CMLS, Cell. Mol. Life Sci. 54 (1998) 935–945
1420-682X/98/090935-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Central nervous system stem cells in the embryo and adult

J. Frisén, C. B. Johansson, C. Lothian and U. Lendahl*

Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institute, S-171 77 Stockholm (Sweden), Fax +46 8 348135, e-mail: Urban.Lendahl@cmb.ki.se

Received 26 March 1998; received after revision 27 April 1998; accepted 27 April 1998

Abstract. The central nervous system is generated from neural stem cells during embryonic development. These cells are multipotent and generate neurons, astrocytes and oligodendrocytes. The last few years it has been found that there are populations of stem cells also in the adult mammalian brain and spinal cord. In this paper, we review the recent development in the field of embryonic and adult neural stem cells.

Key words. Development; differentiation; regeneration; neuron; glia.

Introduction

The central nervous system (CNS) is a complex tissue, both in terms of number of cells and the variety of different cell types. In addition, many billions of neurons have to interact in a very precise manner in order to form functional neuronal networks. The CNS is formed over a short time in embryogenesis, and is rapidly converted from a simple neural plate to a brain and spinal cord. To form the many different types of neurons and glial cells in the adult CNS, embryonic cells have to proliferate and differentiate in a strictly controlled manner, and during the last few years rapid progress has been made in understanding the molecular mechanisms underlying this proliferation and differentiation. It has recently been discovered that CNS stem cells are not confined to the embryo but also exist in the adult CNS. In this review we discuss some recent insights into the biology of embryonic and adult CNS stem cells and how the embryonic CNS is generated.

* Corresponding author.

Origin of the embryonic CNS

In vertebrate embryos, the first overt signs of CNS formation is the appearance of a neural plate from dorsal ectoderm. Classical transplantation experiments by Mangold and Spemann [1] showed that a second nervous system can be ectopically induced by placing mesoderm under ectoderm normally not destined to form nervous system. This experiment spurred the search for factors secreted from mesoderm that would act as positive inducers of neurulation. By assays primarily in embryos of the frog *Xenopus laevis*, a number of factors with these properties, e.g. noggin, follistatin and chordin, have been identified. More recent investigations have demonstrated that these three factors, which are not obviously related to each other, may not exert a positive neuralizing activity, but rather function by inhibiting signalling through the BMP (bone morphogenetic protein) signalling pathway (for a review on BMP and other transforming growth factor β-related molecules, see ref. 2). Noggin, follistatin and chordin bind BMP and block activation of BMP receptors, which leads to increased neurogenesis

[3, 4]; for a review, see ref. 5). Dominant negative versions of the BMP receptor introduced into early *Xenopus* embryos block neurogenesis, which further supports the involvement of BMP signalling in neurulation [6, 7]. The current view based on these experiments is therefore, contrary to the original hypothesis, that neurulation may be the primary or default fate and that BMP signalling is required to diverge cells in the ectoderm to differentiate to nonneural cell types. It is not yet clear whether BMP signalling plays exactly the same role during mammalian neurogenesis, since gene targeting of follistatin has not generated mice in which neurogenesis is altered in a manner expected from the results in *Xenopus* [8, 9]. This discrepancy may, however, reflect the presence of additional highly related gene homologues in mammals, which may have overlapping functions.

The morphology of the early CNS

There is a stereotypic set of morphological alterations during embryogenesis that leads to the formation of the CNS. First, the neural plate, which is a pseudostratified epithelium, folds to form the neural groove, which is then closed to generate the neural tube. This change in shape is accomplished without a substantial increase in cell proliferation and depends rather on changes in cell shape (fig. 1). Experimental depolymerization of microfilaments and microtubules results in failure of the neural tube to close, suggesting involvement of the cytoskeleton in this process [10]; for review see ref. 11. In the newly closed neural tube the neuroepithelium is composed of a single cell layer, and the neuroepithelial cells are attached at both the inner (ventricular) and outer (pial) sides of the neural tube (fig. 2A). The attachment is mediated by endfeet structures. At the pial side there is a basement membrane containing laminin [12] and possibly other proteins, but it is not yet known how the pial endfeet anchor to this structure. The cells in the early neural tube undergo dynamic nuclear movements during the cell cycle, referred to as 'interkinetic movements' [13, 14]. During S phase the nucleus is located near the pial side, while during mitosis the nucleus is found close to the ventricular side. The cell also detaches from the pial side as it undergoes mitosis (fig. 2B).

Shortly after neural tube closure, the neuroepithelial cells begin to proliferate rapidly. Proliferation is largely confined to embryogenesis, with the exception of the cerebellum, the hippocampus and the subventricular zone, in which it continues into postnatal stages. Following mitosis, the daughter cells have two basic options: they can either remain as undifferentiated proliferative cells and stay close to the ventricular side or differentiate to neurons or later glial cells, and migrate out from the neural tube. The migrating neurons build up new cell layers, thus creating a layered CNS. Six distinct layers can be identified in the developing cortex: the ventricular, subventricular, intermediate, subplate, cortical plate and marginal zone. In most regions of the neural tube, neuronal development proceeds according to the 'inside-out' principle, i.e. neurons are born in the ventricular layer and migrate to the cortical plate, where the first neurons settle down in the innermost region and later neurons localize successively further out. Two exceptions from the inside-out principle are the formation of the hippocampus and cerebellum, where

