

Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells

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Received 31 August 2004; accepted 11 April 2005

Abstract

Recent work in neuroscience has shown that the adult central nervous system contains neural progenitors, precursors, and stem cells that are capable of generating new neurons, astrocytes, and oligodendrocytes. While challenging previous dogma that no new neurons are born in the adult mammalian CNS, these findings bring with them future possibilities for the development of novel neural repair strategies. The purpose of this review is to present current knowledge about constitutively occurring adult mammalian neurogenesis, to highlight the critical differences between “neurogenic” and “non-neurogenic” regions in the adult brain, and to describe the cardinal features of two well-described neurogenic regions—the subventricular zone/olfactory bulb system, and the dentate gyrus of the hippocampus. We also provide an overview of currently used models for studying neural precursors in vitro, mention some precursor transplantation models, and emphasize that, in this rapidly growing field of neuroscience, one must take caution with respect to a variety of methodological considerations for studying neural precursor cells both in vitro and in vivo. The possibility of repairing neural circuitry by manipulating neurogenesis is an intriguing one, and, therefore, we also review recent efforts to understand the conditions under which neurogenesis can be induced in non-neurogenic regions of the adult CNS. This work aims toward molecular and cellular manipulation of endogenous neural precursors in situ, without transplantation. We conclude this review with a discussion of what the function might be of newly generated neurons in the adult brain and provide a summary of current thinking about the consequences of disturbed adult neurogenesis and the reaction of neurogenic regions to disease.

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Abbreviations: BDNF, brain-derived neurotrophic factor; BLBP, brain lipid binding protein; BMP, bone morphogenetic protein; BrdU, bromodeoxyuridine; CNS, central nervous system; Dcx, doublecortin; DG, dentate gyrus; EGF, epidermal growth factor; FGF-2, fibroblast growth factor-2 (basic fibroblast growth factor); GABA, gamma amino butyric acid; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; HVC, high vocal center; IGF-I, insulin-like growth factor-I; NeuN, neuron-specific nuclear protein; PDGF, platelet-derived growth factor; PSA-NCAM, polysialylated neural cell adhesion molecule; RMS, rostral migratory stream; SGZ, subgranular zone; SVZ, subventricular zone; TGF α , transforming growth factor- α ; VEGF, vascular endothelial growth factor

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1. Introduction

Contrary to previously held beliefs about the static nature of the adult brain, it is in fact capable of generating new neurons that can integrate into its complex circuitry. However, the recent development of new techniques has resulted in an explosion of research demonstrating that neurogenesis, the birth of new neurons, constitutively occurs in two specific regions of the adult mammalian brain (olfactory bulb and hippocampal dentate gyrus), and that there are significant numbers of multipotent neural precursors, or “stem cells,” in many parts of the adult mammalian brain (Altman and Das, 1965; Altman, 1969; Reynolds and Weiss, 1992; Lois and Alvarez-Buylla, 1993; Palmer et al., 1995) (and see reviews in McKay, 1997; Gage, 2000; van der Kooy and Weiss, 2000; Alvarez-Buylla et al., 2001).

The rise of precursor cell biology has brought new life to neural transplantation and the consideration of cellular replacement strategies to treat diseases of the brain. The idea of “making new neurons” is appealing for neurodegenerative diseases or selective neuronal loss associated with chronic neurological or psychiatric disorders. One goal of neural precursor biology is to learn from this regionally limited, constitutive neurogenesis how to manipulate neural precursors toward therapeutic neuronal or glial repopulation. Elucidation of the relevant molecular controls might allow both control over transplanted precursor cells and the development of neuronal replacement therapies based on the recruitment of endogenous cells.

This review deals with adult neurogenesis; cellular repair of the adult mammalian central nervous system (CNS); what is known about the location, behavior, and function of precursor cells in the adult brain. In the context of CNS regeneration, this information lies at the core of all attempts to guide neuronal or glial development from neural precursors in the adult CNS for therapeutic purposes. These topics are also important for at least two other reasons: (1) adult neurogenesis and precursor cell function might play an important role for the function of the healthy and diseased brain and might underlie particular aspects of neuronal plasticity, as defined by the adaptation of circuitry in response to functional demands; (2) precursor cell biology in the adult CNS also appears to recapitulate some of the molecular, cellular, and other requirements for neuronal development in the developing brain.

A substantial body of research regarding constitutively occurring neurogenesis provides insight into the potential for and limitations of neuronal replacement therapies based on manipulation of neural precursors. Recent work has partially elucidated the normal behavior of endogenous adult precursors, including their ability to migrate to select brain regions, differentiate into neurons, integrate into normal neural circuitry and, finally, functionally integrate into the adult brain. Research is also beginning to identify and describe molecular and activity-related controls over constitutively occurring neurogenesis. The location, identity, and differentiation potential of endogenous adult precursors are beginning to be understood. In this review, we will outline the few examples of normally occurring neurogenesis in the adult mammalian CNS; briefly describe adult neural precursors; discuss a few lines of recent research demonstrating that endogenous neural precursors can be induced to differentiate into neurons in regions of the adult brain that do not normally undergo neurogenesis. Throughout this review, as the many challenges facing studies of adult mammalian neurogenesis are presented, we highlight the fact that constitutive neurogenesis occurs in discrete regions of the adult mammalian brain; that limited neurogenesis can be induced under certain conditions in normally “non-neurogenic” regions; but that any claims of constitutive or induced adult neurogenesis must be supported by rigorous, multi-level, and critical analysis.

2. Defining neural stem cells, progenitors, and precursors

Rigorously defined, adult CNS “stem cells” exhibit three cardinal features: (1) they are “self-renewing,” with the theoretically unlimited ability to produce progeny indistinguishable from themselves; (2) they are proliferative, continuing to undergo mitosis (though perhaps with quite long cell cycles); (3) they are multipotent for the different neuroectodermal lineages of the CNS, including the multitude of different neuronal and glial sub-types. Multipotent progenitors of the adult brain are proliferative cells with only limited self-renewal that can differentiate into at least two different cell lineages (multipotency) (Gage et al., 1995b; Weiss et al., 1996b; McKay, 1997). Lineage-specific precursors or progenitors are cells with restriction to one distinct lineage (e.g., neuronal, astroglial, glial, oligoden-

droglial). Together, CNS stem cells and all precursor/progenitor types are, broadly defined, “precursors.” We favor reserving the term “stem cell” as rigorously defined above and using “precursor” or “progenitor” for most forms of multipotent or lineage-restricted mitotic cells. We use “precursor cell” as a generic term encompassing both stem and progenitor cells.

Adult neurogenesis comprises the entire set of events of neuronal development, beginning with the division of a precursor cell and ending with the presence and survival of a mature, integrated, functioning new neuron. Neurogenesis is sometimes incorrectly used only in the sense of “precursor cell proliferation,” but this definition clearly falls short. Precursor cell proliferation is not predictive of net neurogenesis. True neuronal integration depends on many complex variables and progressive events.

Even in the absence of a fully detailed molecular characterization, neural precursor cells of the adult brain clearly possess features that justify a view of these cells as extremely promising for the goals of CNS cellular repair. They are undifferentiated, often highly mobile cells, relatively resistant to hypoxia and other injury, proliferatively active, and able to produce mature neurons and glia. The most critical caveat, however, is that precursor cells in the adult mammalian brain are heterogeneous, and, while all of these criteria might apply in many cases, it is not clear how many of the sometimes site-specific and condition-specific observations in neural precursor biology can be broadly generalized and how many reflect particulars of a certain brain region or a certain condition.

While the identity of “true” CNS stem cells is as yet unknown, limiting some conclusions, the heterogeneity and seeming variety of neural precursor cells is also an important aspect of the promise of such cells. Their variable specialization and differentiation competence provides great promise for application in medicine. The idea that “one cell fits all” might turn out to be as unrealistic as it is unnecessary. Adult neural precursors with partial fate restriction might, in some cases, allow for more efficient production of desired cell types. The brain itself could provide the environmental determinants for the different forms and sequelae of adult neurogenesis and gliogenesis and may powerfully enable and direct specific lineage development.

3. Functional adult neurogenesis occurs in non-mammalian vertebrates

Adult neurogenesis occurs in many non-mammalian vertebrates. The medial cerebral cortex of lizards, which resembles the mammalian hippocampal dentate gyrus, undergoes postnatal neurogenesis and can regenerate in response to injury (Lopez-Garcia et al., 1992). Newts can regenerate their tails, limbs, jaws, and ocular tissues, and the neurons that occupy these regions (Brockes, 1997). Goldfish

undergo retinal neurogenesis throughout life (Johns and Easter, 1977) and, impressively, can regenerate surgically excised portions of their retina in adulthood (Hitchcock et al., 1992). Although some non-mammalian animals can undergo quite dramatic regeneration of neural tissue, it is unclear how relevant this phenomenon is to mammals. Indeed, it has been proposed that selective evolutionary pressures have led mammals to lose such abilities (Gotz et al., 2002; Rakic, 2002).

Birds, whose brains are much closer to mammals in complexity, also undergo postnatal neurogenesis (Nottebohm et al., 1990; Goldman, 1998; Nottebohm, 2002a, 2002b). Lesioned postnatal avian retina undergoes some neurogenesis, with the new neurons most likely arising from Müller glia (Fischer and Reh, 2001). In songbirds, new neurons are constantly added to the high vocal center (Goldman and Nottebohm, 1983; Nottebohm, 1985; Kim et al., 1991; Alvarez-Buylla et al., 1998), a portion of the brain necessary for the production of learned song (Nottebohm and Arnold, 1976; Simpson and Vicario, 1990), as well as to specific regions elsewhere in the brain (but not all neuronal populations). It is possible to manipulate experimentally the extent to which new neurons are produced in at least one songbird, the zebrafinch (Scharff et al., 2000), which does not normally seasonally replace high vocal center (HVC-RA) projection neurons in the song production network, e.g., as canaries do. Inducing cell death of HVC-RA neurons in zebrafinches leads to deterioration in song. Neurogenesis increases following induced cell death, and birds variably recover their ability to produce song coincident with the formation of new projections from area HVC to area RA, suggesting that induced neuronal replacement can restore a learned behavior.

