERAL CONCLUSION

Data from this thesis demonstrated that the opioid peptide  $\beta$ -endorphin, endogenously released during exercise, increased oligodendrogenesis in cultured adult rat hippocampal stem/progenitor cells. Increased oligodendrogenesis was shown to be mediated by expression of the HLH protein Id1, known to be involved in cell fate. Furthermore,  $\beta$ -endorphin reduced the number of cells expressing Mash-1, a pro-neural bHLH protein expressed in neural progenitors. We also demonstrate that irradiation of the immature mouse brain with a moderate and clinically relevant dose of irradiation significantly reduced the production of new neurons in the adult mouse, acting directly on the dentate gyrus stem cell pool. Furthermore, we show that the given dose altered behaviour in adult age, as well as perturbed orientation of immature neurons in the hippocampal dentate gyrus. Voluntary exercise restored precursor numbers and neurogenesis by affecting the stem cell pool. Also, voluntary exercise ameliorated the effects of irradiation on behaviour, as well as normalised the orientation of new neurons. This thesis also presents data demonstrating that a moderate and clinically relevant dose of irradiation to the immature mouse brain significantly reduced the pool of Olig2-positive cells in the corpus callosum, without affecting the rate of newborn glial progenitors. We also show that the loss of Olig2-positive cells is followed by a significant reduction in corpus callosum volume. In addition, we demonstrate that although the effects of moderate dose irradiation on Olig2-positive cells were persistent, they did not last into adulthood. In contrast to the beneficial effects of voluntary exercise on neurogenesis in the hippocampus, no effects of voluntary exercise on oligodendrogenesis and myelin density in the corpus callosum were found.

### Specific conclusions to given aims:

- (I) Stimulation of AHPs with the opioid-peptide  $\beta$ -endorphin increased mRNA levels of genes associated with oligodendrogenesis, and treatment with opioids for 10 days resulted in increased oligodendrogenesis *in vitro*. We found that opioid-induced oligodendrogenesis required expression of the HLH-protein Id-1, known to be involved in cell fate regulation and activated by the transcription factor Egr-1, which also was found to be upregulated by  $\beta$ -endorphin. Opioid-induced oligodendrogenesis was preceded by downregulation of the bHLH protein Mash-1, a proneuronal transcription factor.
- (II) Voluntary running restored neurogenesis, acting directly on the stem cell pool, and ameliorated irradiation-induced alterations in behaviour. The orientation of immature neurons in the dentate gyrus of the hippocampus was perturbed after irradiation, but restored to normal orientation by voluntary running. These results clearly demonstrate the usefulness of physical exercise for functional and structural recovery from juvenile brain insults, and highlights that despite long-term damage to the hippocampal microenvironment, exercise is able to remodel and reverse brain deficits.

- (III) Voluntary exercise did not significantly affect the number of newborn oligodendrocytes in the corpus callosum of adult mice, regardless of whether the Olig2-positive cell pool was depleted postnatally or not. Although irradiation transiently decreased corpus callosum volume, voluntary exercise did not increase oligodendrogenesis or myelin density in adulthood. This might suggest that a neurogenic niche, such as the hippocampus, is required for pronounced beneficial effects of exercise on cell genesis in the brain.

### SIGNIFICANCE AND OUTLOOK

CNS diseases and injuries provide an enormous challenge to patients and society. For decades, the loss of brain cells and resulting functional deficits have been regarded as an irreversible verdict for the patient, with very limited chances for recovery. Nevertheless, knowledge in the field of neural and glial plasticity has dramatically increased over the past decades. The study of endogenous stem cells and their capacity to generate neurons and glial cells in the brain has given new hope to the prospect of structural and functional CNS repair. Our long-term goal is to control endogenous neural stem cells and progenitor cells *in vivo* to produce new cells and restore functions lost to diseases. Information from the current study could provide knowledge for the development of endogenous cell replacement strategies.

It seems surprising that simple processes, such as optimized physical exercise, could alter the internal milieu of the injured brain to the extent that we observed. However, we have learned over the last years that many neuroplastic changes, including remyelination, synapse and dendrite formation and neurogenesis are more common than previously observed. Physical exercise may help providing a permissive milieu for these mechanisms through release of growth factor and trophic factors and concomitant stem cell activation. In the end, we reach a conclusion that was already put forward at the beginning of our western thinking by the roman poet Juvenal: "a healthy mind lives in a healthy body". And although physical fitness and exercise may not prevent neurological diseases per se, it may provide the best possibility for coping and recovery as well as successful aging.

## REFERENCES

- Abayomi OK (1996) Pathogenesis of irradiation-induced cognitive dysfunction. *Acta Oncol* 35:659-663.
- Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS (2003) IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol Cell Neurosci* 24:23-40.
- Aberg ND, Johansson UE, Aberg MA, Hellstrom NA, Lind J, Bull C, Isgaard J, Anderson MF, Oscarsson J, Eriksson PS (2007) Peripheral infusion of insulin-like growth factor-I increases the number of newborn oligodendrocytes in the cerebral cortex of adult hypophysectomized rats. *Endocrinology* 148:3765-3772.
- Aguirre A, Gallo V (2004) Postnatal neurogenesis and gliogenesis in the olfactory bulb from NG2-expressing progenitors of the subventricular zone. *J Neurosci* 24:10530-10541.
- Alexson TO, Hitoshi S, Coles BL, Bernstein A, van der Kooy D (2006) Notch signaling is required to maintain all neural stem cell populations--irrespective of spatial or temporal niche. *Dev Neurosci* 28:34-48.
- Altman J (1962a) Are new neurons formed in the brains of adult mammals? *Science* 135:1127-1128.
- Altman J (1962b) Autoradiographic study of degenerative and regenerative proliferation of neuroglia cells with tritiated thymidine. *Exp Neurol* 5:302-318.
- Altman J (1963) Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anat Rec* 145:573-591.
- Altman J (1966) Proliferation and migration of undifferentiated precursor cells in the rat during postnatal gliogenesis. *Exp Neurol* 16:263-278.
- Altman J, Das GD (1965a) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol* 124:319-335.
- Altman J, Das GD (1965b) Autoradiographic and histologic evidence of postnatal neurogenesis in rats. *J Comp Neurol* 124:319-335.
- Altman J, Das GD (1965c) Post-natal origin of microneurons in the rat brain. *Nature* 207:953-956.
- Altman J, Das GD (1966) Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. *J Comp Neurol* 126:337-389.
- Altman J, Das GD (1967) Postnatal neurogenesis in the guinea-pig. *Nature* 214:1098-1101.
- Altman J, Bayer SA (1990) Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. *J Comp Neurol* 301:325-342.
- Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD (2001) A unified hypothesis on the lineage of neural stem cells. *Nat Rev Neurosci* 2:287-293.
- Ambrogini P, Lattanzi D, Ciuffoli S, Agostini D, Bertini L, Stocchi V, Santi S, Cuppini R (2004) Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus. *Brain Res* 1017:21-31.
- Andel R, Crowe M, Pedersen NL, Fratiglioni L, Johansson B, Gatz M (2008) Physical exercise at midlife and risk of dementia three decades later: a population-based study of Swedish twins. *J Gerontol A Biol Sci Med Sci* 63:62-66.

- Angelogianni P, Li HL, Gianoulakis C (2000) Ontogenesis of proopiomelanocortin and its processing to beta-endorphin by the fetal and neonatal rat brain. *Neuroendocrinology* 72:231-241.
- Anthony TE, Klein C, Fishell G, Heintz N (2004) Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron* 41:881-890.
- Appenzeller O (1981) What makes us run? *N Engl J Med* 305:578-580.
- Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. *Science* 284:770-776.
- Atkinson SL, Li YQ, Wong CS (2005) Apoptosis and proliferation of oligodendrocyte progenitor cells in the irradiated rodent spinal cord. *Int J Radiat Oncol Biol Phys* 62:535-544.
- Bani-Yaghoob M, Tremblay RG, Lei JX, Zhang D, Zurakowski B, Sandhu JK, Smith B, Ribocco-Lutkiewicz M, Kennedy J, Walker PR, Sikorska M (2006) Role of Sox2 in the development of the mouse neocortex. *Dev Biol* 295:52-66.
- Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, Gage FH, Zhao X (2006) Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. *Stem Cells Dev* 15:407-421.
- Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, Anderson S, Gallo V (2003) Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J Cell Biol* 161:169-186.
- Belka C, Budach W, Kortmann RD, Bamberg M (2001) Radiation induced CNS toxicity--molecular and cellular mechanisms. *Br J Cancer* 85:1233-1239.
- Bellinzona M, Gobbel GT, Shinohara C, Fike JR (1996) Apoptosis is induced in the subependyma of young adult rats by ionizing irradiation. *Neurosci Lett* 208:163-166.
- Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullen F (2005) Extensive piano practicing has regionally specific effects on white matter development. *Nat Neurosci* 8:1148-1150.
- Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M, Lindvall O (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc Natl Acad Sci U S A* 94:10432-10437.
- Berry M, Hubbard P, Butt AM (2002) Cytology and lineage of NG2-positive glia. *J Neurocytol* 31:457-467.
- Bhat RV, Axt KJ, Fosnaugh JS, Smith KJ, Johnson KA, Hill DE, Kinzler KW, Baraban JM (1996) Expression of the APC tumor suppressor protein in oligodendroglia. *Glia* 17:169-174.
- Bick-Sander A, Steiner B, Wolf SA, Babu H, Kempermann G (2006) Running in pregnancy transiently increases postnatal hippocampal neurogenesis in the offspring. *Proc Natl Acad Sci U S A* 103:3852-3857.
- Biebl M, Cooper CM, Winkler J, Kuhn HG (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci Lett* 291:17-20.
- Bjornebekk A, Mathe AA, Brene S (2005) The antidepressant effect of running is associated with increased hippocampal cell proliferation. *Int J Neuropsychopharmacol* 8:357-368.
- Blakemore WF, Gilson JM, Crang AJ (2000) Transplanted glial cells migrate over a greater distance and remyelinate demyelinated lesions more rapidly than endogenous remyelinating cells. *J Neurosci Res* 61:288-294.
- Blakemore WF, Chari DM, Gilson JM, Crang AJ (2002) Modelling large areas of demyelination in the rat reveals the potential and possible limitations of transplanted glial cells for remyelination in the CNS. *Glia* 38:155-168.



