1 INTRODUCTION

1.1 Development and stem cells

Higher organisms of the animal kingdom reproduce sexually, which leads to recombination of their genes in every generation. This mechanism of sexual reproduction requires that every individual starts life as a single cell, the zygote. This zygote is a genetically new individual derived from the fusion of two germ cells, an egg with a sperm. The zygote divides and forms a hollow ball of cells called the blastocyst, which implants into the uterus. Inside the blastocyst is a group of approximately 30 cells (in humans) referred to as the inner cell mass. The cells of the inner cell mass further proliferate and give rise to an enormous number of specialized cell types that together define the organism. Soon after the blastocyst stage, each of these cells is assigned to one of the three germ layers, either to the endoderm, the ectoderm, or the mesoderm. Each germ layer generates specific tissues. The nervous system, for example, is derived from the ectoderm.

A fundamental distinction between two types of cells, germ line cells and somatic cells, was first proposed by August Weismann (1892). During the development of the body, most cells become somatic cells, that is they acquire functional specialization, but loose the ability to generate a complete organism. Only a few cells of the early embryo retain this ability. These cells are called primordial germ cells and represent the germ line. They reside in the reproductive system and, by a process called meiosis, develop into gametes, eggs or sperm cells, as the organism matures. Whereas somatic cells are diploid, cells of the germ line can be either diploid (zygote, blastocyst cells, primordial germ cells) or haploid (gametes).

Stem cells are defined by two important characteristics. First, they can self-renew for long periods by continuous divisions. Second, they have the potential to develop into various specialized cells. The cells of the inner cell mass are called embryonic stem cells. They are totipotent, that is they are able to generate all cells in the adult organism. However, the body contains more cells that fulfill the two criteria mentioned above: self-renewal and differentiation potential. These somatic stem cells reside within the tissues of the developing and adult organism. They are not totipotent, as they cannot form the whole body. Instead, they are classified as being either multipotent cells able to generate different cell types, or as being unipotent. Thus, they have a restricted differentiation potential and were thought to give rise to cells of a single organ or tissue only.

The presence of stem cells in adult tissues has been known for a long time. They generate replacements for cells that are lost through normal wear and tear, injury, or disease. Prominent examples are found in highly regenerative tissues such as blood or skin (Till and McCulloch,

1980; Cotsarelis et al., 1990). Recently, adult stem cells have gained increased attention for a number of reasons. First, the plasticity of these tissue-specific stem cells, that is their ability to generate a specialized cell of another tissue, was underestimated before. Several reports described the differentiation of adult stem cells into cell types normally not found in their tissue of origin. For example, cells from the bone marrow could give rise to cells with neuronal or hepatocyte-like characteristics (Mezey et al., 2000; Schwartz et al., 2002). Although some of these findings have since been questioned, it is clear that a remarkable plasticity enables these cells to aquire multiple different phenotypes. Second, besides the adult stem cells in blood or skin that were known before, many more mammalian tissues and organs were found to contain stem cells long into adulthood. Therefore, many researchers and clinicians as well as the public are excited about the potential use of adult stem cells as replacement for diseased or lost tissue.

1.2 The nervous system

The nervous system is the most complex mammalian organ. It is responsible for perceiving information about the organism and its environment, processing this information, storing some of it in memory for future reference, and coordinating appropriate behavioral responses such as hormonal and metabolic activity, muscle movement, and speech. All of this is essentially accomplished by nerve cells and the connections between them. There are about 100 billion nerve cells (10^{11}) in an adult human brain and although they all share the same basic architecture, at least 1,000 different types of neurons can be distinguished (Kandel, 2000). All these nerve cells, or neurons, can establish hundreds of connections to other neurons by cellular protrusions called axons and dendrites. Electrical charges are transmitted between individual neurons via these axons and dendrites to mediate communication in the nervous system. The specific connections between individual neurons as well as the flexibility in forming and breaking these connections are thought to represent the basis for the outstanding performance of the nervous system.

The nervous system is divided into the central nervous system (CNS), comprising the brain and the spinal cord, and the peripheral nervous system. The major parts of the central and peripheral nervous systems are derived from the ectoderm. Specifically, the neural cells of the nervous system descend from a sheet of ectodermal cells located along the dorsal midline of the early embryo, called the neural plate. Folding of this neural plate generates the neural tube. The caudal region of this neural tube develops into the spinal cord, whereas the rostral region becomes the brain. The cells on the inner wall of the neural tube are the neuroepithelial stem cells. Massive, but highly regulated proliferation of these neuroepithelial stem cells, as well as folding and bending of the expanding neural tube, ultimately forms the adult brain. Deep within

the brain remains a remnant of the folded neural tube, a system of connected cavities called ventricles, which are filled with the cerebrospinal fluid. The cell layer adjacent to the ventricles, which contains the neuroepithelial stem cells, is called the ventricular zone (Fig. 1). The region beneath the ventricular zone is called the subventricular zone (SVZ, also subependymal zone). Both zones are reduced during postnatal development, leaving only an ependymal cell layer with a narrow SVZ in the adult brain (Fig. 1).

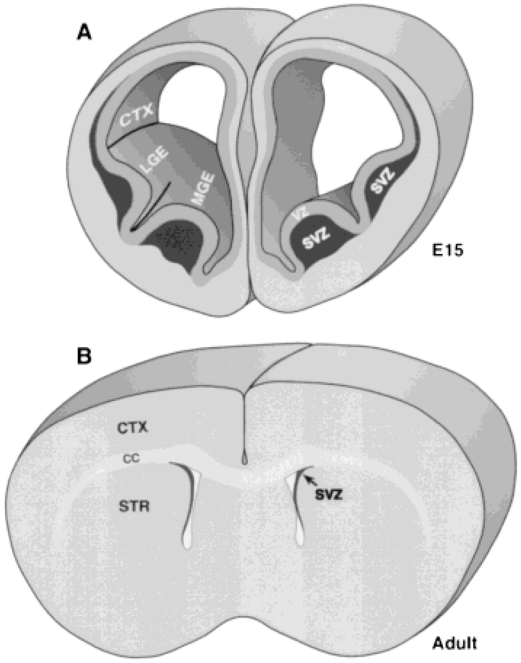

Figure 1. (A) Coronal section through the embryonic mouse forebrain at day 15 postconception. The proliferative ventricular zone (VZ) lines the lateral ventricles. A secondary proliferative region, the subventricular zone (SVZ), is prominent in lateral and medial ganglionic eminences (LGE and MGE) and transiently appears under the VZ of developing neocortex (CTX). (B) Coronal section through the adult mouse telencephalon. The embryonic VZ is transformed into a terminally differentiated ependymal layer lining the brain ventricles. The proliferative SVZ remains adjacent to the ependymal layer of the lateral walls of the lateral ventricles. This region is thought to be derived from the SVZ of the lateral ganglionic eminence (A). CTX: neocortex; CC: corpus callosum; STR: striatum. Adopted from Garcia-Verdugo et al., 1998.