4. The discovery of constitutively occurring adult mammalian neurogenesis

Ramon y Cajal has been widely quoted as writing that “in the adult centers the nerve paths are something fixed, ended and immutable. Everything may die, nothing may be regenerated.” The relative lack of recovery from CNS injury and neurodegenerative disease and the relatively subtle and extremely limited distribution of neurogenesis in the adult mammalian brain resulted in the entire field reaching the conclusion that neurogenesis does not occur in the adult mammalian brain. Joseph Altman was the first to use techniques sensitive enough to detect the ongoing cell division that occurs in the adult brain. Using tritiated thymidine as a mitotic label, he published evidence that neurogenesis constitutively occurs in the hippocampus (Altman and Das, 1965) and olfactory bulb (Altman, 1969) of the adult mammalian brain. These results were later replicated using tritiated thymidine labeling followed by electron microscopy (Kaplan, 1981). However, the absence of neuron-specific immunocytochemical markers at the time

resulted in identification of putatively newborn neurons being made on purely morphological criteria. These limitations led to a widespread lack of acceptance of these results, and made research in the field difficult.

The field of adult neurogenesis was rekindled in 1992, when it was shown that precursor cells isolated from the forebrain can differentiate into neurons in vitro (Reynolds and Weiss, 1992; Richards et al., 1992). A more detailed discussion of these precursors will be presented later in this review. These results, and technical advances such as the development of immunocytochemical reagents that could more easily and accurately identify the phenotype of various neural cells, led to an explosion of research in the field. Normally occurring neurogenesis in the subventricular zone (SVZ)/olfactory bulb and the dentate gyrus sub-region of the hippocampus has now been well characterized in the adult mammalian brain.

5. The location of neural precursor cells and their isolation in vitro

If adult multipotent precursors were limited to the two neurogenic regions of the brain, the SVZ and dentate gyrus, it would severely limit the potential for neuronal replacement therapies based on in situ manipulation of endogenous

precursors (Fig. 1). However, ex vivo studies, in which precursors were isolated from the adult brain, provide evidence for cells with precursor cell properties in the adult brain. Such cells were first found embryonically (Reynolds et al., 1992; Richards et al., 1992), then in the adult hippocampus and the SVZ (in the original publication referred to as striatal) (Reynolds and Weiss, 1992; Palmer et al., 1995). Following these first reports, cells with similar properties have been isolated from many other adult mammalian brain regions as well; they have been cultured in vitro from caudal portions of the SVZ (Palmer et al., 1995), striatum (Palmer et al., 1995), cortex (Palmer et al., 1999), optic nerve, septum, corpus callosum, spinal cord, retina, and hypothalamus (Palmer et al., 1995, 1999; Weiss et al., 1996a; Shihabuddin et al., 1997; Tropepe et al., 2000; Lie et al., 2002). In vitro, it can be shown that these cells exhibit at least limited self-renewal and produce differentiated cells of the three neural lineages: astroglia, oligodendroglia, and neurons.

Neural precursor cells can be propagated in two main forms: in adherent cultures (Richards et al., 1992; Palmer et al., 1995, 1999) and under floating conditions, in which they aggregate to form heterogeneous ball-like structures, termed “neurospheres” (Reynolds and Weiss, 1992). While it has become standard for some groups to use this “neurosphere-forming” assay as a key criterion for “neural

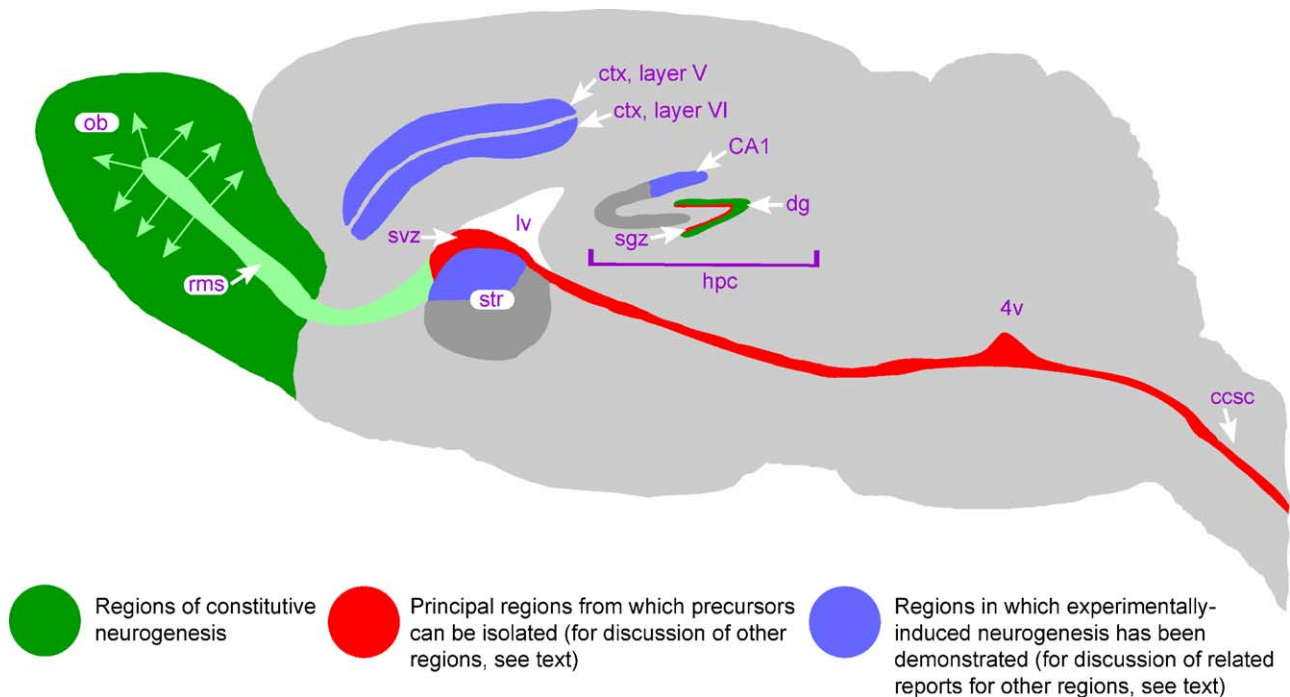


Fig. 1. Schematic diagram showing the two constitutively neurogenic regions of the adult mammalian CNS (green; SVZ/olfactory bulb and hippocampal dentate gyrus), and some of the principal regions where populations of neural precursors have been identified (red; subgranular zone of the dentate gyrus, and rostrally-caudally from the anterior subventricular zone along the neuraxis through the central canal of the spinal cord). Precursors have also been isolated in smaller numbers from several parenchymal regions (see text). The diagram also indicates selected regions in which limited neurogenesis can be induced experimentally (blue; see text). While isolated and sometimes controversial reports of neurogenesis in other regions exist, these are omitted from the figure for simplicity (see text). ccsc: central canal of the spinal cord; ctx: cortex; dg: dentate gyrus of the hippocampus; hpc: hippocampal formation; lv: lateral ventricle; ob: olfactory bulb; rms: rostral migratory stream; sgz: subgranular zone (of the dentate gyrus); str: striatum; svz: subventricular zone; 4v: fourth ventricle.

stem cells,” the ability to form a “neurosphere,” alone, is not a sufficiently reliable hallmark of stem cells and does not fully differentiate between various mitotic and precursor cell populations (Seaberg and van der Kooy, 2003). Clonal analysis provides important additional information. By sub-cloning individual cells, one can test whether an individual cell from these spheres can again give rise to secondary spheres, which upon transfer into differentiation conditions can produce all neural lineages.

Although these studies all contribute useful information, one should not forget that cell culture systems are highly artificial in many respects. *In vivo*, precursor cells are never alone. Their relationship to a neurogenic microenvironment might be inseparable from their inherent properties. Adherent cultures might acknowledge this requirement somewhat better, but the central problem remains. Not surprisingly, the importance of tissue conditions for precursor cell function, for example, factors contributed by astroglia, has become more and more apparent (Song et al., 2002a).

In vitro, many such factors are likely present in the complex media used to culture neural precursors. Epidermal growth factor (EGF) and fibroblast-growth factor 2 (FGF-2) are two key growth factors used in neural precursor cultures to maintain cells in their mitotic and undifferentiated state (Reynolds and Weiss, 1992; Palmer et al., 1995; Craig et al., 1996). The general effectiveness of both factors as mitogens for precursors has also been demonstrated *in vivo* (Craig et al., 1996; Kuhn et al., 1997), and they have been used more recently to enhance induced neurogenesis in the normally non-neurogenic “CA1” region of the hippocampus after focal ischemia (Nakatomi et al., 2002). However, their effects *in vivo* are not identical to those *in vitro* and, consequently, their physiologic relevance for normal neural precursor cell function of the adult brain is not known.

The effectiveness of an individual factor *in vitro* does not prove that it plays a role in neurogenic permissiveness *in vivo*. Conversely, regional differences *in vivo* can be somewhat equalized by treating all precursor cells with the same regimen of growth factors (and other culture conditions) to standardize comparative analyses. Regional differences *in vivo* reinforce the importance of the microenvironment for normal precursor cell function and neurogenesis. Nevertheless, *in vitro* studies are indispensable for many questions in neural precursor biology, including investigation of the transcriptional control of adult neurogenesis and neural differentiation.