- Blumenthal JA, Babyak MA, Moore KA, Craighead WE, Herman S, Khatri P, Waugh R, Napolitano MA, Forman LM, Appelbaum M, Doraiswamy PM, Krishnan KR (1999) Effects of exercise training on older patients with major depression. *Arch Intern Med* 159:2349-2356.
- Blumenthal JA, Babyak MA, Doraiswamy PM, Watkins L, Hoffman BM, Barbour KA, Herman S, Craighead WE, Brosse AL, Waugh R, Hinderliter A, Sherwood A (2007) Exercise and pharmacotherapy in the treatment of major depressive disorder. *Psychosom Med* 69:587-596.
- Boecker H, Sprenger T, Spilker ME, Henriksen G, Koppenhoefer M, Wagner KJ, Valet M, Berthele A, Tolle TR (2008) The Runner's High: Opioidergic Mechanisms in the Human Brain. *Cereb Cortex*.
- Bottenstein JE, Sato GH (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc Natl Acad Sci U S A* 76:514-517.
- Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, von der Behrens W, Kempermann G (2003) Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol Cell Neurosci* 24:603-613.
- Breunig JJ, Silbereis J, Vaccarino FM, Sestan N, Rakic P (2007) Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus. *Proc Natl Acad Sci U S A* 104:20558-20563.
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 467:1-10.
- Bruel-Jungerman E, Rampon C, Laroche S (2007) Adult hippocampal neurogenesis, synaptic plasticity and memory: facts and hypotheses. *Rev Neurosci* 18:93-114.
- Brunner C, Lassmann H, Waehneldt TV, Matthieu JM, Linington C (1989) Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase in the CNS of adult rats. *J Neurochem* 52:296-304.
- Bu J, Banki A, Wu Q, Nishiyama A (2004) Increased NG2(+) glial cell proliferation and oligodendrocyte generation in the hypomyelinating mutant shiverer. *Glia* 48:51-63.
- Buch SK, Khurdayan VK, Lutz SE, Knapp PE, El-Hage N, Hauser KF (2007) Glial-restricted precursors: patterns of expression of opioid receptors and relationship to human immunodeficiency virus-1 Tat and morphine susceptibility *in vitro*. *Neuroscience* 146:1546-1554.
- Buffo A, Vosko MR, Erturk D, Hamann GF, Jucker M, Rowitch D, Gotz M (2005) Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. *Proc Natl Acad Sci U S A* 102:18183-18188.
- Butt AM, Kiff J, Hubbard P, Berry M (2002) Synantocytes: new functions for novel NG2 expressing glia. *J Neurocytol* 31:551-565.
- Cai L, Morrow EM, Cepko CL (2000) Misexpression of basic helix-loop-helix genes in the murine cerebral cortex affects cell fate choices and neuronal survival. *Development* 127:3021-3030.
- Cameron HA, Woolley CS, McEwen BS, Gould E (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience* 56:337-344.
- Campbell K (2003) Dorsal-ventral patterning in the mammalian telencephalon. *Curr Opin Neurobiol* 13:50-56.
- Campos LS (2005) Beta1 integrins and neural stem cells: making sense of the extracellular environment. *Bioessays* 27:698-707.

- Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, During MJ (2004) VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 36:827-835.
- Carles S, Jr., Curnier D, Pathak A, Roncalli J, Bousquet M, Garcia JL, Galinier M, Senard JM (2007) Effects of short-term exercise and exercise training on cognitive function among patients with cardiac disease. *J Cardiopulm Rehabil Prev* 27:395-399.
- Carro E, Trejo JL, Busiguina S, Torres-Aleman I (2001) Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *J Neurosci* 21:5678-5684.
- Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. *Biol Psychol* 54:241-257.
- Cha HJ, Jeong MJ, Kleinman HK (2003) Role of thymosin beta4 in tumor metastasis and angiogenesis. *J Natl Cancer Inst* 95:1674-1680.
- Chang A, Nishiyama A, Peterson J, Prineas J, Trapp BD (2000) NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. *J Neurosci* 20:6404-6412.
- Chari DM, Blakemore WF (2002) Efficient recolonisation of progenitor-depleted areas of the CNS by adult oligodendrocyte progenitor cells. *Glia* 37:307-313.
- Chari DM, Huang WL, Blakemore WF (2003) Dysfunctional oligodendrocyte progenitor cell (OPC) populations may inhibit repopulation of OPC depleted tissue. *J Neurosci Res* 73:787-793.
- Chen CC, Lee WR, Safe S (2004) Egr-1 is activated by 17beta-estradiol in MCF-7 cells by mitogen-activated protein kinase-dependent phosphorylation of ELK-1. *J Cell Biochem* 93:1063-1074.
- Chen XS, Zhou DS, Yao ZX (2007) The inhibitor of DNA binding 2 is mainly expressed in oligodendrocyte lineage cells in adult rat brain. *Neurosci Lett* 428:93-98.
- Chomczynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *Biotechniques* 15:532-534, 536-537.
- Corbett AD, Henderson G, McKnight AT, Paterson SJ (2006) 75 years of opioid research: the exciting but vain quest for the Holy Grail. *Br J Pharmacol* 147 Suppl 1:S153-162.
- Coskun V, Wu H, Blanchi B, Tsao S, Kim K, Zhao J, Biancotti JC, Hutnick L, Krueger RC, Jr., Fan G, de Vellis J, Sun YE (2008) CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain. *Proc Natl Acad Sci U S A* 105:1026-1031.
- Costa MR, Kessar N, Richardson WD, Gotz M, Hedin-Pereira C (2007) The marginal zone/layer I as a novel niche for neurogenesis and gliogenesis in developing cerebral cortex. *J Neurosci* 27:11376-11388.
- Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L (2005) Doublecortin expression levels in adult brain reflect neurogenesis. *Eur J Neurosci* 21:1-14.
- Dash PK, Mach SA, Moore AN (2001) Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. *J Neurosci Res* 63:313-319.
- Davies AM (2003) Regulation of neuronal survival and death by extracellular signals during development. *Embo J* 22:2537-2545.
- Dawson MR, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci* 24:476-488.
- Dayar AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol* 460:563-572.
- Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC (2004) Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* 42:535-552.

- Ding Q, Vaynman S, Akhavan M, Ying Z, Gomez-Pinilla F (2006) Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. *Neuroscience* 140:823-833.
- Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 17:5046-5061.
- Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97:703-716.
- Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A (2002) EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* 36:1021-1034.
- Domokos A, Mergl Z, Barna I, Makara GB, Zelena D (2008) Congenital vasopressin deficiency and acute and chronic opiate effects on hypothalamo-pituitary-adrenal axis activity in Brattleboro rats. *J Endocrinol* 196:113-121.
- Duman CH, Schlesinger L, Russell DS, Duman RS (2008) Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Res* 1199:148-158.
- During MJ, Cao L (2006) VEGF, a mediator of the effect of experience on hippocampal neurogenesis. *Curr Alzheimer Res* 3:29-33.
- Eadie BD, Redila VA, Christie BR (2005) Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *J Comp Neurol* 486:39-47.
- Eckenhoff MF, Rakic P (1984) Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey. *J Comp Neurol* 223:1-21.
- Ehninger D, Kempermann G (2003) Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. *Cereb Cortex* 13:845-851.
- Eisch AJ, Barrot M, Schad CA, Self DW, Nestler EJ (2000) Opiates inhibit neurogenesis in the adult rat hippocampus. *Proc Natl Acad Sci U S A* 97:7579-7584.
- Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* 100:13632-13637.
- Elmi M, Faigle R, Yang W, Matsumoto Y, Rosenqvist E, Funa K (2007) Mechanism of MASH1 induction by ASK1 and ATRA in adult neural progenitors. *Mol Cell Neurosci* 36:248-259.
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. *Nat Med* 4:1313-1317.
- Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, Kuo CJ, Palmer TD (2003) VEGF is necessary for exercise-induced adult hippocampal neurogenesis. *Eur J Neurosci* 18:2803-2812.
- Faigle R, Brederlau A, Elmi M, Arvidsson Y, Hamazaki TS, Uramoto H, Funa K (2004) ASK1 inhibits astroglial development via p38 mitogen-activated protein kinase and promotes neuronal differentiation in adult hippocampus-derived progenitor cells. *Mol Cell Biol* 24:280-293.
- Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR (2004) Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats *in vivo*. *Neuroscience* 124:71-79.
- Ferrer I, Olive M, Blanco R, Ballabriga J, Cinos C, Planas AM (1995) Amoeboid microglial response following X-ray-induced apoptosis in the neonatal rat brain. *Neurosci Lett* 193:109-112.