Besides neurons, glial cells are the second type of cells in the nervous system. They are by definition non-neuronal cells and are more abundant than neurons (approximately 10^{12} to 10^{13} in the adult human brain). Glial cells have traditionally been considered ancillary, satellite cells of the nervous system (Greek gliok = glue, slime), but their importance for proper brain function has also been increasingly appreciated (Nedergaard et al., 2003). Three glial cell types are recognized in the CNS: astrocytes, oligodendrocytes, and microglial cells, and each has a distinct morphology corresponding to its respective function (Kandel, 2000). Astrocytes are the most numerous cells in the CNS and provide metabolic and trophic support for the neurons. In addition, they release and inactivate neurotransmitters and form the blood-brain barrier. A special type of astrocytes are radial glial cells, which send long cellular processes through the brain to guide migrating neurons during development. Oligodendrocytes are the myelinating glial cells of the CNS responsible for insulating axons for efficient propagation of electric potentials during neuronal communication. In addition, there are microglial cells, which are derived from immune cells and become activated in response to injury or disease. Ependymal cells form the epithelial lining of the ventricles in the CNS.

1.3 Stem cells of the nervous system

1.3.1 Neurogenesis in the adult rodent brain

The famous Spanish neuroanatomist Ramón y Cajal (1852-1934) has been quoted as writing: 'In the adult centers the nerve paths are something fixed, ended and immutable. Everything may die, nothing may be regenerated' (1913-1914). It is widely recognized that whereas damage to the peripheral nervous system can be regenerated to some extent, the CNS seems to be incapable of recovering from such insults, may they be due to trauma, ischemia, or to neurodegenerative disorders.

First evidence for production of neurons in the mammalian brain during adulthood was provided by work from Joseph Altman who published evidence for ongoing neurogenesis in the hippocampus and the olfactory bulb of the adult rat brain (*Rattus norvegicus*) (Altman and Das, 1965; Altman, 1969). For the classification of these newborn cells as neurons Altman relied on morphological analysis, but was not able to demonstrate their neuronal identity by immunohistochemical analysis due to a lack of marker proteins at that time. In the early nineties, however, Altman's data was backed by two reports that demonstrated the presence of cells in the adult murine brain (*Mus musculus*) that were able to proliferate *in vitro* in the presence of epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) and that then gave rise to cells positive for neuronal marker proteins, as shown by immunofluorescence analysis (Reynolds and Weiss, 1992; Richards et al., 1992). These cells proliferated in culture without adhering to the dish, but rather formed free-floating clusters of cells called neurospheres. By means of [^{3}H]thymidine birthdating, Lois and Alvarez-Buylla (1993) identified the subventricular zone (SVZ) adjacent to the walls of the lateral ventricles as a site where 'neural stem cells' reside and divide *in situ* in mice.

Since these cells were obviously dividing in the adult brain ([^{3}H]thymidine labeling), the questions arose as to where the newborn cells would integrate. Marla Luskin demonstrated by retroviral labeling of proliferating rat SVZ cells with an X-gal reporter gene, that their descendants migrate anteriorly into the olfactory bulb and acquire the morphology of granule and periglomerular neurons (Luskin, 1993). Shortly after, this extensive migration of newborn cells from the SVZ to the olfactory bulb was demonstrated in the adult mouse brain as well (Lois and Alvarez-Buylla, 1994) (Fig. 2 a). Periglomerular and granule cells are the two major types of interneurons in the *bulbus olfactorius* and they are responsible for the modulation of incoming sensory signals before these are passed on via the *tractus olfactorius* to the olfactory cortex.

In the developing mammalian brain four forms of neuronal migration have been described: radial migration along the radial glial fibers, migration by somal translocation in the

developing cortex, tangential migration parallel to the ventricle's surface, and chain migration (Honda et al., 2003). Newborn neurons from the adult murine SVZ migrate as chains in the so-called rostral migratory stream (RMS) towards the olfactory bulb, and these migrating cells are immunoreactive for the polysialylated form of neural cell adhesion molecule (PSA-NCAM) (Rousselot et al., 1995) and for the early neuronal marker tubulin-β-III (TUBB3) (Thomas et al., 1996). Glial cells ensheath the chains of migrating neuroblasts (Lois et al., 1996) (Fig. 2 b). These cells also synthesize the glial fibrillary acidic protein (GFAP), a widely used marker for astrocytes. The SVZ itself is also organized as an extensive network of chains of migrating neuronal precursors, which are also positive for PSA-NCAM and tubulin-β-III (Doetsch and Alvarez-Buylla, 1996). In the olfactory bulb, neuroblasts exit the cell cycle and start to migrate radially towards their final destination.

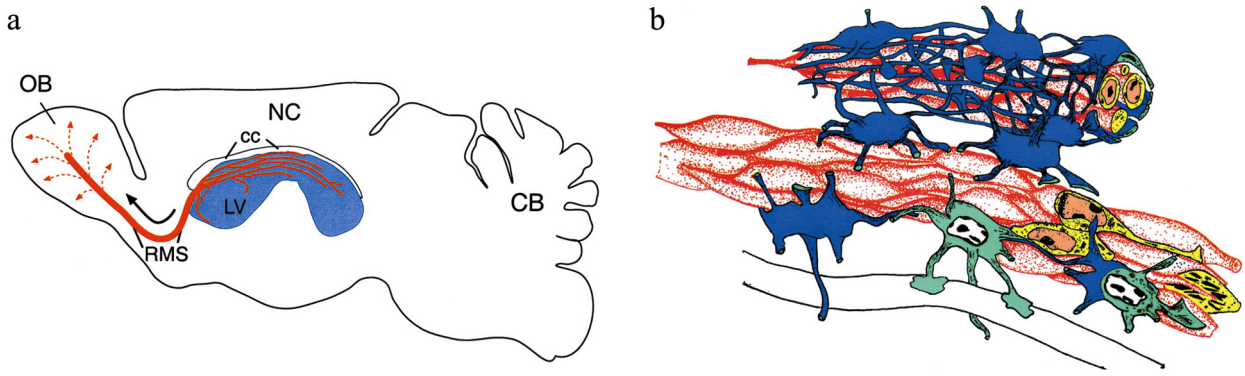

Figure 2. (a) Schematic sagittal view of the adult rodent brain depicting the lateral ventricle (LV) where new neurons are generated throughout the SVZ and aligned into long chains (red lines). The cells migrate as chains within the rostral migratory stream (RMS) towards the olfactory bulb (OB). In the olfactory bulb, cells disperse radially (dotted lines) as individual cells and differentiate into granule and periglomerular interneurons. CB, cerebellum; NC, neocortex; cc, corpus callosum. (b) Chain migration of young neurons (red) within the SVZ and the RMS. The chains of migrating neuroblasts are ensheathed by glial cells (blue) with astrocytic characteristics (connections to blood vessels, immunopositive for GFAP). In the bottom chain only few astrocytes are shown to illustrate the tight organization of neuroblasts into chains. Intracellular characteristics are illustrated in some astrocytes (light blue) and neuroblasts (yellow). Adopted from Alvarez-Buylla and Garcia-Verdugo, 2002.