In addition to the undifferentiated multipotent precursors that are found in various portions of the brain, there are controversial reports that mature neurons themselves can be induced to divide (Brewer, 1999; Gu et al., 2000). While it seems unlikely that a neuron could maintain the elaborate neurochemical and morphologic differentiation state of a mature neuron while replicating its DNA and remodeling its nucleus and soma, it is still theoretically possible. Although it is generally accepted that other neural cells, such as

astroglia, can divide, most reports suggest that any attempt by differentiated neurons to re-enter the cell cycle results in aborted cycling and, ultimately, death (Yang et al., 2001). Significant evidence would need to be presented to demonstrate convincingly that mature neurons in the adult brain are capable of mitosis.

6. Methodological considerations for studying neural precursor cells *in vivo*

There are many methodological considerations to keep in mind when one is assessing the phenomenon of neurogenesis. Methodological problems, and problems of interpretation, can arise if one is not rigorous about the use of various mitotic labels; the correct application of and assessment of double labeling using immunocytochemical labels; the importance of understanding the *in vivo* microenvironment in which neural precursors reside.

In essentially all of this research, the “stemness” of certain cells *in vivo* has been inferred from (1) the fact that after having been labeled as dividing cells, the cells give rise to neurons and glia; (2) *ex vivo* evidence regarding precursor cells in this same brain region. Thymidine analogs are typically used as proliferation markers, to label dividing cells permanently and to pass labeling on to the progeny. Most early studies used tritiated thymidine, which is incorporated into the DNA of dividing cells during S-phase of mitosis and can be detected autoradiographically (Altman and Das, 1965; Cameron et al., 1993). In more recent studies, tritiated thymidine has usually been replaced by the thymidine analog bromodeoxyuridine (BrdU), because BrdU can be detected immunocytochemically and thus allows for double- and triple-labeling with cell type-specific markers and confocal microscopic analysis (Kuhn et al., 1996). The use of thymidine analogs, and BrdU in particular, has raised some technical issues, however. One question is whether BrdU labeling might identify cells undergoing DNA repair, rather than proliferation; this possibility has recently been ruled out for standard BrdU labeling and has been discussed in detail elsewhere (Cooper-Kuhn and Kuhn, 2002). There is no evidence that adult neurogenesis as detected with standard BrdU assessment would confuse cells with ongoing DNA repair (Palmer et al., 2000). However, another issue is the use of BrdU under pathological conditions. There is some evidence that dying neurons can enter into an abortive cell cycle (Yang et al., 2003; Kuan et al., 2004), which includes an S-phase and thus potentially BrdU-incorporation. However, such terminally sick neurons do not survive for long periods (Kuan et al., 2004), so experiments with long survivals do not risk confusion. An additionally important approach is to study development of labeled cells from at least two different times of investigation. If, for example, early after the pathological event, only immature cells or precursors contain BrdU (identified by a combination of immature morphology, migratory location,

and early stage markers), and only at long survival times are mature neurons labeled, this eliminates confusion by ruling out BrdU incorporation by dying cells. Pre-labeling of pre-existing neurons by a variety of methods also eliminates the risk of misinterpreting older cells as newborn. In combination, these and other rigorous analyses can unequivocally identify newborn neurons.

Retroviral labeling can also identify mitotic cells and allow for more complete cellular morphologic and/or electrophysiologic analysis by expression of green fluorescent protein (GFP) family members or other reporter genes. Retroviruses carrying a reporter gene require complete cell division for integration, rather than only S-phase. Unfortunately, labeling efficiency is considerably lower than with BrdU, making quantitative analyses of rare cell divisions difficult. Neurogenesis has been confirmed and further investigated electrophysiologically in the hippocampus (van Praag et al., 2002) and in the olfactory bulb (Carleton et al., 2003; Magavi et al., 2005) by labeling dividing cells with retroviruses.

A second methodological issue concerns criteria for identification of cellular phenotype. Currently, NeuN has become the standard immunocytochemical marker to identify neurons, and GFAP and S100 β are used as astroglial markers. In mice, nestin (currently the best known precursor cell marker *in vivo*) is notoriously difficult to detect within neurogenic regions, except under pathological conditions, when it appears to be up-regulated. The development of transgenic mice expressing GFP under a neural-specific element of the nestin promoter has been very helpful for identifying precursor cells *in vivo* (Yamaguchi et al., 2000; Filippov et al., 2003), but conclusions can only be drawn regarding those cells that are labeled; no conclusions can be drawn about cells in which the nestin promoter is not active. The same caveat applies to other potential precursor cell markers whose sensitivity and specificity *in vivo* are not known and are only inferred from *ex vivo* experiments.

The most problematic point in this context, however, is the potential pitfall in identifying the phenotype of any newborn cell by a single marker, e.g., identification of a BrdU-labeled “neuron” solely by its expression of NeuN is insufficient (Magavi and Macklis, 2002a, 2002b). For the classical neurogenic regions, neurogenesis has been confirmed by other markers, for example, with Prox-1 and calbindin in the dentate gyrus, and with the neurotransmitter GABA and the dopamine-associated enzyme tyrosine hydroxylase in the olfactory bulb. Cellular morphology provides one of the most reliable markers in the nervous system, and subcellular distribution of antigens can be additionally helpful (Magavi et al., 2000; Chen et al., 2004). Electrophysiological studies (Song et al., 2002b; van Praag et al., 2002) and retrograde labeling of axons (Stanfield and Trice, 1988; Hastings and Gould, 1999; Markakis and Gage, 1999; Magavi et al., 2000; Scharff et al., 2000; Catapano et al., 2002; Chen et al., 2004) can powerfully assess and

further confirm the neuronal identity of newly generated cells. Additional important evidence can be obtained by analysis of expression of different markers of progressive neuronal (or other cell type) differentiation over time after the cell division (Magavi et al., 2000; Arvidsson et al., 2002; Magavi and Macklis, 2002a; Parent et al., 2002b; Kronenberg et al., 2003; Chen et al., 2004). Not all of these options can be applied in all experimental contexts, but the use of single markers in isolation is problematic, especially if the only example is at a very early time after the presumed cell division. It is even more problematic if the claim of “neurogenesis” is solely based on the co-localization of BrdU with an immature neuronal marker such as doublecortin (DCX), PSA-NCAM, or beta-III-tubulin. In all of these cases, the specificity of the markers is not clear, and we know from neuronal development and from studies of induced adult neurogenesis that many cells that express these markers do not survive or mature into functionally integrated neurons (Arvidsson et al., 2002; Parent et al., 2002b; Brown et al., 2003; Kronenberg et al., 2003; Chen et al., 2004).

Other aspects of neural precursor cell biology require a closer look at the putative precursor cells in their natural microenvironments *in vivo*. Unfortunately, no marker proteins are yet known that allow for a clear and unambiguous prospective identification of precursor cells in the adult brain. Although nestin is a very useful marker (Lendahl et al., 1990; Yamaguchi et al., 2000), its sensitivity and specificity are only partial, and the limits of each are not fully known. Other markers are of great interest, for example, SOX-2 (D’Amour and Gage, 2003) and brain lipid binding protein (BLBP) (Rousselot et al., 1997), and GFAP in the SVZ (Doetsch et al., 1999). However, these and other reported markers appear to be only partially specific, or selective for as yet otherwise unidentified subpopulations of precursor cells.

7. Neurogenic versus non-neurogenic regions in the adult brain

In the adult mammalian brain, neurogenesis constitutively occurs only in the subventricular zone/olfactory bulb and the dentate gyrus of the hippocampus. In the olfactory system, precursor cells reside in the anterior aspect of the subventricular zone (SVZ; sometimes also called the subependymal zone) in the walls of the lateral ventricles, migrate via “chain migration” through a structure surrounding the remnants of the olfactory ventricle (the rostral migratory stream, RMS) into the olfactory bulb, and differentiate into granule or periglomerular interneurons (Lois and Alvarez-Buylla, 1993; Luskin, 1993; Goldman, 1995; Doetsch et al., 1999). In the hippocampus, the precursor cell population is found in the subgranular zone (SGZ) of the dentate gyrus. Here, new hippocampal granule cell neurons are produced. These two regions are thus

referred to as “neurogenic regions” of the brain (Fig. 1). As will be discussed below in more detail, a report of extremely low-level constitutive neurogenesis in the normal neocortex (Gould et al., 1999c, 2001) has been disputed (Kornack and Rakic, 2001; Koketsu et al., 2003), primarily for methodological reasons. In addition, this same group later modified their interpretation by stating that the existence of these neurons was only transient (Gould et al., 2001). Additionally, there have also been single reports of constitutive adult neurogenesis in the amygdala (Bernier et al., 2002), area CA1 of the hippocampus (Rietze et al., 2000), the dorsal vagal complex of the adult brainstem (Bauer et al., 2005), the spinal cord (Yamamoto et al., 2001), and the substantia nigra (Zhao et al., 2003a), although some of these results have been disputed (Horner et al., 2000; Lie et al., 2002; Frielingsdorf et al., 2004). Overall, these isolated data remain controversial, but not because the possibility of neurogenesis occurring in non-neurogenic regions is remote. Instead, recent isolated reports of such a phenomenon have not sufficiently overcome many of the methodological issues described above.