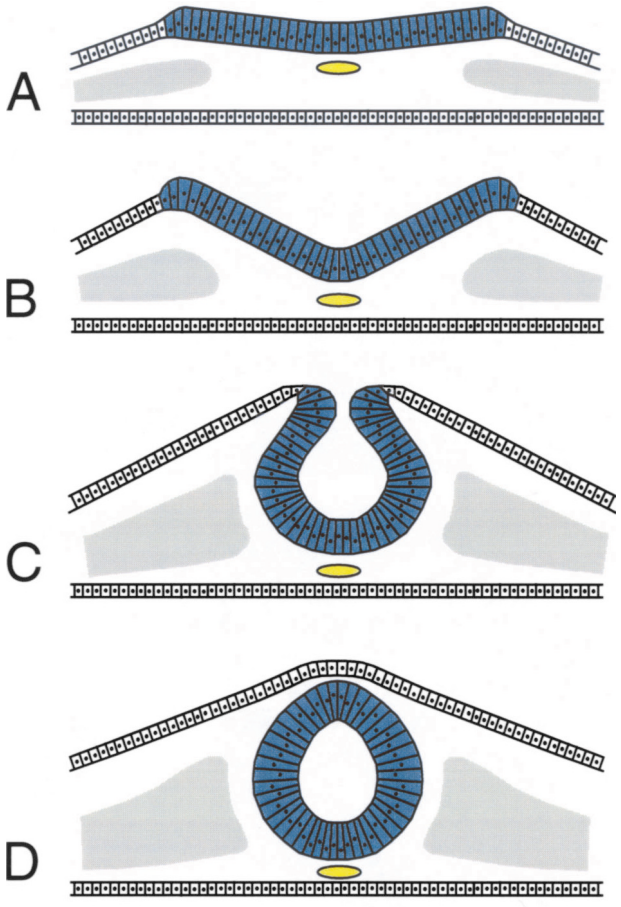

Figure 1. Neural tube formation. (*A*) The first sign of nervous system formation is a thickening of the dorsal ectoderm forming the neural plate (blue). The notochord (yellow) is located in the midline ventral to the neural plate. (*B* and *C*) The neural plate folds and forms a groove in the dorsal ectoderm. (*D*) Continued folding results in the neural folds meeting in the dorsal midline, where they fuse to form the neural tube. The ectoderm lateral to the neural tissue fuses dorsal to the neural tube to form the epidermis.

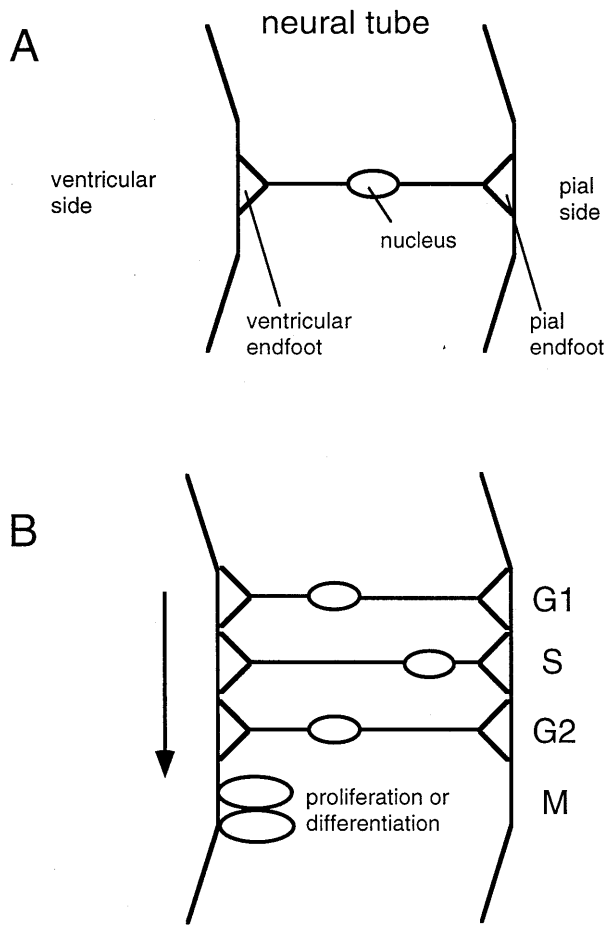

Figure 2. Morphology of neuroepithelial cells. (*A*) A schematic representation of a cell in the early neural tube. The neuroepithelial cell is elongated and attached with endfeet structures to both the ventricular (inner) and pial (outer) side of the neural tube. (*B*) A schematic representation of the morphology of a cell in the early neural tube during the different phases of the cell cycle, i.e. the interkinetic movements. During the G1 phase, the nucleus is located closer to the ventricular side, while during S phase the nucleus is closer to the pial side. During the mitotic phase (M), the cell detaches from the pial side and divides close to the ventricular side. The cell progeny can then remain as undifferentiated cells in the neural tube or migrate out from the neuroepithelium and differentiate to neurons.

undifferentiated cells move out from the neural tube as a cohort and then form localized pools of precursor cells, from which the mature neurons are generated.

As young neurons move out from the inner, ventricular zone to build up the mature brain and spinal cord, it is critical that the correct types of neurons arrive at the correct positions, in order to generate the complex functional networks. It has been a long-standing question whether this precision in neuronal organization stems from a prepatterning already laid down in the neural plate or early neural tube, or whether the neuronal networks are established by functional interactions of cells, and the cells migrating out from the neural tube are more 'plastic' in terms of differentiation. One view, referred to as the 'radial unit' or 'protomap' hypothesis [15], holds that the early neural tube is patterned and that this information is converted to the mature cortex by a very precise, radial migration of the cells. Data from experiments in transgenic mice, in which the clonal distribution of cells is revealed by reporter gene expression, support the notion of a columnar organization of the cells, which is in keeping with this hypothesis [16–18]. Further support comes from the finding that young neurons migrate out from the ventricular zone along a specialized cell type, the radial glial cells. Radial glial cells are a transient population of highly elongated cells which have endfeet structures and span the neuroepithelium from the ventricular to the pial side [19]. Neurons migrating along radial glial cells form columns, which could produce a highly ordered cortex based on the patterns of the underlying neural tube. We are beginning to understand the molecular basis for the interaction between radial glial cells and young neurons [20]. The protein reelin, which is an extracellular matrix glycoprotein secreted at the marginal zone, plays an important role in exiting of neurons from radial glial cells [21], and mutations in the reelin gene, i.e. in the Reeler mouse mutant, or in genes downstream in the signalling cascade [22, 23], severely affect the formation of layers in the cortex.

An alternative hypothesis is based on a less organized mode of migration for a young neuron from the ventricular zone to its final destination. Cells would then be more plastic and receive cues as to phenotypic decisions and interactions from the local environment. This view is in keeping with transplantation experiment showing that cells can take on new fates when transferred to a novel location in the brain [24–26]. Support for this view also comes from experiments in which individual proliferative cells have been labelled with a marker gene following retroviral infection. The clonal progeny is often widely scattered across cortex [27–29], which would argue against a strict radial migration along radial glial cells. It is not yet clear whether migration in a clone is totally randomized in all layers, or whether much of the tangential migration occurs in the ventricular and subventricular zones [27], and is then followed by radial migration. Such a view would incorporate elements of the protomap as well as the randomized hypotheses
[30, 31].

CNS stem cells during neurogenesis

As discussed above, we have a reasonably good understanding of where the proliferating cells reside during different stages of embryonic CNS development. An

important question is whether all or some of the proliferating cells qualify as stem cells. The definition of a stem cell is a controversial matter (for a thorough review of stem cells, see ref. 32), but an operational definition is based on the notion that the stem cell should be capable of self-renewal, so that at least one of the two daughter cells retains the molecular characteristics of the original cell. In addition to self-renewal, the stem cell can also produce differentiated cell types. The term precursor cell could be used for a more general definition of cells which can divide and give rise to differentiated cell progeny, but which may not necessarily do this in a stem-cell mode (see ref. 33 for a review on CNS stem cells and further discussion on the nomenclature of cell populations in the early CNS).