- Fields RD (2005) Myelination: an overlooked mechanism of synaptic plasticity? *Neuroscientist* 11:528-531.
- Filippov V, Kronenberg G, Pivneva T, Reuter K, Steiner B, Wang LP, Yamaguchi M, Kettenmann H, Kempermann G (2003) Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol Cell Neurosci* 23:373-382.
- Fischer SJ, Arguello AA, Charlton JJ, Fuller DC, Zachariou V, Eisch AJ (2008) Morphine blood levels, dependence, and regulation of hippocampal subgranular zone proliferation rely on administration paradigm. *Neuroscience* 151:1217-1224.
- Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron* 23:247-256.
- Franklin RJ (2002) Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci* 3:705-714.
- Franklin RJ, Gilson JM, Blakemore WF (1997) Local recruitment of remyelinating cells in the repair of demyelination in the central nervous system. *J Neurosci Res* 50:337-344.
- Frazer CJ, Christensen H, Griffiths KM (2005) Effectiveness of treatments for depression in older people. *Med J Aust* 182:627-632.
- Friedman B, Hockfield S, Black JA, Woodruff KA, Waxman SG (1989) In situ demonstration of mature oligodendrocytes and their processes: an immunocytochemical study with a new monoclonal antibody, rip. *Glia* 2:380-390.
- Fukuda A, Fukuda H, Swanpalmer J, Hertzman S, Lannering B, Marky I, Bjork-Eriksson T, Blomgren K (2005) Age-dependent sensitivity of the developing brain to irradiation is correlated with the number and vulnerability of progenitor cells. *J Neurochem* 92:569-584.
- Fukuda H, Fukuda A, Zhu C, Korhonen L, Swanpalmer J, Hertzman S, Leist M, Lannering B, Lindholm D, Bjork-Eriksson T, Marky I, Blomgren K (2004) Irradiation-induced progenitor cell death in the developing brain is resistant to erythropoietin treatment and caspase inhibition. *Cell Death Differ* 11:1166-1178.
- Fukuda S, Kato F, Tozuka Y, Yamaguchi M, Miyamoto Y, Hisatsune T (2003) Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *J Neurosci* 23:9357-9366.
- Gage FH (2000) Mammalian neural stem cells. *Science* 287:1433-1438.
- Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, Peterson DA, Suhr ST, Ray J (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proc Natl Acad Sci U S A* 92:11879-11883.
- Gaiano N, Nye JS, Fishell G (2000) Radial glial identity is promoted by Notch1 signaling in the murine forebrain. *Neuron* 26:395-404.
- Gall CM, Hendry SH, Seroogy KB, Jones EG, Haycock JW (1987) Evidence for coexistence of GABA and dopamine in neurons of the rat olfactory bulb. *J Comp Neurol* 266:307-318.
- Gamis AS, Nesbit ME (1991) Neuropsychologic (cognitive) disabilities in long-term survivors of childhood cancer. *Pediatrics* 18:11-19.
- Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV (2004) GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat Neurosci* 7:1233-1241.
- Garcion E, Halilagic A, Faissner A, French-Constant C (2004) Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. *Development* 131:3423-3432.

- Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 439:589-593.
- Gensert JM, Goldman JE (1996) In vivo characterization of endogenous proliferating cells in adult rat subcortical white matter. *Glia* 17:39-51.
- Gensert JM, Goldman JE (1997) Endogenous progenitors remyelinate demyelinated axons in the adult CNS. *Neuron* 19:197-203.
- Gensert JM, Goldman JE (2001) Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. *J Neurobiol* 48:75-86.
- Givogri MI, de Planell M, Galbiati F, Superchi D, Gritti A, Vescovi A, de Vellis J, Bongarzone ER (2006) Notch signaling in astrocytes and neuroblasts of the adult subventricular zone in health and after cortical injury. *Dev Neurosci* 28:81-91.
- Glezer I, Lapointe A, Rivest S (2006) Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *Faseb J* 20:750-752.
- Glickfeld LL, Atallah BV, Scanziani M (2008) Complementary modulation of somatic inhibition by opioids and cannabinoids. *J Neurosci* 28:1824-1832.
- Goldman SA, Nottebohm F (1983) Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci U S A* 80:2390-2394.
- Goto Y, Grace AA (2007) Dopamine Modulation of Hippocampal Prefrontal Cortical Interaction Drives Memory-Guided Behavior. *Cereb Cortex*.
- Gotts JE, Chesselet MF (2005) Vascular changes in the subventricular zone after distal cortical lesions. *Exp Neurol* 194:139-150.
- Gotz M (2003) Glial cells generate neurons--master control within CNS regions: developmental perspectives on neural stem cells. *Neuroscientist* 9:379-397.
- Gotz M, Huttner WB (2005) The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* 6:777-788.
- Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc Natl Acad Sci U S A* 95:3168-3171.
- Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999) Learning enhances adult neurogenesis in the hippocampal formation. *Nat Neurosci* 2:260-265.
- Greenwood K, Butt AM (2003) Evidence that perinatal and adult NG2-glia are not conventional oligodendrocyte progenitors and do not depend on axons for their survival. *Mol Cell Neurosci* 23:544-558.
- Grossman A, Clement-Jones V (1983) Opiate receptors: enkephalins and endorphins. *Clin Endocrinol Metab* 12:31-56.
- Gu F, Hata R, Ma YJ, Tanaka J, Mitsuda N, Kumon Y, Hanakawa Y, Hashimoto K, Nakajima K, Sakanaka M (2005) Suppression of Stat3 promotes neurogenesis in cultured neural stem cells. *J Neurosci Res* 81:163-171.
- Gurwell JA, Duncan MJ, Maderspach K, Stiene-Martin A, Elde RP, Hauser KF (1996) kappa-opioid receptor expression defines a phenotypically distinct subpopulation of astroglia: relationship to Ca<sup>2+</sup> mobilization, development, and the antiproliferative effect of opioids. *Brain Res* 737:175-187.
- Hack MA, Saghatelian A, de Chevigny A, Pfeifer A, Ashery-Padan R, Lledo PM, Gotz M (2005) Neuronal fate determinants of adult olfactory bulb neurogenesis. *Nat Neurosci* 8:865-872.
- Hadley ME, Haskell-Luevano C (1999) The proopiomelanocortin system. *Ann N Y Acad Sci* 885:1-21.

- Halasy K, Racz B, Maderspach K (2000) Kappa opioid receptors are expressed by interneurons in the CA1 area of the rat hippocampus: a correlated light and electron microscopic immunocytochemical study. *J Chem Neuroanat* 19:233-241.
- Hall P, Adami HO, Trichopoulos D, Pedersen NL, Laggiou P, Ekbom A, Ingvar M, Lundell M, Granath F (2004) Effect of low doses of ionising radiation in infancy on cognitive function in adulthood: Swedish population based cohort study. *Bmj* 328:19.
- Harburg GC, Hall FS, Harrist AV, Sora I, Uhl GR, Eisch AJ (2007) Knockout of the mu opioid receptor enhances the survival of adult-generated hippocampal granule cell neurons. *Neuroscience* 144:77-87.
- Hargreaves KM, Flores CM, Dionne RA, Mueller GP (1990) The role of pituitary beta-endorphin in mediating corticotropin-releasing factor-induced antinociception. *Am J Physiol* 258:E235-242.
- Hart IK, Richardson WD, Heldin CH, Westermark B, Raff MC (1989) PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. *Development* 105:595-603.
- Hartfuss E, Galli R, Heins N, Gotz M (2001) Characterization of CNS precursor subtypes and radial glia. *Dev Biol* 229:15-30.
- Hattiangady B, Shetty AK (2008) Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus. *Neurobiol Aging* 29:129-147.
- Hauser KF, Gurwell JA, Bhat NR (1993) Endogenous opioid systems and the growth of oligodendrocyte progenitors: paradoxical increases in oligodendrogenesis as an indirect mechanism of opioid action. *Glia* 9:157-162.
- Hauser KF, Houdi AA, Turbek CS, Elde RP, Maxson W, 3rd (2000) Opioids intrinsically inhibit the genesis of mouse cerebellar granule neuron precursors in vitro: differential impact of mu and delta receptor activation on proliferation and neurite elongation. *Eur J Neurosci* 12:1281-1293.
- Heine VM, Zareno J, Maslam S, Joels M, Lucassen PJ (2005) Chronic stress in the adult dentate gyrus reduces cell proliferation near the vasculature and VEGF and Flk-1 protein expression. *Eur J Neurosci* 21:1304-1314.
- Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M (2002) Glial cells generate neurons: the role of the transcription factor Pax6. *Nat Neurosci* 5:308-315.
- Herz A, ed (1993) Opioids II. Berlin Heidelberg: Springer verlag.
- Highfield DA, Hu D, Amsel A (1998) Alleviation of x-irradiation-based deficit in memory-based learning by D-amphetamine: suggestions for attention deficit-hyperactivity disorder. *Proc Natl Acad Sci U S A* 95:5785-5788.
- Hinks GL, Franklin RJ (1999) Distinctive patterns of PDGF-A, FGF-2, IGF-I, and TGF-beta1 gene expression during remyelination of experimentally-induced spinal cord demyelination. *Mol Cell Neurosci* 14:153-168.
- Hinks GL, Chari DM, O'Leary MT, Zhao C, Keirstead HS, Blakemore WF, Franklin RJ (2001) Depletion of endogenous oligodendrocyte progenitors rather than increased availability of survival factors is a likely explanation for enhanced survival of transplanted oligodendrocyte progenitors in X-irradiated compared to normal CNS. *Neuropathol Appl Neurobiol* 27:59-67.
- Hodge C, Liao J, Stofega M, Guan K, Carter-Su C, Schwartz J (1998) Growth hormone stimulates phosphorylation and activation of elk-1 and expression of c-fos, egr-1, and junB through activation of extracellular signal-regulated kinases 1 and 2. *J Biol Chem* 273:31327-31336.