Transplantation studies support the principle of defining neurogenic and non-neurogenic regions and provide evidence for the role of the microenvironment in influencing the potential of neural precursors. If precursor cells are transplanted into neurogenic regions, they can differentiate into neurons in a region-specific manner (Gage et al., 1995a; Suhonen et al., 1996; Takahashi et al., 1998; Shihabuddin et al., 2000). SVZ precursor cells generate hippocampal neurons when transplanted into the hippocampus, and SGZ precursor cells generate olfactory interneurons after transplantation into the RMS (Suhonen et al., 1996). When implanted outside the neurogenic regions, both types of precursor cells generate only glia. These data further support the interpretation that neurogenesis is dependent upon a permissive microenvironment, rather than on the regionally different properties of precursor cells. Local microenvironmental influences on the behavior of precursors and their ability to differentiate into neurons support the argument that it will be critical for the goal of neuronal repopulation to understand the cellular and molecular controls over cell type-specific precursor cell differentiation in the adult CNS.

It came as a surprise to many in the field that neurogenic regions are defined by the neurogenic permissiveness of the local microenvironment, rather than by the presence of neural precursor cells. Neural precursor cells have been found in a large number of brain regions, including white matter tracts (Palmer et al., 1999; Kondo and Raff, 2000). It is possible that the entire brain contains neural precursor cells (albeit at very low density), just as it contains partially restricted glial precursors that act as progenitor cells (Reynolds and Hardy, 1997; Belachew et al., 2003; Dawson et al., 2003). These widely distributed neural precursor cells do not seem to differ fundamentally from one another, although they are far from homogeneous, with notably different growth kinetics and differentiation potential. Their

function outside the classical neurogenic regions is not known, nor is their relation to the precursor cells of the neurogenic regions. For example, neural precursor cells from the spinal cord behave *in vitro* much like their counterparts from the SGZ and SVZ (Shihabuddin et al., 1997) and are multipotent after implantation into the hippocampus, producing granule cell neurons, but generate only glial cells and no neurons *in situ* or when transplanted back to their original site in the spinal cord (Shihabuddin et al., 2000). *In vivo*, no adult neurogenesis can be found in the rodent spinal cord (Horner et al., 2000). In sum, the extent of neurogenesis in a region is a function of the local microenvironment on precursor cells with (varying) neurogenic potential.

There is, therefore, an important conceptual distinction between neurogenic and non-neurogenic regions, but it might turn out that this distinction reflects complex molecular and functional states rather than a fixed cellular environment. Can non-neurogenic regions be molecularly modified to promote neurogenesis? Could this change in neurogenic permissiveness be induced under certain pathological conditions? There are strong natural precedents in other vertebrate systems, such as the avian telencephalon and the olfactory epithelium, for the concept that selective neuronal death can alter the differentiation of precursors, thereby inducing or increasing neurogenesis (Herzog and Otto, 1999; Scharff et al., 2000). While not yet proven, it is hypothesized that this change in neurogenesis occurs via alterations in the local molecular microenvironment that resemble conditions seen during nervous system development and in adult neurogenic regions.

Several groups have recently demonstrated the induction of modest levels of neurogenesis in normally non-neurogenic regions in response to selective neuronal death or degeneration (Fig. 1). As will be described later in this review, we found that endogenous multipotent precursors normally located in the adult brain can be induced to differentiate into neurons in the normally non-neurogenic adult mammalian neocortex, a result recently extended to cortico-spinal motor neurons (Magavi et al., 2000; Chen et al., 2004). More recently, other groups have reported similar and complementary results in a normally non-neurogenic region of the hippocampus (Nakatomi et al., 2002) and in the striatum (Arvidsson et al., 2002; Parent et al., 2002b), confirming and further supporting this direction of research. These reports of seemingly regenerative neurogenesis in the vicinity of targeted cortical neuron degeneration and cerebral ischemia suggest that the lack of normal neurogenic permissiveness in these regions is not unalterable (Magavi et al., 2000; Arvidsson et al., 2002; Nakatomi et al., 2002; Parent et al., 2002b; Chen et al., 2004). The as-yet controversial examples of reportedly extremely low-level constitutive neurogenesis outside the classical neurogenic regions might also support this possibility, potentially as “leakage” from normally tightly inhibited systems. Are precursor cells resident in non-

neurogenic regions “waiting” for either instructive neurogenic signals or release from inhibition to “awaken” them to develop into new, function-restoring neurons?

The vast majority of studies investigating potential neurogenesis in the neocortex of the well-studied rodent brain, however, do not report normally occurring adult cortical neurogenesis. Rigorous studies employing serial-section analysis and three-dimensional confocal reconstruction demonstrate a complete absence of constitutively occurring neurogenesis in the murine neocortex (Magavi et al., 2000; Ehninger and Kempermann, 2003; Chen et al., 2004). These experiments provide evidence that satellite glial cells, closely apposed to neurons in normal neocortex, could be mistakenly interpreted as adult-born neurons when, in fact, a newborn glial cell overlies a pre-existing neuronal nucleus. However, a few studies report low level, constitutively occurring neurogenesis in specific regions of the neocortex of adult primates (Gould et al., 1999c, 2001) and in the visual cortex of adult rat (Kaplan, 1981). In Gould et al. (1999), neurogenesis of 2–3 new neurons/mm³ was reported in prefrontal, inferior temporal, and posterior parietal cortex of the adult macaque, but not in striate cortex, interpreted by the authors as being because this is a simpler primary sensory area. These authors later reported that these cells are transient and do not survive (Gould et al., 2001). In contrast, other more recent reports analyzed with more rigorous methods as noted above have been unable to reproduce these findings and report a complete absence of constitutive cortical neurogenesis in both rodents and primates (Magavi et al., 2000; Kornack and Rakic, 2001; Ehninger and Kempermann, 2003; Koketsu et al., 2003; Chen et al., 2004). There exists a single report of neurogenesis in the visual cortex of the adult rat (Kaplan, 1981), but this study used tritiated thymidine and purely morphological cell type identification and has not been confirmed by any other group. It is unclear whether even very low level neurogenesis occurs normally in the neocortex of any mammals (though most data indicate none in rodents or primates), but further examination of the potential for constitutive neurogenesis in what are considered normally non-neurogenic regions will be required to assess definitively the potential existence of perhaps extremely low-level neurogenesis.

8. Neurogenesis in the adult olfactory bulb

Of the two neurogenic regions in the adult mammalian CNS, the SVZ generates by far the larger number of new neurons, and these are destined for the olfactory bulb (reviewed in Garcia-Verdugo et al., 1998; Luskin, 1998). The cells contributing to olfactory bulb neurogenesis originate in the anterior SVZ and migrate to their final position in the olfactory bulb. Adult olfactory bulb neurogenesis has been most extensively studied in the rodent, with some studies conducted in non-human primates

(Lois and Alvarez-Buylla, 1993; Kornack and Rakic, 1999; Pencea et al., 2001a). In vitro (Kirschenbaum et al., 1994; Pincus et al., 1998) and in vivo (Sanai et al., 2004) evidence suggests that such neuronal precursors also exist in humans. When retroviruses (Lois and Alvarez-Buylla, 1994), tritiated thymidine (Lois and Alvarez-Buylla, 1994), vital dyes (Lois and Alvarez-Buylla, 1994; Doetsch and Alvarez-Buylla, 1996), or virally labeled SVZ cells (Luskin and Boone, 1994; Doetsch and Alvarez-Buylla, 1996) are microinjected into the anterior SVZ of postnatal animals, labeled cells are eventually found in the olfactory bulb. Upon reaching the olfactory bulb, these labeled neurons differentiate into interneurons specific to the olfactory bulb (olfactory granule neurons) and, to a much lesser extent, peri-glomerular interneurons (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Betarbet et al., 1996; Winner et al., 2002). To reach the olfactory bulb, the immature neurons undergo tangential chain migration through the RMS into the olfactory bulb (Lois and Alvarez-Buylla, 1994; Rousselot et al., 1995), although this may occur differently in humans (Sanai et al., 2004). Once in the olfactory bulb, the neurons migrate radially away from the RMS before differentiating into interneurons.

Most cells born in the SVZ die before they mature fully into neurons (Winner et al., 2002). Thus, a principle of embryonic development is repeated here: new neurons (or cells with neuronal potential) are generated in surplus, and only a subset of them survives to maturation and functional integration (Oppenheim, 1991; Biebl et al., 2000). Adult neurogenesis in the olfactory bulb appears to involve both the replacement and addition of new neurons. In contrast to the hippocampus (to be discussed below), there is evidence of both neuronal turnover and a net increase in neurons in the olfactory bulb (Winner et al., 2002).

Although neural precursors residing in the SVZ in rodents and in non-human primates have been found to undergo tangential chain migration through the RMS and into the olfactory bulb, recent evidence in humans has raised the possibility that, despite the presence of progenitors in the SVZ, these cells may not undergo chain migration through the RMS in humans (Sanai et al., 2004). While this evidence in humans is compelling, it does not yet suggest an alternative mechanism, and it underlines the fact that direct comparisons cannot always be made between mice and humans or even between non-human primates and humans. The complexity of the human brain is considerably greater than that of rodents, and the introduction of new neurons into such a complex pre-existing system may be a far more challenging feat. Understanding the factors that contribute to normal SVZ precursor migration will be critical for developing approaches to induce such precursors to migrate to injured or degenerating regions of the brain.

Efforts to identify and characterize the neural precursors that contribute to olfactory bulb neurogenesis have generated a great deal of controversy. The predominant precursor cell population of the adult SVZ appears to be

astrocyte-like cells, termed “B-cells,” which reside in the subependymal layer and send out one ciliated process touching the ventricular surface (Doetsch et al., 1999). These multipotent neural precursors have astrocytic morphology and express glial fibrillary acidic protein (GFAP) (Doetsch et al., 1999; Alvarez-Buylla et al., 2001; Seri et al., 2001). Additional independent reports provide support for the concept that multipotent neural precursors with similarities to astrocytes contribute to adult neurogenesis (Laywell et al., 2000). Other investigators suggest that a subset of ependymal cells themselves act as the precursors in this region (Johansson et al., 1999), although this interpretation has been challenged (Chiasson et al., 1999). Because it is potentially difficult to distinguish a true ependymal cell from a subependymal cell with a process in the ependymal layer, both sets of observations might actually correspond to the identical cell type. B-cells give rise to immature neurons, still able to divide, that migrate in the ventricular wall and later in the RMS.