1.3.2 Identity of the proliferating cells in the adult rodent brain

Soon after the finding of ongoing adult neurogenesis in the SVZ of adult rodents, the question arose which cells in the SVZ would represent the stem cells. The cellular composition and three-dimensional organization of the SVZ in adult mice was described in great detail by means of electron microscopy, [^{3}H]thymidine autoradiography, and immunocytochemistry (Doetsch et al., 1997). This study identified four major cell types in this region: migrating neuroblasts (type A

cells), astrocytes (type B cells), undifferentiated putative precursor cells (type C cells), and ependymal cells. The type C cells were most actively dividing and found in close proximity to clusters of type A neuroblasts which were organized in chains. This suggested that type C cells are the highly proliferative precursor cells that produce the neuroblasts. However, it remained unclear whether type C cells are the actual 'stem' cells, or only descendants of a slowly dividing 'stem' cell population. Morshead et al. had demonstrated earlier, that selective killing of the constitutively proliferating cells in the SVZ did not abolish the capacity of SVZ explants to form neurospheres *in vitro* (1994). They concluded that a relatively quiescent SVZ cell can both repopulate the constitutively proliferating population *in situ* and give rise to neurospheres *in vitro*.

In 1999, the lab of Jonas Frisen claimed that the ependymal cells, which separate the ventricular lumen from the SVZ, are the neural 'stem' cells which generate new neurons *in situ* and neurospheres *in vitro* (Johansson et al., 1999a). However, their results have been questioned since. Arturo Alvarez-Buylla and colleagues provided evidence that the SVZ astrocytes (type B cells) are the slowly-dividing 'stem' cells *in situ* (Doetsch et al., 1999). Other studies confirmed these findings (Chiasson et al., 1999; Laywell et al., 2000; Capela and Temple, 2002). Therefore, the current model is that GFAP-positive SVZ astrocytes are the 'stem' cells in the adult rodent brain. They generate transit-amplifying cells (type C cells), which are highly proliferative precursors that give rise to neuroblasts (type A cells) (Fig. 3). These neuroblasts align in chains and migrate anteriorly along the lateral ventricles through the SVZ. The chains fuse to build up the RMS, in which the neuroblasts migrate further to the olfactory bulb, where they stop dividing, differentiate, and functionally integrate as interneurons (Fig. 2).

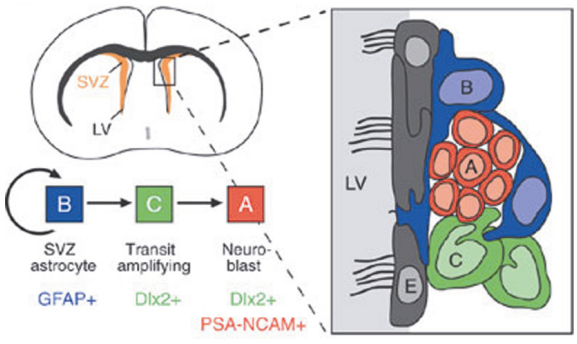

Figure 3. Organization of the adult rodent SVZ and cell types therein. Frontal scheme of the mouse brain showing the SVZ (orange), adjacent to the lateral ventricle (LV), and the SVZ cell types. Multiciliated ependymal cells (E, gray) line the lateral ventricle. The chains of neuroblasts (A, red) migrate through glial tunnels formed by SVZ astrocytes (B, blue). Rapidly dividing transit-amplifying cells (C, green) are scattered in small foci along the network of chains. Some SVZ astrocytes extend a process between ependymal cells that contacts the lateral ventricle. SVZ astrocytes ($GFAP^+$) are 'stem' cells in this region and generate neuroblasts ($GFAP^-$, $Dlx2^+$, PSA-$NCAM^+$) via the C cells ($GFAP^-$, $Dlx2^+$, PSA-$NCAM^-$). Adopted from Doetsch, 2003.

The second region in the mammalian brain with substantial adult neurogenesis is the subgranular zone in the dentate gyrus of the hippocampus. In this subgranular zone of adult mice hundreds of cells divide daily and give rise to neurons which migrate into the adjacent granule cell layer, and become morphologically indistinguishable from the other surrounding granule cells (Kempermann et al., 1997a). Interestingly, paralleling the results for proliferating SVZ cells, it was shown that new neurons in the adult hippocampus were derived from GFAP-positive astrocytes (Seri et al., 2001). These cells can be propagated *in vitro* after attachment to an adhesive surface and exposure to bFGF (Gage et al., 1998).

1.3.3 Adult neurogenesis in primates

In the late nineties it became clear that in the adult primate brain neurogenesis takes place as well. First evidence for the production of new neurons in the hippocampus of adult macaque monkeys was found by Kornack and Rakic (1999). The generation of neuroblasts in the SVZ and chain migration of these cells towards the olfactory bulb was demonstrated in adult rhesus macaques (*Macaca mulatta*) and long-tailed macaques (*Macaca fascicularis*) (Kornack and Rakic, 2001; Pencea et al., 2001). Concerning the adult brain of *Homo sapiens*, first indications for the presence of progenitor cells with the potential for production of neurons *in vitro* was already presented in 1994 (Kirschenbaum et al.). These scientists kept human SVZ explants *in vitro* in the presence of [^{3}H]thymidine and found rare newborn cells double-positive for [^{3}H]thymidine and neuronal marker proteins. Treatment with bFGF and brain-derived neurotrophic factor (BDNF) strongly promoted neuronal outgrowth and survival of these cultured cells (Pincus et al., 1998). Fred Gage and colleagues published evidence for ongoing neurogenesis in the hippocampus and the lateral ventricle wall of adult humans (Eriksson et al., 1998). They had analyzed postmortem tissue samples of cancer patients aged 57 to 72 years who had received BrdU to label dividing cells for diagnostic purposes. The newborn cells were frequently positive for the neuronal marker NeuN and negative for the astrocyte marker GFAP. Johansson et al. (1999b) were able to generate neurosphere cultures from the lateral ventricle and the hippocampus of two female patients aged 16 and 19. These neurosphere cells were passaged as single cells and their progeny differentiated into neurons, astrocytes, and oligodendrocytes. This demonstrated the existence of self-renewing cells in the adult human brain which are multipotent *in vitro*. Such cells from the ventricular wall of adult humans were also shown to develop the distinct electrophysiological properties of neurons and glial cells *in vitro* (Westerlund et al., 2003). Taken together, it was established that, analogous to rodents, some cells proliferate in the SVZ and the hippocampus of adult humans, and that explants of these regions contain progenitors that can give rise to neurons and glial cells *in vitro*.