- Holmes MM, Galea LA (2002) Defensive behavior and hippocampal cell proliferation: differential modulation by naltrexone during stress. *Behav Neurosci* 116:160-168.
- Horner PJ, Thallmair M, Gage FH (2002) Defining the NG2-expressing cell of the adult CNS. *J Neurocytol* 31:469-480.
- Hsieh J, Aimone JB, Kaspar BK, Kuwabara T, Nakashima K, Gage FH (2004) IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. *J Cell Biol* 164:111-122.
- Hu S, Sheng WS, Lokensgard JR, Peterson PK (2002a) Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology* 42:829-836.
- Hu VW, Black GE, Torres-Duarte A, Abramson FP (2002b) 3H-thymidine is a defective tool with which to measure rates of DNA synthesis. *Faseb J* 16:1456-1457.
- Hunsberger JG, Newton SS, Bennett AH, Duman CH, Russell DS, Salton SR, Duman RS (2007) Antidepressant actions of the exercise-regulated gene VGF. *Nat Med* 13:1476-1482.
- Irvine KA, Blakemore WF (2007) A different regional response by mouse oligodendrocyte progenitor cells (OPCs) to high-dose X-irradiation has consequences for repopulating OPC-depleted normal tissue. *Eur J Neurosci* 25:417-424.
- Ivanova A, Nakahira E, Kagawa T, Oba A, Wada T, Takebayashi H, Spassky N, Levine J, Zalc B, Ikenaka K (2003) Evidence for a second wave of oligodendrogenesis in the postnatal cerebral cortex of the mouse. *J Neurosci Res* 73:581-592.
- Jana A, Pahan K (2007) Oxidative stress kills human primary oligodendrocytes via neutral sphingomyelinase: implications for multiple sclerosis. *J Neuroimmune Pharmacol* 2:184-193.
- Jessberger S, Kempermann G (2003) Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur J Neurosci* 18:2707-2712.
- Jiang W, Wan Q, Zhang ZJ, Wang WD, Huang YG, Rao ZR, Zhang X (2003) Dentate granule cell neurogenesis after seizures induced by pentylenetetrazol in rats. *Brain Res* 977:141-148.
- Jiao J, Chen DF (2008) Induction of Neurogenesis in Non-conventional Neurogenic Regions of the Adult CNS by Niche Astrocyte-Produced Signals. *Stem Cells*.
- Jin N, Chen W, Blazar BR, Ramakrishnan S, Vallera DA (2002) Gene therapy of murine solid tumors with T cells transduced with a retroviral vascular endothelial growth factor-immunotoxin target gene. *Hum Gene Ther* 13:497-508.
- Joels M (2007) Role of corticosteroid hormones in the dentate gyrus. *Prog Brain Res* 163:355-370.
- Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD (1996) Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes Dev* 10:3129-3140.
- Kahn L, Alonso G, Normand E, Manzoni OJ (2005) Repeated morphine treatment alters polysialylated neural cell adhesion molecule, glutamate decarboxylase-67 expression and cell proliferation in the adult rat hippocampus. *Eur J Neurosci* 21:493-500.
- Kanzawa T, Iwado E, Aoki H, Iwamaru A, Hollingsworth EF, Sawaya R, Kondo S, Kondo Y (2006) Ionizing radiation induces apoptosis and inhibits neuronal differentiation in rat neural stem cells via the c-Jun NH(2)-terminal kinase (JNK) pathway. *Oncogene*.
- Kaplan MS (1983) Proliferation of subependymal cells in the adult primate CNS: differential uptake of DNA labelled precursors. *J Hirnforsch* 24:23-33.
- Kaplan MS, Hinds JW (1977) Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* 197:1092-1094.
- Kaplan MS, McNelly NA, Hinds JW (1985) Population dynamics of adult-formed granule neurons of the rat olfactory bulb. *J Comp Neurol* 239:117-125.

- Kempermann G (2006) Adult Neurogenesis Stem Cells and Neuronal Development in the Adult Brain. New York: Oxford University Press.
- Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386:493-495.
- Kempermann G, Krebs J, Fabel K (2008) The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. *Curr Opin Psychiatry* 21:290-295.
- Kempermann G, Jessberger S, Steiner B, Kronenberg G (2004) Milestones of neuronal development in the adult hippocampus. *Trends Neurosci* 27:447-452.
- Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130:391-399.
- Kerler R, Rabes HM (1994) Rat tumor cytogenetics: a critical evaluation of the literature. *Crit Rev Oncog* 5:271-295.
- Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD (2006) Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci* 9:173-179.
- Khurdayan VK, Buch S, El-Hage N, Lutz SE, Goebel SM, Singh IN, Knapp PE, Turchan-Cholewo J, Nath A, Hauser KF (2004) Preferential vulnerability of astroglia and glial precursors to combined opioid and HIV-1 Tat exposure in vitro. *Eur J Neurosci* 19:3171-3182.
- Kim SH, Kim HB, Jang MH, Lim BV, Kim YJ, Kim YP, Kim SS, Kim EH, Kim CJ (2002) Treadmill exercise increases cell proliferation without altering of apoptosis in dentate gyrus of Sprague-Dawley rats. *Life Sci* 71:1331-1340.
- Kintner C (2002) Neurogenesis in embryos and in adult neural stem cells. *J Neurosci* 22:639-643.
- Knapp PE, Hauser KF (1996) mu-Opioid receptor activation enhances DNA synthesis in immature oligodendrocytes. *Brain Res* 743:341-345.
- Knapp PE, Maderspach K, Hauser KF (1998) Endogenous opioid system in developing normal and jimpy oligodendrocytes: mu and kappa opioid receptors mediate differential mitogenic and growth responses. *Glia* 22:189-201.
- Knapp PE, Itkis OS, Zhang L, Spruce BA, Bakalkin G, Hauser KF (2001) Endogenous opioids and oligodendroglial function: possible autocrine/paracrine effects on cell survival and development. *Glia* 35:156-165.
- Knowles WD (1992) Normal anatomy and neurophysiology of the hippocampal formation. *J Clin Neurophysiol* 9:252-263.
- Koehl M, Meerlo P, Gonzales D, Rontal A, Turek FW, Abrous DN (2008) Exercise-induced promotion of hippocampal cell proliferation requires  $\beta$ -endorphin. *FASEB J*.
- Kolodziej A, Stumm R, Becker A, Holtt V (2008) Endogenous opioids inhibit ischemia-induced generation of immature hippocampal neurons via the mu-opioid receptor. *Eur J Neurosci* 27:1311-1319.
- Komitova M (2005) Neurogenesis and gliogenesis after focal brain ischemia. In: Institute of Neuroscience and Physiology, p 103. Göteborg: Göteborg University.
- Komitova M, Eriksson PS (2004) Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. *Neurosci Lett* 369:24-27.
- Komitova M, Perfilieva E, Mattsson B, Eriksson PS, Johansson BB (2002) Effects of cortical ischemia and postischemic environmental enrichment on hippocampal cell genesis and differentiation in the adult rat. *J Cereb Blood Flow Metab* 22:852-860.
- Koo JW, Duman RS (2008) IL-1 $\beta$  is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A* 105:751-756.



- Kornblum HI, Loughlin SE, Leslie FM (1987a) Effects of morphine on DNA synthesis in neonatal rat brain. *Brain Res* 428:45-52.
- Kornblum HI, Hurlbut DE, Leslie FM (1987b) Postnatal development of multiple opioid receptors in rat brain. *Brain Res* 465:21-41.
- Kosaka T, Kosaka K, Heizmann CW, Nagatsu I, Wu JY, Yanaihara N, Hama K (1987) An aspect of the organization of the GABAergic system in the rat main olfactory bulb: laminar distribution of immunohistochemically defined subpopulations of GABAergic neurons. *Brain Res* 411:373-378.
- Krischke N, Petermann F (1995) [Quality of life: assessment instruments and results]. *Z Klin Psychol Psychopathol Psychother* 43:34-56.
- Kronenberg G, Bick-Sander A, Bunk E, Wolf C, Ehninger D, Kempermann G (2005a) Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. *Neurobiol Aging*.
- Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G (2003) Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol* 467:455-463.
- Kronenberg G, Wang LP, Synowitz M, Gertz K, Katchanov J, Glass R, Harms C, Kempermann G, Kettenmann H, Endres M (2005b) Nestin-expressing cells divide and adopt a complex electrophysiologic phenotype after transient brain ischemia. *J Cereb Blood Flow Metab* 25:1613-1624.
- Krum JM, Mani N, Rosenstein JM (2002) Angiogenic and astroglial responses to vascular endothelial growth factor administration in adult rat brain. *Neuroscience* 110:589-604.
- Kuhn HG, Cooper-Kuhn CM (2007) Bromodeoxyuridine and the detection of neurogenesis. *Curr Pharm Biotechnol* 8:127-131.
- Kuhn HG, Dickinson-Anson H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 16:2027-2033.
- Kukekov VG, Laywell ED, Suslov O, Davies K, Scheffler B, Thomas LB, O'Brien TF, Kusakabe M, Steindler DA (1999) Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. *Exp Neurol* 156:333-344.
- Kurita H, Kawahara N, Asai A, Ueki K, Shin M, Kirino T (2001) Radiation-induced apoptosis of oligodendrocytes in the adult rat brain. *Neurol Res* 23:869-874.
- Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, Donovan MH, Fischer SJ, Farnbauch LA, Beech RD, DiLeone RJ, Greer CA, Mandyam CD, Eisch AJ (2007) Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci* 27:12623-12629.
- Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K (2001) Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch Neurol* 58:498-504.
- Lazar SW, Kerr CE, Wasserman RH, Gray JR, Greve DN, Treadway MT, McGarvey M, Quinn BT, Dusek JA, Benson H, Rauch SL, Moore CI, Fischl B (2005) Meditation experience is associated with increased cortical thickness. *Neuroreport* 16:1893-1897.
- Lee SM, Tole S, Grove E, McMahon AP (2000) A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127:457-467.
- Levison SW, Chuang C, Abramson BJ, Goldman JE (1993) The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. *Development* 119:611-622.
- Li YQ, Wong CS (1998) Apoptosis and its relationship with cell proliferation in the irradiated rat spinal cord. *Int J Radiat Biol* 74:405-417.

- Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH (2005) Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 437:1370-1375.
- Ligon KL, Kesari S, Kitada M, Sun T, Arnett HA, Alberta JA, Anderson DJ, Stiles CD, Rowitch DH (2006) Development of NG2 neural progenitor cells requires Olig gene function. *Proc Natl Acad Sci U S A* 103:7853-7858.
- Lillien L, Raphael H (2000) BMP and FGF regulate the development of EGF-responsive neural progenitor cells. *Development* 127:4993-5005.
- Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A (2000) Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 28:713-726.
- Limoli CL, Giedzinski E, Rola R, Otsuka S, Palmer TD, Fike JR (2004) Radiation response of neural precursor cells: linking cellular sensitivity to cell cycle checkpoints, apoptosis and oxidative stress. *Radiat Res* 161:17-27.
- Lin TN, Sun SW, Cheung WM, Li F, Chang C (2002) Dynamic changes in cerebral blood flow and angiogenesis after transient focal cerebral ischemia in rats. Evaluation with serial magnetic resonance imaging. *Stroke* 33:2985-2991.
- Lind D, Franken S, Kappler J, Jankowski J, Schilling K (2005) Characterization of the neuronal marker NeuN as a multiply phosphorylated antigen with discrete subcellular localization. *J Neurosci Res* 79:295-302.
- Liu X, Wang Q, Haydar TF, Bordey A (2005) Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* 8:1179-1187.
- Lledo PM, Saghatelian A (2005) Integrating new neurons into the adult olfactory bulb: joining the network, life-death decisions, and the effects of sensory experience. *Trends Neurosci* 28:248-254.
- Lo LC, Johnson JE, Wuenschell CW, Saito T, Anderson DJ (1991) Mammalian achaete-scute homolog 1 is transiently expressed by spatially restricted subsets of early neuroepithelial and neural crest cells. *Genes Dev* 5:1524-1537.
- Lois C, Alvarez-Buylla A (1993) Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci U S A* 90:2074-2077.
- Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. *Science* 264:1145-1148.
- Loughlin SE, Massamiri TR, Kornblum HI, Leslie FM (1985) Postnatal development of opioid systems in rat brain. *Neuropeptides* 5:469-472.
- Luo CX, Jiang J, Zhou QG, Zhu XJ, Wang W, Zhang ZJ, Han X, Zhu DY (2007) Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. *J Neurosci Res* 85:1637-1646.
- Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* 11:173-189.
- Lyden D, Young AZ, Zagzag D, Yan W, Gerald W, O'Reilly R, Bader BL, Hynes RO, Zhuang Y, Manova K, Benezra R (1999) Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. *Nature* 401:670-677.
- Ma Q, Wang J, Liu HT, Chao FH (2002) [Attenuation of chronic stress-induced hippocampal damages following physical exercise]. *Sheng Li Xue Bao* 54:427-430.
- Magnus T, Coksaygan T, Korn T, Xue H, Arumugam TV, Mughal MR, Eckley DM, Tang SC, Detolla L, Rao MS, Cassiani-Ingoni R, Mattson MP (2007) Evidence that nucleocytoplasmic Olig2 translocation mediates brain-injury-induced differentiation of glial precursors to astrocytes. *J Neurosci Res* 85:2126-2137.

- Malatesta P, Appolloni I, Calzolari F (2008) Radial glia and neural stem cells. *Cell Tissue Res* 331:165-178.
- Malatesta P, Hack MA, Hartfuss E, Kettenmann H, Klinkert W, Kirchhoff F, Gotz M (2003) Neuronal or glial progeny: regional differences in radial glia fate. *Neuron* 37:751-764.
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20:9104-9110.
- Mandyam CD, Norris RD, Eisch AJ (2004) Chronic morphine induces premature mitosis of proliferating cells in the adult mouse subgranular zone. *J Neurosci Res* 76:783-794.
- Mandyam CD, Wee S, Eisch AJ, Richardson HN, Koob GF (2007) Methamphetamine self-administration and voluntary exercise have opposing effects on medial prefrontal cortex gliogenesis. *J Neurosci* 27:11442-11450.
- Manger TA, Motta RW (2005) The impact of an exercise program on posttraumatic stress disorder, anxiety, and depression. *Int J Emerg Ment Health* 7:49-57.
- Marchetti E, Chaillan FA, Dumuis A, Bockaert J, Soumireu-Mourat B, Roman FS (2004) Modulation of memory processes and cellular excitability in the dentate gyrus of freely moving rats by a 5-HT<sub>4</sub> receptors partial agonist, and an antagonist. *Neuropharmacology* 47:1021-1035.
- Marques-Mari AI, Nacher J, Crespo C, Gutierrez-Mecinas M, Martinez-Guijarro FJ, Blasco-Ibanez JM (2007) Loss of input from the mossy cells blocks maturation of newly generated granule cells. *Hippocampus* 17:510-524.
- Marshall CA, Novitsch BG, Goldman JE (2005) Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. *J Neurosci* 25:7289-7298.
- McKay R (1997) Stem cells in the central nervous system. *Science* 276:66-71.
- Meilandt WJ, Barea-Rodriguez E, Harvey SA, Martinez JL, Jr. (2004) Role of hippocampal CA3 mu-opioid receptors in spatial learning and memory. *J Neurosci* 24:2953-2962.
- Menezes JR, Smith CM, Nelson KC, Luskin MB (1995) The division of neuronal progenitor cells during migration in the neonatal mammalian forebrain. *Mol Cell Neurosci* 6:496-508.
- Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006) Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* 26:7907-7918.
- Mennerick S, Zorumski CF (2000) Neural activity and survival in the developing nervous system. *Mol Neurobiol* 22:41-54.
- Merkle FT, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A (2004) Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci U S A* 101:17528-17532.
- Mezey E, Chandross KJ, Harta G, Maki RA, McKeicher SR (2000) Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290:1779-1782.
- Miller MW, Nowakowski RS (1988) Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. *Brain Res* 457:44-52.
- Minami M, Satoh M (1995) Molecular biology of the opioid receptors: structures, functions and distributions. *Neurosci Res* 23:121-145.
- Minamisawa T, Hirokaga K (1996) Long-term changes in open field activity of male mice irradiated with low levels of gamma rays at late stage of development. *J Radiat Res (Tokyo)* 37:117-124.
- Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. *Annu Rev Neurosci* 28:223-250.

- Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD, Fike JR (2003) Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. *Cancer Res* 63:4021-4027.
- Mizutani K, Saito T (2005) Progenitors resume generating neurons after temporary inhibition of neurogenesis by Notch activation in the mammalian cerebral cortex. *Development* 132:1295-1304.
- Mockett BG, Guevremont D, Williams JM, Abraham WC (2007) Dopamine D1/D5 receptor activation reverses NMDA receptor-dependent long-term depression in rat hippocampus. *J Neurosci* 27:2918-2926.
- Monge M, Kadiiski D, Jacque CM, Zalc B (1986) Oligodendroglial expression and deposition of four major myelin constituents in the myelin sheath during development. An *in vivo* study. *Dev Neurosci* 8:222-235.
- Monje ML, Toda H, Palmer TD (2003) Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302:1760-1765.
- Monje ML, Mizumatsu S, Fike JR, Palmer TD (2002) Irradiation induces neural precursor-cell dysfunction. *Nat Med* 8:955-962.
- Monje ML, Vogel H, Masek M, Ligon KL, Fisher PG, Palmer TD (2007) Impaired human hippocampal neurogenesis after treatment for central nervous system malignancies. *Ann Neurol* 62:515-520.
- Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D (1994) Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* 13:1071-1082.
- Mulhern RK, Merchant TE, Gajjar A, Reddick WE, Kun LE (2004) Late neurocognitive sequelae in survivors of brain tumours in childhood. *Lancet Oncol* 5:399-408.
- Mulhern RK, Palmer SL, Reddick WE, Glass JO, Kun LE, Taylor J, Langston J, Gajjar A (2001) Risks of young age for selected neurocognitive deficits in medulloblastoma are associated with white matter loss. *J Clin Oncol* 19:472-479.
- Nagesh V, Tsien CI, Chenevert TL, Ross BD, Lawrence TS, Junick L, Cao Y (2008) Radiation-induced changes in normal-appearing white matter in patients with cerebral tumors: a diffusion tensor imaging study. *Int J Radiat Oncol Biol Phys* 70:1002-1010.
- Nait-Oumesmar B, Picard-Riera N, Kerninon C, Decker L, Seilhean D, Hoglinger GU, Hirsch EC, Reynolds R, Baron-Van Evercooren A (2007) Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. *Proc Natl Acad Sci U S A* 104:4694-4699.
- Nakashima K, Takizawa T, Ochiai W, Yanagisawa M, Hisatsune T, Nakafuku M, Miyazono K, Kishimoto T, Kageyama R, Taga T (2001) BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis. *Proc Natl Acad Sci U S A* 98:5868-5873.
- Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H, Michaud N, Hafezi-Moghadam A, Miller JW, Benowitz LI (2006) Tumor necrosis factor- $\alpha$  mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci* 26:12633-12641.
- Naylor AS, Persson AI, Eriksson PS, Jonsdottir IH, Thorlin T (2005) Extended voluntary running inhibits exercise-induced adult hippocampal progenitor proliferation in the spontaneously hypertensive rat. *J Neurophysiol* 93:2406-2414.
- Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins. *Nature* 373:109.
- Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 726:49-56.