While the majority of currently available evidence suggests that GFAP-expressing cells in the SVZ are a proximal source of olfactory bulb neurogenesis, it is important to distinguish between true astroglia and a distinct class of precursor cells that may also express GFAP. GFAP, while generally a reliable marker for activated astrocytes, has been used along with electron microscopic characteristics as a sole antigenic marker in reports suggesting that astrocytes are multipotent neural precursors, or “stem cells.” It is quite possible that at least some multipotent neural precursors may also express GFAP, while remaining distinct from astroglia. However, these findings are additionally compelling when combined with recent data from embryogenesis that a subset of glia-like cells serves as neural precursors. Radial glia not only guide migrating neurons to their proper position in the cortex, but a subset possesses multipotent neural precursor properties and generate neurons during neocortical development (Mala-testa et al., 2000; Heins et al., 2002; Noctor et al., 2002).

Although the identity of the adult multipotent neural precursors in the SVZ is still controversial, many experiments have been performed to manipulate their fate and examine their potential, both *in vitro* and *in vivo*. These results will guide attempts to manipulate endogenous precursors for brain repair. *In vitro*, SVZ precursors have been exposed to a number of factors to determine their responses. Generally, precursor cells have been removed from the brain, dissociated, and cultured in epidermal growth factor (EGF) or basic fibroblast growth factor (FGF-2). The growth factor is then removed, and the cells are exposed to differentiation conditions. The details of this process, including the particular regions from which the cells are derived, the media in which they are grown, and the substrates on which they are plated, can have significant effects on their fate. EGF and FGF-2 (Gritti et al., 1995, 1996, 1999) both induce the proliferation of SVZ precursors and can influence their differentiation. EGF tends to direct

cells to a glial fate, and FGF-2 more toward a neuronal fate (Whittemore et al., 1999). Bone morphogenetic proteins (BMPs) promote differentiation of SVZ precursors into astroglial fates (Gross et al., 1996), while platelet-derived growth factor (PDGF) (Williams et al., 1997; Whittemore et al., 1999) and insulin-like growth factor-I (IGF-I) (Arsenijevic and Weiss, 1998) promote SVZ precursors to differentiate into neurons. There are ambiguous results regarding whether brain-derived neurotrophic factor (BDNF) promotes the survival (Kirschenbaum and Goldman, 1995) or differentiation of SVZ precursors *in vitro* (Ahmed et al., 1995). Remaining questions notwithstanding, *in vitro* results show that it might be possible to influence the proliferation and differentiation of adult SVZ precursors.

The effects of several growth factors have also been tested *in vivo* to investigate their effects under physiological conditions. Intracerebroventricularly (ICV) infused EGF or transforming growth factor- α (TGF- α) induces a dramatic increase in SVZ precursor proliferation, and FGF-2 induces a smaller increase in proliferation (Craig et al., 1996; Kuhn et al., 1997). Even subcutaneously delivered FGF-2 can induce the proliferation of SVZ precursors (Tao et al., 1996). But despite the fact that mitogen-induced newborn cells disperse into regions of the brain surrounding the ventricles, these newborn cells generally do not differentiate into neurons (Kuhn et al., 1997).

Other factors have also emerged as potentially important regulators of neurogenesis, and these factors include, most notably, Noggin (Lim et al., 2000), vascular endothelial growth factor (VEGF) (Jin et al., 2002) and BDNF (Zigova et al., 1998; Pencea et al., 2001b). Intraventricularly infused BDNF increased the number of newly born neurons found in the olfactory bulbs of adult animals (Zigova et al., 1998). Further studies have shown that intraventricularly administered BDNF is not only capable of increasing the proliferation of SVZ precursors but also is additionally able to promote neuronal migration into ectopic areas such as the neostriatum, septum, thalamus, and hypothalamus (Benraiss et al., 2001; Pencea et al., 2001b). These findings indicate that it may be possible to use growth factors to manipulate endogenous precursors in the adult *in vivo* to replace neurons lost to diseases or degeneration in brain areas that do not normally undergo neuronal replacement. Some skepticism regarding the feasibility of such approaches is also justified, however; for example, intracerebroventricular EGF infusions have also been reported to cause massive hyperplasia of the ventricular walls (Kuhn et al., 1997).

In addition to growth and neurotrophic factors, a number of other molecular and extracellular control pathways have been identified that can influence neural precursor behavior in the SVZ (others will be discussed below in relation to precursor behavior and constitutive neurogenesis in the dentate gyrus). For example, transcription factors such as E2F1 (Cooper-Kuhn et al., 2002), and the homeobox gene *Vax1* (Soria et al., 2004) have both been implicated in

regulating neurogenesis in the adult SVZ. In addition, the mRNA binding protein, Musashi1, has been implicated in regulating adult SVZ proliferation and differentiation (Sakakibara et al., 2001), as have the glycosylated form of cystatin C (CCg) (Taupin et al., 2000) and the orphan nuclear receptor TLX (Shi et al., 2004).

Several studies have attempted to establish the differentiation potential of SVZ multipotent precursors, but these studies have yielded conflicting results. Postnatal mouse SVZ precursors can differentiate into neurons in a number of regions in the developing neuraxis (Lim et al., 1997), yet their fate is more limited to astroglia when they are transplanted into adult brain (Herrera et al., 1999). Adult mouse SVZ precursors injected intravenously into sublethally irradiated mice have been reported to differentiate into hematopoietic cells, interpreted as demonstrating the broad potential of neural precursors for differentiation and interlineage “trans-differentiation.” However, it is possible that either cell fusion or a chance transformation of cultured SVZ cells led to a single transformant precursor accounting for this finding (Alvarez-Dolado et al., 2003). Labeled multipotent neural precursors, derived from adult mouse and transplanted into stage 4 chick embryos or developing mouse morulae or blastocysts, have been reported to integrate into the heart, liver, and intestine, and express proteins specific for each of these sites (Clarke et al., 2000), although the same alternate interpretations of fusion and transformation might apply here. Adult multipotent neural precursors may not be pluripotent, but they appear to be capable of differentiating into a variety of cell types under appropriate conditions. These results indicate that the local cellular and molecular environment in which SVZ neural precursors are located can play a significant role in their differentiation. Providing the cellular and molecular signals for appropriate differentiation and integration of new neurons will be critical for neuronal replacement therapies in which endogenous neural precursors are either transplanted or manipulated in situ.

9. Neurogenesis in the adult hippocampus

Neurogenesis in the dentate gyrus of the adult hippocampus has been extensively studied, due at least partially to the tantalizing connection between the hippocampus and the formation of memory. Does hippocampal neurogenesis play a part in memory formation? This question has only begun to be answered, but our understanding of hippocampal neurogenesis is already quite significant (Kempermann et al., 2004). Of particular interest is the fact that hippocampal neurogenesis can be modulated by physiological and behavioral events such as aging, stress, seizures, learning, and exercise. These properties of hippocampal neurogenesis may provide novel methods for studying neurogenesis and may help to elucidate broader influences that may be relevant to neuronal replacement therapies.

Adult hippocampal neurogenesis has been described in vivo in rodents (Altman and Das, 1965), monkeys (Gould et al., 1998, 1999b; Kornack and Rakic, 1999), and humans (Eriksson et al., 1998). Newborn cells destined to become neurons are generated along the innermost aspect of the granule cell layer, the subgranular zone (SGZ) of the dentate gyrus. The cells migrate a short distance into the granule cell layer, send dendrites into the molecular layer of the hippocampus, and send their axons into the CA3 region of the hippocampus (Stanfield and Trice, 1988; Hastings and Gould, 1999; Markakis and Gage, 1999). Adult-born hippocampal granule neurons are morphologically indistinguishable from surrounding granule neurons (Kempermann et al., 2003), and they develop mature electrophysiological properties (van Praag et al., 2002). The precursor cells appear to mature rapidly, and they extend their processes into the CA3 region as early as 4 days after division (Hastings and Gould, 1999). The properties of both the precursor cells and the hippocampal environment likely contribute to the rapid maturation observed.

In the adult hippocampus, precursor cells reside in the SGZ, the border between the granule cell layer and the hilus (or CA4). This region is highly vascularized, and a proximal spatial relationship between blood vessels and the dividing precursor cells has been noted (Palmer et al., 2000). Based on morphological criteria and antigenic properties, three different types of proliferative cells have been distinguished by multiple groups (Kempermann et al., 2004): (1) radial glia-like precursors termed “B cells” or “type-1 cells;” (2) nestin-expressing “type-2 cells,” which lack glial features; (3) doublecortin-positive, nestin-negative “type-3 cells.” The rarely dividing cells with radial glia-like appearance have been identified as the predominant precursors of this region (Seri et al., 2001), although the degree of “stemness” of these hippocampal cells is still controversial. Specifically, it has been suggested that neuron-specific progenitors, with a restricted capacity for self-renewal, generate new neurons in the adult dentate gyrus (Seaberg and van der Kooy, 2002). These cells, termed B-cells or “type-1 cells” by different groups, have astrocytic properties, among them characteristic electrophysiological properties and vascular end-feet, but do not express S100 β , a marker of mature astrocytes (Seri et al., 2001; Filippov et al., 2003; Fukuda et al., 2003). These cells are thought to give rise to progenitor cells via asymmetric divisions. Type-2 cells can be subdivided into doublecortin-positive “type-2a” and -negative “type-2b” cells (Kronenberg et al., 2003).