There are several lines of evidence suggesting that stem cells exist in the embryonic CNS. Experiments on cells cultured in vitro and labelled with lineage markers show that many cells can both self-renew and give rise to differentiated progeny [34] (see ref. 35 for review). Analysis of clones originating from single cells supports this view [36]. It is, however, important to remember that experiments on cultured primary cells in vitro reveal the potential to stay undifferentiated or to differentiate under specific conditions, but that all these possibilities may not be available to the cell in vivo. In vivo analysis, carried out using retroviral infection of reporter genes, supports the notion that there are cells capable of both self-renewal and differentiation to distinct cell types. Differentiation to multiple cell types as well as to only a single type of neuron or glial cells has been observed [28, 29].

To objectively discuss the issue of stem cells vs. differentiated cells, access to reliable molecular markers is a prerequisite. The sequencial expression of different members of the intermediate filament gene family has proven useful to identify various cell types in the early CNS. The intermediate filaments nestin [37] and vimentin are expressed in the proliferating cells of the early neural tube, and then replaced by glial fibrillary acidic protein (GFAP) and neurofilament expression in astrocytes and neurons, respectively [38]. In addition to these markers, there is an increasing number of markers identifying distinct subpopulations of cells or specific differentiation steps. A useful marker for newly differentiated neurons is TuJ1 (a class-III β-tubulin: see ref. 39 and references therein).

Factors influencing the choice between proliferation and differentiation in the embryonic CNS

To arrive at the correct number of differentiated cells in the adult brain and spinal cord, there has to be a strictly controlled balance between proliferation and differentiation in the embryonic CNS. To generate a sufficient number of cells, it seems reasonable to assume that proliferation may be predominant in the early phases, and that more cells differentiate during later stages. This line of reasoning implies that there is a higher probability for generating two undifferentiated daughter cells at early stages, and that later divisions favour the production of neurons or later glial cells. Rapid progress is being made in identifying factors that control how undifferentiated or differentiated cells are generated following proliferation, and these factors can be of two principally different types: intrinsic or extrinsic. In a system based on intrinsic factors, the decision to generate two identical or nonidentical daughter cells is based on molecules operating within the cell, while an extrinsic mechanism relies on signals influencing the cell from the outside.

Intrinsic mechanisms and asymmetric cell division

One way to control the choice between proliferation and differentiation could conceivably be based on a system which is completely cell-autonomous, i.e. which is independent of signals from outside the cell. Such a system could be driven by a precisely timed activation of key regulatory genes, for example genes encoding transcription factors important for differentiation. In this way, differentiation could occur at the appropriate time point, independent of the environment of the cells. There are clearly cell fate choices which are highly stereotypic and reproducible, in particular in less complex organisms like the nematode *Caenorhabditis elegans*, and which may be executed by a cell-autonomous mechanism. But it seems unlikely that the complex mammalian CNS would be constructed by this type of 'hardwired' differentiation decision process.

Intrinsic mechanisms may, however, be operating in the context of asymmetric cell division. One means of generating two nonidentical daughter cells is to provide the cell that undergoes cell division with a compartmentally localized protein that is important for differentiation and ensure that this protein is unequally distributed in the two daughter cells. In *Drosophila*, the molecular machinery for this type of asymmetric cell division is now being revealed and involves the function of the genes *Inscuteable*, *Miranda*, *Prospero* and *Numb* (see refs. 40–42 and references therein).

Less is known about asymmetric cell division in mammalian systems, but a mammalian homologue to Numb has been characterized, which is asymmetrically localized in cells of the developing CNS [43]. This finding, in conjunction with the observation that the cleavage plane in dividing cells is altered as neurogenesis proceeds, opens up an interesting possibility of regulating the proportion of symmetric vs. asymmetric cell division, and hence the possibility of generating proliferat-

ing or differentiating cells. In early CNS development, most cells have their cleavage plane perpendicular to the surface of the neural tube, i.e. generating two symmetric cells with equal amounts of Numb (fig. 3). In contrast, at later stages an increasing fraction of the cells exhibit a cleavage plane more in parallel with the surface of the neural tube [44]. This results in the generation of two unequal cells: one undifferentiated cell, which receives more Numb and remains in the ventricular zone, and another cell, which has low Numb levels, and rapidly migrates away from the ventricular zone, presumably to differentiate into a neuron.

Extrinsic factors

The majority of cell differentiation events in the developing CNS are likely to depend on various types of interactions between cells and/or on secreted factors acting on cells from a distance. It has been convincingly demonstrated that neural cells can change their mode of differentiation in response to an altered environment, for example after transplantation to new regions of the CNS [24–26].

Direct cell-cell communication is important for cellular differentiation in the early CNS, and the Notch signalling pathway plays a critical role in this process. From a group of initially equipotent cells, a cell that becomes committed to the neuronal fate will repress the cells in immediate contact from taking on the same fate and instead remain undifferentiated; this mechanism is called lateral inhibition. This is executed by expression of high levels of ligand on the differentiating cells, which signals to Notch receptors on the surrounding cells not to differentiate (for a review on Notch signalling in the nervous system, see ref. 45).

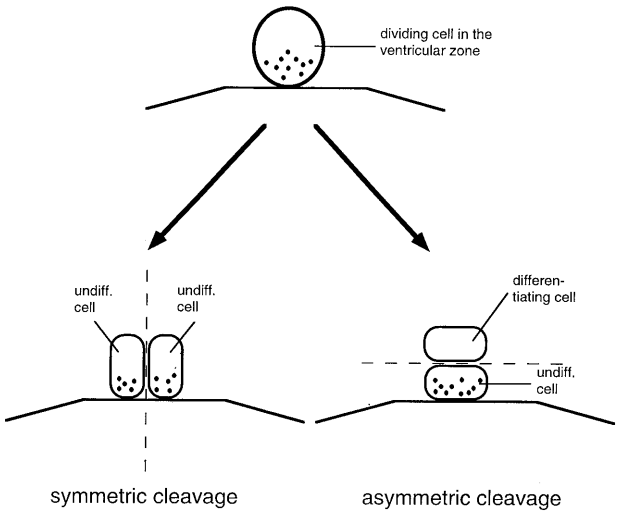

Figure 3. Asymmetric cell division. A cell in the ventricular zone of the developing CNS expresses a protein (small black circle), which is localized predominantly to the part of the cell located close to the ventricular side. If the cell undergoes symmetric cleavage during mitosis (lower left) the protein will be symmetrically distributed to the two daughter cells, which will remain as undifferentiated cells. Alternatively, if the cell undergoes asymmetric cleavage (lower right), the protein will be sorted only to the cell closest to the ventricular side. This cell will then remain as an undifferentiated cell, while the other cell can migrate out and differentiate to a neuron.