- Nilsson M (2005) Movement pattern analysis of hypoglutamatergic mice. A model for cognitive deficits of schizophrenia and autism? In: Institute of Clinical Neurosciences, p 75. Göteborg: Gothenburg.
- Nishiyama A (2007) Polydendrocytes: NG2 cells with many roles in development and repair of the CNS. *Neuroscientist* 13:62-76.
- Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB (1996) Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. *J Neurosci Res* 43:299-314.
- Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR (2004) Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci* 7:136-144.
- Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409:714-720.
- Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR (2002) Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J Neurosci* 22:3161-3173.
- Nottebohm F (1985) Neuronal replacement in adulthood. *Ann N Y Acad Sci* 457:143-161.
- Nottebohm F (1989) From bird song to neurogenesis. *Sci Am* 260:74-79.
- O'Callaghan RM, Ohle R, Kelly AM (2007) The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. *Behav Brain Res* 176:362-366.
- Oberholzer M, Ostreicher M, Christen H, Bruhlmann M (1996) Methods in quantitative image analysis. *Histochem Cell Biol* 105:333-355.
- Ohab JJ, Fleming S, Blesch A, Carmichael ST (2006) A neurovascular niche for neurogenesis after stroke. *J Neurosci* 26:13007-13016.
- Oi S, Kokunai T, Ijichi A, Matsumoto S, Raimondi AJ (1990) Radiation-induced brain damage in children--histological analysis of sequential tissue changes in 34 autopsy cases. *Neurol Med Chir (Tokyo)* 30:36-42.
- Olivier C, Cobos I, Perez Villegas EM, Spassky N, Zalc B, Martinez S, Thomas JL (2001) Monofocal origin of telencephalic oligodendrocytes in the anterior entopeduncular area of the chick embryo. *Development* 128:1757-1769.
- Olson AK, Eadie BD, Ernst C, Christie BR (2006) Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus* 16:250-260.
- Ong WY, Levine JM (1999) A light and electron microscopic study of NG2 chondroitin sulfate proteoglycan-positive oligodendrocyte precursor cells in the normal and kainate-lesioned rat hippocampus. *Neuroscience* 92:83-95.
- Oomen CA, Mayer JL, de Kloet ER, Joels M, Lucassen PJ (2007) Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. *Eur J Neurosci* 26:3395-3401.
- Paakko E, Harila-Saari A, Vanionpaa L, Himanen S, Pyhtinen J, Lanning M (2000) White matter changes on MRI during treatment in children with acute lymphoblastic leukemia: correlation with neuropsychological findings. *Med Pediatr Oncol* 35:456-461.
- Palmer TD, Takahashi J, Gage FH (1997) The adult rat hippocampus contains primordial neural stem cells. *Mol Cell Neurosci* 8:389-404.
- Palmer TD, Willhoite AR, Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425:479-494.

- Palmer TD, Markakis EA, Willhoite AR, Safar F, Gage FH (1999) Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J Neurosci* 19:8487-8497.
- Panagiotakos G, Alshamy G, Chan B, Abrams R, Greenberg E, Saxena A, Bradbury M, Edgar M, Gutin P, Tabar V (2007) Long-term impact of radiation on the stem cell and oligodendrocyte precursors in the brain. *PLoS ONE* 2:e588.
- Parent JM, Tada E, Fike JR, Lowenstein DH (1999) Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *J Neurosci* 19:4508-4519.
- Parras CM, Schuurmans C, Scardigli R, Kim J, Anderson DJ, Guillemot F (2002) Divergent functions of the proneural genes Mash1 and Ngn2 in the specification of neuronal subtype identity. *Genes Dev* 16:324-338.
- Parras CM, Hunt C, Sugimori M, Nakafuku M, Rowitch D, Guillemot F (2007) The proneural gene Mash1 specifies an early population of telencephalic oligodendrocytes. *J Neurosci* 27:4233-4242.
- Parras CM, Galli R, Britz O, Soares S, Galichet C, Battiste J, Johnson JE, Nakafuku M, Vescovi A, Guillemot F (2004) Mash1 specifies neurons and oligodendrocytes in the postnatal brain. *Embo J* 23:4495-4505.
- Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC (1999) Structural maturation of neural pathways in children and adolescents: *in vivo* study. *Science* 283:1908-1911.
- Peirce JL, Chesler EJ, Williams RW, Lu L (2003) Genetic architecture of the mouse hippocampus: identification of gene loci with selective regional effects. *Genes Brain Behav* 2:238-252.
- Peissner W, Kocher M, Treuer H, Gillardon F (1999) Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. *Brain Res Mol Brain Res* 71:61-68.
- Pellegrino RG, Ritchie JM (1984) Sodium channels in the axolemma of normal and degenerating rabbit optic nerve. *Proc R Soc Lond B Biol Sci* 222:155-160.
- Penit C (1986) *In vivo* thymocyte maturation. BUdR labeling of cycling thymocytes and phenotypic analysis of their progeny support the single lineage model. *J Immunol* 137:2115-2121.
- Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, Sloan R, Gage FH, Brown TR, Small SA (2007) An *in vivo* correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci U S A* 104:5638-5643.
- Perreau VM, Adlard PA, Anderson AJ, Cotman CW (2005) Exercise-induced gene expression changes in the rat spinal cord. *Gene Expr* 12:107-121.
- Persson AI, Bull C, Eriksson PS (2006) Requirement for Id1 in opioid-induced oligodendrogenesis in cultured adult rat hippocampal progenitors. *Eur J Neurosci* 23:2277-2288.
- Persson AI, Thorlin T, Bull C, Eriksson PS (2003a) Opioid-induced proliferation through the MAPK pathway in cultures of adult hippocampal progenitors. *Mol Cell Neurosci* 23:360-372.
- Persson AI, Naylor AS, Jonsdottir IH, Nyberg F, Eriksson PS, Thorlin T (2004) Differential regulation of hippocampal progenitor proliferation by opioid receptor antagonists in running and non-running spontaneously hypertensive rats. *Eur J Neurosci* 19:1847-1855.
- Persson AI, Thorlin T, Bull C, Zarnegar P, Ekman R, Terenius L, Eriksson PS (2003b) Mu- and delta-opioid receptor antagonists decrease proliferation and increase neurogenesis in cultures of rat adult hippocampal progenitors. *Eur J Neurosci* 17:1159-1172.

- Petryniak MA, Potter GB, Rowitch DH, Rubenstein JL (2007) Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron* 55:417-433.
- Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Evercooren AB (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci U S A* 99:13211-13216.
- Pleasure SJ, Collins AE, Lowenstein DH (2000a) Unique expression patterns of cell fate molecules delineate sequential stages of dentate gyrus development. *J Neurosci* 20:6095-6105.
- Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, Lowenstein DH, Rubenstein JL (2000b) Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. *Neuron* 28:727-740.
- Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Romer B, Rodriguez GR, Kronenberg G, Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci* 7:77.
- Pringle NP, Mudhar HS, Collarini EJ, Richardson WD (1992) PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* 115:535-551.
- Przewlocki R, Przewlocka B (2001) Opioids in chronic pain. *Eur J Pharmacol* 429:79-91.
- Purves D (2004) *Neuroscience*, Third Edition: Sinauer Associates.
- Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, Rao M, Sussel L, Rubenstein J, Qiu M (2001) Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. *Development* 128:2723-2733.
- Ra SM, Kim H, Jang MH, Shin MC, Lee TH, Lim BV, Kim CJ, Kim EH, Kim KM, Kim SS (2002) Treadmill running and swimming increase cell proliferation in the hippocampal dentate gyrus of rats. *Neurosci Lett* 333:123-126.
- Raber J, Rola R, LeFevour A, Morhardt D, Curley J, Mizumatsu S, VandenBerg SR, Fike JR (2004) Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat Res* 162:39-47.
- Racz B, Halasy K (2002) Kappa opioid receptor is expressed by somatostatin- and neuropeptide Y-containing interneurons in the rat hippocampus. *Brain Res* 931:50-55.
- Raffin-Sanson ML, de Keyser Y, Bertagna X (2003) Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. *Eur J Endocrinol* 149:79-90.
- Rakic P (1971) Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in Macacus Rhesus. *J Comp Neurol* 141:283-312.
- Rakic P (1974) Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science* 183:425-427.
- Rakic P (1978) Neuronal migration and contact guidance in the primate telencephalon. *Postgrad Med J* 54 Suppl 1:25-40.
- Rakic P (1985) DNA synthesis and cell division in the adult primate brain. *Ann N Y Acad Sci* 457:193-211.
- Ramirez-Castillejo C, Sanchez-Sanchez F, Andreu-Agullo C, Ferron SR, Aroca-Aguilar JD, Sanchez P, Mira H, Escribano J, Farinas I (2006) Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 9:331-339.
- Ray J, Gage FH (2006) Differential properties of adult rat and mouse brain-derived neural stem/progenitor cells. *Mol Cell Neurosci* 31:560-573.