Proliferation of precursor cells is an important part of neurogenic regulation. Although there is evidence of progression through the four types, or stages, of dividing cells, from type-1 to type-2a and b to type-3, these cell classes do not contribute evenly to the overall proliferative activity (Kronenberg et al., 2003). Under resting conditions, type-2a cells are most actively dividing, and physiologic mitogenic stimuli (such as physical activity) seem to affect

primarily these early types of precursors. Type-1 cells rarely divide, and their proliferation is not induced by the experimental manipulations studied so far. Behavioral stimuli such as in environmental enrichment (discussed below), which are thought to be more specific for the dentate gyrus in the sense that they are closer to presumed hippocampal functions, have a limited effect on dividing progenitors, and affect later, nestin-negative stages. A subset of type-3 cells becomes post-mitotic and begins to express neuronal markers such as neuron-specific nuclear protein (NeuN). The maturing granule cells also transiently express calretinin, and it is believed that this phase is the one during which the new neurons extend their axons along the mossy fiber tract to CA3, as do all other older granule cells (Brandt et al., 2003). The cells also send dendrites into the molecular layer of the dentate gyrus. In mice, adult-born granule neurons mature functionally and become electrophysiologically similar or identical to older granule neurons over 1 or 2 months (van Praag et al., 2002). The newly generated granule neurons later exchange their calcium-binding protein calretinin for calbindin (Brandt et al., 2003). During this early postmitotic stage, the new neurons apparently are recruited into a functional role, or otherwise die (Kempermann et al., 2004).

Much research on hippocampal precursors has been performed *in vivo*, but many *in vitro* results are also useful for understanding the effects of growth factors on the differentiation of hippocampal precursors. Hippocampal precursors are studied *in vitro* much like SVZ precursors: they are removed from the brain, dissociated, and typically cultured in EGF or FGF-2; the mitogen is then removed, and the cells are exposed to differentiation-inducing agents such as retinoic acid. Hippocampal precursors proliferate in response to FGF-2 and can differentiate into astroglia, oligodendroglia, and neurons *in vitro* (Gage, 1998). Further demonstrating the existence of precursors in the adult human, multipotent precursors derived from the adult human hippocampus can be cultured *in vitro* (Kukekov et al., 1999; Roy et al., 2000).

Hippocampal neurogenesis occurs throughout adulthood but declines with age (Kuhn et al., 1996). This age-related decline could result from depletion of multipotent precursors, a change in precursor cell properties, or a change in the levels of molecular factors that influence neurogenesis. Understanding what causes this age-related decrease in neurogenesis might be important in assessing the possible utility of potential future neuronal replacement therapies based on manipulation of endogenous precursors. Although aged rats have dramatically lower levels of neurogenesis than young rats, adrenalectomized aged rats have levels of neurogenesis comparable to those of young adrenalectomized rats (Cameron and McKay, 1999; Montaron et al., 1999). These results suggest that it is at least partially increased corticosteroids, produced by the adrenal glands, and not a decrease in the number of multipotent precursors, that leads to an age-related decrease in neurogenesis.

Seizure activity can increase hippocampal neurogenesis. However, it appears that seizure-induced neurogenesis may contribute to inappropriate plasticity, highlighting the fact that newly introduced neurons need to be appropriately integrated into the brain in order to have beneficial effects. Chemically or electrically induced seizures induce the proliferation of SGZ precursors, the majority of which differentiate into neurons in the granule cell layer (Bengzon et al., 1997; Parent et al., 1997). However, some newborn cells differentiate into granule cell neurons in ectopic locations in the hilus or molecular layers of the hippocampus and form aberrant connections to the inner molecular layer of the dentate gyrus, in addition to the CA3 pyramidal cell region (Parent et al., 1997; Huang et al., 1999). It is hypothesized that these ectopic cells, with their aberrant connections, might contribute to the phenomenon of hippocampal kindling (Parent et al., 1999, 2002a, 1997; Represa et al., 1995).

Hippocampal cell death or activity-related signals resulting from seizures may modify signals that lead to increased neurogenesis. Induced seizures lead to both the degeneration of hippocampal neurons (Magloczky and Freund, 1993; Pollard et al., 1994) and hippocampal neurogenesis (Bengzon et al., 1997; Parent et al., 1997). Excitotoxic or physical lesions of the hippocampal granule cell layer induce precursor cell proliferation within the dentate gyrus and the formation of neurons that have the morphological and immunocytochemical properties of granule cell neurons. These results suggest that hippocampal granule cells either suppress neurogenesis or that they or surrounding cells produce signals that induce neurogenesis as they die. However, since neurogenesis is also increased by less pathological levels of electrophysiological activity (Derrick et al., 2000; Deisseroth et al., 2004), it is also possible that signals induced by electrophysiological activity play a role in seizure-induced hippocampal neurogenesis.

Events occurring in the hippocampus dramatically demonstrate that behavior and environment can have a direct influence on the brain's microcircuitry. Animals living in an enriched environment containing toys and social stimulation have more newborn cells in their hippocampus than control mice living in standard cages (Kempermann et al., 1997). Experiments to assess further which aspects of the enriched environment contribute to increased neurogenesis revealed that part of the increase can be attributed to simply exercise via running (van Praag et al., 1999). Associative learning tasks that involve the hippocampus also appear to increase neurogenesis (Gould et al., 1999a). Stress, on the other hand, can reduce neurogenesis in both rodents (Tanapat et al., 1998) and primates (Gould et al., 1998), as can inflammation due to *x*-irradiation (Monje et al., 2003). While still quite speculative, it is possible that the processes mediating these effects on neurogenesis may underlie some of the benefits that physical and social therapies provide for patients with stroke and brain injury.

In adult dentate gyrus neurogenesis, there is some evidence that essentially all neurons that have survived the

initial 2 weeks will mature and persist (Kempermann et al., 2003). In other words, regulation of adult hippocampal neurogenesis occurs at the level of differentiation and survival of newly generated cells. Therefore, overall proliferative activity is a poor predictor of net neurogenesis. Most of the newly generated cells in the SGZ die, presumably by apoptosis (Kempermann et al., 2003). This cell death is not unlike the process found during development, where a surplus of neurons is generated and only a proportion of these are recruited into function. Clearly, genetic factors and external influences together determine the kinetics in this system (Kempermann and Gage, 2002).

Some of the molecular mechanisms that mediate behavioral influences on hippocampal neurogenesis have begun to be elucidated. For instance, IGF-I, which increases adult hippocampal neurogenesis (Aberg et al., 2000), is preferentially transported into the brain in animals that are allowed to exercise. Blocking IGF-I activity in exercising animals reduces hippocampal neurogenesis (Carro et al., 2000), suggesting that IGF-I at least partially mediates the effects of exercise on neurogenesis (Trejo et al., 2001). Stress increases systemic adrenal steroid levels and reduces hippocampal neurogenesis (Tanapat et al., 1998). Adrenalectomy, which reduces adrenal steroids, including corticosteroids, increases hippocampal neurogenesis (Gould et al., 1992; Cameron and Gould, 1994), suggesting that adrenal hormones at least partially mediate the effects of stress on hippocampal neurogenesis. Serotonin is a strong positive regulator of adult hippocampal neurogenesis (Brezun and Daszuta, 1999, 2000). Drugs that increase serotonin action, such as antidepressants, increase neurogenesis (Malberg et al., 2000). Together, these results demonstrate that adult neurogenesis can be modified by systemic signals, suggesting that modifying such systemic signals, and not only local ones, may be useful in developing potential future neuronal replacement therapies involving manipulation of endogenous precursors. In addition to those discussed earlier with regard to precursor behavior and constitutive neurogenesis in the SVZ, a number of molecular control pathways are now being elucidated for the SGV and dentate gyrus. Transcription factors and chromatin proteins, such as the methyl-CpG binding protein (Zhao et al., 2003b) and HMGB1 chromatin protein (Guazzi et al., 2003), respectively, may regulate adult hippocampal neurogenesis. Wnt signaling and other astroglial factors might also play critical roles in controlling the fate of hippocampal neural precursors (Lie et al., 2004). In addition, the developmentally critical basic helix-loop-helix (bHLH) transcription factor *NeuroD* has been shown to be involved in adult hippocampal neurogenesis (Hsieh et al., 2004). Considerable work is ongoing in this direction.

After transplantation, adult hippocampal multipotent precursors can adopt a variety of fates, suggesting that they may be able to integrate appropriately into neuronal microcircuitry outside of the dentate gyrus. Hippocampal

precursors transplanted into neurogenic regions of the brain can differentiate into neurons, whereas precursors transplanted into non-neurogenic regions do not. Adult rat hippocampal precursors transplanted into the RMS migrate to the olfactory bulb and differentiate into a neuronal subtype not found in the hippocampus, tyrosine-hydroxylase-positive neurons (Suhonen et al., 1996). However, although adult hippocampal precursors transplanted into the retina can adopt neuronal fates and extend neurites, they do not differentiate into photoreceptors, demonstrating at least conditional limitations of their differentiation fate potential (Takahashi et al., 1998; Young et al., 2000). These findings further support the importance of the local cellular and molecular microenvironment in determining the fate of multipotent precursors. These results also emphasize that, although adult hippocampal precursors can adopt a variety of neuronal fates, the capacity of adult neural stem cells to form the majority of neuronal phenotypes remains to be systematically tested. Recent correlative evidence suggests that newly generated neurons in the adult hippocampus may participate in some way in hippocampal-dependent memory. Non-specifically inhibiting hippocampal neurogenesis using a systemic mitotic toxin has been suggested to impair trace conditioning in a manner not seen in relevant controls, implying a role for newly born neurons in the formation of memories (Shors et al., 2001). These correlative results, along with direct analysis of electrophysiologic integration by newborn granule neurons (van Praag et al., 2002) and an increasing responsiveness of new neurons to external stimuli (Jessberger and Kempermann, 2003), suggest that adult-born hippocampal neurons integrate functionally into the adult mammalian brain. Ongoing research in multiple laboratories is exploring the precise role they play in adult hippocampal circuitry.