In addition to mechanisms for direct cell-cell communication, there is also a growing list of secreted signalling molecules that act at a distance to instruct cells to differentiate to particular fates. Important work from McKay's research group [46] demonstrates that specific signalling molecules can promote stem cell fate or, alternatively, convert embryonic CNS stem cells to neuronal and glial fates in large numbers. They found that T3 promotes differentiation to oligodendrocytes, CNTF (ciliary neurotrophic factor) to astrocytes and PDGF (platelet-derived growth factor) to neurons. EGF (epidermal growth factor) and bFGF (basic fibroblast growth factor) allowed the CNS stem cells to remain in an undifferentiated state [46]. The same group has also worked out conditions for the transition from a more undifferentiated cellular state to the CNS stem cell by analysing how embryonal stem cells (ES cells) differentiate to express markers typical of CNS stem cells [47].

Signalling molecules important for dorsoventral signalling have been identified and provide an example of how an extrinsic signal is converted to an intrinsic signal. Sonic Hedgehog is first expressed in the notochord and later also in the floor plate, and it plays an important role in ventralizing cells in the embryonic spinal cord and brain. The *Sonic Hedgehog* gene encodes a secreted protein, which is cleaved by an autoproteolytic mechanism to generate two moieties, and the N-terminal fragment contains the biological activity (for review, see ref. 48). From the localized source of Sonic Hedgehog production in the notochord and floor plate, a gradient with decreasing levels is found towards more dorsal regions in the spinal cord. Experiments on explant cultures of dissected tissue from various positions in the chick neural plate and early neural tube show that different levels of Sonic Hedgehog concentration induce different fates [49]. Differentiation of motoneurons thus requires higher concentrations of Sonic Hedgehog than differentiation of more dorsally located interneurons [49]. A recent important observation is that the gradient of Sonic Hedgehog is converted into a reverse gradient of the levels of the transcription factor Pax 6 in the nuclei of cells in the spinal cord, i.e. there are higher levels of Pax 6 in the more dorsal regions, where Sonic Hedgehog levels are low and vice versa in the ventral regions [49]. Although the molecular mechanisms for how Sonic Hedgehog regulates the levels of Pax 6 are not

yet understood, these experiments provide a conceptually important example of how a gradient of a secreted, extracellular factor can be translated into distinct levels of a nuclear transcription factor.

Neurogenesis in the adult CNS

Neurogenesis in the adult organism is a well-documented phenomenon in many nonmammalian vertebrates and has been most extensively studied in birds (reviewed in ref. 50). In mammals, neurogenesis was for a long time considered to be restricted to the embryonic and early postnatal period. However, labelling of neurons after injection of radioactive nucleotide analogues revealed that neurogenesis does occur in the adult mammalian nervous system [51]. As discussed in detail below, it is now firmly established that new neurons are added continuously in the hippocampus and in the olfactory bulb. Another region where neurogenesis is seen in the adult is the olfactory epithelium; olfactory sensory neurons have an average life span of about 30 to 60 days, after which they die by apoptosis (programmed cell death) and are replaced by new neurons. Neurogenesis in the olfactory epithelium has been the subject of other recent reviews (see e.g. ref. 52), and since it is fundamentally different from the neurogenesis seen in the CNS, it will not be discussed further in this review. Neurogenesis in the adult brain has been documented in a variety of mammals and seems to be an evolutionarily well-conserved phenomenon. Generation of neurons in cultures of adult human brain tissue [53] suggests that there may be continuous neurogenesis also in the adult human brain, although this issue is difficult to address. New neurons are added throughout adulthood in rodents, although there seems to be a decrease in neurogenesis in senescent animals [54, 55].

Isolation of a CNS stem cell from the adult nervous system

Neurogenesis in adult animals implies the presence of undifferentiated progenitor cells. Reynolds and Weiss were the first to describe the isolation of such a cell from the adult mammalian CNS [56]. In serum-free medium, but in the presence of EGF, they were able to propagate a population of cells which could generate neurons, astrocytes and oligodendrocytes in vitro [56, 57].

Under the conditions used by Reynolds and Weiss, single cells proliferate in vitro, and the progeny forms a cluster of aggregated cells [56, 57]. Such cell clones detach from the culture dish after a few days in vitro. The cells continue to proliferate and form a spheroid cell aggregate, referred to as a neurosphere, of tightly clustered cells, all of which are derived from a single cell (fig. 4A). Most of the cells in the neurosphere express nestin but not markers typical for differentiated cells [56]. These undifferentiated cells rapidly differentiate if plated on an adhesive substrate or if serum is added to the culture medium (fig. 4B–D). Importantly, a clone of cells derived from a single cell can generate neurons, astrocytes and oligodendrocytes, demonstrating that at least the initial cell was multipotent [57]. Moreover, if a cell clone is dissociated, many of the cells will form new clusters of undifferentiated multipotent cells [57], thus fulfilling the criteria for being stem cells (as discussed above). The possibility of culturing neural stem cells from the adult CNS has greatly improved the means of studying these cells and has led to major breakthroughs in this field.

Localization and identity of the adult neural stem cell

To determine the localization of the adult CNS stem cells, different parts of the adult rodent forebrain were carefully dissected and cultured to test for the capacity of neurogenesis. These studies have demonstrated that stem cells are most abundant in the wall of the lateral ventricle and in the hippocampus [58–61]. Furthermore, stem cells can be isolated from the walls of the third and fourth ventricles as well as from the adult spinal cord, suggesting the presence of stem cells adjacent to the ventricular system along the entire neuraxis [62]. Under certain conditions, a very small number of cells with the capacity to generate neurons in vitro can be isolated also from the striatum and septum [60], although it has not been tested if these cells have stem cell properties or if they are committed neuronal progenitors.

It is not clear whether the stem cells located in association with the ventricular system and in other regions derive from a common cell population, or whether they represent independent germinal centres. It is noteworthy that the lateral ventricles in young animals extend over the hippocampus, but are later withdrawn. However, groups of ependymal cells remain in association with the hippocampus after the lateral ventricle has withdrawn [63], suggesting that the stem-cell population in association with the hippocampus may derive from the wall of the lateral ventricle.