Importantly, a detailed study of the human brain revealed no chains of migrating neuroblasts in the SVZ or the pathway to the olfactory bulb (Sanai et al., 2004). Instead, the human SVZ cytoarchitecture was found to be strikingly different from that of primates, rodents, dogs, cows, and sheep. A gap that contains no cell bodies separated the ependymal cells from a band of astrocytes referred to as an SVZ astrocyte ribbon (Fig. 4). No chains of migrating neuroblasts and only few single tubulin-β-III-positive cells were observed in the SVZ. However, astrocytes in the ribbon co-expressed GFAP and the cell-division marker Ki-67, and they gave rise to neurospheres *in vitro*. These neurospheres generated secondary neurospheres, whose cells differentiated into astrocytes, neurons, and oligodendrocytes analogous to rodent neurosphere cells. Therefore, the proliferating cells in the human SVZ, like in rodents and monkeys, seem to be astrocytes. This study by Arturo Alvarez-Buylla and colleagues also highlighted the importance of verifying findings in humans, although rodents can serve as valuable model organisms.

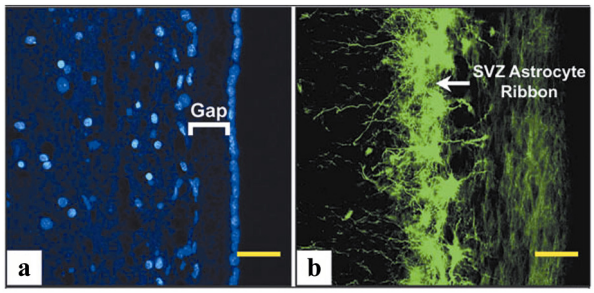

Figure 4. Dense ribbon of SVZ astrocytes in the adult human brain. (a) Coronal sections (6 µm) stained with the nuclear marker DAPI reveals a region of high cellularity that is separated from the ependyma by a gap. (b) Vibratome section showing GFAP expression in the SVZ astrocyte ribbon and GFAP-positive fibre bundles filling the subependymal gap. Adopted from Sanai et al., 2004.

Another recent study reported that some PSA-NCAM-positive cells were found in the adult human SVZ, although these were not aligned in chains. In addition, a few PSA-NCAM and nestin double-positive cells were detected in the olfactory bulb, indicating that these cells had migrated there in analogy to the rodent system (Höglinger et al., 2004). This is contradictory to the study by Arturo Alvarez-Buylla and colleagues. Only further and more elaborate experiments can settle this conflict. However, one could speculate that migration of newborn cells from the SVZ towards the olfactory bulb does happen in humans, but is strongly reduced compared to other primates or rodents. It is well known that humans rely a lot less on their olfactory sense, and correspondingly both the size of the olfactory bulb and the number of functional olfactory receptor genes are significantly decreased in humans compared to other mammals (Kornack and Rakic, 2001; Gilad et al., 2003). Irrespective of whether there is migration to the olfactory bulb, it is established that a substantial number of mitotically active cells exist in the adult human brain. However, the fate of these newborn cells from the human SVZ is unclear.

1.3.4 Stem cells or progenitor cells?

A stem cell exhibits two features: the capacity for unlimited self-renewal and the ability to generate cells of different types (multipotentiality). A progenitor cell, in contrast, displays a limited self-renewal and a restricted differentiation potential. The multipotentiality of CNS stem cells was defined as their ability to give rise to neurons, astrocytes, and oligodendrocytes *in vitro* (McKay, 1997; Gage, 2000).

During development neuroepithelial and radial glial cells give rise to billions of CNS cells of all neural lineages (neurons, astrocytes, oligodendrocytes) and therefore are considered as stem cells. However, the situation is not clear for the neurogenic cells from the adult SVZ or the dentate gyrus. Are they multipotent *in situ*? This has not been demonstrated so far. It is unclear, whether they generate cells of different lineages or specific cell types such as olfactory bulb interneurons *in situ*. Conflicting reports were published concerning the nature of the adult neurogenic cells from the dentate gyrus (see Seaberg and van der Kooy, 2002; Markakis et al., 2004; and references therein). Also for the SVZ cells it is not known whether they generate astrocytes or oligodendrocytes *in situ*. Therefore, if these neurogenic cells are lineage- or fate-restricted, the weaker term 'progenitor cells' should be applied to them as long as their fate *in situ* is unclear. Nonetheless, these cells are frequently referred to as adult CNS 'stem cells' in the literature.

Irrespective of the situation *in situ*, the progenitor cells from the adult SVZ display self-renewal and multipotentiality *in vitro*, when they are kept in specific media supplemented with EGF. Single cell suspensions of explanted SVZ tissue generate neurospheres within one to two weeks (Reynolds and Weiss, 1992; Morshead et al., 1994). These neurospheres can be dissociated and replated, and a small, but significant proportion of these cells will produce neurospheres again. Upon EGF removal the neurosphere cells differentiate into neurons, astrocytes, and oligodendrocytes. Therefore, these cells behave as stem cells *in vitro*.

There is no consensus yet about the nature of the neurospheres-generating cells. It was proposed that the majority of EGF-responsive cells from the SVZ that give rise to neurospheres *in vitro* are the highly proliferative GFAP-negative transit-amplifying C cells (Fig. 3) (Doetsch et al., 2002; Maslov et al., 2004). Other reports propose the slowly dividing GFAP-positive B cells (Morshead et al., 2003; Morshead and van der Kooy, 2004). In fact, neurospheres in culture might be derived from both type B and type C cells. In addition, although all cells in a neurosphere are clonally derived from a single cell, the cells within the neurosphere are heterogeneous. The position of a cell within a neurosphere influences its biology. The more immature and dividing cells are located close to the surface, whereas cellular differentiation takes place in the center (Campos et al., 2004).

1.3.5 Descent of the adult neural progenitors

To understand why there are neurogenic progenitor cells in the adult mammalian CNS, it is important to take a look at the lineage of these cells to determine their developmental origin. As stated above, neuroepithelial stem cells from the ventricular zone (VZ) generate almost all cells in the CNS. At the onset of embryonic neurogenesis in the VZ, some neuroepithelial stem cells give rise to so-called radial glial cells. These are an ubiquitous cell type in the developing vertebrate CNS characterized by the expression of a variety of glial marker proteins and by long radial processes from the VZ through the developing cerebral cortex to the basement membrane (the pia mater) (reviewed in Götz et al., 2002). Newborn neurons from the VZ migrate along the processes of the radial glial cells to form the multi-layered cortex (Rakic, 1972). In most vertebrates the radial glial cells persist into adulthood, but in mammals they transform into mature astrocytes during the first two postnatal weeks and cannot be found in the VZ thereafter (Schmechel and Rakic, 1979; Voigt, 1989; Gaiano et al., 2000). Recent findings demonstrated that in contrast to previous assumption, radial glial cells from rodents do not just serve as guidance structures to migrating neuroblasts, but themselves give rise to neurons and astrocytes *in vitro* and *in situ* (Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001; Tamamaki et al., 2001). This implies that radial glial cells act as stem cells during embryonic and early postnatal development of rodents, and thereafter become astrocytes. Since astrocytes were also shown to serve as progenitor cells in the adult mammalian SVZ, it has been suggested that there is a continuum of a neural stem/progenitor cell compartment that links radial glial cells to astrocytes, which serve as neural progenitors in the adult mammalian brain (Fig. 5) (Alvarez-Buylla et al., 2001; Tramontin et al., 2003, Doetsch, 2003; Götz, 2003).