An interesting, but as yet unproven, hypothesis concerning the role of hippocampal neurogenesis in human depression has been proposed. Jacobs et al. and later others suggested that insufficient hippocampal neurogenesis causally underlies depression (Jacobs et al., 2000; D'Sa and Duman, 2002). Consistent with this hypothesis, stress-related glucocorticoids are associated with a decrease in neurogenesis, and increased serotonin levels are associated with an increase in neurogenesis (Brezun and Daszuta, 1999; Herrlinger et al., 2000). If adult neurogenesis is blocked by irradiation, the behavioral effects of fluoxetine treatment (a serotonin reuptake inhibitor) have been found to be abolished (Santarelli et al., 2003). However, the hippocampus is generally thought to be involved in memory consolidation, and less involved in the generation of mood, suggesting that altered hippocampal neurogenesis may be secondary to, rather than causative of, depression. For example, disturbed adult neurogenesis in depression might be only one aspect of a more general failure of neural plasticity (Kempermann and Kronenberg, 2003). Additionally and importantly, adult hippocampal neurogenesis is limited to the dentate gyrus sub-region and does not occur

throughout the hippocampus, so interpretations drawn from more global hippocampal changes in disease states should be considered with caution.

10. Induction of neurogenesis in non-neurogenic regions of the adult brain

Induced neurogenesis in non-neurogenic regions can be envisioned in two basic forms: (1) local parenchymal precursor cells might be activated to generate new neurons upon a stimulus associated either with a pathological event or with a set of directed molecular and/or genetic interventions or (2) precursor cells from normally neurogenic regions, e.g., the SVZ, might respond to either type of stimulus and migrate into the perenchyma. There are already examples for both possibilities.

The first evidence that regenerative neurogenesis is generally possible was reported by our lab (Magavi et al., 2000) in the neocortex; this work served as a “proof of concept” for the induction of adult mammalian neurogenesis. Work by this and other groups has extended this general concept importantly in new directions, in the striatum, hippocampus, and corticospinal system (Arvidsson et al., 2002; Nakatomi et al., 2002; Parent et al., 2002b; Chen et al., 2004). Whereas multipotent precursors are concentrated in the anterior SVZ and the dentate gyrus, they can be found in lower densities in several other regions of the adult brain. As discussed above, these precursors have broad potential; they can differentiate into astroglia, oligodendroglia, and neurons; receive afferents; extend axons to their targets, given an appropriate *in vitro* or *in vivo* environment. Prior results demonstrated that cortex undergoing targeted apoptotic degeneration could direct multipotent precursors to integrate into adult cortical microcircuitry (Snyder et al., 1997; Sheen et al., 1999). The population-specificity of neuronal death was found to be essential, allowing for maintenance of local circuitry and survival of surrounding cells that alter gene expression to direct precursors (Wang et al., 1998; Shin et al., 2000; Fricker-Gates et al., 2002). These properties of endogenous multipotent precursors led us to investigate the fate of these precursors in an adult cortical environment manipulated in a manner previously demonstrated to support neurogenesis (Wang et al., 1998; Scharff et al., 2000).

In agreement with prior data, it was found that endogenous multipotent precursors could be induced to differentiate into neurons in the adult mouse neocortex, without transplantation (Magavi et al., 2000). These experiments induced synchronous apoptotic degeneration of corticothalamic projection neurons and examined the fates of newborn cells within cortex, using markers of progressive neuronal differentiation. The results demonstrated that endogenous neural precursors (mostly from the SVZ, and possibly some from the parenchyma of the cortex itself) could be induced *in situ* to differentiate into cortical

neurons, survive for many months, and form appropriate long-distance connections in the adult mammalian brain. These results indicated that the normal absence of constitutive cortical neurogenesis does not reflect an intrinsic limitation of the endogenous neural precursors’ potential, but more likely results from a lack of appropriate microenvironmental signals necessary for neuronal differentiation or survival. Elucidation of these signals could enable CNS repair.

Other groups have reported similar and complementary results in hippocampus (Nakatomi et al., 2002) and striatum (Arvidsson et al., 2002; Parent et al., 2002b), confirming and further supporting this direction of research. Nakatomi and colleagues used an *in vivo* ischemia model by which it was found that repopulation of neurons in the CA1 region of the hippocampus is possible following the elimination of CA1 neurons (Nakatomi et al., 2002). It was found that the overwhelming majority of adult-born neurons repopulating the damaged CA1 region originated from a proliferative response in the posterior periventricle, and that this proliferative response could be augmented by infusion of high levels of EGF and FGF-2, dramatically increasing the number of neurons able to migrate into and repopulate the damaged CA1 region. While the levels of EGF and FGF-2 substantially exceeded those reasonable for human application, these experiments serve as a proof-of-principle for enhancement of an endogenous neurogenic response. Recently, we demonstrated induction of low-level neurogenesis of corticospinal motor neurons, with progressive extension of axons into the cervical spinal cord over 3–4 months (Chen et al., 2004). Ultimately, a considerable and critical challenge will be to assess true functional integration of recruited adult-born neurons.

Taken together, these results demonstrate that endogenous neural precursors can be induced to differentiate into CNS neurons in a region-specific manner and at least partially replace neuronal populations that do not normally undergo neurogenesis. It appears that these results are generalizable to at least several classes of projection neurons, including the clinically relevant striatal medium spiny neurons and corticospinal motor neurons. Microenvironmental factors appear capable of supporting and instructing the neuronal differentiation and axon extension of adult-born neurons derived from endogenous precursors.

11. Function of adult neurogenesis and neural precursor cells in the adult brain

Given the restricted regional occurrence of neurogenesis in the adult mammalian brain, it is reasonable to hypothesize that there are specific contributions of adult neurogenesis to the functions of the hippocampus and the olfactory system (Kempermann et al., 2004; Magavi et al., 2005). Possibly, these functions differ fundamentally from those found elsewhere in the brain.

For the most part, the adult brain seems to do just fine without the ability to generate or regenerate neurons. Obviously, this characteristic of the mammalian CNS presents a disadvantage in cases of neuronal loss, but one theory is that this is the price paid during evolution for increasingly powerful and complex computing power (Rakic, 1985). This reasoning posits that the cardinal requirement for stability in the system precludes the capability for neuronal replacement. Despite its appeal and clarity, this theory has for years been at odds with the fact that, even without considering actual cellular replacement or addition via neurogenesis, there is amazing structural and synaptic plasticity in the brain. Nevertheless, the fact remains that there is normally no (or extremely little) neurogenesis outside the two specific neurogenic regions of the adult brain. This striking restriction of neurogenesis to these two regions suggests that we should be asking the question differently: What are the special functions of the dentate gyrus and olfactory bulb that evolutionarily maintain adult neurogenesis?

In simple terms, the hippocampus and olfactory system would appear to have little in common functionally. Whereas the hippocampus is one of the best-studied areas of the brain, and has been named the “gateway to memory” because of its important and indispensable role in learning and memory, much less is known about the olfactory system. However, when one considers evolutionarily important “olfactory perceptual learning,” and the similar output modulatory roles of dentate gyrus granule neurons and olfactory bulb granule neurons that have been postulated, the functions of neurogenesis in each system might indeed be more closely related.

A “gatekeeper” theory has been proposed to help explain the potential function of adult-born dentate gyrus granule neurons (Kempermann, 2002). This theory hypothesizes that new granule cells allow for optimization of the mossy fiber connections between the dentate gyrus and CA3 in accordance with the level of complexity and novelty frequently encountered by an individual. Because adult neurogenesis occurs at a bottleneck connection, even a few new neurons could have a large impact. Selectively eliminating the adult-born neurons would be the method of choice to test this idea. However, both irradiation (Santarelli et al., 2003) and treatments with cytostatic drugs (Shors et al., 2001) as a means of killing dividing precursor cells are less selective than would be desired, with many structural and potentially functional side effects (Monje et al., 2002). Therefore, despite being highly intriguing, results of such studies have remained ambiguous and difficult to interpret (Shors et al., 2002).

Similarly, while the precise function of new neurons in the olfactory bulb is not yet known, one theory is that high turnover of receptor neurons in the olfactory epithelium requires a similar degree of plasticity at the level of the synaptic targets in the olfactory bulb (Petreanu and Alvarez-Buylla, 2002). Recent evidence has identified an entirely

novel function of adult-born olfactory bulb granule neurons – they are especially responsive to novel odorants and undergo experience-dependent modification in response to familiarization with novel odorants presented as the neurons are integrating into functional circuitry (Magavi et al., 2005). Selective survival of activated neurons might underlie at least some of this change in population response. These results indicate that adult-born granule neurons provide a unique form of synaptic plasticity via insertion of entirely new cellular units into existing functional circuitry.

12. Consequences of disturbed adult neurogenesis and the reaction of neurogenic regions to disease

Because so little is known about the physiological function of adult neurogenesis, it has been difficult to evaluate the possible consequences of disturbed neurogenesis, although there are now several studies characterizing the response of the SVZ and dentate gyrus to disease conditions. Nevertheless, altered adult hippocampal neurogenesis has been proposed to play a role in a number of medical conditions; the most sound data supporting this role come from studies of temporal lobe epilepsy.