The exact localization and identity of the neural stem cell have remained enigmatic. The wall of the lateral ventricles has been the subject of detailed morphological studies [64, 65]. The ventricular system is lined by a single layer of ependymal cells. Beneath the ependymal layer is the subependymal layer, also known as the subventricular zone. This area harbors astrocytes, neuroblasts and progenitor cells [64]. The progenitor cells in the subependymal layer have a high proliferation rate [66]. Generally, stem cells proliferate very slowly [32], and when the rapidly proliferating subependymal cells were selectively killed, it did not deplete the stem-cell

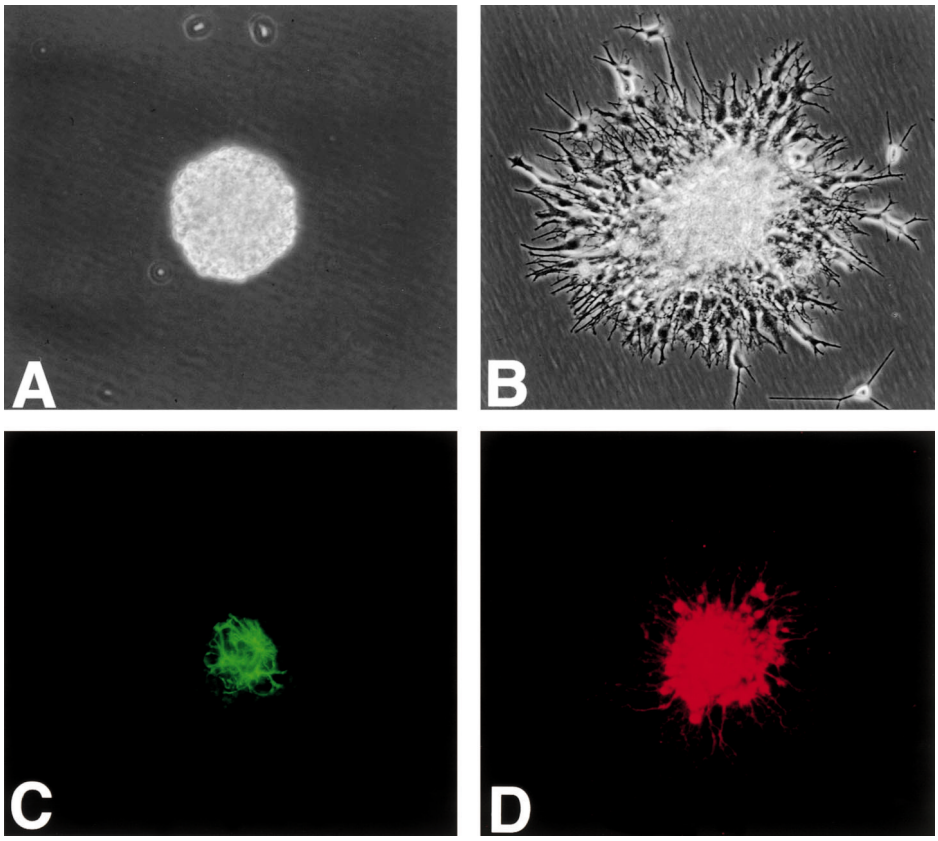

Figure 4. Undifferentiated and differentiated neurospheres. Phase-contrast views of a neurosphere derived from a single neural stem cell from the adult rat spinal cord cultured for 10 days (*A*) and 2 days after plating on an adhesive substrate which triggers differentiation (*B*). (*C* and *D*) A differentiated neurosphere labelled with antibodies against the astrocyte marker GFAP (*C*) and neuronal βIII-tubulin (*D*).

population, arguing against these cells being stem cells [59]. Ependymal cells express the highest nestin levels, followed by the progenitor cells in the subventricular zone [64]. Data from salamanders and birds suggest that the neural stem cells in these species are located in the layer facing the ventricles [50, 67], corresponding to the localization of ependymal cells in mammals. However, mammalian ependymal cells are generally considered to be highly differentiated cells forming a barrier between the nervous tissue and the cerebrospinal fluid [68], which may argue against these cells being undifferentiated stem cells. Determining the exact localization and identity of adult CNS stem cells remains an outstanding question in neurobiology.

Continuous addition of neurons in the olfactory bulb and hippocampus

The stem cells located adjacent to the lateral ventricles predominantly generate olfactory bulb interneurons in the adult animal [53]. Neuroblasts are generated in the subventricular zone, from where they migrate towards the olfactory bulb [69]. The neuroblasts migrate on top of each other in rows, representing a novel type of cell migration termed 'chain migration' [70, 71], which has not been described in other parts of the brain. All the chains of migrating cells in the subventricular zone of one hemisphere merge to form the rostral migratory stream [72], a dense tract of migrating neuroblasts, where the neuroblasts migrate closely together surrounded by a scaffold of astrocytes [70]. Polysialic acid associated with neural cell adhesion molecule (NCAM) is required for the migration of neuroblasts from the subependymal zone to the olfactory bulb, and mutant mice lacking NCAM have abnormally small olfactory bulbs due to reduced cell migration along the rostral migratory stream [73]. In the hippocampus, progenitor cells proliferate at the border between the hilus and the granule cell layers. Newborn cells migrate into the granular cell layer, where they begin to express neuronal markers (reviewed in ref. 74).

Continuous death of neurons is seen in both the olfactory bulb and hippocampus throughout adult life [74, 75], and it is tempting to speculate that neurogenesis in these locations primarily serves to replace lost neurons. However, the total number of granule neurons in both the olfactory bulb and the hippocampus increases substantially through adulthood [75, 76]. The significance of the increase in the numbers of neurons in these regions is unclear, but one hypothesis is that they may meet a need for increased information storage capacity with time.

Factors which influence the proliferation and differentiation of adult neural stem cells

A question of significant interest is how the proliferation and differentiation of the adult neural stem cell are regulated. Several studies have emphasized the need for at least one mitogen, which can be either EGF or bFGF, for the proliferation of adult neural stem cells in vitro [56, 77]. In line with this, injection of EGF or bFGF into the forebrain stimulates the proliferation of progenitor cells [78, 79]. However, it is not known whether EGF or bFGF are required mitogens for the adult neural stem cells in vivo.

Interestingly, the external environment seems to affect the rate of neurogenesis. Thus, mice which are kept in an enriched environment compared with standard laboratory conditions for 1 month (from 3 weeks of age) have approximately 15% more granular neurons in the hippocampus [80]. This was mainly a result of increased survival of newly generated neurons [80]. In contrast, exposure of adult tree shrews to psychological stress reduced cell proliferation in the dentate gyrus [81]. Synaptic activity has been found to regulate neurogenesis in the adult hippocampus and may perhaps explain in part how the number of neurons may be affected by the environment the animals are kept in [82, 83]. Furthermore, adrenal hormones negatively regulate cell proliferation in the hippocampus [74], and it is likely that the reduced proliferation in animals exposed to stress may to some extent be explained by elevated levels of adrenal hormones [81].