There seems to be an intriguing correlation between the loss of regenerative potential of the brain and phylogeny. Radial glial cells persist in most vertebrates into adulthood (fish, amphibians, reptiles), and some of these animals display a remarkable capacity for brain regeneration. Many fish and amphibians can regenerate entire brain regions after removal (reviewed in Götz et al., 2002; Doetsch, 2003). In contrast, brain regeneration is poor in rodents and primates, and they lose their radial glial cells after establishment of the brain architecture in early postnatal development. Therefore, the ability to generate neurons for brain regeneration and repair could be linked to the presence of radial glial cells. The progenitor cells in the adult brain of rodents and primates could correspond to former radial glia, now astrocytes, which have reduced duties such as the generation of interneurons for the olfactory bulb or granule cells of the hippocampus. Thus, the stem cell properties displayed by adult SVZ progenitors *in vitro* (self-renewal and multipotentiality) seem to reflect the potential that these cells have as a consequence of their ontogeny, but the adult organism does not make full use of this potential.

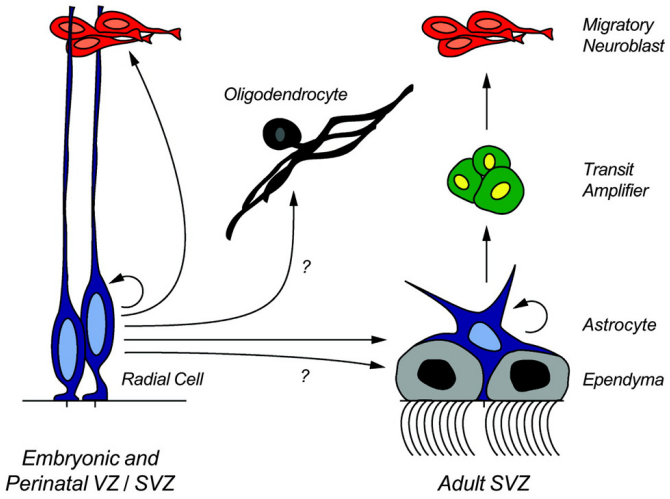

Figure 5. Radial glia as putative stem cells in the neonatal brain. Radial glia in the prenatal VZ appear to produce several major brain cell types including neurons and astrocytes. Preliminary results suggest that radial glia might also produce oligodendrocytes and ependymal cells (Tramontin et al., 2002). Adopted from Tramontin et al., 2003.

It remains unclear, how this reduced activity of the SVZ progenitors is achieved on the molecular level. However, more and more evidence points to an important regulatory role of the cell's microenvironment (for example: Tramontin et al., 2003; Fuchs et al., 2004). In addition, it remains mysterious why the regenerative potential was given up during mammalian evolution. Mammalian behavior depends more on learning and individual experience and therefore, their neuronal circuitry appears to be more plastic and shaped by the environment compared to amphibians and reptiles, whose behavior is dominated by reflexes and whose neuronal circuitry is more determined by intrinsic genetic factors. Such a plastic brain with many neuronal connections formed during life might have difficulty to precisely integrate newborn neurons because of the higher complexity.

1.3.6 Newborn neurons in the adult CNS

In the olfactory system activated sensory neurons transmit primary sensory information to mitral cells, which in turn send long axons through the *tractus olfactorius* to the olfactory cortex. Interneurons in the olfactory bulb, granule and periglomerular neurons, modulate sensory information before its projection to the olfactory cortex by selective inhibition of mitral cells (Fig. 6). In addition, they seem to participate in the formation of odor memory by developing specific connection patterns. These processes are not completely understood yet (for review see Saghatelyan et al., 2003).

Newborn neuroblasts migrate anteriorly into the olfactory bulb and acquire the morphology of mature granule cells and periglomerular interneurons within two to four weeks (Luskin, 1993; Petreanu and Alvarez-Buylla, 2002). A long-term study revealed that approximately half the newborn cells of the olfactory bulb died during the first three months after arrival, whereas the remaining cells survived up to 19 months (Winner et al., 2002). Thus,

the SVZ is a source for neuroblasts, but not all of them are stably integrated into the olfactory bulb circuitry. Removal of the olfactory bulb or transsection of the olfactory peduncle did not affect proliferation and migration in the SVZ (Kirschenbaum et al., 1999; Jankovski et al., 1998).