- Ray J, Peterson DA, Schinstine M, Gage FH (1993) Proliferation, differentiation, and long-term culture of primary hippocampal neurons. *Proc Natl Acad Sci U S A* 90:3602-3606.
- Redwine JM, Armstrong RC (1998) *In vivo* proliferation of oligodendrocyte progenitors expressing PDGF $\alpha$ R during early remyelination. *J Neurobiol* 37:413-428.
- Revest JM, Di Blasi F, Kitchener P, Rouge-Pont F, Desmedt A, Turiault M, Tronche F, Piazza PV (2005) The MAPK pathway and Egr-1 mediate stress-related behavioral effects of glucocorticoids. *Nat Neurosci* 8:664-672.
- Reynolds R, Hardy R (1997) Oligodendroglial progenitors labeled with the O4 antibody persist in the adult rat cerebral cortex *in vivo*. *J Neurosci Res* 47:455-470.
- Reynolds R, Dawson M, Papadopoulos D, Polito A, Di Bello IC, Pham-Dinh D, Levine J (2002) The response of NG2-expressing oligodendrocyte progenitors to demyelination in MOG-EAE and MS. *J Neurocytol* 31:523-536.
- Rickmann M, Amaral DG, Cowan WM (1987) Organization of radial glial cells during the development of the rat dentate gyrus. *J Comp Neurol* 264:449-479.
- Rogers RL, Meyer JS, Mortel KF (1990) After reaching retirement age physical activity sustains cerebral perfusion and cognition. *J Am Geriatr Soc* 38:123-128.
- Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR, Fike JR (2004) Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. *Exp Neurol* 188:316-330.
- Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006) Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 24:1850-1856.
- Rousselot P, Lois C, Alvarez-Buylla A (1995) Embryonic (PSA) N-CAM reveals chains of migrating neuroblasts between the lateral ventricle and the olfactory bulb of adult mice. *J Comp Neurol* 351:51-61.
- Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, Soininen H, Nissinen A, Kivipelto M (2005) Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *Lancet Neurol* 4:705-711.
- Roy NS, Wang S, Jiang L, Kang J, Benraiss A, Harrison-Restelli C, Fraser RA, Couldwell WT, Kawaguchi A, Okano H, Nedergaard M, Goldman SA (2000) *In vitro* neurogenesis by progenitor cells isolated from the adult human hippocampus. *Nat Med* 6:271-277.
- Rushton WA (1951) A theory of the effects of fibre size in medullated nerve. *J Physiol* 115:101-122.
- Sainz IF, Holinka LG, Lu Z, Risatti GR, Borca MV (2008) Removal of a N-linked glycosylation site of classical swine fever virus strain Brescia Erns glycoprotein affects virulence in swine. *Virology* 370:122-129.
- Samanta J, Kessler JA (2004) Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. *Development* 131:4131-4142.
- Sano K, Morii K, Sato M, Mori H, Tanaka R (2000) Radiation-induced diffuse brain injury in the neonatal rat model--radiation-induced apoptosis of oligodendrocytes. *Neurol Med Chir (Tokyo)* 40:495-499; discussion 499-500.
- Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301:805-809.



- Sargeant TJ, Day DJ, Mrkusich EM, Foo DF, Miller JH (2007) Mu opioid receptors are expressed on radial glia but not migrating neuroblasts in the late embryonic mouse brain. *Brain Res* 1175:28-38.
- Sato G, Tanaka R, Akiyama K, Yamanaka R, Sato M (2003) Immunohistochemical analysis of myelination following hemispheric irradiation in neonatal rats. *Neurosci Lett* 353:131-134.
- Scarlato M, Beesley J, Pleasure D (2000) Analysis of oligodendroglial differentiation using cDNA arrays. *J Neurosci Res* 59:430-435.
- Schanzer A, Wachs FP, Wilhelm D, Acker T, Cooper-Kuhn C, Beck H, Winkler J, Aigner L, Plate KH, Kuhn HG (2004) Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. *Brain Pathol* 14:237-248.
- Schindler MK, Forbes ME, Robbins ME, Riddle DR (2008) Aging-dependent changes in the radiation response of the adult rat brain. *Int J Radiat Oncol Biol Phys* 70:826-834.
- Schmidt HD, Duman RS (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. *Behav Pharmacol* 18:391-418.
- Schnitzer J, Schachner M (1982) Cell type specificity of a neural cell surface antigen recognized by the monoclonal antibody A2B5. *Cell Tissue Res* 224:625-636.
- Seki T (2002) Hippocampal adult neurogenesis occurs in a microenvironment provided by PSA-NCAM-expressing immature neurons. *J Neurosci Res* 69:772-783.
- Seki T, Arai Y (1991) The persistent expression of a highly polysialylated NCAM in the dentate gyrus of the adult rat. *Neurosci Res* 12:503-513.
- Seki T, Arai Y (1995) Age-related production of new granule cells in the adult dentate gyrus. *Neuroreport* 6:2479-2482.
- Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J Neurosci* 21:7153-7160.
- Seri B, Garcia-Verdugo JM, Collado-Morente L, McEwen BS, Alvarez-Buylla A (2004) Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol* 478:359-378.
- Seri B, Herrera DG, Gritti A, Ferron S, Collado L, Vescovi A, Garcia-Verdugo JM, Alvarez-Buylla A (2006) Composition and organization of the SCZ: a large germinal layer containing neural stem cells in the adult mammalian brain. *Cereb Cortex* 16 Suppl 1:i103-111.
- Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 304:1338-1340.
- Shetty AK, Hattiangady B, Shetty GA (2005) Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes. *Glia* 51:173-186.
- Shi F, Hoekstra D (2004) Effective intracellular delivery of oligonucleotides in order to make sense of antisense. *J Control Release* 97:189-209.
- Shier D, Butler J, Lewis R. (2004) *Hole's Human Anatomy & Physiology*, 10th Edition. New York: Lange, M. J./McGraw Hill.
- Shinomiya N (2001) New concepts in radiation-induced apoptosis: 'premitotic apoptosis' and 'postmitotic apoptosis'. *J Cell Mol Med* 5:240-253.
- Sievers J, Hartmann D, Pehlemann FW, Berry M (1992) Development of astroglial cells in the proliferative matrices, the granule cell layer, and the hippocampal fissure of the hamster dentate gyrus. *J Comp Neurol* 320:1-32.

- Silasi G, Diaz-Heijtz R, Besplug J, Rodriguez-Juarez R, Titov V, Kolb B, Kovalchuk O (2004) Selective brain responses to acute and chronic low-dose X-ray irradiation in males and females. *Biochem Biophys Res Commun* 325:1223-1235.
- Singh VK, Bajpai K, Biswas S, Haq W, Khan MY, Mathur KB (1997) Molecular biology of opioid receptors: recent advances. *Neuroimmunomodulation* 4:285-297.
- Skoff RP, Knapp PE (1991) Division of astroblasts and oligodendroblasts in postnatal rodent brain: evidence for separate astrocyte and oligodendrocyte lineages. *Glia* 4:165-174.
- Skup M, Dwornik A, Macias M, Sulejczak D, Wiater M, Czarkowska-Bauch J (2002) Long-term locomotor training up-regulates TrkB(FL) receptor-like proteins, brain-derived neurotrophic factor, and neurotrophin 4 with different topographies of expression in oligodendroglia and neurons in the spinal cord. *Exp Neurol* 176:289-307.
- Smart I, Leblond CP (1961) Evidence for division and transformation of neuroglia cells in the mouse brain as derived from radioautography after injection of thymidine-H3. *J Comp Neurol* 116:349-367.
- Smart IH (1976) A pilot study of cell production by the ganglionic eminences of the developing mouse brain. *J Anat* 121:71-84.
- Snyder SH, Pasternak GW (2003) Historical review: Opioid receptors. *Trends Pharmacol Sci* 24:198-205.
- Sommer L, Shah N, Rao M, Anderson DJ (1995) The cellular function of MASH1 in autonomic neurogenesis. *Neuron* 15:1245-1258.
- Sousa N, Almeida OF, Wotjak CT (2006) A hitchhiker's guide to behavioral analysis in laboratory rodents. *Genes Brain Behav* 5 Suppl 2:5-24.
- Sprinkle TJ (1989) 2',3'-cyclic nucleotide 3'-phosphodiesterase, an oligodendrocyte-Schwann cell and myelin-associated enzyme of the nervous system. *Crit Rev Neurobiol* 4:235-301.
- Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* 99:195-231.
- Staugaitis SM, Zerlin M, Hawkes R, Levine JM, Goldman JE (2001) Aldolase C/zebrin II expression in the neonatal rat forebrain reveals cellular heterogeneity within the subventricular zone and early astrocyte differentiation. *J Neurosci* 21:6195-6205.
- Steiner B, Kronenberg G, Jessberger S, Brandt MD, Reuter K, Kempermann G (2004) Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. *Glia* 46:41-52.
- Steiner B, Klempin F, Wang L, Kott M, Kettenmann H, Kempermann G (2006) Type-2 cells as link between glial and neuronal lineage in adult hippocampal neurogenesis. *Glia* 54:805-814.
- Stiene-Martin A, Hauser KF (1993) Morphine suppresses DNA synthesis in cultured murine astrocytes from cortex, hippocampus and striatum. *Neurosci Lett* 157:1-3.
- Stiene-Martin A, Zhou R, Hauser KF (1998) Regional, developmental, and cell cycle-dependent differences in mu, delta, and kappa-opioid receptor expression among cultured mouse astrocytes. *Glia* 22:249-259.
- Stiene-Martin A, Knapp PE, Martin K, Gurwell JA, Ryan S, Thornton SR, Smith FL, Hauser KF (2001) Opioid system diversity in developing neurons, astroglia, and oligodendroglia in the subventricular zone and striatum: impact on gliogenesis *in vivo*. *Glia* 36:78-88.
- Stranahan AM, Khalil D, Gould E (2006) Social isolation delays the positive effects of running on adult neurogenesis. *Nat Neurosci* 9:526-533.
- Stummer W, Weber K, Tranmer B, Baethmann A, Kempfski O (1994) Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia. *Stroke* 25:1862-1869.