There is increasing evidence that nervous system injuries may affect stem cells in the adult CNS. After both spinal cord and brain injuries, nestin expression is increased in cells lining the central canal and in the subventricular zone, respectively [84, 85]. With time, nestin-expressing cells are seen progressively further from the central canal and the lateral ventricle, and these cells express astrocytic markers [84, 85]. These data have led to the suggestion that stem cells or progenitor cells residing by the ventricular system are induced to proliferate, migrate towards the site of the injury and differentiate to astrocytes. Furthermore, hippocampal lesions increase the proliferation of hippocampal progenitor cells and the number of granular neurons in the hippocampus [86].

When it comes to molecules affecting the differentiation of adult stem cells to different cell types, i.e. neurons, astrocytes or oligodendrocytes, current data suggest that stem cells derived from the adult brain are very similar to those derived from the embryonic brain and that a similar set of molecules instructively can induce the differentiation to certain cell fates ([46]; see also above). Less is known about the regulation of the differentiation of adult stem cells to different mature neuronal subtypes producing, for example, certain transmitters. Injection of stem cells from the wall of the lateral ventricle of perinatal mice into the ventricles of embryonic mice resulted in integration of neurons derived from the donor in various parts of the forebrain, emphasizing that at least cells from perinatal animals can take on many different neuronal fates [87]. This also indicates that the stem cells from young postnatal animals respond to the same cues as their embryonic counterparts. As the molecules directing the differentiation to certain neuronal fates during embryogenesis are unveiled, it will be possible to test whether these factors may be used to direct the differentiation of neural stem cells in the adult to specific neuronal subtypes.

Potential therapeutic utilization of adult neural stem cells

A large variety of approaches have been tested to restore the function after CNS injury in animal models, most of them attempting either to replace lost neurons by transplantation of cells from a donor, to support the survival of affected neurons or to stimulate axonal regrowth. Several different strategies have been successful in animal models, and a few have been tested in humans in clinical trials (reviewed in ref. 88).

The presence of neural stem cells in the adult CNS may open up new avenues to replace neurons lost due to injuries or various diseases. One may envisage two main strategies to utilize a patient's own stem cells to generate new neurons; to induce neuronal differentiation from stem cells in situ, or to remove stem cells from the patient, propagate and manipulate them in vitro, and transplant them back in to the appropriate region of the CNS. Utilization of neural stem cells is a tantalizing possibility which would offer considerable advantages compared with transplantation of cells from donors. All immunological concerns regarding rejection of a graft or graft vs. host reactions would be circumvented, since the new cells would derive from the patient and thus be immunologically identical to the other cells in the body. The majority of transplantation studies in humans have

thus far involved grafting of tissue from aborted human foetuses to Parkinson's disease patients (reviewed in ref. 89). An interesting alternative to transplantation of human tissue is xenotransplantation (see e.g. ref. 90); but the technique has considerable immunological drawbacks and remains controversial (recently discussed in ref. 91). Transplantation of tissue from foetuses as well as from animals also raises a number of ethical questions which would be eliminated if neurogenesis could be induced from the stem cells of the patient.

A key issue is whether new neurons can integrate in the adult brain in a functional way, i.e. Are there cues in the adult nervous system that may instruct new neurons how to integrate? Although not yet analysed in detail, it should be noted that grafted undifferentiated embryonic cells in many cases can differentiate to region-specific neuronal types and integrate when transplanted to the adult brain (reviewed in ref. 92). Furthermore, grafting of cultured undifferentiated cells from the adult hippocampus back to the hippocampus of adult animals demonstrated that these cells survived transplantation and differentiated to neurons [93]. In addition, cultured hippocampal cells grafted to the rostral migratory stream migrated to the olfactory bulb and differentiated to site-specific neurons distinct from the cell types normally generated by stem cells in the hippocampus [39]. In contrast, hippocampal cells transplanted to the adult cerebellum survived but failed to generate neurons [39]. This difference may be related to the fact that neurogenesis is absent in the adult cerebellum, while the rostral migratory stream and the olfactory bulb represent areas of ongoing neuronal differentiation. The studies discussed above have focused on grafting undifferentiated cells. Transplantation of more differentiated embryonic cells has demonstrated a large potential for such cells to integrate in the adult nervous system. No studies have yet tested the possibility of transplanting neuroblasts or differentiated neurons derived from adult stem cells. Further studies are needed to assess the potential for using adult stem cells to replace specific neuronal populations which may be affected in neurodegenerative diseases.

Acknowledgements. The authors' work is supported by the Swedish Medical Research Council (project no. 12183), the Wenner-Gren foundations, Kapten Artur Erikssons stiftelse and Cancerfonden.