Experimental seizures are a robust inducer of cell proliferation and net neurogenesis (Bengzon et al., 1997; Parent et al., 1997). Because of this, dysregulated adult neurogenesis might provide an explanation for the development of the “granule cell dispersion” found in many cases of temporal lobe epilepsy. There is some evidence that ectopic neurons can form in seizure-induced neurogenesis and that they might contribute to seizure activity (Scharfman et al., 2000). In addition, there have been recent reports of increased neurogenesis in Alzheimer’s disease, as well as in a transgenic model of Alzheimer’s disease (Jin et al., 2004a,b). There is also reported increased cell proliferation and subsequent neurogenesis in the brains of Huntington’s disease patients (Curtis et al., 2003). Further, experimental depletion of dopamine in a rodent model of Parkinson’s disease has been reported to decrease precursor cell proliferation in both the SVZ and dentate gyrus (Hoglinger et al., 2004). The reproducibility and functional significance of these phenomena in neurological disorders has yet to be evaluated. For example, it is not yet clear whether and why neurogenesis increases in Huntington’s disease, yet a seemingly opposite response occurs in a model of Parkinson’s disease. It remains to be investigated whether these neurogenic changes are in direct response to pathogenic conditions, or whether they are downstream responses to a variety of pathological changes occurring in the brain.

Studies on the potential interaction of dysregulated adult hippocampal neurogenesis in epilepsy have suggested to some a possible role for disturbed neurogenesis in the pathogenesis of dementias. For example, presenilin mutations alter both amyloid processing and processing of the

developmentally important molecule Notch. It has been reported that such dysregulation leads to premature neuronal differentiation from precursors and depletion of the adult precursor population (Handler et al., 2000). Although there might be a connection, it is difficult to merge this idea with the overt lack of normal mammalian forebrain neurogenesis, and very few studies specifically deal with this quite speculative hypothesis.

Various forms of experimental data have been gathered to support another quite speculative hypothesis, that a failure of adult hippocampal neurogenesis might underlie major depression (Jacobs et al., 2000; D'Sa and Duman, 2002; Kempermann and Kronenberg, 2003). Patients with long-lasting depression have global hippocampal atrophy; however, it should be stressed that this change is not limited to the neurogenic dentate gyrus (Sheline et al., 1996). Furthermore, essentially all modes of antidepressant therapy appear to increase at least some aspect of adult dentate gyrus neurogenesis (Malberg et al., 2000). Santarelli et al. eliminated cell proliferation in the SGZ by selective irradiation and showed that this manipulation attenuated the effects of antidepressants on performance on an anxiety task (Santarelli et al., 2003). However, the interpretation of these experiments is somewhat controversial, because this behavioral test is not widely accepted as a model for depression, and because irradiation causes damage to the microvasculature of the SGZ (and potentially other structures) and thus alters this brain region beyond affecting neurogenesis (Monje et al., 2002). Further, in general, depression is not thought to be primarily a hippocampal disorder, and the presumed link to adult neurogenesis would need to depend strongly on the functional contribution of new dentate gyrus neurons to hippocampal function (Kempermann and Kronenberg, 2003). The fact that neurogenesis occurs only in the dentate gyrus, while hippocampal atrophy in depression and positive effects of therapies are widespread throughout the hippocampus, argues that these phenomena are not directly linked. Rather, the gross hippocampal changes are more likely diffuse alterations to dendrites and the neuropil. The most important aspect of the “neurogenesis theory” of depression might be that it brings neurons and hippocampal circuitry into a context that has been largely dominated by biochemical considerations and transmitter dysbalances. In any case, this theory of depression should still be considered speculative and in need of further rigorous analyses.

13. Conclusions and future prospects

Neurogenesis and neural precursor/stem cell biology in the adult CNS are important both toward potential future therapeutic applications for CNS repair, and regarding the fundamental function of the CNS. First, from the point of view of CNS repair, adult brain precursor/stem cell biology is highly relevant, because the generation, insertion, and

functional integration of new neurons is possible in the adult mammalian brain, and we might be able to direct the processes of in situ neurogenesis and ex vivo precursor differentiation toward therapies using cellular and molecular controls of cell-type specific differentiation (Arlotta et al., 2005). Second, from a more fundamental neuroscience point of view, it is important to learn about the normal role of precursor cells and the normal function of neurogenesis in the neurogenic regions of the adult CNS. This information might have immense implications for our understanding of brain function in development, normal adulthood, and disease states. Circuit plasticity at the level of individual cells, neuronal or glial, adds a new layer of complexity to our understanding of how brain structure and function interact. Better understanding of these issues may enable the prevention of disease and dysfunction beyond the already evident goals of trying to repair what is already degenerating or damaged. Such protection of normal brain function may offer important therapeutic options complementary to those possible by cellular repair. Indeed, maintaining cellular plasticity might be one of the most straightforward concepts in neuroprotection.

A better understanding of the cellular and molecular controls over differentiation of neural precursor cells along specific cellular lineages during development and in the adult CNS will be critical to potential cellular therapeutic approaches for repopulating damaged or diseased areas of the nervous system (Wichterle et al., 2002; Arlotta et al., 2005). The future prospect of directing the development and integration of precursors in the adult mammalian brain, toward the replacement of lost neurons or glia, is exciting indeed, and several recent lines of work provide remarkable progress toward this aim. Specifically, recent findings regarding the presence of neural precursors in a number of areas in the adult mammalian brain, ongoing adult mammalian neurogenesis, the possibility of activating even limited neurogenesis in normally non-neurogenic regions of the adult brain, and molecular/genetic controls over lineage-specific differentiation are advancing the field toward the goal of cellular repopulation and repair.

Although constitutive neurogenesis normally occurs in only two areas of the adult mammalian brain (SVZ and DG), recent research suggests that it may be possible to manipulate endogenous neural precursors in situ to undergo neurogenesis in other regions of the adult brain, toward future neuronal (or oligodendroglial) replacement therapy for neurodegenerative disease and other CNS injury. Multipotent precursors capable of differentiating into neurons, astroglia, and oligodendroglia exist in many regions of the adult brain. These precursors have considerable plasticity, and, although they may have limitations in their integration into some areas of the CNS, they appear capable of differentiating into neurons appropriate to a wide variety of regions, when either heterotopically transplanted, or, more recently, recruited in situ. Many adult precursors are capable of migrating long distances, using both

tangential and radial forms of migration. Endogenous adult neural precursors are also capable of extending axons significant distances through the adult brain. In addition, *in vitro* and *in vivo* experiments have begun to elucidate the responses of endogenous precursors to growth factors, molecular/genetic controls, and behavioral manipulations, and are beginning to provide key information toward control of their proliferation, specification, differentiation, and functional integration. For example, recent experiments demonstrate that endogenous precursors can differentiate into neurons, extend long distance axonal projections, and survive for long periods of time in regions of the adult brain that do not normally undergo neurogenesis. Taken together, these results indicate that there exists a sequence and combination of molecular signals by which neurogenesis can be induced in regions of the adult mammalian brain where it does not normally occur.

These results suggest that neuronal replacement therapies based on manipulation of endogenous precursors may be possible in the future. However, many questions must be answered before neuronal replacement therapies using endogenous precursors become a reality. The multiple signals that are responsible for endogenous precursor division, migration, differentiation, axon extension, circuit integration, and survival will need to be elucidated in order for such therapies to be developed efficiently. These challenges also exist for neuronal replacement strategies based upon transplantation of precursors, because donor cells, whatever their source, must interact with an extremely complex and intricate mature CNS environment in order to integrate into the brain. In addition, while it remains an open question, recent results in the field suggest that potential therapies manipulating endogenous precursors *in situ* would not necessarily be limited to portions of the brain near adult neurogenic regions. It appears that neural precursor cells, albeit in low numbers, might be much more widely distributed in the parenchyma of the adult CNS than previously thought. Another relatively unexplored theoretical possibility for cellular repopulation in the mammalian CNS is the mechanism used so successfully by urodeles and other amphibians: de-differentiation of seemingly mature cells and subsequent repopulation of damaged areas. While there are not yet data to directly support such de-differentiation strategies in mammals, it might be theoretically possible to reconstruct complex structures in the adult CNS as well. Although precursor cells might be widely distributed in the adult mammalian CNS, this population of cells might include precursors that exist as seemingly differentiated cells, expressing mature neural markers, while still retaining the ability to act as precursor cells in response to a highly orchestrated and regulated set of molecular signals. However, with the exception of the SVZ and the inner granular layer of the dentate gyrus, the adult mammalian CNS still appears to be a highly restrictive environment for neuronal production and integration under normal physiological conditions.

Looking forward, it might be possible to induce the cellular repopulation of the diseased brain and spinal cord via specific activation and differentiation of endogenous neural precursors along desired neuronal or glial lineages. Even if multipotent precursors are not located in large numbers outside of normally neurogenic regions of the brain, it might be feasible to induce them to proliferate and differentiate from small populations that are more widely distributed throughout the neuraxis. However, this field is just at the beginning of understanding the complex interplay between neural precursors' potential and signals in their local microenvironment; much can be learned about precursor heterogeneity and how to take advantage of what might be partial cell-type restriction, permissive and instructive developmental signals, and modulation of specific aspects of neuronal differentiation and survival. Progress over the past decade has been great, and the coming decades promise to offer significant insight into these and other critical issues for the field.

Acknowledgements

This work was partially supported by grants from the NIH (NS41590, NS45523, NS49553, MRRC HD18655), the Christopher Reeve Paralysis Foundation, the ALS Association, and the National Science Foundation to J.D.M. J.G.E. was partially supported by a Heart and Stroke Foundation of Canada Fellowship, a grant from the Paralyzed Veterans of America/Travis Roy Foundation, and a grant from the Children's Neurobiological Solutions Foundation to J.D.M. B.D.M. was partially supported by a LifeBridge-United Sydney Association Fellowship. We also wish to thank the organizers and participants from past Route 28 Summits in Neurobiology conferences for helpful and stimulating discussions related to this topic.

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