- Sturrock RR, Smart IH (1980) A morphological study of the mouse subependymal layer from embryonic life to old age. *J Anat* 130:391-415.
- Sugimori M, Nagao M, Bertrand N, Parras CM, Guillemot F, Nakafuku M (2007) Combinatorial actions of patterning and HLH transcription factors in the spatiotemporal control of neurogenesis and gliogenesis in the developing spinal cord. *Development* 134:1617-1629.
- Sundholm-Peters NL, Yang HK, Goings GE, Walker AS, Szele FG (2004) Radial glia-like cells at the base of the lateral ventricles in adult mice. *J Neurocytol* 33:153-164.
- Sweatt JD (2004) Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* 14:311-317.
- Tada E, Parent JM, Lowenstein DH, Fike JR (2000) X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. *Neuroscience* 99:33-41.
- Takagi Y, Nozaki K, Takahashi J, Yodoi J, Ishikawa M, Hashimoto N (1999) Proliferation of neuronal precursor cells in the dentate gyrus is accelerated after transient forebrain ischemia in mice. *Brain Res* 831:283-287.
- Takahashi J, Palmer TD, Gage FH (1999) Retinoic acid and neurotrophins collaborate to regulate neurogenesis in adult-derived neural stem cell cultures. *J Neurobiol* 38:65-81.
- Tamura Y, Kataoka Y, Cui Y, Takamori Y, Watanabe Y, Yamada H (2007) Multi-directional differentiation of doublecortin- and NG2-immunopositive progenitor cells in the adult rat neocortex *in vivo*. *Eur J Neurosci* 25:3489-3498.
- Tanaka M, Kadokawa Y, Hamada Y, Marunouchi T (1999) Notch2 expression negatively correlates with glial differentiation in the postnatal mouse brain. *J Neurobiol* 41:524-539.
- Taniguchi M, Miura K, Iwao H, Yamanaka S (2001) Quantitative assessment of DNA microarrays--comparison with Northern blot analyses. *Genomics* 71:34-39.
- Tansey FA, Cammer W (1991) A pi form of glutathione-S-transferase is a myelin- and oligodendrocyte-associated enzyme in mouse brain. *J Neurochem* 57:95-102.
- Tatsumi K, Haga S, Matsuyoshi H, Inoue M, Manabe T, Makinodan M, Wanaka A (2005) Characterization of cells with proliferative activity after a brain injury. *Neurochem Int* 46:381-389.
- Thakker-Varia S, Krol JJ, Nettleton J, Bilimoria PM, Bangasser DA, Shors TJ, Black IB, Alder J (2007) The neuropeptide VGF produces antidepressant-like behavioral effects and enhances proliferation in the hippocampus. *J Neurosci* 27:12156-12167.
- Thoren P, Floras JS, Hoffmann P, Seals DR (1990) Endorphins and exercise: physiological mechanisms and clinical implications. *Med Sci Sports Exerc* 22:417-428.
- Tolwani RJ, Buckmaster PS, Varma S, Cosgaya JM, Wu Y, Suri C, Shooter EM (2002) BDNF overexpression increases dendrite complexity in hippocampal dentate gyrus. *Neuroscience* 114:795-805.
- Tomita K, Moriyoshi K, Nakanishi S, Guillemot F, Kageyama R (2000) Mammalian achaete-scute and atonal homologs regulate neuronal versus glial fate determination in the central nervous system. *Embo J* 19:5460-5472.
- Tong Y, Chabot JG, Shen SH, O'Dowd BF, George SR, Quirion R (2000) Ontogenic profile of the expression of the mu opioid receptor gene in the rat telencephalon and diencephalon: an *in situ* hybridization study. *J Chem Neuroanat* 18:209-222.
- Torii M, Matsuzaki F, Osumi N, Kaibuchi K, Nakamura S, Casarosa S, Guillemot F, Nakafuku M (1999) Transcription factors Mash-1 and Prox-1 delineate early steps in differentiation of neural stem cells in the developing central nervous system. *Development* 126:443-456.

- Tournay O, Benezra R (1996) Transcription of the dominant-negative helix-loop-helix protein Id1 is regulated by a protein complex containing the immediate-early response gene Egr-1. *Mol Cell Biol* 16:2418-2430.
- Tramontin AD, Garcia-Verdugo JM, Lim DA, Alvarez-Buylla A (2003) Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. *Cereb Cortex* 13:580-587.
- Trapp BD, Andrews SB, Cootauco C, Quarles R (1989) The myelin-associated glycoprotein is enriched in multivesicular bodies and periaxonal membranes of actively myelinating oligodendrocytes. *J Cell Biol* 109:2417-2426.
- Trejo JL, Carro E, Torres-Aleman I (2001) Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. *J Neurosci* 21:1628-1634.
- Trejo JL, Llorens-Martin MV, Torres-Aleman I (2008) The effects of exercise on spatial learning and anxiety-like behavior are mediated by an IGF-I-dependent mechanism related to hippocampal neurogenesis. *Mol Cell Neurosci* 37:402-411.
- Tropepe V, Sibilina M, Ciruna BG, Rossant J, Wagner EF, van der Kooy D (1999) Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. *Dev Biol* 208:166-188.
- Tryoen-Toth P, Gaveriaux-Ruff C, Labourdette G (2000) Down-regulation of mu-opioid receptor expression in rat oligodendrocytes during their development *in vitro*. *J Neurosci Res* 60:10-20.
- Tulving E, Markowitsch HJ (1998) Episodic and declarative memory: role of the hippocampus. *Hippocampus* 8:198-204.
- Tzeng SF (2003) Inhibitors of DNA binding in neural cell proliferation and differentiation. *Neurochem Res* 28:45-52.
- Tzeng SF, de Vellis J (1997) Expression and functional role of the Id HLH family in cultured astrocytes. *Brain Res Mol Brain Res* 46:136-142.
- Uda M, Ishido M, Kami K (2007) Features and a possible role of Mash1-immunoreactive cells in the dentate gyrus of the hippocampus in the adult rat. *Brain Res* 1171:9-17.
- Vallieres E (2002) Adjuvant radiation therapy after complete resection of non-small-cell lung cancer. *J Clin Oncol* 20:1427-1429.
- Van der Borght K, Wallinga AE, Luiten PG, Eggen BJ, Van der Zee EA (2005) Morris water maze learning in two rat strains increases the expression of the polysialylated form of the neural cell adhesion molecule in the dentate gyrus but has no effect on hippocampal neurogenesis. *Behav Neurosci* 119:926-932.
- Van Praag H, Kempermann G, Gage FH (1999a) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2:266-270.
- Van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999b) Running enhances neurogenesis, learning and long-term potentiation in mice. *Proc Natl Acad Sci USA* 96:13427-13431.
- van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. *J Neurosci* 25:8680-8685.
- Vaynman S, Ying Z, Gomez-Pinilla F (2004) Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci* 20:2580-2590.
- Vinals F, Reiriz J, Ambrosio S, Bartrons R, Rosa JL, Ventura F (2004) BMP-2 decreases Mash1 stability by increasing Id1 expression. *Embo J* 23:3527-3537.
- Wang JW, David DJ, Monckton JE, Battaglia F, Hen R (2008) Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci* 28:1374-1384.

- Watterson JM, Watson DG, Meyer EM, Lenox RH (2002) A role for protein kinase C and its substrates in the action of valproic acid in the brain: implications for neural plasticity. *Brain Res* 934:69-80.
- Weickert CS, Webster MJ, Colvin SM, Herman MM, Hyde TM, Weinberger DR, Kleinman JE (2000) Localization of epidermal growth factor receptors and putative neuroblasts in human subependymal zone. *J Comp Neurol* 423:359-372.
- Weiss B, Davidkova G, Zhang SP (1997) Antisense strategies in neurobiology. *Neurochem Int* 31:321-348.
- Whittemore SR, Morassutti DJ, Walters WM, Liu RH, Magnuson DS (1999) Mitogen and substrate differentially affect the lineage restriction of adult rat subventricular zone neural precursor cell populations. *Exp Cell Res* 252:75-95.
- Widmer H, Ferrigan L, Davies CH, Cobb SR (2006) Evoked slow muscarinic acetylcholinergic synaptic potentials in rat hippocampal interneurons. *Hippocampus* 16:617-628.
- Wilson HC, Scolding NJ, Raine CS (2006) Co-expression of PDGF alpha receptor and NG2 by oligodendrocyte precursors in human CNS and multiple sclerosis lesions. *J Neuroimmunol* 176:162-173.
- Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG (2002) Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur J Neurosci* 16:1681-1689.
- Wojtowicz JM (2006) Irradiation as an experimental tool in studies of adult neurogenesis. *Hippocampus* 16:261-266.
- Wong CS, Van der Kogel AJ (2004) Mechanisms of radiation injury to the central nervous system: implications for neuroprotection. *Mol Interv* 4:273-284.
- Yang HK, Sundholm-Peters NL, Goings GE, Walker AS, Hyland K, Szele FG (2004) Distribution of doublecortin expressing cells near the lateral ventricles in the adult mouse brain. *J Neurosci Res* 76:282-295.
- Yang SH, Whitmarsh AJ, Davis RJ, Sharrocks AD (1998) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *Embo J* 17:1740-1749.
- Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO, Moskowitz MA (2003) FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. *J Clin Invest* 112:1202-1210.
- Yosida TH (1983) Karyotype evolution and tumor development. *Cancer Genet Cytogenet* 8:153-179.
- Yuen T, Wurmbach E, Pfeffer RL, Ebersole BJ, Sealfon SC (2002) Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. *Nucleic Acids Res* 30:e48.
- Zadina JE, Hackler L, Ge LJ, Kastin AJ (1997) A potent and selective endogenous agonist for the mu-opiate receptor. *Nature* 386:499-502.
- Zalc B, Monge M, Dupouey P, Hauw JJ, Baumann NA (1981) Immunohistochemical localization of galactosyl and sulfogalactosyl ceramide in the brain of the 30-day-old mouse. *Brain Res* 211:341-354.
- Zhang RL, Zhang ZG, Wang Y, LeTourneau Y, Liu XS, Zhang X, Gregg SR, Wang L, Chopp M (2007) Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain. *J Cereb Blood Flow Metab* 27:1201-1212.
- Zhao S, Chai X, Frotscher M (2007) Balance between neurogenesis and gliogenesis in the adult hippocampus: role for reelin. *Dev Neurosci* 29:84-90.

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Zhu X, Bergles DE, Nishiyama A (2008) NG2 cells generate both oligodendrocytes and gray matter astrocytes. *Development* 135:145-157.