1 Spemann H. (1938) Embryonic development and induction. Yale University Press, New Haven, CT
2 Heldin C.-H., Miyazono K. and ten Dijke P. (1997) TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature **390:** 465–471
3 Piccolo S., Sasai Y., Lu B. and De Robertis E. (1996) Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. Cell **86:** 589–598
4 Zimmerman L. B., De Jesús-Escobar J. M. and Harland R. M. (1996) The Spemann Organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell **86:** 599–606
5 Hemmati-Brivanlou A. and Melton D. (1997) Vertebrate embryonic cells will become nerve cells unless told otherwise. Cell **88:** 13–17
6 Hawley S. H., Wunnenberg-Stapleton K., Hashimoto C., Laurent M. N., Watabe T., Blumberg B. W. et al. (1995) Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. Genes Dev. **9:** 2923–2935
7 Suzuki A., Theis R. S., Yamaji N., Song J. J., Wozney J., Murakami K. et al. (1994) A truncated BMP receptor affects dorsal-ventral patterning in the early *Xenopus* embryo. Proc. Natl. Acad. Sci. USA **91:** 10255–10259
8 Matzuk M. M., Lu N., Vogel H., Sellheyer K., Roop D. R. and Bradley A. (1995) Multiple defects and perinatal death in mice deficient in follistatin. Nature **374:** 360–363
9 Smith J. (1995) Angels on activin's absence. Nature **374:** 311–312
10 Karfunkel P. (1971) The role of microtubules and microfilaments in neurulation in *Xenopus*. Dev. Biol. **25:** 30–56
11 Schoenwolf G. C. and Smith J. L. (1990) Mechanisms of neurulation: traditional viewpoint and recent advances. Development **109:** 243–270
12 Thomas T. and Dziadek M. (1993) Genes coding for basement membrane glycoproteins laminin, nidogen and collagen IV are differentially expressed in the nervous system and by epithelial, endothelial, and mesenchymal cells of the mouse embryo. Exp. Cell Res. **208:** 54–67
13 McConnell S. K. (1995) Constructing the cerebral cortex: neurogenesis and fate determination. Neuron **15:** 761–768
14 Sauer F. C. (1935) Mitosis in the neural tube. J. Comp. Neurol. **62:** 377–405
15 Rakic P. (1988) Specification of cerebral cortical areas. Science **1988:** 170–176
16 Soriano E., Dumesnil N., Auladell C., Cohen-Tannoudji M. and Sotelo C. (1995) Molecular heterogeneity of progenitors and radial migration in the developing cerebral cortex revealed by transgene expression. Proc. Natl. Acad. Sci. USA **92:** 11676–11680
17 Tan S.-S. and Breen S. (1993) Radial mosaicism and tangential cell dispersion both contribute to mouse neocortical development. Nature **362:** 638–640
18 Tan S.-S., Faulkner-Jones B., Breen S. J., Walsh M., Bertram J. F. and Reese B. E. (1995) Cell dispersion patterns in different cortical regions studied with an X-inactivated transgenic marker. Development **121:** 1029–1039
19 Rakic P. (1972) Mode of cell migration to the superficial layers of monkey neocortex. J. Comp. Neurol. **145:** 61–84
20 Anton E. S., Cameron R. S. and Rakic P. (1996) Role of neuron-glial junctional domain proteins in the maintenance and termination of neuronal migration across the embryonic cerebral wall. J. Neurosci. **16:** 2283–2293
21 Goffinet A. M. (1997) Unscrambling a disabled brain. Nature **389:** 668–669
22 Howell B. W., Hawkes R., Soriano P. and Cooper J. A. (1997) Neuronal position in the developing brain is regulated by *mouse disabled-1*. Nature **389:** 733–737
23 Sheldon M., Rice D. S., D'Arcangelo G., Yoneshima H., Nakajima K., Mikoshiba K. et al. (1997) *Scrambler* and *yotari* disrupt the disabled gene and produce a *reeler*-like phenotype in mice. Nature **389:** 730–733
24 Brüstle O., Maskos U. and McKay R. D. G. (1995) Host-guided migration allows targeted introduction of neurons into the embryonic brain. Neuron **15:** 1275–1285
25 Campbell K., Olsson M. and Björklund A. (1995) Regional incorporation and site-specific differentiation of striatal precursors transplanted to the embryonic forebrain ventricle. Neuron **15:** 1259–1273
26 Fishell G. (1995) Striatal precursors adopt cortical identities in response to local cues. Development **121:** 803–812
27 Fishell G., Mason C. A. and Hatten M. E. (1993) Dispersion of neural progenitors within the germinal zones of the forebrain. Nature **362:** 636–638
28 Reid C. B., Liang I. and Walsh C. (1995) Systematic widespread clonal organization in cerebral cortex. Neuron **15:** 299–310

29 Reid C. B., Tavazoie S. F. and Walsh C. A. (1997) Clonal dispersion and evidence for asymmetric cell division in ferret cortex. Development **124:** 2441–2450
30 Kornack D. R. and Rakic P. (1995) Radial and horizontal deployment of clonally related cells in the primate neocortex: relationship to distinct mitotic lineages. Neuron **15:** 311–321
31 Rakic P. (1995) Radial versus tangential migration of neuronal clones in the developing cerebral cortex. Proc. Natl. Acad. Sci. USA **92:** 11323–11327
32 Morrison S. J., Shah N. M. and Anderson D. J. (1997) Regulatory mechanisms in stem cell biology. Cell **88:** 287–298
33 McKay R. (1997) Stem cells in the central nervous system. Science **276:** 66–71
34 Williams B. P. and Price J. (1995) Evidence for multiple precursor cell types in the embryonic rat cerebral cortex. Neuron **14:** 1181–1188
35 Temple S. and Qian X. (1996) Vertebrate neural progenitor cells: subtypes and regulation. Curr. Opin. Neurobiol. **6:** 11–17
36 Temple S. and Davis A. A. (1994) A self-renewing multipotential stem cell in embryonic rat cerebral cortex. Nature **372:** 263–266
37 Lendahl U., Zimmerman L. B. and McKay R. D. G. (1990) CNS stem cells express a new class of intermediate filament protein. Cell **60:** 585–595
38 Dahlstrand J., Lardelli M. and Lendahl U. (1995) Nestin mRNA expression correlates with the CNS progenitor cell state in many, but not all, regions of developing CNS. Dev. Brain Res. **84:** 109–129
39 Suhonen J. O., Peterson D. A., Ray J. and Gage F. H. (1996) Differentiation of adult hippocampus-derived progenitors into olfactory neurons in vivo. Nature **383:** 624–627
40 Broadus J., Fuerstenberg S. and Doe C. Q. (1998) Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate. Nature **391:** 792–795.
41 Ikeshima-Kataoka H., Skeath J. B., Nabeshima Y., Doe C. Q. and Matsuzaki F. (1997) Miranda directs Prospero to a daughter cell during *Drosophila* asymmetric divisions. Nature **390:** 625–629
42 Shen C.-P., Jan L. Y. and Jan Y. N. (1997) Miranda is required for the asymmetric localization of Prospero during mitosis in *Drosophila*. Cell **90:** 449–458
43 Zhong W., Feder J. N., Jiang M.-M., Jan L. Y. and Jan Y. N. (1996) Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron **17:** 43–53
44 Chenn A. and McConnell S. K. (1995) Cleavage orientation and the asymmetric inheritance of Notch1 immunoreactivity in mammalian neurogenesis. Cell **82:** 631–641
45 Lewis J. (1996) Neurogenic genes and vertebrate neurogenesis. Curr. Opin. Neurobiol. **6:** 3–10
46 Johe K. K., Hazel T. G., Muller T., Dugich-Djordjevic M. and McKay R. D. G. (1996) Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes Dev. **10:** 3129–3140
47 Okabe S., Forsberg-Nilsson K., Spiro A. C., Segal M. and McKay R. D. G. (1996) Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. Mech. Dev. **59:** 89–102
48 Hammerschmidt M., Brook A. and McMahon A. P. (1997) The world according to hedgehog. Trends Genet. **13:** 14–21
49 Ericson J., Rashbass P., Schedl A., Brenner-Morton S., Kawakami A., van Heyningen V. et al. (1997) Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signalling. Cell **90:** 169–180
50 Alvarez-Byulla A. and Kirn J. R. (1997) Birth, migration, incorporation and death of vocal control neurons in adult songbirds. J. Neurobiol. **33:** 585–601
51 Altman J. and Das G. D. (1965) Autoradiographic and histological evidence of postnatal neurogenesis in rats. J. Comp. Neurol. **124:** 319–335
52 Calof A. L., Hagiwara N., Holcomb J. D., Mumm J. S. and Shou J. (1996) Neurogenesis and cell death in olfactory epithelium. J. Neurobiol. **30:** 67–81
53 Kirschenbaum B., Nedergaard M., Preuss A., Barami K., Fraser R. A. R. and Goldman S. A. (1994) In vitro neuronal production and differentiation by precursor cells derived from the adult human forebrain. Cereb. Cortex **6:** 576–589
54 Kuhn P. G., Dickinson-Anson H. and Gage F. H. (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. **16:** 20–27
55 Tropepe V., Craig C. G., Morshead C. M. and van der Kooy D. (1997) Transforming growth factor-α null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. J. Neurosci. **17:** 7850–7859
56 Reynolds B. A. and Weiss S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science **255:** 1707–1710
57 Reynolds B. A. and Weiss S. (1996) Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic precursor is a stem cell. Dev. Biol. **175:** 1–13
58 Lois C. and Alvarez-Buylla A. (1993) Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc. Natl. Acad. Sci. USA **90:** 2074–2077
59 Morshead C. M., Reynolds, B. A., Craig C. G., McBurney M. W., Staines W. A., Morassutti D. et al. (1994) Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron **13:** 1071–1082
60 Palmer T. D., Ray J. and Gage F. H. (1995) FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. Mol. Cell. Neurosci. **6:** 474–486
61 Palmer T. D., Takahashi, J. and Gage F. H. (1997) The adult rat hippocampus contains primordial neural stem cells. Mol. Cell. Neurosci. **8:** 389–404
62 Weiss S., Dunne C., Hewson J., Wohl C., Wheatley M., Peterson A. C. et al. (1996) Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J. Neurosci. **16:** 7599–7609
63 Kawamata S., Stumpf W. E. and Bidmon H. J. (1995) Adhesion and fusion of ependyma in rat brain. Acta Anat. (Basel) **152:** 205–214
64 Doetsch F., Garcia-Verdugo J. M. and 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
65 Gates M. A., Thomas L. B., Howard E. M., Laywell E. D., Sajin B., Faissner A. et al. (1995) Cell and molecular analysis of the developing and adult mouse subventricular zone of the cerebral hemispheres. J. Comp. Neurol. **361:** 249–266
66 Morshead C. M. and van der Kooy D. (1992) Postmitotic death is the fate of constitutively proliferating cells in the subependymal layer of the adult mouse brain. J. Neurosci. **12:** 249–256
67 Holder N., Clarke J. D. W., Stephens N., Wilson S. W., Orsi C., Bloomer T. et al. (1991) Continous growth of the motor system in the axolotl. J. Comp. Neurol. **303:** 534–550
68 Del Bigio M. R. (1995) The ependyma: a protective barrier between brain and cerebrospinal fluid. Glia **14:** 1–13
69 Lois C. and Alvarez-Buylla A. (1994) Long-distance neuronal migration in the adult mammalian brain. Science **264:** 1145–1148
70 Lois C., García-Verdugo J.-M. and Alvarez-Buylla A. (1996) Chain migration of neuronal precursors. Science **271:** 978–981
71 Wichterle H., Garcia-Verdugo J. M. and Alvarez-Buylla A. (1997) Direct evidence for homotypic, glia-independent neuronal migration. Neuron **18:** 779–791
72 Doetsch F. and Alvarez-Buylla A. (1996) Network of tangential pathways for neuronal migration in adult mammalian brain. Proc. Natl. Acad. Sci. USA **93:** 14895–14900
73 Hu H., Tomasiewicz H., Magnusson T. and Rutishauser U. (1996) The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. Neuron **16:** 735–743