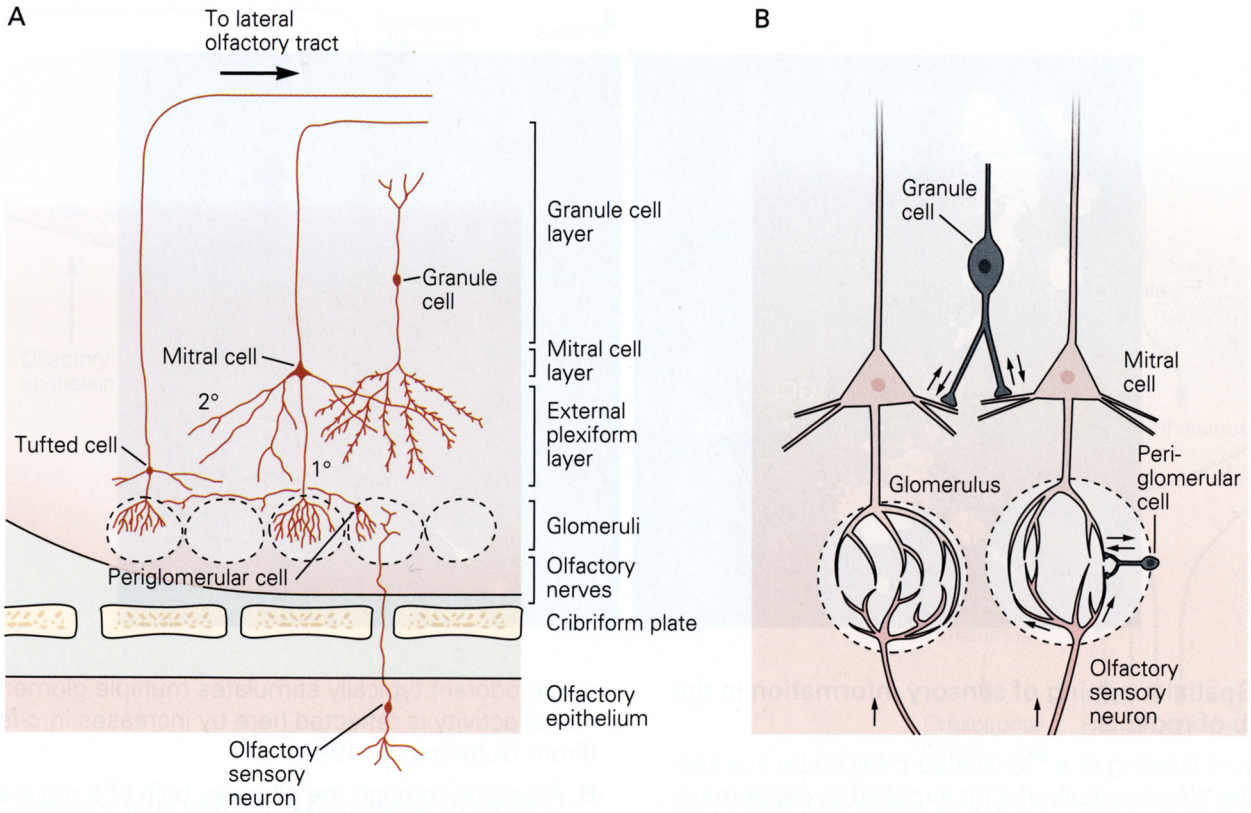

Figure 6. The neurons and the circuitry in the olfactory bulb. (A) The olfactory bulb receives signals from olfactory sensory neurons. Each sensory axon terminates in a single glomerulus, forming synapses with the dendrites of periglomerular interneurons and mitral and tufted relay neurons. The primary dendrite of each mitral and tufted cell enters a single glomerulus, where it arborizes extensively. Mitral and tufted cells also extend secondary dendrites into the external plexiform layer, where granule cell interneurons make reciprocal synapses with these secondary dendrites. The output of the bulb is carried by the mitral cells and the tufted cells, whose axons project in the lateral olfactory tract. (B) Within each glomerulus, periglomerular cells form inhibitory dendrodendritic synapses with mitral cell dendrites. The periglomerular cells also sometimes make inhibitory contacts with mitral cells that receive input in nearby glomeruli. The secondary dendrites of mitral and tufted cells form excitatory synapses on the dendrites of granule cell interneurons, which form inhibitory synapses on numerous secondary dendrites. These inhibitory connections may provide a curtain of inhibition that must be penetrated by the peaks of excitation generated by odorant stimuli. They may also serve to sharpen or refine sensory information prior to transmission to the olfactory cortex. Adult neurogenesis in the SVZ of rodents continuously supplies periglomerular and granule interneurons. Adopted from Buck, 2000.

Reduction of the number of newborn interneurons in a mouse model results in an impairment of discrimination between odors (Gheusi et al., 2000). In odor-deprived rodents, elevated neuronal death takes place in the olfactory bulb (Frazier-Cierpial and Brunjes, 1989; Najbauer and Leon, 1995). In contrast, when mice are exposed to an odor-enriched environment, survival of newborn cells in the olfactory bulb is doubled, and these mice display an improved odor memory (Rochefort et al., 2002). This indicates that sensory-driven activity in the olfactory system may play an important role in the survival and functional integration of neuroblasts and in the formation of odor memory in the adult olfactory bulb.

The hippocampus, the second region of the adult CNS that displays substantial ongoing neurogenesis, is generally thought of as the 'gatekeeper to memory'. This structure does not store information itself, but rather defines what is stored. Several studies have linked adult neurogenesis in this area with hippocampus-dependent learning behaviors. It was therefore proposed that the function of adult neurogenesis in the hippocampus is to provide a structural basis in form of new interneurons to enable the hippocampus to process a continuous influx of novel experiences throughout life (for review see Gould et al., 1999; Kempermann, 2002; Kempermann et al., 2004). The hippocampi of mice that were kept under the stimulating conditions of an enriched environment contained more newborn neurons compared to their littermates housed in standard cages (Kempermann et al., 1997b). This effect was attributed to improved survival of newborn cells rather than to increased proliferation. Also, adult neurogenesis in the dentate gyrus steadily declined during aging of rats (Kuhn et al., 1996). However, the precise role of hippocampal neurogenesis is not known.

1.4 Neural progenitors and CNS diseases

Several publications have reported a connection between neural progenitor cells and diseases. This section presents different aspects of neural progenitor cells in the adult CNS in relation to human health.

1.4.1 Neural progenitors as a source of brain tumors

Brain tumors are very aggressive, difficult to treat, and often fatal. Different types of brain tumors can be distinguished, classified by their aggressiveness, cellular composition, and age of onset. Interestingly, cells derived from brain tumors such as medulloblastoma, astrocytoma, and glioblastoma multiforme resemble neural progenitor cells *in vitro*. Cells derived from these tumors form neurosphere-like aggregates in culture, contain several proteins whose combination

is rather specific for neural progenitors (CD133, nestin, SOX2, musashi-1, BMI-1). Like neural progenitors, after induction of differentiation the tumor-derived cells lost expression of CD133 and nestin, and acquired expression of tubulin-β-III and GFAP (Ignatova et al., 2002; Singh et al., 2003; Hemmati et al., 2003). Based on these similarities, it has been suggested that brain tumors arise from endogenous stem cells (reviewed in Recht et al., 2003; Oliver and Wechsler-Reya, 2004; Berger et al., 2004).

Many tumors in the hematopoietic system are now thought of as stem cell diseases (Al-Hajj et al., 2004). Misregulated stem cells or commited progenitors escape their cell cycle control and proliferate massively, ultimately causing leukemia. These newborn cells do not necessarily have the phenotype of their mother cell, but rather differentiate according to their intrinsic program, giving rise to more mature cell types. Consequently, therapeutic ablation of these mature cells is not sufficient to eradicate tumors, since the 'root cell' will reestablish the disease. Thus, it is critical to identify the cell of origin.

This concept of cancer as a stem cell disease might translate well into neuro-oncology. Besides growth characteristics and marker gene expression *in vitro*, other features of neural progenitors are reminiscent of brain tumor cells. For example, both cell types are capable of differentiating into cells of the neuronal and the glial lineage. In addition, both are known to migrate in the CNS. Brain tumors spread through migration, and this could explain why removal of tumor tissue frequently does not prevent the reoccurance of the cancer. The 'root cell' or its descendants might reside elsewhere. It is not known, whether brain tumor cells really arise from endogenous stem or progenitor cells, or whether more differentiated cells acquire immature characteristics. However, for the development of future treatments it will be important to better understand the genesis of brain tumors.

1.4.2 CNS neurons and regeneration

In the human peripheral nervous system nerves are regenerated after an insult. In contrast, neurons of the CNS do not regenerate properly. This is obvious in the case of CNS injury. Damage to specific areas of the brain due to trauma is usually followed by loss of specific functions. In fact, this phenomenon permitted scientists to correlate brain regions with functions for which their integrity is essential. Damage to the spinal cord results in paraplegia. Occlusion of the blood circulation, e.g. by a stroke, leads to loss of specific brain functions due to local neuronal death following insufficient oxygen supply.

Several diseases, commonly classifed as neurodegenerative diseases, involve the death of neurons of the CNS. The most important examples in terms of incidence are Alzheimer's disease and Parkinson's disease. Both most frequently affect the elderly and become more and more

relevant to human health with the increasing life expectancy in developed countries. Especially Alzheimer's disease dramatically increases in frequency, affecting about one third of all people above 90 years of age (Deutsche Alzheimer Gesellschaft, www.deutsche-alzheimer.de). In Alzheimer's disease neurons of the hippocampus and the cerebral cortex are lost, whereas in Parkinson's disease mainly dopaminergic neurons of the substantia nigra die (reviewed in Bossy-Wetzel et al., 2004).

Besides the lack of proper neuronal regeneration, there is accumulating evidence that during the course of such disorders, neurogenesis itself can be affected. The amyloid beta-peptide, a hallmark of Alzheimer's disease, reduces progenitor cell proliferation and neuroblast migration in mice *in situ*, as well as differentiation of human neurosphere cells *in vitro* (Haughey et al., 2002). Dopamine depletion, the dominant feature of Parkinson's disease, also reduces progenitor proliferation in mice and man (Höglinger et al., 2004).

The adult human brain can compensate tissue loss to some extent by redistributing functions to other areas. However, the examples mentioned above demonstrate that the CNS is not able to sufficiently regenerate lost neurons to compensate for such insults, although it contains mitotically active neural progenitor cells. In other words, the adult human brain lacks proper progenitor cell activity. The evolutionary processes that lead to this situation are not understood. Nonetheless, there is an evident need for treatments to cope with brain injury or neurodegenerative disorders.

1.4.3 Therapeutic applications of neural progenitors

Transplanted stem cells, with their capability to proliferate and differentiate into diverse cell types, could in principle replace diseased or lost tissue. Such transplantation therapies have been realized in the hematopoietic system. Patients with leukemias or immunodeficiencies have received hematopoietic stem cells to reconstitute the blood or the immune system after chemo- or radiation therapy. Further therapies based on stem cell transplantation have been proposed for many diseases. Of great interest are injuries and neurodegenerative disorders of the CNS.

Two principal ideas dominate current thinking about future therapeutic strategies. First, if the brain lacks stem or progenitor cells that compensate for neuronal loss, these cells could be supplied from outside by transplantation. Second, if the endogenous progenitors do not take care of the problem properly, they could be manipulated to do so by using genetic or biochemical tools. Both strategies could be combined, such as the transplantation of genetically modified cells that secrete a specific cytokine.

Transplantation therapies can sometimes compensate for the loss of tissue, e.g. in the hematopoietic system. Loss of CNS neurons is currently not treatable by transplantation therapy, since no donor cells capable of functional integration into the neuronal circuitry are available. Stem cells have been suggested as a source for such transplantable cells, which could be implanted and then take over neuronal functions in the diseased brain (Björklund and Lindvall, 2000). Embryonic and adult stem cells with varying degrees of differentiation have been tested in animal models, and these studies showed that, in principle, neuronal replacement and partial reconstitution of the circuitry are possible (reviewed in Temple, 2001). So far, clinical trials have only been reported for Parkinson's disease, where human embryonic mesencephalic tissue was transplanted into patients' brains with moderate success (Kordower et al., 1995; Piccini et al., 1999; Freed et al., 2001; reviewed in Lindvall, 2003). Up to now, mainly embryonic stem cells and embryonic tissue containing immature cells have been proposed for therapeutic use. However, efficient predifferentiation of these cells *in vitro* to support grafting and to prevent teratoma formation was recommended (Lindvall, 2003; Lindvall and McKay, 2003). Rejection of foreign transplanted tissue might also complicate this approach.

Whether adult neural progenitor cells can serve as a source of transplantable tissue is unclear. In theory, they could be expanded *in vitro* and produce significant numbers of neurons. Either undifferentiated progenitors or neurons derived thereof could be transplanted and might functionally integrate into a host brain. Animal studies demonstrated the differentiation and functional integration of adult neural progenitor cells after transplantation in the nervous system (e.g. Gage et al., 1995; Herrera et al., 1999; Shihabuddin et al., 2000; Pluchino et al., 2003). No teratoma formation was detected in the animals as has been observed after the transplantation of embryonic stem cells. Pluchino et al. injected neural progenitors from the adult SVZ intravenously or intrathecally into a mouse model for chronic multiple sclerosis, and the transplanted cells promoted multifocal remyelination and functional recovery. Transplantation trials with humans have not been reported yet.

In addition to their neurogenic potential, adult neural progenitors have been reported to differentiate into hematopoietic and skeletal muscle cells *in vitro* (Björnson et al., 1999; Galli et al., 2000), to produce blood cells in mice whose hematopoietic system was destroyed by irradiation (Björnson et al., 1999), and to generate multiple different cells of all three germlayers after integration into developing chick and mouse embryos (Clarke et al., 2000). Thus, their plasticity appears to be higher than expected. Some of these studies, however, were later refuted, and the results have been explained by contamination of progenitor cell preparations with circulating hematopoietic stem cells and by genetic instability of neural progenitors due to long-term cultivation (Morshead et al., 2002; Magrassi et al., 2003). In fact, this high level of

plasticity might depend on *in vitro* conditions, especially on the high doses of mitogens, such as EGF or bFGF (Morrison, 2001). In addition, it was recently reported that fusion of transplanted cells with host cells occurs (Terada et al, 2002; Ying et al., 2002), which complicates the interpretation of studies involving transplantation.

An alternative to cell transplantation might be the activation of endogenous progenitors. Indeed, the neural progenitors seem to respond to pathological incidents. Proliferation and neurogenesis in the SVZ was shown to increase after induced apoptosis of neocortical neurons (Magavi et al., 2000), seizure-induced brain injury (Parent et al., 2002), and neuronal death after cerebral ischemia (Jin et al., 2003; Arvidsson et al., 2002). These and other reports described that the respective insults caused an increase in proliferation, migration of progenitors via unusual tracks towards the affected site, and finally local differentiation of newborn cells. However, the functional integration of newborn neurons was rather low in these studies. Neural progenitors were also shown to migrate towards brain tumors and to negatively affect tumor growth (Aboody et al., 2000; Staflin et al., 2004). Importantly, all these experiments with endogenous progenitors were done in mice or rats. It is unclear whether comparable responses take place in humans.