74 Gould E. and Cameron H. A. (1996) Regulation of neuronal birth, migration and death in the rat dentate gyrus. Dev. Neurosci. **18:** 22–35
75 Kaplan M. S., McNelly N. A. and Hinds J. W. (1985) Population dynamics of adult-formed granule neurons of the rat olfactory bulb. J. Comp. Neurol. **239:** 117–125
76 Bayer S. A., Yackel J. W. and Puri P. S. (1982) Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science **216:** 890–892
77 Gritti A., Parati E. A., Cova L., Frolichsthal P., Galli R., Wanke E. et al. (1996) Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J. Neurosci. **16:** 1091–1100
78 Craig C. G., Tropepe V., Morshead C. M., Reynolds B. A., Weiss S. and van der Kooy D. (1996) In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. J. Neurosci. **16:** 2649–2658
79 Kuhn H. G., Winkler J., Kempermann G., Thal L. J. and Gage F. H. (1997) Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. J. Neurosci. **17:** 5820–5829
80 Kempermann G., Kuhn H. G. and Gage F. H. (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature **386:** 493–495
81 Gould E., McEwen B. S., Tanapat P., Galea L. A. M. and Fuchs E. (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. **17:** 2492–2498
82 Cameron H. A., McEwen B. S. and Gould E. (1995) Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. J. Neurosci. **15:** 4687–4692
83 Parent J. M., Yu T. W., Leibowitz R., Geschwind D. H., Sloviter R. S. and Lowenstein D. H. (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult hippocampus. J. Neurosci. **17:** 3727–3738
84 Frisén J., Johansson C. B., Török C., Risling M. and Lendahl U. (1995) Rapid, widespread and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. J. Cell Biol. **131:** 453–464
85 Holmin S., Almzvist P., Lendahl U. and Mathiesen T. (1997) Adult nestin-expressing subependymal cells differentiate to astrocytes in response to brain injury. Eur. J. Neurosci. **9:** 65–75
86 Gould E. and Tanapat P. (1997) Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience **80:** 427–436
87 Lim D. A., Fishell G. J. and Alvarez-Buylla A. (1997) Postnatal mouse subventricular zone neuronal precursors can migrate and differentiate within multiple levels of the developing neuraxis. Proc. Natl. Acad. Sci. USA **94:** 14832–14836
88 Olson L. (1997) Regeneration in the adult central nervous system: experimental repair strategies. Nature Med. **3:** 1329–1335
89 Lindvall O. (1997) Neural transplantation: a hope for patients with Parkinson's disease. Neuroreport **8:** III–X
90 Deacon T., Schumacher J., Dinsmore J., Thomas C., Palmer P., Kott S. et al. (1997) Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Med. **3:** 350–353
91 Butler D., Wadman M., Lehrman S. and Schirmeier Q. (1998) Last chance to stop and think on risks of xenotransplantation. Nature **391:** 320–325
92 Björklund A. (1994) Long distance axonal growth in the adult central nervous system. J. Neurol. **242:** S33–S35
93 Gage F. H., Coates P. W., Palmer T. D., Kuhn H. G., Fisher L. J., Suhonen J. O. et al. (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc. Natl. Acad. Sci. USA **92:** 11879–11883

.