The scientists who first described the cultivation of murine SVZ progenitors hoped that 'identification of factors that induce or inhibit the *in situ* proliferation and differentiation of these cells may allow for their eventual manipulation in the intact adult mammalian CNS to replace cells lost to injury or disease' (Reynolds and Weiss, 1992). This is an appealing concept, but difficult to realize. Little is known about the *in situ* biology of adult neural progenitor cells. The molecular mechanisms that control the activity and fate of these progenitor cells are poorly understood. The cells reside in a specific niche, frequently referred to as the 'stem cell niche,' in which regulation is thought to take place. Cells adjacent to the progenitors, such as ependymal cells, seem to contribute to the maintenance of this niche (Fuchs et al., 2004; Alvarez-Buylla and Lim, 2004). The whole system is dynamic and reacts to varying needs for newborn cells, at least in rodents. Promisingly, it has already been shown, that simple intraventricular infusion of the growth factors EGF or bFGF into rats that suffered from an ischemic brain injury was sufficient to stimulate endogenous progenitors from the dentate gyrus to give rise to new functional pyramidal neurons (Nakatomi et al., 2002). Nonetheless, a detailed understanding of the molecular biology of adult neurogenesis is not yet realized.

Besides laborious attempts to dissect the complex molecular mechanisms, high-throughput screening of large molecular libraries can help to identify molecules capable of inducing or repressing progenitor cell proliferation and differentiation, or modifying fate choice during differentiation. These molecular libraries generated by combinatorial chemistry and the correponding screening techniques are well established methods in pharmaceutical research. In

the context of neural differentiation, such screening analyses are currently performed by pharmaceutical companies, and the target proteins are frequently involved in signal transduction (for review see Ding and Schultz, 2004).

It is important to keep in mind that model organisms do not always sufficiently reflect the situation in humans. Research on humans, however, is difficult to conduct for technical and ethical reasons. Therefore, the analysis of adult neurogenesis in rodent and primate model organisms is valuable, but results have to be confirmed, before a transfer to the clinic is possible.

Irrespective of whether cell transplantation or endogenous progenitor activation are attempted, it is important to realize the crucial influence of the local microenvironment within the targeted CNS area (e.g. Tramontin et al., 2003; Fuchs et al., 2004; Alvarez-Buylla and Lim, 2004). This microenvironment, much of it astrocytic, is generally non-permissive for neurogenesis (Goldman, 2003). Interestingly, neural progenitor cells lie close to blood vessels (Palmer et al., 2000; Capela and Temple, 2002), and endothelial cells stimulate their self-renewal and proliferation by secretable factors (Shen et al., 2004). Thus, a more thorough understanding of the developmental and molecular processes that control cell fate in the CNS is necessary before successful brain repair becomes feasible.

1.4.4 Ethical issues

Stem cell research, especially work on embryonic stem cells, is heavily debated in the public. For many people this research is morally questionable, the chief reason being the source of the embryonic stem cells. These are derived from cells of the inner cell mass of the blastocyst. *Homo sapiens* embryos go through the blastocyst stage during the fifth and sixth day of their embryonic development. Removal of these totipotent embryonic stem cells is fatal for the embryo. Important questions arise when attempting to work with these cells. Does an embryo at this very early stage deserve the same protection and human rights (granted, for example, by a democratic constitution) as an adult human being? Is it justifiable to use (terminate) embryos that were left over from *in vitro* fertilization procedures for biomedical research? Can the opportunity to improve therapies for neurodegenerative disorders counterbalance the rights of a human blastocyst? These questions are interwined with other moral issues of modern bioethics and biomedicine. It is difficult if not impossible to find a common ground on these issues. Only for one problem a consensus position seems achievable, that is a ban on reproductive cloning of human beings.

Adult stem cells are a potential alternative for embryonic stem cells. They are not totipotent, therefore not able to generate a human being. No moral complications arise when working with or therapeutically applying these cells. Therapeutic interventions with adult stem

(progenitor) cells might involve manipulation of the patient's endogenous cells or tissue transplantation, both being unproblematic in moral terms. However, it remains to be seen whether the therapeutic potential of adult stem (progenitor) cells matches that of embryonic stem cells.

1.5 Microarrays

Microarray experiments are used to quantify and compare gene expression on a large scale. DNA microarrays comprise different types of probes: oligonucleotides, complementary DNA (cDNA), or genomic regions. In case of a cDNA microarray, thousands of PCR-amplified cDNAs can be spotted and immobilized onto a glass slide (Schena et al., 1995) (definition of probe and target adopted from Duggan et al., 1999). The RNA of interest (target) is reverse-transcribed into cDNA, while incorporating either fluorescent molecules or a tag that is later bound to a fluorescent molecule. These targets are hybridized to the microarray, and the fluorescence at each spot is detected using a laser scanner. To compare gene expression levels from two RNA samples, they are co-hybridized to a single microarray and detected with different fluorescent dyes. Since every sequence has distinct hybridization characteristics, this method does not provide an absolute measurement for the presence of a given RNA molecule. Instead, it compares the co-hybridized sample pools and determines the relative abundance for each RNA species (reviewed in Duggan et al., 1999; Butte, 2002).

A cDNA microarray can be composed of a few, well-known clones for the analysis of isolated biological systems. It can also contain thousands of clones to cover as much of the genome as possible. Such large arrays are used for screening studies, when little is known about the molecular biology of the examined system. They allow the simultanous identification of thousands of expression patterns, effectively generating a snapshot of transcriptome differences between the two compared samples. The transcriptome is the entirety of all RNA molecules (transcripts) in a cell at a particular time point. The targets of interest are usually derived from cells or tissues, and comparisons are frequently drawn between disease versus control samples, treated versus untreated samples, or different biological states. Whereas most experimental setups are static, time course experiments permit observation of gene expression changes over time (reviewed in Yang and Speed, 2002).

1.6 Question and approach

Very little is known about the molecular processes that govern the proliferation and migration of neural progenitors and their differentiation into specific cell types. A more thorough understanding of the molecular biology of these cells and their niche in the CNS is of crucial importance. To shed more light on these molecular mechanisms and to discover the genes involved in these processes, neural progenitor cells should be cultured, expanded, and differentiated *in vitro*, providing a model system for their behavior *in situ*. The transcriptomes of neural progenitors and of differentiated cells derived from these were to be compared by means of cDNA microarray analysis. This approach should identify many genes involved in the biological processes linked to the maintenance and differentiation of these cells. Special attention was given to genes whose products take part in the regulation of cellular fate by participating in signal transduction and gene expression, and in genes whose products control and facilitate the migration of neuroblasts in the adult brain. Gaining experimental control over fate regulation and guided cell migration will be crucial to successfully use endogenous progenitor cells in therapy. Therefore, this study aimed at identifying candidate genes and proteins involved in the functioning of adult neural progenitor cells .